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Research articles in medicine & pharmacy

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Contents

Internal Medicine

Surgery

Medical Basic Sciences

CONTENTS

Patients' Perception: Hepatitis C Virus can be Transmitted during Medical Manipulations

Ieva Tolmane, Baiba Rozentale [1, 2], *Jazeps Keiss* [1], *Ludmila Ivancenko* [1], *Zaiga Reinholde* [1], *Nadezda Subnikova* [1], *Nina Sumlaninova* [1], *Ieva Reinholde* [1], *Raimonds Simanis* [2]

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Abstract

Prevalence of hepatitis C virus (HCV) infection in Latvia is relatively high – antibodies are found in 2.4% and HCV RNA in 1.7% of general population. The HCV spreads mainly via blood. The risk of infection is for intravenous (also intranasal) drug users, during tattooing, manicure and pedicure procedures, the virus can also be transmitted vertically from mother to child, sexually, in dialysis patients, an increased risk of infection is also observed in alcohol consumers. There are publications of increased risk of getting infected during different medical manipulations. There are no studies performed about patients' own perception on how they obtained infection.

The objective of this study was to assess patients' opinion on HCV transmission routes in Latvia.

There were 224 questionnaires filled-in by patients attending outpatient department of the Infectology Center of Latvia. There were 113 men (42%), 87 women (39%) and 24 patients who did not indicate sex (11%) among responders. Mean age of responders was 37 years (range 19–68). 129 patients (58%) were questioned prior and 95 (42%) patients after specific therapy.

Data were analyzed using Microsoft Excel 2003 and SPSS 14.0 programs.

Among all patients 96 (40%) had an opinion that they have been infected during different medical procedures – mainly surgical operations – 43 patients' opinion, 18 (7%) in the cosmetics saloon or during tattooing procedure, 4 (2%) got infected by a sex partner, 13 (5%) through intravenous drug use and 11 (4%) during work duty. Nevertheless, a big part of patients 96 (40%) did not have any opinion on where they obtained infection. Mean time from infection until questioning time according to patients' opinion was 8.7 years (range 1–35 years).

Our study draws a conclusion that majority of chronic VHC patients, based on their own opinion, have been infected during different medical procedures – surgical operations, visiting dentist or through blood transfusion.

Keywords: hepatitis C virus, medical manipulations, patients' perception.

Introduction

Chronic viral hepatitis C (VHC), due to its prevalence and clinical course, has become one of the most discussed infectious diseases worldwide. Currently, the number of infected persons worldwide is approximately 170 million [Chen, 2008], but in Europe it exceeds 9 million. Prevalence of hepatitis C in the countries of the European region according to the World Health Organization is from 0.1 to 4.5%.

Prevalence of hepatitis C virus (HCV) infection in Latvia also is relatively high – antibodies are found in 2.4% and HCV RNA in 1.7% of general population [Tolmane, 2009].

The HCV is the main reason for the development of chronic hepatitis, liver cirrhosis, hepatocellular carcinoma and for the necessity of liver transplantation. In 20% of the cases the infection progresses to cirrhosis and decompensated liver disease within 10 to 20 years. Currently only combination therapy and virus eradication can stop the progression of the disease. With the combination of pegylated interferon and ribavirin, sustained virological response (SVR) can be achieved in 54% to 63% of patients [Lettmeier, 2008].

The HCV spreads mainly via blood. The risk of infection is for intravenous (also intranasal) drug users, during tattooing, manicure and pedicure procedures, the virus can also be transmitted vertically from mother to child, sexually, in dialysis patients, an increased risk of infection is also observed in alcohol consumers [Heintges, 1997]. There are publications of increased risk of getting infected during different medical manipulations [Martinez-Bauer, 2008].

There are no studies performed about patients' own perception on how they got infected.

The aim

The objective of this study was to assess patients' opinion on HCV transmission routes in Latvia.

Material and methods

There were 224 questionnaires filled-in by patients attending open care unit of the Infectology Center of Latvia. The questionnaires consisted of 23 different questions on person's habits (smoking, alcohol consumption, medical treatment of other diseases, surgical, blood transfusion anamnesis, usage of narcotics, tattooing etc.). Additionally, the questions included in the survey allowed patients to analyse their opinions on when and where they obtained infection of HCV. There were 113 men (42%), 87 women (39%) and 24 patients who did not indicate sex (11%) among responders. Mean age of responders was 37 years (range 19–68). 129 (58%) patients were questioned prior and 95 (42%) patients after specific hepatitis C therapy with pegylated alpha interferon and ribavirin.

The study is approved by the Independent Ethics Committee for clinical investigation of drugs and pharmaceutical products.

Data were analyzed using Microsoft Excel 2003 and SPSS 14.0 programs.

Results

Among all patients 96 (40%) had an opinion that they have been infected during different medical procedures, 18 (7%) in the cosmetics saloon or while tattooing, 4 (2%) got infected by a sex partner, 13 (5%) through intravenous drug use and 11 (4%) during work duty. Nevertheless, a big part of patients 96 (40%) did not have any opinion on where they got infected (Figure 1).

Out of all respondents who indicated medical manipulation as a possible route of HCV transmission, 41 (43%) associated it with surgical operation, 38 (40%) with dentist's service, 10 (10%) with blood transfusion and 7 (7%) – other medical manipulations (Figure 2).

30 patients declared intravenous drug use in anamnesis, but only 11 (37%) presumed it as a possible route of infection, 10 patients (30%) had no idea how they got infected and 6 patients indicated surgery, dental procedures or sexual way as a possible route of HCV transmission (Figure 3).

Mean time from infection until questioning time according to patients' opinion was 8.7 years (range 1–35 years).

Figure 1. Patients' opinion on possible routes of HCV transmission

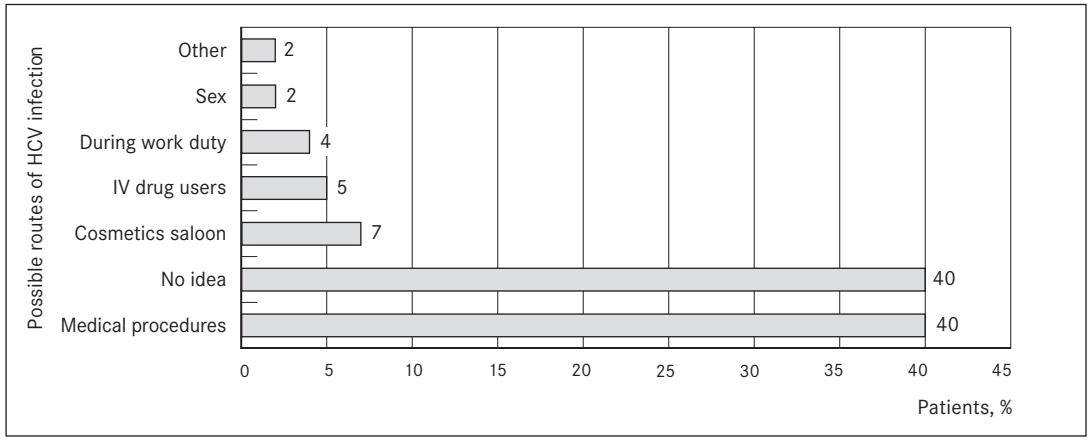


Figure 2. Patients' opinion on medical manipulations as a route of HCV transmission

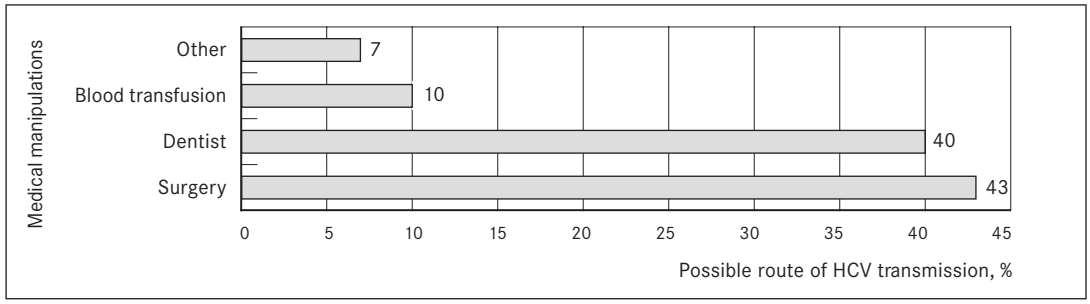
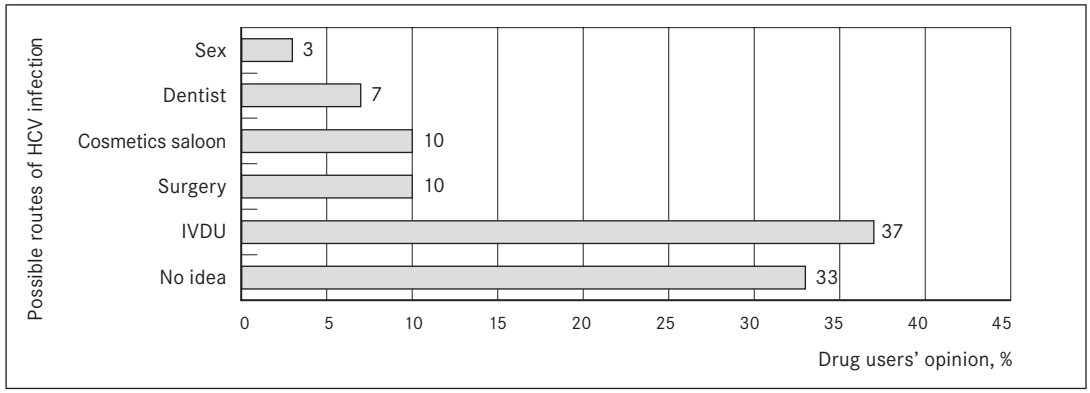


Figure 3. IV drug users' opinion on possible routes of HCV transmission



Discussion

This is the first study in Latvia done on patients' opinion about possible routes of transmission. Results revealed that majority of VHC patients concede – they have been infected during different medical manipulations – surgical operations, visiting dentist or through blood transfusion.

Although patients' opinion cannot be entirely accepted as true reasons of possible routes of transmission, there is very little room for contradiction as well. Transmission of hepatitis C and B viruses during medical procedures is the problem all over the world, also in developed countries. In Spain, during a study of possible ways of infection with acute VHC, it has been established that more than half of the patients were hospitalized during incubation period, thus infection with HCV was possibly

acquired during different medical processes [Martinez-Bauer, 2008]. There has been a report from hemodialysis center – the reduction of HCV distribution started right after strict request to change gloves before every patient.

Interesting data comes from intravenous drug users – only one third (37%) pointed intravenous drug usage as a possible way of getting infected, 33% had no idea, but the rest (30%) – indicated different medical procedures. This fact probably indicates very low patients' awareness of possible HCV transmission routes. But patient is the only information source about epidemiological anamnesis, due to the lack of test systems to detect when and how a patient has been infected. So patients' awareness of possible spread of disease and compliance is essential during treatment and monitoring process, also for epidemiological purposes and prevention of blood transmitted diseases.

Results of this study should be assessed critically due to their e subjectivity. Nevertheless the data are considerable and we can use them to improve patients' care and prophylaxis.

Conclusions

Our study draws a conclusion that majority of chronic VHC patients, based on their own opinion, have been infected during different medical manipulations – surgical operations, visiting dentist or through blood transfusion.

References

1. Chen W., Wong T., Tomlinson G., et al. Prevalence and predictors of obesity among individuals with positive hepatitis C antibody in a tertiary referral clinic // J of Hepatol, 2008; 49(5): 711–717.
2. Heintges T., Wands J. R. Hepatitis C Virus: Epidemiology and transmission // Hepatology, 1997; 26(3): 521–526.
3. Lettmeier B., Muhlberger N., Schwarzer R., et al. Market uptake of new antiviral drugs for the treatment of hepatitis C // J of Hepatol, 2008; 49(4): 528–536.
4. Martinez-Bauer E., Forns X., Armelles M., et al. Hospital admission is a relevant source of hepatitis C virus acquisition in Spain // J of Hepatol, 2008; 48(1): 20–27.
5. Tolmane I., Rozentale B., Keiss J., Arsa F., Brigis G. Prevalence of viral hepatitis C in Latvia: a population-based survey's results // RSU Scientific Papers 2009; 100–105.

HLA B27 Allele Types and DMARDs Therapy in Juvenile Idiopathic Arthritis Patients in Latvia

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Abstract

Juvenile idiopathic arthritis (JIA) is a heterogeneous condition and therapeutic strategies vary in different JIA types. The routinely accepted practice, to start with Sulphasalazine (SS) as the first line treatment in patients with HLA B27 positive JIA, proves to be ineffective in a large proportion of children.

In order to investigate HLA B27 positive JIA patient's clinical characteristics, determine HLA B27 allele types and their connection with antirheumatic treatment in homogenous patient groups, 56 patients with JIA were diagnosed and observed over the period from 2006 to 2009. HLA B27 allele types were determined using PCR (polymerase chain reaction) method.

In HLA B27 positive JIA patients mean disease onset was 12.34 ± 3.3 years. Most common (44%) JIA type was enthesitis related arthritis. Positive response to the treatment with SS was found in 32% of patients, Methotrexate (MTX) – in 43%, combined treatment – SS with MTX was effective in 12.5%. 12.5% of patients required combination of MTX with Enbrel.

Eight HLA B27 allele types were found in JIA patients in Latvia: *2702, *2703, *2704, *2705, *2710, *2715, *2717, *2728. The most common was *2705 – in 55% of cases. Among all the patients, enthesitis related arthritis most commonly occurred in patients with HLAB*2705 allele (OR = 2.01, $p < 0.02$), oligoarthritis in patients with *2710 allele (OR = 3.0, $p < 0.04$) and polyarthritis with *2717 allele (OR = 3.0, $p < 0.05$). In patients with *2705 allele effective treatment was MTX (OR = 1.13, $p < 0.03$) and MTX with SS (OR = 2.02, $p < 0.05$), but in patients having *2703 allele – MTX with Enbrel (OR = 2.94, $p < 0.02$).

There are 8 different HLA B27 alleles in JIA patients in Latvia and the most common is *2705. To improve JIA patient's quality of life achieving rapid disease control, it is necessary to determine HLA B27 type at the onset of treatment with DMRDs.

Keywords: HLA B27 allele, juvenile idiopathic arthritis, antirheumatic treatment, DMARDs, JIA.

Introduction

Juvenile idiopathic arhtritis (JIA) is the most common chronic rheumatic juvenile disease, causing substantial invalidity, social and psychological problems both for children and their parents. According to information sources, the incidence of the disease is 1–22 per 100,000 children/year and prevalence 8–150 per 100,000 children [1].

Nearly half of the patients continue carrying the disease also in adult age [2]. Radiology tests have proved that bone damages among most of the patients with systemic or polyarticular arthritis appear already within the first 2 years, and among patients with oligoarthritis – within 5 years of the disease. 50–70% of patients with systemic arthritis and 40–50% of patients with oligoarthritis in adult age, preserve the disease in its active phase. 30–40% of patients suffer from long-term joint dysfuncion, invalidity or physical incapacity, unemployment and 25–50% needs surgical help, including joint prosthetics [3]. JIA extra-articular manifestations are uveitis, mainly iridocyclitis, which occurs in 21% of oligoathritis patients, and in 10% of patients with polyarthritis.

During last 15 years, the tactics of medical care of JIA patients has remarkably changed. The approach now is early and aggressive, based on disease-modifying medication (DMARDs) to suppress the inflammation promptly and effectively. Thanks to this approach, the number of patients with functional disability has considerably decreased.

JIA is not a single disease, but a group of heterogeneous diseases that combines all kinds of chronic arthritis of unknown aetiology, which persist for more than 6 weeks and the onset is before 16 years of age. Heterogeneity of JIA becomes apparent in many ways: in the onset of disease, in clinical features, symptoms, in the progress of disease as well as in genetic basis. Up to now the origin of the disease is unclear, pathogenesis mechanism of the disease is still under research, but what matters are environmental factors (psychological stress, traumas, injuries, viral and bacterial infections) as well as different genes, which determine a person's characteristic respondent (counter-action) features to immunity and inflammation. Several studies have proved the importance of certain genes being a risk factor in the development of the disease. One of the most investigated genetic associations with certain rheumatic diseases is HLA alleles, which were first described already in 1973, when it was proved that there is an association between HLA B27 allele and ankylosing spondylitis (AS) among adults [5, 6]. It is documented that JIA with oligoarthritis has a positive association with several HLA class I and II gene alleles: HLA-A2, HLA-DRB1*11 (HLA-DR5 subtype) and HLA-DRB1*08. JIA with positive rheumatoid factor (RF) is considered to be an equivalent of adult RF positive rheumatoid arthritis (RA) and, like for adults, is associated with HLA-DR4. JIA type with negative RF is heterogeneous and HLA associations are not as distinct as of other types. Arthritis with enthesitis is associated with the presence of HLA B27 allele [8]. Arthritis with enthesitis is a disease, similar to spondyloarthropathies, but children, unlike adults for whom the dominating symptom is inflammatory backache, have their extra-axial joints involved in the process – mainly lower extremities. For some patients with arthritis with enthesitis the disease may advance and affect sacroiliac joints and spine, and within 5–10 years it may develop AS clinical symptoms; however, usually it takes many years before sacroilitis and spondylitis get visible radiologically [9]. It is known that in children HLA B27 alleles are connected with other JIA types, like oligo- and polyarthritis, but especially with arthritis with enthesitis and juvenile spondyloarthropathies [10–12].

Human leukocyte antigen HLA (Human Leukocyte Antigen) B27 or allele HLA B27 is a cell-surface protein and genetically the main cell compatibility complex MHC class I molecule [13]. It possesses several proven specific functions within immune system, but many functions stay still unclear. One of the main functions of this molecule is the ability to present peptide antigens, which result from intracellular degradation of proteins, to other cells of the immune system, mainly to CD8 T cells. Recent researches revealed the importance of this molecule in the function of natural killer cells [14].

It has been discovered that HLA B27 is not a single allele, but it represents a highly homologous HLA B alleles family, called types of alleles and denoted as HLA B*2701, B*2702 etc. These alleles differ from each other by one or several amino acids in peptide binding site, and by their diverse race/ethnic prevalence in the world population [15, 16].

In Latvia, according to the data of the Latvian Rheumatic Disease Patient Registry, in 2006 the JIA prevalence was in 861 patients, among which 119 carried positive HLA B27 allele. Each of HLA B27 positive patients may have a different type of disease (polyarhritis, oligoarthritis, arthritis with enthesitis), as well as a different degree of disease activity. Usually the presence of this allele determines the primary choice of therapy – treatment with Sulphasalazine (SS). SS is one of the first anti-rheumatic drugs and

the first drug that has been developed specially for RA treatment, today SS is considered as DMARDS group drug. In the 1930s Prof. Nanna Svartz, a rheumatologist at the Karolinska Institute in Stockholm, advanced a postulate that RA is a disease of bacterial aetiology and it should be treated with preparations of sulphonamide group, which at that were already available. Prof. Svartz noticed that in RA treatment, combining the antibiotic sulphanilamide with the salicylates of anti-inflammatory therapy did not bring any solid benefit, and produced significant gastrointestinal side effects. Svartz was of an opinion that these two medical products when combined chemically could at least decrease the gastrointestinal intolerance and asked Swedish Pharmaceutical enterprise Pharmacia in Upsala to merge chemically these two preparations. Thus linking sulphapyiridine to 5-aminosalicyilic acid, a new medication was derived with the name Sulphasalazine [17]. First reports on using SS in JIA therapy appeared in 1986, when Özdogan in his research work published the results on 18 JIA patients [18]. Since then the efficacy of SS in JIA therapy has been proved in several other studies [19–29].

In rheumatology efficacy of the therapy can be judged in every 3 months after the beginning of the treatment. Van Rossum, et al. in double-blind placebo-controlled trial showed, that SS is a safe and effective medication both managing the symptoms of arthritis, and improving the inflammation data in patients with oligo- and polyarticular JIA, though one third of the patients developed intolerance [19, 28]. SS is indicated also for patients with HLA B27 associated spondiloarthropathies [24]. Exact mechanism of SS performance is still not fully clear and is under continuous research.

The clinical praxis shows that SS therapy efficacy may vary with different JIA patients: in one part of patients SS takes a good control over the progress of arthritis, as well as decreases laboratory figures of inflammation, in other part of patients the SS efficacy is insufficient and it is necessary to combine or change the medication. Though, so far there is no scientifically grounded explanation found for such differences. Patients with insufficient therapeutic effect to SS receive a different basic drug – Methotrexate (MTX) instead. In severe cases of disease, combined therapy of MTX and SS is prescribed. If such conventional therapy, however, proves to be ineffective, the patient receives treatment with glycocorticosteroids or biological medication, such as TNF alpha receptor blocker – Etanercept.

Taking into account that JIA is a heterogeneous group of diseases, the therapy tactics for different types of the disease may differ. If it were possible already in early stage of the disease to predict, for which HLA B27 positive patients the SS therapy would be effective, and for which patients the therapy would not achieve the desired efficacy, then patients could be prescribed the most appropriate and effective medication from the very beginning, to get early control of the disease, because, as studies of long-term observations show, suppressing the activity of the disease already at an early stage of the disease has a favourable influence on the further progress of the disease, and the positive therapeutic effect can persist in a longer period of time [19].

In Latvia, for children with JIA no genetic studies have been performed on the incidence of allele types HLA B27, as well as their possible connection with different JIA types and choice of DMARDs therapy.

Hereby, hypothesis is raised that there might exist a connection between allele types HLA B27, JIA types and patient's reaction to anti-rheumatic therapy.

Material and methods

The study design was compound – prospective and retrospective, 56 HLA B27 positive JIA children at the age from 3 to 18 years were included in the study during the period of 2006–2009.

The JIA diagnosis and type of the disease was confirmed according to ILAR criteria. The efficacy of the treatment was evaluated and measured for every 3 months according to ACR Pediatric 30 clinical response and improvement criteria [20, 21]. The ACR Pediatric 30 uses the following 6 basic clinical response and improvement criteria: physician global assessment of overall disease activity; parent or patient global assessment of overall well-being; functional ability; number of joints with active arthritis; number of joints with limited range of motion and erythrocyte sedimentation rate. To consider a therapeutic response in JIA patients, the improvement has to be in at least 3 out of 6 criteria for 30% [21].

HLA class I HLA B27 allele was determined by polymerase chain reaction (PCR). 31 type of HLA B27 were defined (from B*2701-B*2727; *2705 type 5 sybtypes – *270502-270507).

DNA samples were taken starting with peripheral blood cells using the "salting-out" method [30, 31]. The genetic amplification was performed through PCR [30, 31]. MHC classes I antigens subtype identifying by PCR-SSP method that apply MHC class I allele: HLA-B*2701 to HLA-B *2727 specificity detection. These DNA samples were processed by SSP DYNAL Invitrogen Corporation USA.

DNA was extracted from whole blood using a "salting-out" technique. HLA-B loci were typed according to the DNA typing method described by Bunce et al. [32]. This polymerase chain reaction (PCR) technique uses sequence-specific primer (SSP) reactions to simultaneously identify alleles of the class I locus in an allele-specific or group-specific manner.

To identify class I alleles each PCR reaction consisted of in final concentration, 0.02_g DNA, 0.42 units Taq DNA polymerase, 1_L of 10X Buffer IV, 3.4 mM MgCl2, 250_M of each dNTP, 0.54_L glycerol, 0.01_L 100 mg/mL Cresol Red, and 5_L of the allele-specific and control primer mixes at a concentration of 1–4_M each. The PCR reaction mix was plated out into 96-well plates, and amplifications were carried. The cycling parameters used were as follows: 96 °C for 1 minute, followed by 14 cycles of 96 °C for 30 seconds, 65 °C for 50 seconds, and 72 °C for 20 seconds, followed by 25 cycles of 96 °C for 30 seconds, 62 °C for 50 seconds, 72 °C for 20 seconds, followed by 72 °C for 4 minutes. The PCR products were electrophoresed in a 1.5% agarose gel for approximately 35 minutes at 150 V in 0.5 Tris-borate-EDTA (TBE) and visualized using UV illumination.

The HLA-B*2701 to HLA-B *2727 allele frequencies in patients with juvenile idiopathic arthritis and control subjects were compared using the chi-square (χ^2) test and gene frequency (gf). The p value and odds ratio (OR) were calculated using EPI INFO software, version 06, with 95% confidence intervals and Fisher exact correction for small numbers [33].

Any experimental research that is reported in the manuscript has been performed with the approval of Central Medicine Ethics Committee of Latvia on March 26, 2006, act No 3 signed by the chairman of the committee Prof. Aivars Lejnieks. The study is in compliance with the Helsinki Declaration. Parents or legal guardians of participants had been given their informed consent before taking part in the study.

Results

The study included 56 white children – 25 boys (45%) and 31 girls (55%). Patient mean age was 12.34 ± 3.32 years (from 3 to 16 years). At the onset of disease 7% of patients were under 7 years and over 7 years – 93%. In the process of evaluation for received therapy we found 24 patients (43%) to be effective with MTX, 18 patients (32%) with SS. 7 patients (12.5%) required combined SS with MTX therapy, but in 7 patients (12.5%) due to ineffective standard therapy treatment with biological drug Etanercept with MTX were started.

According to ILAR criteria all JIA patients were divided in 3 groups: 23% of oligoarthritis, 33% – polyarthritis seronegative and 44% – enthesitis related arthritis.

In 96% of HLA B27 positive JIA boys was diagnosed enthesitis related arthritis and in 4% – polyarthritis. There were no oligoarthritis in boys diagnosed. In 58% of HLA B27 positive JIA girls polyarthritis was diagnosied, in 39% – oligoarthritis, but enthesitis related arthritis in 3%.

In JIA patients by typing HLA class I B27 with PCR *B2705 type (OR = 2.01, $p < 0.02$) was found more frequently (55%) in enthesitis related arthritis group, *B2710 (OR = 3.0, $p < 0.04$) in oligoarthritis group (11%) and *B2717 (OR = 3.0, $p < 0.05$) in polyarthritis patient group (9%). Other HLA B27 types were found respectively less (Table 1).

By analyzing the frequency of HLA B27 allele types between male and female, the most frequently (68%) found type in boys was *B2705, but *B2703 type in 12% of cases and just in boys.

In girls the most frequently found HLA B27 allele type was *B2705 – in 45% of all female patients, but *2704 type in 3% of cases and *B2715 in 6% of cases and was diagnosed just in girls.

In HLA B27 positive patients positive ANA was found in 18 cases (32%) with *2715 type (OR = 2.18, p < 0.05), but significantly less positive ANA cases in patients with *2702 (OR = 0.69, p < 0.01) type and *2717 (OR = 0.21, p <0.00) type (Table 2).

In evaluation the efficacy of treatment in JIA groups and HLA B27 types, the data showed statistically significant therapeutic effect of MTX (OR = 1.13, p < 0.03) and MTX with SS (OR = 2.02, p < 0.05) in *2705 type positive patients, but MTX with Etanercept combined therapy was necessary for *2703 type (OR = 2.94, p < 0.02) (Table 3).

Therapy with SS, which is the recommended start therapy in HLA B27 positive JIA patients, did not show significantly positive efficacy of treatment in any of HLA B27 allele type (Table 3).

Table 1. Association between JIA type and HLA B27 allele type

HLA B27 subtypes	JIA subgroups			All children with JIA (n = 56), *gf*
	Arthritis and enthesitis (n = 25), OR / χ^2 / p	Oligoarthritis (n = 12), OR / χ^2 / p	Polyarthritis (n = 19), OR / χ^2 / p	
*2702	0.04	–	0.16	0.07
*2703	0.08	–	–	0.05
*2704	–	0.08	–	0.03
*2705	**2.01/5.25/0.02**	0.39/0.021**	0.39/0.021**	0.56
*2710	0.04	**3.0/0.710/0.04**	0.11	0.03
*2715	–	0.08	0.05	0.07
*2717	0.04	–	**3.0/0.81/0.05**	0.07
*2728	0.08	–	0.11	0.10

* HLA B27 subtypes.

** Type protective statistically significant association for patients all JIA subjects.
Results are only shown for those alleles where there was evidence for a difference between JIA subgroups.
Bold-face type highlights statistically significant associations for patients all JIA subjects.

JIA – juvenile idiopathic arthritis.

gf – allele frequency; p – probability; OR – odds ratio and χ^2 values of OR are reported only for significant associations (p < 0.05).

n – number of patients.

Table 2. Association between ANA and HLA B27 allele type

HLA B27 subtypes	All children with JIA (n = 56)		OR	p <
	ANA positive, n = 18	ANA negative, n = 38		
*2702	1	3	0.69**	0.01
*2703	1	2	1.06	0.09
*2704	1	–	ND	–
*2705	11	20	1.41	0.59
*2710	1	1	0.39	0.01
*2715	1	1	2.18	0.05
*2717	–	4	0.21**	0.00
*2728	2	3	1.46	0.28

* HLA B27 subtypes.

** Type protective statistically significant association for patients all JIA subjects.
Results are only shown for those alleles where there was evidence for a difference between JIA subgroups.
Bold-face type highlights statistically significant associations for patients all JIA subjects.

JIA – juvenile idiopathic arthritis.

p – probability; OR – odds ratio and χ^2 values of OR are reported only for significant associations (p < 0.05).

n – number of patients.

ND – not defined.

Table 3. Association between effective treatment and HLA B27 allele type

HLA B27 subtypes	Treatment groups				
	SS + MTX (n = 7), OR / χ^2 / p	MTX (n = 24), OR / χ^2 / p	SS (n = 18), OR / χ^2 / p	MTX + Enbrel (n = 7), OR / χ^2 / p	All children with JIA (n = 56), *gf*
*2702	–	0.57/0.13/0.01**	2.26/1.75/0.65	–	0.07
*2703	–	0.08	–	**2.94/0.50/0.02**	0.05
*2704	–	–	3.24/0.38/0.22	–	0.02
*2705	**2.02/0.16/0.05**	**1.13/0.54/0.03**	0.05	0.6/0.35/0.02**	0.55
*2710	0.29	0.08	0.49/0.35/0.05**	0.14	0.11
*2715	–	0.04	3.24/0.38/0.22	–	0.04
*2717	–	0.08	0.06	0.14	0.07
*2728	–	0.08	0.11	0.14	0.10

* HLA B27 subtypes.

** Type protective statistically significant association for patients all JIA subjects.

Results are only shown for those alleles where there was evidence for a difference between JIA subgroups.

Group SS + MTX indicates combination therapy with Sulfasalazine and Methotrexate.

Group MTX indicates therapy with Methotrexate.

Group MTX + Enbrel indicates combination therapy with Methotrexate and Enbrel.

Bold-face type highlights statistically significant associations for patients all JIA subjects.

JIA – juvenile idiopathic arthritis.

gf – allele frequency; p – probability; OR – odds ratio and χ^2 values of OR are reported only for significant associations ($p < 0.05$).

n – number of patients.

Discussion

Genetic studies are of significant importance in order to comprehend the etiology and pathogenesis of JIA. Different studies have shown that there exists the correlation between HLA genes and certain JIA types [8]. The present study in Latvia is the first of this kind regarding analysis of HLA B27 allele types and their significance in children with JIA. Murray et al. having studied HLA data in 680 JIA patients, came to a conclusion that there exists a HLA specific age of years regarding onset of the disease, and it is unique for every JIA type [34]. In 1973 it was suggested that there was the association between incidence of AS and HLA B27. Since then onwards a great amount of scientific research has been performed on HLA B27, but no explicit knowledge of the role of this antigen in the disease pathogenesis, has been found up till now [6]. To date it has been found that HLA B27 is a heterogeneous allele group.

According to the published data, the most common HLA B27 allele types are B*2705 (in representatives of Caucasian race and American Indians), B*2704 (Asiatic), and HLA–B*2702 (Mediterranean population) [35]. All these alleles are linked also with AS. It has been found out that there are also other HLA B27 allele types associated with AS, like HLA–B*2701, B*2703, B*2707, B*2708, B*2710, B*2713, B*2714, B*2715, B*2719, and B*2725 [23]. The incidence of the largest part of these other alleles is small and rare, and their possible linkage with the disease has not yet been discovered completely. It has been suggested that there are 2 alleles, which do not have any connection with AS progression – HLA-B*2706, prevailing in Southeast Asia, and HLA-B*2709, which is found to be a prevailing allele on the Italian island Sardinia [36]. The two above mentioned alleles have not been detected in JIA patients in Latvia.

It is known, that HLA B27 allele has its role also in the paediatric population. 76% of children with arthritis and enthesitis have HLA B27 positive [37]. This type of JIA is associated with enthesitis and arthritis, and affects mainly boys after 6 years of age. In the neighbouring country Estonia, 28.6% of children with JIA have positive HLA B27 [38]. There are 13.8% HLA B27 positive JIA patients in Latvia. Bersntson et al. prospective studies with 305 JIA patients from three Nordic countries (Sweden, Norway

and Denmark) concluded that HLA B27 is of great significance for boys with JIA. For HLA B27 positive boys, unlike patients with negative HLA B27 allele, it is typical that JIA is more active during the first three years of affection, with involvement of lower extremity joints [39].

According to the data of our studies in Latvia, boys and girls, primarily after 7 years of age, equally frequently develop HLA B27 positive JIA, the most common JIA type being arthritis with enthesitis (44%). However, analysing the JIA type incidence among boys and girls, it is boys after 7 years of age who most often develop arthritis and enthesitis (96%), whereas girls – polyarthritis (58%) and oligoarthritis (39%). Other published research data confirm these correlations [5].

It was detected that in Latvia in children with HLA B27 positive JIA is associated with 8 HLA B27 allele types: *2702, *2703, *2704, *2705, *2710, *2715, *2717, *2728; however, credibly the most frequent types are HLA B*2705,*2710 and *2717. Analyzing the data about the relationship between JIA type and HLA B27 allele type, credibly positive association was defined in such correlations as arthritis with enthesitis with *2705 type ($OR = 2.01$, $p < 0.02$), polyarthritis with *2717 ($OR = 3.0$, $p < 0.05$) and oligoarthritis with *2710 type ($OR = 3.0$, $p < 0.04$). These findings once again show that according to the published data, JIA is a heterogeneous disease and genetic predisposition varies with different JIA types [17].

There exists HLA B27 association with acute anterior uveitis (AAU) [40]. In our study uveitis was diagnosed in 3 patients and all three patients had B*2705 type. AAU involves serious complications like glaucoma, cataract, ocular hypotony, formation of synechia, and consequently may lead to visual impairment and deterioration in the quality of life [41]. Frequently AAU progress may be asymptomatic, for that reason a timely diagnostics and treatment are of utmost importance in prevention of complications. The number of patients with uveitis involved in this study is insufficient to be able to develop any recommendations, but we believe that patients with *B2705 type need more frequent slit lamp examinations at oculist's.

Today MTX is considered to be the basic medication from among DMARDs in JIA therapy [42], and during our study, when treating with MTX, therapeutic effect was reached in 43% of JIA patients. But guidelines of JIA therapy recommend that the treatment of HLA B27 positive JIA cases should be started with SS, but evaluating the efficacy of treatment according to ACR Pediatric 30 criteria, our findings revealed that with SS the therapeutic effect was reached only in 32% cases, and still 12.5% of patients needed SS combination with MTX, but for 12.5% of patients it was necessary to remove SS, and therapeutic effect was reached only by MTX or MTX in combination with Etanercept.

Further comparing the efficacy of treatment in homogeneous JIA type and HLA B27 allele type groups, it was found that boys after the age of 7, who most frequently developed arthritis and enthesitis and who credibly most often had B*2705 type, after 3 months of insufficient efficacy of treatment with SS produced therapeutic efficacy with MTX and SS with MTX.

Analyzing the girl group separately, we found out that also for girls who in 3% of all cases developed arthritis and enthesitis and where also B*2705 was present, credible therapeutic effect could be reached with SS with MTX, but for girls in cases with the association of *2710, SS does not produce any therapeutic effect. Likewise in cases of polyarthritis, which are frequent in girls, and where the association with *2717 was found a credibly effective DMARDs therapy was not proved.

Patients with *2703 need combined therapy – biological with MTX.

The obtained data of the efficacy evaluation suggest that the conventional primary JIA therapy with SS does not produce the desired therapeutic effect, and in order to keep control of the disease and to improve the quality of life for children, MTX should be chosen as primary therapy. However, in order to apply the most appropriate therapy from the early onset of the disease, it would be really necessary to determine the HLA B27 allele type, and aimfully launch a therapy with MTX, SS, MTX with SS or MTX with Enbrel. Moreover, to make the obtained data more exact and credible, it would be necessary to survey all HLA B27 positive JIA patients in Latvia.

Analyzing ANA connection with HLA B27 allele types and therapy efficacy in homogeneous JIA type groups, no credibility was found, it can be concluded that ANA most probably indicates the prognosis of the disease and not disease severity as it has already been proved in rheumatology with other autoimmune diseases, when, for instance, autoantibody, positive RF, indicates to a prognosis of worsening and faster progressing course of disease in the case of seropositve JIA [10].

The study shows again, that JIA is a group of heterogeneous diseases with diverse genetic basis. It explains also the fact that patients with different JIA types have varied reaction to the therapy. It would be very useful to continue genetic studies on JIA and HLA B27 allele types as nuzzling of the genetic material is a most likely way to find the most suitable therapy, which, in its turn, is a key to more favourable prognosis of the disease.

Conclusions

Our findings indicate a trend to have HLA B27 positive JIA in children over 7 years of age and the most common type is enthesitis related arthritis. Girls tend to have HLA B27 positive oligo- and polyarthritis, where boys most often have enthesitis related arthritis.

In HLA B27 positive JIA patients the most effective disease modifying antirheumatic drug is MTX – in 43% of the study patients, SS – 32%, but SS with MTX – 12.5% of the study patients. Combined biologic therapy with MTX and Etanercept was necessary for 12.5% of the patients.

There are 8 different HLA B27 alleles in JIA patients in Latvia and the most common significantly are *2705, *2710, *2717.

HLA B*2705 allele is significantly often found to enthesitis related arthritis group, *2710 in oligoarthritis group and *2717 – in polyarthritis patient group.

The data revealed a statisticcaly significant efficacy of treatment of MTX and MTX with SS in *2705 allele type patients, but MTX with Etanercept was effective in *2703 allele type patients.

To improve children's quality of life achieving rapid disease control, the first line treatment in HLA B27 positive patients should be MTX. In order to start with the most appropriate drug: MTX, SS, MTX with SS or MTX with Enbrel, it is necessary to determine HLA B27 type at the onset of disease. But in order to assert the above mentioned data, more extensive studies are needed; and this cannot be considered as a statement, it is only a supposition. In order to develop the supposition into a statement, it is necessary to continue the experiments by increasing the group under the study, including all HLA B27 positive JIA patients in Latvia which would lead to the development of good practice guidelines.

Competing interests

There are no financial and non-financial interests of all the authors.

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References

1. Gäre B. A. Juvenile arthritis: who gets it, where and when? A review of current data on incidence and prevalence // Clin Exp Rheumatol, 1999; 17: 367–374.
2. Cassidy J. T., Petty R. E., Laxer R. M. Textbook of pediatric rheumatology. – 5th ed. – Philadelphia: Elsevier, 2005.
3. Packham J. C., Hall M. A. Long-term follow-up of 246 adults with juvenile idiopathic arthritis: functional outcome // Rheumatology, 2002; 41: 1428–1435.
4. Petty R. E., Southwood T. R., Baum J., et al. Revision of the proposed criteria for idiopathic arthritis: Durban 1997 // Journal of Rheumatology, 1998; 25: 1991–1994.
5. Brewerton D. A., Hart F. D., Nicholls A., Caffrey M., et al. Ankylosing spondylitis and HL-A 27 // Lancet, 1973; 1: 904–907.
6. Schlosstein L., Terasaki P. I., Bluestone R., Pearson C. M. High association of an HL-A antigen, W27, with ankylosing spondylitis // N Engl J Med, 1973; 288: 704–706.
7. Ball E.J., Khan M.A. HLA-B27 polymorphism // Joint Bone Spine, 2001; 68: 378–382.

8. Thomson W., Donn R. Juvenile idiopathic arthritis genetics – what's new? What's next? // Arthritis Res, 2002; 4: 302–306.
9. Gensler L., Davis J. C. Jr. Recognition and treatment of juvenile-onset spondyloarthritis // Curr Opin Rheumatol, 2006; 18(5): 507–511.
10. Ravelli A., Martini A. Juvenile idiopathic arthritis // Lancet, 2007; 369: 767–778.
11. Burgos-Vargas R., Pacheco-Tena C., Vazquez-Mellado J. Juvenile-onset spondyloarthropathies // Pediatr Rheumatol, 1997; 23: 569–598.
12. Rachelefsky G. S., Teresaki P. I., Katz R. Increased prevalence of Bw27 in juvenile rheumatoid arthritis // N Engl J Med, 1974; 290: 892–893.
13. Taurog Joel D. The Mystery of HLA-B27: if it isn't one thing, it's another // Arthritis & Rheumatism, Aug 2007, 56(8): 2478–2481.
14. McMichael A., Bowness P. HLA-B27: natural function and pathogenic role in spondyloarthritis // Arthritis Res, 2002; 4(suppl 3): S153–S158.
15. Hülsmeyer M., Hillig R. C., Volz A., Rühl M., et al. HLA-B27 subtypes differentially associated with disease exhibit subtle structural alterations // J Biol Chem, 2002; 277(49): 47844–47853.
16. Akkoc N., Khan M. A. Etiopathogenic role of HLA-B27 alleles in ankylosing spondylitis // APLAR Journal of Rheumatology, 2005; 8: 146–153.
17. Brooks C. D. Sulphasalazine for the management of juvenile rheumatoid arthritis // J Rheumatol, 2001; 28: 845–853.
18. Özdogan H., Turunc M., Deringol B., et al. Sulphasalazine in the treatment of juvenile rheumatoid arthritis: a preliminary open trial // J Rheumatol, 1986; 13: 124–125.
19. Van Rossum M., et al. Long-term outcome of juvenile idiopathic arthritis following a placebo-controlled trial: sustained benefits of early sulphasalazine treatment // Ann Rheum Dis, 2007; 66: 1518–1524.
20. Ringold S., Wallace C. A., Hart J. A. Measuring clinical response and remission in juvenile idiopathic arthritis: measures of disease activity // Curr Opin Rheumatol CME, 2007; 19(5): 471–476.
21. Giannini E. H., Ruperto N., Ravelli A., Lovell D. J. Preliminary definition of improvement in juvenile arthritis // Arthritis Rheum, 1997; 40(7): 1202–1209.
22. Reveille J. D. HLA-B27 and the seronegative spondyloarthropathies // Am J Med Sci, 1998; 316: 239–249.
23. Frosch M., Ganser G., Herter B., et al. Treatment of juvenile rheumatoid pauciarthritis with sulphasalazine // Clin Exp Rheumatol, 1995; 13: 560–566.
24. Imundo L. F., Jacobs J. C. Sulphasalazine therapy for juvenile rheumatoid arthritis // J Rheumatol, 1996; 23: 360–366.
25. Huang J. L., Chen L. C. Sulphasalazine in the treatment of children with chronic arthritis // Clin Rheumatol, 1998; 17: 359–363.
26. Gedalia A., Barash J., Press J., Buskila D. Sulphasalazine in the treatment of pauciarticular-onset juvenile chronic arthritis // Clin Rheumatol, 1993; 12: 511–514.
27. Romicka A. M., Siemienska-Rywik S., Rostropowicz-Denisiewicz K. An assessment of the results of sulphasalazine treatment in patients with juvenile rheumatoid arthritis // Reumatologia, 1994; 32: 221–225.
28. Van Rossum M. A. J., Fiselier T. J. W., Fransen M. J. A. M., et al. Sulphasalazine in the treatment of juvenile chronic arthritis: a randomized, double-blind, placebo controlled, multi-center study. Dutch Juvenile Chronic Arthritis Study Group // Arthritis Rheum, 1998; 41: 808–816.
29. Burgos-Vargas R., Vazquez-Mellado J., Pacheco-Tena C., Hernandez-Garduno A., et al. A 26 week randomised double blind, placebo controlled exploratory study of sulphasalazine in juvenile onset spondyloarthropathies // Ann Rheum Dis, Oct 2002; 61(10): 941–942.
30. Olerup O., Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: An alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation // Tissue Antigens, 1992; 39: 225–235.
31. Miller S. A., Dykes D. D., Polesky H. F. A simple salting out procedure for extracting DNA from human nucleated cells // Nucleic Acid Res, 1988; 16: 1215–1218.
32. Bunce M., O'Neill C. M., Barnardo M. C. N., Krausa P., et al. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilising sequence-specific primers (PCR-SSP) // Tissue Antigens, 1995; 46: 355–367.
33. Mehta C. R., Patel N. R., Gay R. J. Pascal program by ELF Franco & N Compos-Filho Ludwig Cancer Institute: Mathematics Software StatCalc. São Paulo, Brazil: Am. Stat. Assoc, 1985; 78: 969–973.
34. Murray K. J., Moroldo M. B., Donnelly P., et al. Age specific effects of juvenile rheumatoid arthritis-associated alleles // Arthritis Rheum, 1999; 42: 1843–1853.

35. Blanco-Gelaz M. A., Lopez-Vazquez A., Garcia-Fernandez S., et al. Genetic variability, molecular evolution, and geographic diversity of HLA-B27 // Hum Immunol, 2001; 62: 1042–1050.
36. Ren E. C., Koh W. H., Sim D., et al. Possible protective role of HLA-B*2706 for ankylosing spondylitis // Tissue Antigens, 1997; 49: 67–69.
37. Thomson W., Barrett J. H., Donn R., et al. Juvenile idiopathic arthritis classified by the ILAR criteria: HLA associations in UK patients // Rheumatology, 2002; 41: 1183–1189.
38. Pruunsild C., Uibo K., Liivamägi H., Tarraste S., et al. Incidence of juvenile idiopathic arthritis in children in Estonia: a prospective population-based study // Scand J Rheumatol, 2007; 36: 7–13.
39. Berntson L., Damgard M., et al. For the Nordic Paediatric Rheumatology Study HLA-B27 predicts a more extended disease with increasing age at onset in boys with juvenile idiopathic arthritis // J Rheumatol, 2008; 35(10): 2055–2061.
40. Chang J. H., Mc Cluskey P. J., Wakefield D. Acute anterior uveitis and HLA-B27 // Surv Ophthalmol, Jul-Aug 2005; 50(4): 364–388.
41. Huhtinen M., Karma A. HLA-B27 typing in the categorisation of uveitis in a HLA-B27 rich population // Br J Ophthalmol, 2000; 84(4): 413–416.
42. Hashkes P. J., Laxer R. M. Medical treatment of juvenile idiopathic arthritis // JAMA, 2005; 294: 1671–1684.

Non-pharmacological Treatment and Rehabilitation of Depressive Patients: Effectiveness of Methods and Availability of Acute Mental Hospitals for Adults in Latvia

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Abstract

Depression is not only medical, but also a social problem. Patient's quality of life decreases, and there is an increase in rates of disability. That is why it is necessary to use pharmacological and non-pharmacological methods of treatment and rehabilitation. The aim of this study is to analyze the scientific literature about the effectiveness of non-pharmacological methods of treatment and rehabilitation and availability of these methods in psychiatric hospitals of Latvia. The obtained data of this study show evidence based efficacy of psychotherapeutic methods of treatment, and also music therapy, relaxation techniques and physical exercises. Non-pharmacological methods of treatment are used only partially in acute psychiatric hospitals for adults in Latvia. Thus, it is necessary to include non-pharmacological methods of treatment in rehabilitation of depressive patients and to expand their availability in psychiatric hospitals of Latvia.

Keywords: depression, rehabilitation of depressed patients, non-pharmacological treatment of depression.

Introduction

Clinical depression is a syndrome characterized by a number of behavioural, cognitive and emotional features. Depressed patients often exhibit signs of depressed (low) mood, loss of interest in normally enjoyable things, self neglect and social withdrawal, poor appetite or overeating, insomnia or hypersomnia, fatigue or loss of energy, low self esteem, poor concentration or difficulty making decisions, and feelings of hopelessness. Depression is recognized as a major public health problem, which has a substantial impact on individuals and society. In the United States life time prevalence of a major depressive disorder (MDD) has been reported at 16.2% [Kessler, 2009]. The number of people affected by major depressive disorder in Europe has reached 30.3 million and the best estimate for 12-month prevalence has been reported at 6.9% in 2010 [Wittchen, 2011]. The World Health Organisation has described depression as an "unseen burden" [Beach, 1992; Kessler, 2009]. MDD is associated with a significant loss of work days, but also substantial role impairment in relation to household responsibilities, social life, and personal relationships [Kessler, 2009]. Mood disorders have, in addition, been shown to have a greater impact on quality of life compared with conditions such as hypertension and cardiac disease. There is a range of treatments that can be effective for people with depression. Treatments may include psychological therapies or/and medication. Depression is a well-recognized reason for seeking alternative therapies. Whilst this

may reflect dissatisfaction with conventional treatments, another possibility is that alternative therapies may be more in line with people's own beliefs and philosophies [DOH, 2001]. There has been increasing interest in the potential role of alternative therapies such as music therapy, light therapy, acupuncture, family therapy, marital therapy, relaxation and exercise for the management of depression.

That is why it is important to analyze the publications related to using alternative therapies in rehabilitation of depressive patients and conduct the data of accessibility of rehabilitation approaches in mental in-patient departments of Latvia.

The aim

The aim of the study is to determine the effectiveness of non-pharmacological treatment and rehabilitation methods in the treatment of depression based on publication data of systematic reviews, meta-analytical data and to research the data of availability of non-pharmacological treatment and rehabilitation approaches in mental in-patient departments of Latvia.

Material and methods

Medline, Dynamed, Embase, Sports Discus, PsycINFO, the Cochrane Controlled Trials Register, and the Cochrane Database of Systematic Reviews were used for eligible study of April, 2010. In addition, hand-search of several relevant journals was carried out, and experts in the field were contacted. Selection criteria included published systematic reviews, meta-analytical data and randomised controlled trials, in which methods of non-pharmacological treatment and rehabilitation of depressed patients were described.

The data of availability of non-pharmacological treatment and rehabilitation approaches in mental in-patient departments of Latvia were researched by interviewing administration of hospitals and analysing documentations of mental hospitals (staff employee schedule, descriptions of staff employee and functional responsibilities and technologies and certifications of structural departments in April, 2010). Regional hospital's names will appear in the text as "A", "B", "C", "D" and "E" mental hospitals.

Results

Depressive disorders can be reliably diagnosed and effectively treated. Current practice guidelines for the treatment of major depressive disorder recommend pharmacotherapy, psychotherapy or a combination of both [APA, 1994]. Pharmacologic treatment for major depressive disorder includes tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRI), monoamine oxidase inhibitors, and selective norepinephrine reuptake inhibitors. With regard to psychotherapy, a wide range of psychotherapeutic models are now being used, including cognitive behavioural therapy (CBT) and interpersonal therapy (IPT) [Churchill, 2001]. These interventions are all directed at the individual worker and target the depressive symptoms. Alternative treatments and practices vary in how much they have been studied and how effective they have been found to be in helping people with depression disorders. A recent study reviewed a range of practices: evidence base of alternative treatments for depression: Cognitive behaviour therapy, St John's wort (herb), physical exercise, self-help books involving some evidence: acupuncture, light therapy (for non-seasonal depression), massage therapy, negative air ionisation (for winter depression), relaxation therapy, yoga breathing exercises.

Psychotherapy for the treatment of depression. Cognitive behavioural therapy and interpersonal psychotherapy reduce symptoms of mild to moderate depression, although many of the trials have been insignificant [Butler, 2006]. Cognitive behavioural therapy Brief (6–20 sessions over 12–16 weeks) is a structured treatment incorporating elements of cognitive therapy and behavioural therapy. Behavioural therapy is based on learning theory and concentrates on changing behaviour. Cognitive therapy Brief is a structured treatment aimed at changing the dysfunctional beliefs and negative automatic thoughts that characterise depressive disorders. It requires a highly trained therapist.

Interpersonal psychotherapy is a standardised form of individual Brief psychotherapy (usually 12–16 weekly sessions) primarily intended for outpatients with unipolar depressive disorders without psychotic features. It focuses on improving a person's interpersonal functioning and identifying the problems associated with the onset of the depressive episode. Combining psychological treatment with antidepressant drugs may be more effective than either treatment alone.

The aim of psychodynamic supportive psychotherapy is to facilitate change by detecting and resolving underlying psychological conflicts. The treatment aims to be less challenging by incorporating supportive elements.

Problem solving therapy consists of three stages: identifying the main problems for a person, generating solutions, and trying out the solutions. This therapy is potentially briefer and simpler than cognitive therapy and may be feasible in primary care. Non-directive counselling may also be effective, but we do not know whether problem-solving therapy or befriending is beneficial. Care pathways may improve the effectiveness of treatment for depression [Bower, 2003]. Use of patient-centred, motivational approaches involves encouraging people to actively participate in their own care.

Individuals suffering from depression often report significant problems in multiple areas of their family functioning, suggesting the need for a more family-oriented approach such as family therapy, or combinations of family therapy with individual psychotherapy and pharmacotherapy in the treatment of depression [Keitner, 2003]. Family therapy may be defined as any psychotherapeutic endeavour that explicitly focuses on altering interactions between or among family members and seeks to improve the functioning of the family as a unit, or its subsystems, and/or the functioning of the individual members of the family [Cottrell, 2002]. The goal of family therapy for depression is to work with patients and their families to disengage from destructive forms of communication, and through that process, reduce the symptoms of depression. Recent Cochrane review identified three high-quality and three low-quality studies, involving 519 people with depression [Henken, 2009]. The author's conclusions were that the current evidence base is too heterogeneous and sparse to draw conclusions on the overall effectiveness of family therapy in the treatment of depression. At this point, use of psychological interventions for the treatment of depression for which there is already an evidence-base would seem to be more preferable to family therapy.

Six studies were selected on a priori determined criteria in Cochrane collaboration review of efficacy of family therapy for depression. The authors emphasize, that these studies were largely non-combinable from several aspects, including intervention (participants were treated from very different approaches such as cognitive, behavioural, systemic, psychoeducational or other more unfamiliar theories of family therapy intervention), participants (adults, adolescents and children), and the disorder (measurement instruments). Consequently, an overall conclusion concerning the effectiveness of family therapy for depression could not be drawn. Despite the existence of some good quality studies and some positive findings, evidence for the effectiveness of family therapy for depression does not exceed level 3 (limited or conflicting evidence), except for moderate evidence (level 2) that family therapy is more effective than no treatment or waiting list condition in reducing depression levels and in increasing family functioning. It should be noted that good evidence (two or more high quality trials with consistent findings) could not be established.

Marital therapy, which is mainly based on social learning theory, has been suggested, on the basis of several converging lines of evidence, as an approach for couples where there is a depressed spouse [Beach, 1992]. Marital therapy has the two-fold aim of modifying negative interactional patterns and increasing mutually supportive aspects of couple relationships. In recent Cochrane review, authors concluded that there is no evidence to consider marital therapy as being more or less effective than individual psychotherapy or drug therapy for depression, the evidence for improvement in couple relationships due to marital therapy may favour the choice of marital therapy when marital distress is perceived as a major problem [Barbato, 2009]. Although the large effect favouring marital therapy versus no treatment may suggest that marital therapy could be included among psychological approaches considered effective in the treatment of depression, the finding is only based on two insignificant studies. The results of

the comparison with drug therapy are greatly influenced by a single study [Leff, 2000], in which there is a strong suggestion of a selection bias, possibly leading to the inclusion of patients seeking an alternative to drug treatment. Even in the two studies by Emanuels [Emanuels, 1996; Emanuels, 1997], where two different models of marital therapy were used with distressed and non-distressed couples, no difference in reduction of depressive symptoms was acknowledged, despite the obvious difference regarding the reduction of marital distress in distressed couples. Moreover, even in the studies showing a clear effect on marital distress, this was not coupled by a corresponding improvement in depression. The available evidence suggests that there is no reason to consider marital therapy more or less effective than individual therapy in the treatment of depression, even when associated with marital distress. However, an improvement in couples' relationship, at least in distressed couples, can be expected from marital therapy. This finding may justify the choice of marital therapy when marital distress is a major problem or attention to the quality of marital relations is warranted. Moreover, the available data cannot be applied to the elderly and to people with major depression, because the samples studied included only people with mild to moderate depression.

Booklets or videos, which deliver information about the illness, its prognosis, its treatment, and simple cognitive and behavioural self treatment approaches, may be made available for patients and cares [Butler, 2006]. One or more professionals may deliver group teaching sessions on depression and how to recover.

Alternative treatments and practices for the treatment of depression. The effect of exercise on depression has been the subject of research for several decades and is believed by a number of researchers and clinicians to be effective in the treatment of depression [Beesley, 1997]. Research indicates that exercise improves mood, making people with depression less negative, angry, tired, and anxious. It can also have a positive effect on self-esteem and improve sleep. A recent study concluded that just 30 minutes of moderate exercise on most or all days of the week can help both prevent and treat depression. Recent Cochran systematic review authors' conclusions were: exercise seems to improve depressive symptoms in people with diagnosis of depression, but when only methodologically robust trials are included, the effect sizes are only moderate and not statistically significant [Mead, 2010]. However, it is likely that this result is biased in favour of exercise for methodological reasons. When only those trials with blinded outcome assessments were included in the analysis, the effect size was only moderate, though of borderline statistical significance. When only those trials with intention to treat analyses were included, the effect size was similarly moderate but only of borderline statistical significance. When only those trials fulfilling all three methodological criteria, including allocation concealment, were included, the effect size was moderate and not statistically significant. Effect sizes were higher for mixed exercise and resistance exercise than aerobic exercise alone, but confidence intervals were wide and other methodological aspects of the trials may have confounded these analyses. The authors excluded trials which compared two types of exercise intervention without a non-exercising control group, as these were not relevant to our particular research question. When compared with other established treatments (CBT and antidepressants), there was no difference between exercise and the established intervention. The effect of exercise on depressive symptoms in those trials which included long-term follow-up was only moderate; suggesting that the benefits of exercise may gradually be lost after the intervention is completed, implying that exercise may need to be continued long-term in order to maintain the initial benefits. The authors suggest that the mechanisms by which exercise may improve depression remain uncertain. There was no clear relationship between the duration of the exercise intervention and outcomes. If exercise improves mood via improvements in fitness, authors would have expected the trials which were longer in duration to demonstrate the bigger effect sizes. Only half of the trials which related mood to indices of physical fitness found significant correlations. One trial found that more intense exercise led to larger improvements in mood [Singh, 2005]. All the trials except one provided supervised exercise, so authors cannot determine whether the improvements in mood may have been mediated by social contact with the person supervising the exercise. One trial found no difference between supervised and home-based exercise [Blumenthal, 2007].

Acupuncture has a long history of use in China and Japan. Traditional Chinese Medicine (TCM) and Classical Acupuncture are based on theoretical concepts of Yin and Yang and the Five Elements and explain disease and physiological function. Medical acupuncture may involve the application of acupuncture based on the principles of neurophysiology and anatomy, rather than TCM principles and philosophy. Auricular therapy involves the use of the ear to make a diagnosis and subsequent needling to points on the ear. There are studies indicating a preference for treatment with self-help and complementary therapies for depression. Thirty trials, and 2,812 participants were included in the review and meta-analysis, however there was insufficient evidence that acupuncture can assist with the management of depression. The recent Cochrane systematic review contains data from 30 studies [Smith, 2010]. Thirty trials with 2,812 participants were included in the Cochrane collaboration meta-analysis "Acupuncture for depression". There was a wide diversity in acupuncture interventions and comparator groups used in the evaluation of acupuncture to treat depression. A number of single trials reported significant findings. In two small trials, manual acupuncture reduced the severity of depression compared with a wait list control (WMD –0.73, 95% CI –1.18, –0.29); however, one of these trials had a high risk of bias. One trial of acupuncture compared with sham acupuncture found a reduction in depression (RR 3.67, 95% CI 1.36, 9.91). One small trial found a reduction in the severity of depression from laser acupuncture compared with sham laser but had a high risk of bias due to attrition. Two trials where acupuncture was used in combination with standard medication suggest some benefit with reducing symptoms of depression. One small trial of manual acupuncture plus sertraline, and/or venlafaxine / mirtazepine versus medication found a reduction in the severity of depression (SMD –1.06, 95% CI –1.69, –0.43). One small trial of electro-acupuncture plus SSRI compared with SSRI alone found a reduction in the severity of depression compared with medication alone (SMD –0.70, 95% –1.32, –0.07). Majority of trials comparing manual or electro-acupuncture with medication found no evidence in difference between groups with reducing symptoms of depression. Acupuncture appeared to perform as well as medication with reducing the severity of depression. Overall, given the small numbers of trials and participants studied there is currently insufficient evidence that acupuncture was more effective than sham acupuncture, or non-specific acupuncture. Eight trials with 935 participants were included in meta-analysis. There was evidence that acupuncture may benefit this sub-clinical group of participants. Three trials (94 participants) found an improvement in depression for participants receiving manual acupuncture compared with SSRIs (RR 1.66, 95% CI 1.03, 2.68). The majority of included studies did not report on outcomes other than depression, and a small number of trials suggest there were no serious adverse events found in the review. Only a small number of trials was included for each per comparison. This limits the power of the review to detect meaningful differences between groups and analyses suggesting the benefit should be interpreted with caution. The authors found insufficient evidence to recommend the use of acupuncture for people with depression.

Music therapy has been defined as "an interpersonal process in which the therapist uses music and all of its facets to help patients to improve, restore or maintain health" [Bruscia, 1998]. Music therapy approaches across the world have emerged from diverse traditions such as behavioural, psychoanalytic, educational or humanistic models of therapy. While techniques used in music therapy are also diverse, they can be broadly categorized as "active", in which people re-create, improvise or compose music, and "receptive", in which they listen to music [Bruscia, 1991]. A recent Cochrane review analysed five studies on music therapy [Mead, 2010]. Four out of five studies individually reported greater reduction in symptoms of depression among those randomised to music therapy than to those in standard care conditions. The fifth study, in which music therapy was used as an active control treatment, reported no significant change in mental state for music therapy compared with standard care. Dropout rates from music therapy conditions appeared to be low in all studies.

Five studies met the inclusion criteria for Cochrane collaboration review "Music therapy for depression". Of these, four [Chen, 1992; Hanser, 1994; Hendricks, 1999; Radulovic, 1997] reported clinically significant positive effects. One [Zerhusen, 1995] in which music therapy was used as a control treatment, showed no effect. Few studies provided sufficient numerical data to be included in meta-analysis and marked heterogeneity resulting from differences in the type of intervention used and in the populations studied meant that quantitative synthesis would be inappropriate [Fletcher, 2007].

The authors emphasize that the range of interventions included guided imagery to music; ”prescribed” music to induce particular emotional states, for example, relaxation or motivation; reflective discussions around pre-composed music chosen by the patient or therapist; and joint music making between therapist and participants. Two out of five studies describe a similar approach [Hanser, 1994; Hendricks, 1999], but Hendricks in 1999 modified the approach taken by Hanser in 1994 with individual older adults, for groups of adolescents. Only one of the studies examined the effects of an active approach [Chen, 1992]. The authors conclude that findings from individual randomized trials suggest that music therapy is accepted by people with depression and is associated with improvements in mood.

In an earlier qualitative review of complementary and self-help interventions, relaxation techniques were found to be as effective as the standard treatments they were compared to [Biddle, 2000]. Relaxation techniques including progressive muscle relaxation, relaxation imagery, autogenic training, combined or enhanced versions of these, as well as relaxation adjunctive to other treatments, aimed at treating depression directly or via its influence on some other variable, e.g. anxiety. The Cochrane Collaboration Review of Relaxation for depression conducted 15 trials with 11 included in meta-analysis. The authors detect, that clinician-rated depressive symptoms were less clear because of significant heterogeneity, with the fixed-effects model finding relaxation more effective, but the random-effects model demonstrating no statistically significant difference (2 studies, 52 participants). In this and other cases where heterogeneity was observed, none of our sub-grouping factors could account for the heterogeneity. However, relaxation training was not as effective as psychological (mainly cognitive-behaviour) treatment in reducing self-rated depressive symptoms. These differences were found both at the end of treatment (9 studies, 286 participants) and at follow-up several months later (6 studies, 192 participants). Results from clinician-rated depressive symptoms were partially supportive, with non-response/remission post-intervention favouring psychological treatment (3 studies, 104 participants), but no significant difference in clinician-rated depression scores post-intervention (3 studies, 72 participants). There were inconsistent effects found when comparing relaxation training to medication (2 studies, 115 participants). The comparisons with medication did not lead to clear conclusions because of inconsistencies between the post-test and follow-up data. Similar inconsistencies were also found by the authors in the trials comparing relaxation plus-medication to medication alone (2 studies, 40 participants), which also showed inconsistency between self-report and clinician-rated data. There were too little data to draw any conclusions about relaxation in comparison with complementary and lifestyle treatments. Three out of four trials that compared relaxation to complementary or lifestyle therapies did not have data that could be pooled. The authors conclude that relaxation techniques were more effective at reducing self-rated depressive symptoms than no or minimal treatment. However, they were not as effective as psychological treatment.

Availability of non-pharmacological treatment in acute mental in-patient departments for adults of Latvia. There are four acute mental hospitals for adults in Latvia and one of them is a mental department in somatic hospital for adults [Taube, 2010]. The staff involved in treatment and rehabilitation process of depressed patients is psychiatrists, psychologists, psychotherapists, occupational therapists, physiotherapists, movement therapists, social workers, nurses and aids of nurses. Psychotherapeutic methods are used in 4 hospitals, music therapy active method in 3, receptive in 3, exercise in 4, relaxation techniques in 2 hospitals.

The usage of rehabilitation methods of treatment depends on organization of mental help for patients in hospitals. “A” and “E” mental hospitals have rehabilitation departments and use music therapy (active, receptive methods), relaxation training, exercise and psychotherapy for rehabilitation. However, only exercise and music therapy (receptive) are used in mental hospital “D”, psychotherapy and exercise in “C” and psychotherapy and music therapy (active method) in mental hospital “B”.

Discussion

Psychotherapy and alternative treatments and practices for the treatment of depression. The main result of this study was done based on Cochrane collaboration systematic reviews and meta-analytical data. Systematic reviews by R. Butler about psychotherapy for depression were classified as valid source of

analyses [Butler, 2006]. Interpersonal psychotherapy shown in repeated randomized controlled trials has efficacy both as an acute and a maintenance treatment for major depressive disorder [Weissman, 2003]. Cognitive and behavioural therapies have also demonstrated efficacy in repeated randomized controlled trials for depression [Hollon, 2005]. IPT and CBTs have already been included in numerous professional and national treatment guidelines [van den Broek, 2005; Tylee, 2006]. The main result of Cochrane collaboration meta-analysis about the effectiveness of marital therapy in treatment of depression is that there is no evidence to suggest that marital therapy is more or less effective than individual psychotherapy in such treatment.

The Cochrane collaboration review "Exercise for depression" found that exercise has a large effect (SMD –0.82, 95% CI –1.12, –0.51) on depressive symptoms in people with diagnosis of depression when compared with no treatment (waiting list/placebo). The recent UK National Institute of Clinical Excellence guideline for depression recommended structured, supervised exercise programs, three times a week (45 minutes to 1 hour) for 10 to 12 weeks for mild depression [NICE, 2007]. Exercise programs can be offered in the UK through Exercise Referral Systems [DOH, 2001]. These schemes direct someone to a service offering an assessment of need, development of a tailored physical activity program, monitoring of progress and follow up.

The Cochrane collaboration review "Relaxation for depression" data showed that relaxation training was more effective at reducing self-rated depressive symptoms than waitlist or no or minimal treatment (5 studies, 136 participants).

National Institute for Health and Clinical Excellence gives recommendation in guideline "Treatment and management of depression in adults" [NICE, 2009]. For people with mild to moderate depression it is considered to offer one or more of the following interventions, guided by a person's preference: individual guided self-help based on the principles of cognitive behavioral therapy, computerized CBT (CCBT), a structured group physical activity program. For people with moderate or severe depression provision of a combination of antidepressant medication and a high-intensity psychological intervention (CBT or interpersonal therapy) is suggested.

People with depression who are considered to be at significant risk of relapse (including those who have relapsed despite antidepressant treatment or who are unable or choose not to continue antidepressant treatment) or who have residual symptoms should be offered one of the following psychological interventions: individual CBT for people who have relapsed despite antidepressant medication and for people with a significant history of depression and residual symptoms despite treatment, mindfulness-based cognitive therapy for people who are currently well but have experienced three or more previous episodes of depression.

But Latvian Psychiatric Association's guideline "Treatment and management of depression" gives recommendation to use CBT, IP, psychodynamic psychotherapy for treatment of mild depression, combination of psychotherapy and antidepressants for the treatment of moderate severe depression and antidepressants combination with support psychotherapy for the treatment of severe depression [Terauds, 2009]. Music therapy, acupuncture and relaxation have not been included in the guidelines for the treatment of depression.

Availability of non-pharmacological treatment in acute mental in-patient departments for adults of Latvia. There were 36 049 hospitalisations due to psychiatric and behavioural disorders in Latvia in 2009. 4.8% of the patients with affective disorders (F30–39, ICD 10) among all patients with psychiatric diagnosis has been admitted and treated in all mental hospitals of Latvia in 2009. 1809 patients with depressive episode and 2661 with recurrent depression were in the psychiatric register of patients in 2009 [Taube, 2010]. The data of availability of non-pharmacological treatment and rehabilitation approaches in mental in-patient departments of Latvia were conducted by interviewing administration of hospitals and analysing documentations of mental hospitals (staff employee schedule, descriptions of staff employee and functional responsibilities and technologies and certifications of structural departments in April, 2010). However, the hospitals do not have documentation about the frequency of using non-pharmacological methods and especially about using these methods for depressive patients.

Conclusions

Combining psychological treatment with antidepressant drugs may be more effective than either treatment alone for moderate severe and severe depression. Care pathways may improve the effectiveness of treatment for depression. In mild to moderate depression, there is no reliable evidence that any one of alternative treatments is superior in improving symptoms of depression, but the strength of evidence supporting different treatments varies. Use of patient-centred, motivational approaches involves encouraging people to actively participate in their own care. Music therapy is accepted by people with depression and is associated with improvements in mood. Relaxation techniques were more effective at reducing self-rated depressive symptoms than no or minimal treatment. Exercise seems to improve depressive symptoms in people with diagnosis of depression. Acupuncture has insufficient evidence to recommend the use of it for people with depression. The data of availability of non-pharmacological treatment and rehabilitation approaches in acute mental in-patient departments of Latvia for adults suggest that the useage of these methods is unequal in different hospitals and it depends on the organization of mental help in concert regional hospital. It is necessary to include non-pharmacological methods of treatment in rehabilitation of depressive patients and to expand their availability in some regional mental hospitals.

References

1. APA 1994 American Psychiatric Association diagnostic and statistical manual of mental disorders. – 4th ed. – Washington, DC: American Psychiatric Association, 1994.
2. Barbato A., D'Avanzo B. B. D. Cochrane review: Marital therapy for depression // The Cochrane Collaboration. – JohnWiley & Sons, Ltd., 2009.
3. Bower P., Rowland N., Hardy R. The clinical effectiveness of counselling in primary care: a systematic review and meta-analysis // Psychol Med, 2003; 33: 203–215. [An update of the Bower, et al. Cochrane Review; 7 RCTs, 824 patients; search date not reported].
4. Beach S. R., O'Leary K. Treating depression in the context of marital discord: outcome and predictors of response of marital therapy versus cognitive therapy // Behaviour Therapy // 1992; 23: 507–528.
5. Beesley S., Mutrie N. Exercise is beneficial adjunctive treatment in depression // BMJ Publishing Group Ltd., 1997; 315(7121): 1542–1543.
6. Biddle S. J. H. Emotion, mood and physical activity // Physical activity and psychological well-being. – London: Routledge, 2000.
7. Blumenthal J. A., Babyak M. A., Doraiswamy P. M., et al. Exercise and pharmacotherapy in the treatment of major depressive disorder // Psychosomatic Medicine, 2007; 69: 587–596.
8. Bruscia K. E. Defining music therapy. – Barcelona: Gilsum, NH, 1998.
9. Bruscia K. E. Case studies in music therapy. – Barcelona: Gilsum, NH, 1991.
10. Butler R., Hatcher S., Price J., von Korff M. Depression in adults: psychological treatments and care pathways // BMJ Publishing Group Ltd., 2007; 08: 1016.
11. Chen X. Active music therapy for senile depression // Chinese Journal of Neurology and Psychiatry 1992; 25: 208–210.
12. Churchill R., Hunot V., Corney R., et al. A systematic review of controlled trials of the effectiveness and cost-effectiveness of brief psychological treatments for depression // Health Technology Assessment, 2001; 5(35): 1–173.
13. Cottrell D., Boston P. Practitioner review: the effectiveness of systemic family therapy for children and adolescents // Journal of Child Psychology and Psychiatry, 2002; 43(5): 573–586.
14. DOH 2001 Department of Health // Exercise referral systems: A National quality assurance framework // http:// www.dh.gov.uk/en/ (accessed: April, 2010).
15. Emanuels-Zuurveen L., Emmelkamp P. M. Individual behavioural cognitive therapy v. marital therapy for depression in marital distressed couples // British Journal of Psychiatry, 1996; 169: 181–188.
16. Emanuels-Zuurveen L., Emmelkamp P. M. Spouse-aided therapy with depressed patients // Behavior Modification, 1997; 21: 62–77.
17. Eshleman S. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States: results from the National Comorbidity Survey // Archives of General Psychiatry, 1994; 51: 8–19.

18. Fletcher J. What is heterogeneity and why is it important? // BMJ Publishing Group Ltd., 2007; 334: 94–96.
19. Hanser S. B. Effects of music therapy strategy on depressed older adults // Journal of Gerontology, 1994; 49: 265–269.
20. Hendricks C. B. A study of the use of music therapy techniques in a group for the treatment of adolescent depression // Dissertation Abstracts International, 2001; 62(2-A): 472.
21. Henken T., Huibers M. J., Churchill R., et al. Cochrane review: Family therapy for depression // The Cochrane Collaboration. – John Wiley & Sons, Ltd., 2009.
22. Hollon S. D., Jarrett R. B., Nierenberg A. A., et al. Psychotherapy and medication in the treatment of adult and geriatric depression; which monotherapy or combined treatment? // Journal of Clinical Psychiatry, 2005; 66: 455-468.
23. Keitner G. I., Archambault R., Ryan C. E. Family therapy and chronic depression // JCLP, 2003; 59(8): 873–884.
24. Kessler C., McGonagle K. A, Zhao S., et al. Cochrane review: music therapy for depression. Cochrane Library // Issue 1. – John Wiley & Sons, Ltd., 2009.
25. Leff J., Vearnals S., Brewin C. R., et al. The London Depression Intervention trial // Randomised controlled trial of antidepressants v. couple therapy in the treatment and maintenance of people with depression living with the partner: clinical outcome and costs // British Journal of Psychiatry, 2000; 177: 95–100.
26. Mead G. E., Morley W., Campbell P., et al. Cochrane review: Exercise for depression // The Cochrane Library / Issue 1. – JohnWiley & Sons, Ltd., 2010.
27. NICE 2007 National Institute for Health and Clinical Excellence // Management of depression in primary and secondary care. // http://www.nice.org.uk/nicemedia/pdf/CG023fullguideline.pdf 2007. (accessed: April, 2010)
28. Radulovic R., Cvetkovic M., Pejovic M. Complementary musical therapy and medicamentous therapy in treatment of depressive disorders // WPA Thematic Conference, Jerusalem, Nov 1997.
29. Singh N. A., Clements K. M., Singh M. A. F. The efficacy of exercise as a long-term anti-depressant- elderly subjects. A randomized controlled trial // Journals of Gerontology – Series A: Biological Sciences and Medical Sciences, 2001; 56(8): 497–504.
30. Smith C. A., Hay P. P. J., Mac Pherson H. Cochrane review: Acupuncture for depression // The Cochrane Library / Issue 1. – John Wiley & Sons, Ltd., 2010.
31. Taube M., Pelne A., Pulmanis T., et al. Mental health care in Latvia // Statistical Yearbook. – 10th issue. – Health and Economic Centre, 2010; 77: 20–50.
32. Tylee A. Identifying and managing depression in primary care in the United Kingdom // Journal of Clinical Psychiatry, 2006; 67(6): 41-45.
33. Terauds E., Andrezina R., Kupca B., Ancane G. Depresijas norise un ārstēšanas vadlīnijas (Course of depression and treatment guidelines) // Latvijas psihiatru asociācija (Latvian Phyciatric Association), 2009; 64: 47.
34. Van den Broek W. W. The practice guideline "Depressive disorder" // Nederlands Tijdschrift Voor Geneeskunde, 2005; 149: 509–510.
35. Weisman M. M., Markowitz J. C., Klerman G. L. Comprehensive guide for interpersonal psychotherapy: basic book. – New York, 2000. Pp.70–94.
36. Wittchen H. U., Jacobi F., Rehm J. The size and burden of mental disorders and other disorders of the brain in Europe 2010 // European Neuropsychopharmacology, 2011; 21: 655–679.
37. Zerhusen J. D., Boyle K., Wilson W. Out of the darkness: Group cognitive therapy for depressed elderly // Journal of Military Nursing Research, 1995; 1: 28–32.

Indoor Tanning Facilities and Their Complications in Citizens of Latvia

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Abstract

Melanoma and non-melanoma skin cancer has risen gradually during the last 25 years and reached a peak in 2010. Skin cancer is higher than that of any other human malignancy; one of the crucial, proved and better understood causes of this is ultraviolet radiation. Moreover, the World Health Organization classifies ultraviolet radiation as group I carcinogen. Risk factors of skin cancer in the population of Latvia are explored by analyzing the main acute and chronic effects from overexposure to UV radiation and tanning in inappropriate facilities.

The aim of the study is to analyze main acute and chronic complications, associated with ultraviolet radiation in citizens of Latvia. The study explores how UV radiation impacts human skin from skin ageing to cancerogenesis.

There is a significant association between ever-use of indoor tanning facilities and an increased risk of skin malignancy. Past years of studies have presented the dramatic data. The association between indoor tanning facilities and skin malignancy is strong among those who started to use indoor tanning facility in young adulthood.

The research justifies the necessity to understand the main behavior and habits using indoor tanning facilities by the citizens of Latvia; it also characterizes the average user of indoor tanning beds. It is already clear that indoor tanning bed facilities represent an unavoidable risk factor for skin damages such as skin premature ageing hence to developing of skin benign tumors and skin malignancy.

Keywords: ultraviolet radiation, melanoma, non-melanoma skin cancer.

Introduction

Every year around the world, about 130 000 melanoma cases are diagnosed and about 37 000 people die from this disease. New melanoma cases grow per 0% to 8% every year.

Comparing data form Europe and the world data, lots of differences between them are revealed.

1. People of Mediterranean countries with darker skin color have a lower incidence in European countries – per 100 000 population (Greece: male – 2.8; female – 3.9).
2. In males per 100 000 population the highest incidence in Europe is in Norway (16.1) and Switzerland (15.3), in females in Denmark (16.9) and Norway (15.7).
3. The highest rates are in Australia and in New Zealand (males – 39.8; females – 32.3).
4. The lowest incidence rates are in Asian and African population where dark skin dominates.

The comparison of European countries shows that the highest firstly diagnosed melanoma cases (incidence) are in females, while the highest mortality is in males.

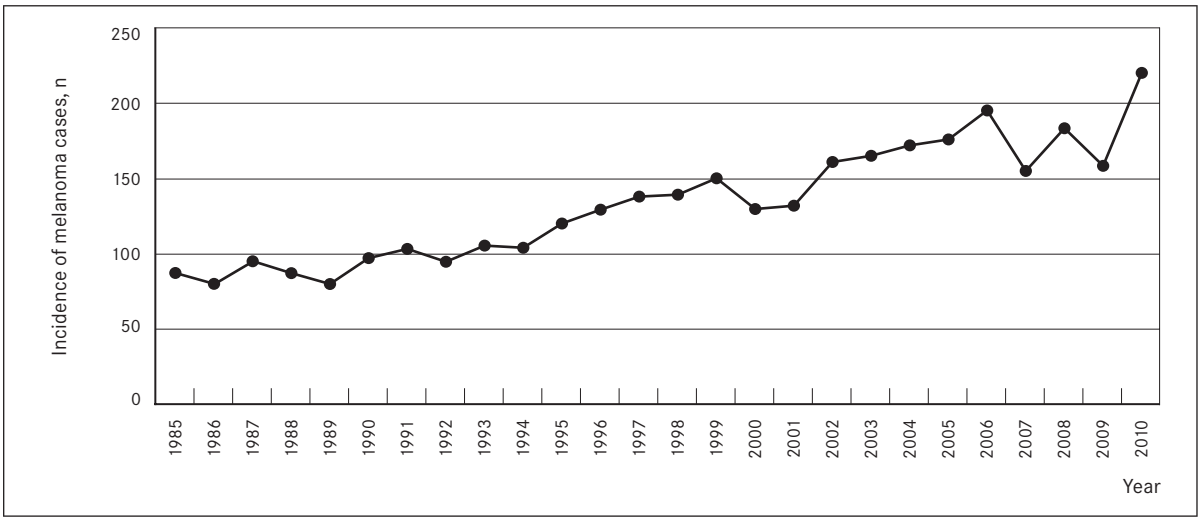
An increased number of melanoma cases per year is observed in Latvia (Figure 1). High frequency of skin cancer all over the world continues to exist despite the knowledge of the main etiological risk factor – UV radiation. Numerous studies have documented an association between the use of indoor tanning devices and an increased risk of skin cancer, especially in young women. Studies show that UV overexposure, even due to the absence of erythema or burn, results in DNA damage. Countries and regulatory bodies worldwide have recognized health risks associated with indoor tanning. Latvia has about 2.5 thousand indoor tanning beds, which are in high demand especially amongst young people. However, dramatic numbers of skin cancer cases per year do not impact social opinion about UV radiation overexposure.

According to the latest calculations, there are more than 1100 patients in Latvia with new skin cancer cases per year and millions of cases worldwide. However, the number of exacerbations and severe complications causing such irreversible changes as premature skin ageing, developing of *lenigo* type pigmentation, arising of solar (actinic) keratoses, which is a pre-cancerous lesion for squamous cell carcinoma, still remains very high. The main skin changes under the UV radiation are melanoma, non-melanoma skin cancer and photo-ageing, which results in loss of skin elasticity and appearance of skin elastosis. Non-melanoma (NMSC) skin cancer (cutaneous epithelial cancer) includes two types of cancer: basal cell carcinoma and squamous cell carcinoma. The most common etiology of NMSC in fair-skinned individuals is sunlight and indoor tanning facilities, which causes DNA damages and mutations. Comparing data about morbidity and mortality, taken into account the place of residence (south / north), depending on work characteristics (indoor / outdoor), socio-economic factors, physical activity, the highest number of skin malignancy was in regions with high level of UV radiation and in the group of patients who work outdoors. Besides, according to a different research, cancer risk level does not depend on skin photo-type. Most authors point to the frequent occurrence of basal cell carcinoma on the most sun exposed areas of the body (nose, forehead, ears).

As evidence of the dangers of UV overexposure mounts, the indoor tanning industry launches an aggressive public relations campaign, which appears designed to reassure the general public that the advantages of UV radiations outweigh health risks. This public relations campaign reached a peak on March 26, 2008 when the industry published a full-page advertisement in *The New York Times*, claiming that an association between tanning and skin malignancy was “hype”.

UV radiation induces specific mutations in cellular and skin genome, such as UV-signature and triplet mutations, and induces DNA damage. Cutaneous epithelial cancers (NMSC) are the easiest of all cancers to diagnose and treat. They originate most commonly in the epidermal keratinocytes and adnexal structures. SCC often has its origin in situ lesion that can be treated before frank invasion occurs.

Figure 1. Incidence of melanoma in Latvia over a period of 25 years (1985–2010)



Epithelial precancerous lesions, dysplasia of epidermal keratinocytes in epidemis and squamous mucosa, can involve the lower portion of epidermis or the full thickness. Basal cells mature into dysplastic keratinocytes resulting in a hyperkeratotic papule, clinically identified as "keratosis".

Solar (actinic) keratosis is formed by single or multiple, dry, and rough lesions that may occur on the habitually sun-exposed skin of adults. They can progress in SCC.

It is still not clear whether regular use of topical sunscreens can prevent melanoma of the skin, but there is a reasonable proof that topical sunscreens reduce the induction of solar keratoses and probably squamous cell carcinoma.

Solar *lentigo* is circumscribing 1–3 centimeter brown maculae resulting from a localized proliferation of melanocytes due to acute or chronic exposure to sunlight. It usually appears in adults after 40 years of age, but may be in 30-year-olds, in sunny climates, and susceptible persons. The correlation between skin photo-types I and III and the duration and intensity of solar exposure can be observed. The amount of *lentigo* type pigmentation depends on the time of UV overexposure and frequency of it.

Cutaneous ageing may be the result of one's skin chronically damaged by UVR during childhood, which thereafter looks old. This photo-ageing effect causes skin to become thin. Chronic overexposure to UV radiation causes freckles and roughness of skin. Skin ages even in sun-protected areas, but at a slower rate. If buttock skin is compared to the skin on the grace, forearms or back, the thinning of dermis can be traced; also, skin collagen falls by 1% per year throughout adult life and becomes more stable.

Abundant epidemiologic data have been examined to asses potential connections between indoor tanning and both melanoma and non-melanoma skin cancers. According to a 2006 meta-analyses by International Agency for Research on Cancer (IARC), among people who first used indoor tanning before 35 years of age the relative risk of melanoma was 1.75 – a finding that prompted the Worlds Health Organization to classify tanning beds as a group I carcinogen.

The aim

The goal of the study is to analyze main acute and chronic complications associated with ultraviolet radiation in the citizens of Latvia. The study also explores how UV radiation impacts human skin from skin ageing to cancerogenesis.

Material and methods

This study was carried out at the clinic of esthetic dermatology at "Health Center 4" in Rīga, Latvia. 73 patients in different age groups were examined. Patients came to a dermatologist with diverse problems, and during the visit the interview was conducted with them.

The examination included an interview and clinical inspection. First part of the study entailed answering the questions in a questionnaire with various questions about a patient's age, educational level, life habits, oral contraception intake (for females), their habitude in facial procedures. The overall point of view about indoor tanning facilities and sunbathing in general was defined. The main users of indoor tanning facilities were clarified. Past history of sunburns, which were obtained because of UV over-exposure naturally or due to tanning bed facilities was detected. During the interviewing the quantity of water intake per day and smoking habits was revealed.

Second part of the survey was an objective inspection of a patient. First of all, a patient's phototype, skin wrinkle accordance to its age and state of skin in general were determined. The second step also involved the inspection of skin pigmentation intensity on the body (which included nevus and other hyper-pigmented regions on the body) which included a closer study of the most sun exposed body areas (face, neck, back, shoulders, palm dorsal surface). During the examination seborrheic keratosis, actinic keratosis and *lentigo* type pigmentation was evaluated. Skin dryness level, facial skin wrinkles and creases were also assessed. Also, telangiectasia existence on the facial skin as well as its visibility, from minimally visible telangiectasia to clearly visible one was identified. The main chronic diseases of a patient and possible positive cancer anamnesis in the family were detected. The results were processed under SPSS 16.0 for Windows. Spearman correlation and Colmogorov-Smirnov's test was carried out.

Results

The examination of 73 patients was carried out. 72.6% (n = 53) were females and 27.4% (n = 20) were males (Table 1). The mean age for women was 42.5 and for men – 41.8 years.

Most frequently identified phototype in citizens of Latvia was phototype II (using Fitzpatrick classification) – 52.2%. Type III was identified in 27.4% participants and phototype I was common in 15.1% of the patients; no phototypes IV and V were discovered with any participant of the study. 58.9% of the study group at least once in their lifetime had experienced sunburn. The number of patients that have been exposed to sunburn at least 1–2 times in their life was 34% women and 30% men; those who have had sunburn more than three times in their life was 35% of females and 22.6% of males. Patients, who have had more than 3 sunburns in their life, had much more *lentigo* type pigmentation in dorsal surface of the palm (p = 0.01) compared to patients, who were not overexposed to UV.

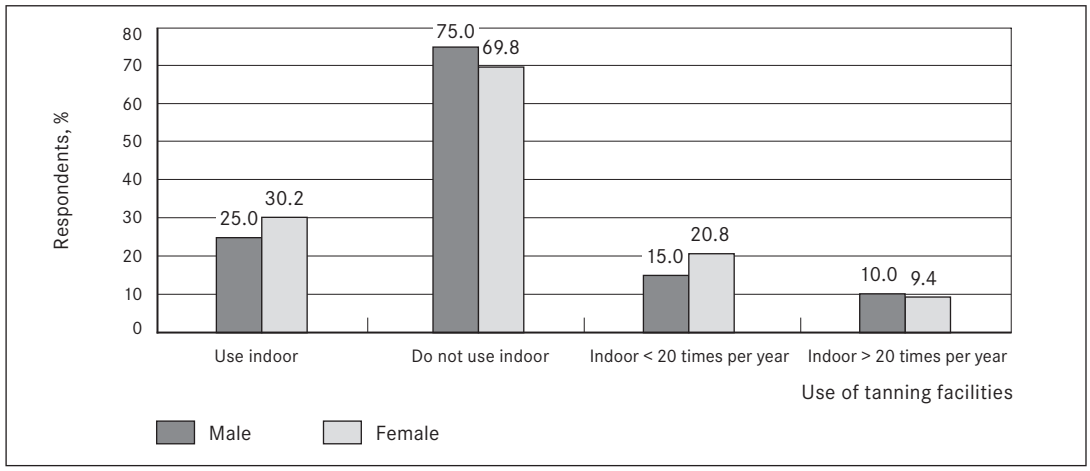
In general, 30.2% of women and 25% of men use indoor tanning facilities (Figure 2). 75% of men and 69.8% of women do not use indoor tanning beds. 20.8% of females and 15% of males visit indoor tanning session less than 20 times a year. 10% of males and 9.4% of females visit indoor tanning session more than 20 times a year.

Furthermore, there are several aspects of impact revealed. Men with secondary level education are more likely to use indoor tanning facilities than men with higher education. There is a positive correlation amongst males and their level of education (p = 0.08). Even so, there is a positive correlation amongst women who use indoor tanning facilities and smoke (p = 0.02). Nevertheless, in the middle-age patient group, who visit indoor tanning facilities, the average UV overexposure time using indoor tanning for women, mean age 29, is 6 years and for men, mean age 34, is 5 years. Approximately 74% of females and 55% of males always use protective sunscreens before sunbathing or tanning bed session; however, 45% of men and 26% of women never use this kind of protection.

Table 1. Premature skin ageing and tumor main risk factors

Gender	Female, %	Male, %
Smoking	18.9	25.0
Using indoor tanning	30.2	25.0
Sun burns in whole life	56.6	65.0
Do not use sunscreens	26.4	45.0
Water intake per day less than 1.5 l	77.4	60.0
Other chronic diseases	39.6	30.0

Figure 2. Use of indoor tanning facilities



There are quite high numbers of patients who do not use sunscreens despite the fact of harmful effects on skin due to UV overexposure. For this reason, 11 women and 3 men were observed having discrepancy between the actual age and visual one. All patients who had UV overexposure look older than they actually are, which means that their skin condition is more changed under the chronic impact of UV. The skin dryness and minimal desquamation was observed in those patients who use indoor tanning facilities, in 37% of women and 44% of men.

Table 2. Intensity of skin pigmentation

Pigmented lesions on the body	Less than 25%	25–50%	50–100%	More than 100%
Percentage of lesion	13.7	23.3	34.2	49.3

Table 3. *Lentigo* type pigmentation and seborrheic keratosis existence on the body

Pigmentation	Chest, %	Shoulders, %	Back, %	Face, %	Dorsal surface of the palm, %
Lentigo type pigmetation	24.3	23.5	24.3	17.6	10.3
Seborrheic keratosis	26.7	31.1	20.0	4.4	17.8

In 37.3% of patients teleangiectasia existence on facial skin was observed. The most common facial region is forehead (24.2%), nose (23%) and skin of the cheeks (13.7%), the less common region was chin. A person with phototype II has more teleangiectasia on the chin compared with other phototype.

Discussion

While sun exposure is a known risk factor for developing skin cancer, particularly squamous cell carcinoma and basal cell carcinoma, comparatively less attention has been directed at indoor tanning as a risk factor. The age for the first use of tanning device is also a significant risk factor; the age younger than 20 years is associated with the highest risk for both BCC and SCC. It is important to admit that numerous laboratory models suggest that a relatively low-dose UV exposure, which would not be sufficient to generate erythema, is still able to induce skin carcinogenesis. Countries and regulatory bodies worldwide have recognized health risks associated with indoor tanning. Latvia has about 2.5 thousand of indoor tanning beds, which are in high demand especially amongst young people. According to the latest calculations, there are more than 1100 patients in Latvia with new skin cancer cases per year and millions of cases worldwide. According to 2006 meta-analyses by International Agency for Research on Cancer (IARC), among people who first used indoor tanning before 35 years of age the relative risk of melanoma was 1.75 – a finding that prompted the Worlds Health Organization to classify tanning beds as a group I carcinogen.

Conclusions

1. Our study shows that people who are overexposed to UV irradiation have more sunburn in comparison to patients who are not overexposed to UV; 58.9% have had sunburn.
2. Moreover, seborrheic keratosis and *lentigo* type pigmentations are more common in patients who use indoor tanning facilities ($p = 0.034$).
3. UV radiation accelerates skin ageing and makes skin look older than it actually is. In addition, it makes skin drier and wrinkles deeper ($p = 0.017$).
4. It is necessary for medical specialists and beauty workers to provide more information about the impact of UV radiation overexposure, such as premature ageing and the increased risk of skin cancer.

References

1. Schulman J. M., Fisher D. E. Indoor UV tanning and skin cancer: health risks and opportunities // Curr Opin. Oncol, 2009; 21(2): 144–149.
2. Nilsen L. T. N., Nakken A. T., Hannevik M., Veierod M. B. UVB and UVA irradiances from indoor tanning devices // Photochem Photobiol Sci, 2011; 10: 1039.
3. Lim H. W., James W. D., Rigel D. S. Adverse effects of ultraviolet radiation from the use of indoor tanning equipment: time to ban the tan // J Am Acad Dermatol, 2011; 64(4): e51–60.
4. Ikehata H., Ono T. The mechanisms of UV mutagenesis // J Radiat Res (Tokyo), 2011; 52(2): 115–125.
5. Svobodova A. R., Galandakova A., Sianska J., Doležal D., et al. Acute exposure to solar simulated ultraviolet radiation affects oxidative stress-related biomarkers in skin, liver and blood of hairless mice // Biol Pharm Bull, 2011; 34(4): 471–479.
6. Fisher E. D., James W. D. Indoor tanning – science, behavior, and policy // N Engl J Med, 2010; 2(9): 901–903.
7. Veierød M. B., Weiderpass E., Thörn M., et al. A prospective study of pigmentation, sun exposure, and risk of cutaneous malignant melanoma in women. // J Nat Cancer Inst, 2003; 95: 1530–1538.
8. World Health Organization. Environmental health criteria 160. Ultraviolet radiation. – Geneva, 1994.
9. World Health Organization. Artificial tanning sunbeds – risks and guidance. – Geneva, 2003.
10. Young A. Tanning devices – fast track to skin cancer // Pigment Cell Res, 2004; 17: 2–9.
11. Latvian statistics about melanoma and non-melanoma skin malignancy // http://euromelanoma.lv/lv/articles/view/ne-melanoma/ne-melanomu-statistika

Differences in Outcomes and Guidelines-based Management in Hemodynamically Stable Patients with non-ST-segment Elevation Myocardial Infarction:
Data from Latvian Registry of Acute Coronary Syndromes

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Abstract

Only some factors responsible for in-hospital mortality in patients with acute coronary syndromes without ST-elevation (NSTEMI) are well known. Therefore, the aim of the present study was to analyse which factors could influence in-hospital mortality in NSTEMI patients presenting without cardiogenic shock as well as to estimate adherence to guidelines recommended management.

1426 hemodynamically stable consecutive patients were studied who were admitted with diagnosis of NSTEMI at three hospitals in Rīga with the availability of cardiac catheterisation laboratory and specialised coronary care unit.

During hospital stay 95 (6.7%) of patients died. Hospital non-survivors were elderly; more of them had positive myocardial necrosis markers and cerebral infarction in history, many of them were smokers and diabetics. Survivors were more managed with percutaneous coronary intervention, had more PCI in history, had more hypertension and hypercholesterolemia and received better guidelines recommended pharmacotherapy except glycoprotein IIb/IIIa inhibitors and low-molecular-weight heparins. In multivariate analyses interventional management, smoking status, hypercholesterolemia, use of clopidogrel, cerebral infarction and PCI in history lost their statistical significance.

In patients with unstable coronary-artery disease, guidelines recommended pharmacotherapy was underused. In-hospital mortality was dependent on positive myocardial necrosis markers, age, *diabetes mellitus*, hypertension, use of aspirin, statins, nitrates, beta-blockers and ACI-inhibitors.

Keywords: acute coronary syndrome, coronary intervention, mortality.

Introduction

Patients with acute coronary syndromes without ST-elevation (NSTEMI) account for large proportion of hospital admissions as well as mortality annually and are at high risk for adverse cardiac events. Optimal treatment consists of intensive medical therapy followed by coronary revascularization [Bhatt, 2004].

It is still discussed which patients presenting with NSTEMI should be directly sent to catheterization lab, and which ones should first be non-invasively risk stratified and treated medically. Some trials have shown that a routine invasive strategy improves outcomes in patients with NSTEMI [Fox, et al., 2002; Roger, et al., 2000; Mehta, et al., 2005; Bavry, et al., 2006]; however, others have generated conflicting results [Neumann, et al., 2003; Riezebos, et al., 2009].

Though it is well known from the surveys and registries performed so far that the management of acute coronary syndrome patients differs a lot not only among countries but hospitals and that a wide gap exists between guidelines and current clinical practice [Mandelzweig, et al., 2006; Peterson, et al., 2006].

Some factors, such as cardiogenic shock and critical three artery disease, responsible for in-hospital morality in patients presenting with NSTEMI are clear, while others remain uncertain.

The aim

The aim of the present analyses of Latvian Registry of Acute Coronary Syndromes data was to evaluate which factors (coronary risk factors, guidelines recommended pharmacotherapy, patient history and coronary intervention) could influence in-hospital mortality in NSTEMI patients presenting without cardiogenic shock as well as to estimate adherence to guidelines recommended management.

Material and methods

The Latvian Registry of Acute Coronary Syndromes is a prospective, multicentre, observational registry designed to examine current epidemiology, in-hospital management and outcome of patients with acute coronary syndrome (ACS) in Latvia. A total of 34 hospitals participated in the registry during the study period from 1st January, 2008 to 31st December, 2008. To minimise selection bias, all consecutive patients with a suspected diagnosis of acute coronary syndrome were included regardless of the treatment strategy or outcome. During the index hospitalisation, data concerning baseline demographic and clinical characteristics, relevant laboratory results, pharmacotherapy during hospital stay and adverse cardiovascular outcomes were recorded on a standardised, electronic web page based case report form. Standardised definitions were used for adverse events and final diagnosis. Data were collected in a central electronic database. Cardiogenic shock was defined as reduced blood pressure (systolic blood pressure < 90 mmHg or a drop of a mean arterial pressure > 30 mmHg) and/or low urine output (< 0.5 ml/kg/h), with a pulse rate > 60 beats per minute with or without evidence of organ congestion [Dickstein, et al., 2008]. The primary end point was in-hospital mortality. Occurrence of other ischemic or bleeding events was not analysed. For the purpose of the present analysis patients were classified as NSTEMI and STEMI based on their final diagnosis. STEMI was diagnosed if ST-segment elevation more than or equal to 1 mm occurred in at least one lead or new left bundle branch block was found in the electrocardiogram with biochemical evidence of myocardial necrosis. NSTEMI was diagnosed in patients with typical symptoms and without new ST-segment elevation in the electrocardiogram. In current study we analysed only NSTEMI patients without cardiogenic shock admitted to hospitals with percutaneous coronary intervention (PCI) availability in Rīga, capital city of Latvia (in total three hospitals). We compared adherence to guidelines based therapies, interventional strategy, coronary risk factors and patient history data among in-hospital survivors and non-survivors. Separate analyses were done for patient subgroups with positive and negative myocardial necrosis markers (Troponin I or T, or creatinin kinase – MB (CK-MB)).

Special definitions. Hyperholesterolemia was defined as total blood cholesterol above 4.5 mmol/l. Hypertension was defined as blood pressure above 140/90 mmHg without medications or use of antihypertensive drugs in history.

Statistical analyses. Statistical analyses were performed with SPSS software (version 12.0, Chicago, Illinois, USA). Continuous values are expressed as mean ± SD. Categorical variables are presented as percentages. Categorical values were compared with hi-quare (χ^2) test and continuous values with

ANOVA test. Multivariate linear regression analyses were conducted to evaluate adjusted effect estimates among different factors possibly influencing in-hospital mortality. For those analyses linear regression was used, for which in-hospital death was the dependent variable for interest. The following variables were used as independent factors: positive myocardial necrosis markers, interventional management, age, smoking, cerebral infarction and PCI in history, *diabetes mellitus*, hypercholesterolemia, hypertension, use of aspirin, clopidogrel, statins, nitrates, beta-blockers and ACI-inhibitors. Odds ratios and 95% confidence intervals (CI) were presented in univariate and multivariate analyses. P values under 0.05 were considered statistically significant.

Results

1426 hemodynamically stable consecutive patients were studied who were admitted with diagnosis of NSTEMI at three hospitals with the availability of cardiac catheterisation laboratory and specialised coronary care unit. From all patients 95 (6.7%) died during hospital stay. Baseline demographic and clinical characteristics for both groups of patients are shown in Table 1. Hospital survivors were elderly; many of them had positive myocardial necrosis markers (91.6% vs. 60.0%) and cerebral infarction in history, and were smokers or diabetics. From all survivors 31.9% were managed with PCI but only 17.9% from non-survivors were managed interventionally (p = 0.004). Most survivors had PCI in history, had hypertension and hypercholesterolemia.

Pharmacological treatment during hospital stay is represented in Table 2. Survivors were more likely to receive more aggressive antiplatelet therapy with aspirin and clopidogrel. Glycoprotein IIb/IIIa (GP IIb/IIIa) inhibitors were better used in survivors; however, not statistically significant. Use of low-molecular-weight heparins did not differ among groups. Also, survivors received better guidelines based anti-ischemic and anti-atherosclerotic therapy with beta-blockers, angiotensin-converting enzyme (ACE) inhibitors, statins and nitrates. However, guidelines recommended pharmacotherapy was underconsumed especially the use of GP IIb/IIIa inhibitors, low-molecular-weight heparins and clopidogrel.

Multivariate analyses showed (Table 3) that in-hospital mortality was dependent on positive myocardial necrosis markers, age, *diabetes mellitus*, hypertension, and use of aspirin, statins, nitrates, beta-blockers and ACI-inhibitors. Smoking status, PCI, cerebral infarction and PCI in history, hypercholesterolemia and use of clopidogrel lost their statistical significance on in-hospital mortality in multivariate analyses.

Table 1. Baseline demographic and clinical characteristics

Characteristics	Survivors	Non-survivors	p value
Age, mean ± SD	70.37 ± 11.20	76.75 ± 8.48	< 0.0001
Gender (male), n (%)	760 (57.1)	47 (49.5)	0.164
Positive myocardial necrosis marcers, n (%)	798 (60.0)	87 (91.6)	< 0.0001
Transferred patients, n (%)	86 (6.5)	2 (2.1)	0.118
PCI, n (%)	425 (31.9)	17 (17.9)	0.004
Myocardial infarction in history, n (%)	955 (71.8)	68 (71.6)	1.000
Cerebral infarction in history, n (%)	100 (7.5)	16 (16.8)	0.003
PCI in history, n (%)	321 (24.1)	12 (12.6)	0.011
CABG in history, n (%)	106 (8.0)	5 (5.3)	0.430
Smoking, n (%)	9 (9.5)	241 (18.1)	0.035
Diabetes mellitus, n (%)	278 (20.9)	31 (32.6)	0.010
Hypertension, n (%)	920 (69.1)	49 (51.6)	0.001
Hypercholesterolemia, n (%)	644 (48.4)	33 (34.7)	0.011

Table 2. Pharmacological treatment during hospital stay

Method of treatment	Survivors	Non-survivors	p value
Low-molecular-weight heparins, n (%)	952 (71.5)	71 (74.7)	0.557
Clopidogrel, n (%)	974 (73.2)	46 (48.4)	< 0.0001
Aspirin, n (%)	1247 (93.7)	69 (72.6)	< 0.0001
GP IIb/IIIa inhibitors, n (%)	222 (16.7)	12 (12.6)	0.389
Statins, n (%)	1157 (86.9)	58 (61.1)	< 0.0001
Beta-blockers, n (%)	1150 (86.4)	53 (55.8)	< 0.0001
ACE-inhibitors, n (%)	1088 (81.7)	42 (44.2)	< 0.0001
Nitrates, n (%)	1161 (87.2)	68 (71.6)	< 0.0001

Table 3. Multivariate analyses for in-hospital mortality*

Characteristics	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value
Positive myocardial necrosis markers	7.260 (3.49–15.11)	< 0.0001	0.082 (0.056–0.107)	< 0.0001
PCI	0.465 (0.272–0.795)	0.006	–	–
Age, years	76.75 (75.02–78.48)	< 0.0001	0.001 (0.0001–0.003)	0.014
Smoking	0.473 (0.235–0.954)	0.046	–	–
Cerebral infarction in history	2.490 (1.40–4.43)	0.003	–	–
PCI in history	0.455 (0.245–0.844)	0.015	–	–
Diabetes mellitus	1.840 (1.17–2.84)	0.011	0.031 (0.001–0.060)	0.041
Hypercholesterolemia	0.568 (0.367–0.878)	0.014	–	–
Hypertension	0.476 (0.313–0.723)	0.001	0.037 (0.010–0.063)	0.008
Aspirin	0.179 (0.108–0.295)	< 0.0001	0.089 (0.041–0.136)	< 0.0001
Clopidogrel	0.344 (0.226–0.524)	< 0.0001	–	–
Statins	0.236 (0.152–0.367)	< 0.0001	0.066 (0.030–0.101)	< 0.0001
Nitrates	0.369 (0.230–0.592)	< 0.0001	0.059 (0.023–0.094)	< 0.0001
Beta-blockers	0.199 (0.129–0.307)	< 0.0001	0.086 (0.052–0.120)	< 0.0001
ACI inhibitors	0.177 (0.115–0.272)	< 0.0001	0.101 (0.071–0.132)	< 0.0001

* Multivariate analysis of significant univariate risk factors $p < 0.0001$.

Discussion

Meta-analyses of previous randomised trials that compared an invasive strategy with a conservative strategy in patients with acute coronary syndrome have shown a benefit for an invasive strategy [Mehta, et al., 2005; Bavry, et al., 2006]. For example the Third Randomised Intervention Treatment of Angina (RITA-3) study showed that an interventional strategy in NSTEMI patients resulted in a significant reduction in the combined endpoint of death, non-fatal myocardial infarction, or refractory angina with the main effect on the last. An interventional strategy halved the frequency of refractory angina at 4 months (risk ratio 0.47, 95% CI 0.32–0.68) with the absolute difference of about 5 per 100, which persisted at 1 year [Fox, et al., 2002]. However, recently published data from randomised, multicenter TIMACS (The Timing of Intervention in Acute Coronary Syndromes) trial showed that early intervention did not differ greatly from delayed intervention in patients with NSTEMI in preventing the primary outcome, but it did reduce the rate of the composite secondary outcome of death, myocardial infarction, or refractory ischemia and was superior to delayed intervention in high-risk patients [Mehta, et al., 2009].

The obtained data revealed that PCI almost halved in-hospital mortality in univariate analyses; however, this association was lost in multivariate analyses. We did not randomise patients for interventional or conservative management; therefore, we cannot exclude selection bias – that possibly more perspective, younger, male patients were more dedicated to PCI as older patients with co-morbidities. Our analyses showed that from all male patients 36.6% were managed with PCI vs. 23.7% female patients ($p < 0.0001$) as well as patients managed with PCI were younger (67.75 ± 11.11 vs. 72.16 ± 10.91 years, $p < 0.0001$). Moreover, 25.6% of diabetics were sent to PCI in comparison with 32.5% non-diabetics ($p = 0.022$) and only 17.8% of patients with atrial fibrillation and 21.6% with cerebral infarction in history were managed interventionally compared to 34.6% of patients with sinus rhythms ($p < 0.0001$) and 31.8% of patients without cerebral infarction in history ($p = 0.021$).

Antithrombotic and antiplatelet therapy plays an important role in the pathophysiology of NSTEMI ACS [Hamm, et al., 2011] and outcomes after PCI in NSTE-ACS have been markedly improved with the use of contemporary antithrombotic and antiplatelet therapy. Global Registry of Acute Coronary Events (GRACE) showed that the use of triple therapy with aspirin, clopidogrel and statins was associated with the lowest mortality in NSTEMI patients [Fox, et al., 2007; Lim, et al., 2005]. Our study proved that use of statins, aspirin and clopidogrel were better in hospital survivors; however, clopidogrel lost its statistical significance in multivariate analyses.

In the light of strong recommendations by the European Society of Cardiology supported by extensive evidence from clinical trials and already published registry data suggest that practical use of GP IIb/IIIa inhibitors is suboptimal [Hamm, et al., 2011; Fox, et al., 2004; Topol, et al., 1998; Fitchett, et al., 2006]. Our study proved the use of particularly GP IIb/IIIa inhibitors unsatisfactory, from all studied NSTEMI patients only 16.41% ($n = 234$) were managed with GP IIb/IIIa inhibitors. However, the use of GP IIb/IIIa inhibitors in patients managed with PCI was comparable with published data. Our results showed that 43.7% ($n = 193$) of patients managed with intervention received GP IIb/IIIa inhibitors opposed to 4.2% ($n = 41$) patients who were managed conservatively ($p < 0.0001$). The CRUSADE registry consisted of 65424 NSTEMI patients in which only 35.5% received GP IIb/IIIa inhibitors [Hoekstra, et al., 2005]. Patients in the CRUSADE registry who received early GP IIb/IIIa inhibitors had 2% absolute reduction in overall mortality. In the NRMI-4 registry only 35% of patients received GP IIb/IIIa inhibitors. Treated patients had lower in-hospital mortality even after adjustment for patient risk, treatment propensity and hospital characteristics (3.3% vs. 9.6%, odds ratio, 0.88; 95% confidence interval, 0.79–0.97; $p < 0.0001$) [Peterson, et al., 2003].

Use of low molecular heparins was performed low in the study. Nevertheless, the results did not differ among hospital survivors and non-survivors.

The obtained data presented surprisingly low adherence to guidelines recommended antiplatelet and antithrombotic therapy; however, the literature data [Fox, et al., 2007; Lim, et al., 2005] and current analyses show that better compliance with those medications could improve overall short term survival in NSTEMI patients. It has been estimated that one possible reason for underuse of antithrombotic therapy was inadequate patient coronary risk stratification. We assume that coronary risk for many patients was stratified lower as it actually was. Another assumption for underconsumption of antithrombotics for NSTEMI patients could be physician's fear from bleeding complications; however, in our registry we do not possess data about real bleeding complications. Lastly, physician's education and competence play very important role in adequate patient management.

Use of all guidelines recommended pharmacotherapy groups except GP IIb/IIIa inhibitors and low molecular weight heparins were significantly better in hospital survivors compared with non-survivors. Therefore, it can be concluded that there is a reasonable potential for the reduction of in-hospital mortality in Latvia with better implementation of guidelines recommended pharmacotherapy in real life practice.

Moreover, we did see that in-hospital survival was lower in older NSTEMI patients with positive myocardial necrosis markers and diabetics. However, all those factors are well known high-coronary risk factors [Hamm, et al., 2011]. Paradoxically, we found that hypertension was associated with lower in-hospital mortality. This finding could be explained by the consumption that elevated blood pressure

becoming the factor which does not have high influence on short term mortality after NSTEMI, which does not mean that hypertension does not have a negative effect on long term survival even in the same data set of patients.

Study limitations. Our analysis has several limitations and should be interpreted accordingly. First, as an observational cohort analysis, we adjusted for observed clinical differences between patient cohorts. Unaccounted differences may have remained and influenced our findings. Secondly, the selection process used may be taken into account. We did not perform any randomisation for interventional management or pharmacotherapy, each individual choice was physician based.

Moreover, the definition for hypercholesterolemia (total cholesterol above 4.5 mmol/l) is not absolutely correct according to the guidelines; however, we do not have any scalar data for blood cholesterol in our cohort and redefinition of hypercholesterolemia is not possible.

This study was a registry study based on a prospectively assembled database, part of a quality-improvement programme and since the analyses itself were retrospective, a formal power analysis was not performed. For this reason, we cannot exclude some not-significant results due to a too small sample size.

Finally, despite data set was build using patient admission registries as a starting point in order to include all patient data with NSTEMI diagnosis in our registry, there is still a possibility that some patient data could be missing.

Conclusions

The study demonstrated that in-hospital survival in hemodynamically stable NSTEMI patients was associated with better use of guidelines' recommended pharmacotherapy, particularly, with the use of ACI-inhibitors, beta-blockers, statins, nitrates and aspirin. However, in-hospital mortality was associated with age, positive myocardial necrosis markers and *diabetes mellitus*.

The Latvian Registry of Acute Coronary Syndromes showed that guidelines recommended therapy was underused, especially the use of GP IIb/IIIa inhibitors and clopidogrel were low. Better compliance with guidelines recommended anti-aggregate as well as anti-ischemic therapies are needed in patients presenting with acute coronary syndromes without ST segment elevation.

Analysis of NSTEMI patients of Latvian Acute coronary syndrome register is still continuing. The plans include comparative mortality analysis in dynamics, as well as an expansion of the patients' group included in the study.

References

1. Bavry A. A., Kumbhani D. J., Rassi A. N., et al. Benefit of early invasive therapy in acute coronary syndromes: a meta-analysis of contemporary randomized clinical trials // J Am Coll Cardiol, 2006; 48: 1319–1325.
2. Bhatt D. L., Roc M. T., Peterson E. D., et al. Utilization of early invasive management strategies for high-risk patients with non-ST-segment elevation acute coronary syndromes // JAMA, 2004; 292: 2096–2104.
3. Dickstein K., Cohen-Solal A., Filippatos G., et al. ECS guidelines for the diagnosis and treatment of acute and chronic heart failure // Eur Heart J, 2008; 29: 2388–2442.
4. Fitchett D. H., Langer A., Armstrong P. W., et al. Randomized evaluation of the efficacy of enoxaparin versus unfractionated heparin in high-risk patients with non-ST-segment elevation acute coronary syndromes receiving the glycoprotein IIb/IIIa inhibitor eptifibatide. Long-term results of the integrilin and enoxaparin randomized assessment of acute coronary syndrome treatment (INTERACT) trial // Am Heart J, 2006; 151: 373–379.
5. Fox K. A. A., Poole-Wilson P. A., Henderson R. A., et al. Interventional versus conservative treatment for patients with unstable angina or non-ST-elevation myocardial infarction: the British Heart Foundation RITA 3 randomised trial // Lancet, 2002; 360: 743–751.
6. Fox K. A. A., Mehta S. R., Peters R., et al. Benefits and risks of the combination of clopidogrel and aspirin in patients undergoing surgical revascularization for non-ST-elevation acute coronary syndrome. The clopidogrel in unstable angina to prevent recurrent ischemic events (CURE) trial // Circulation, 2004; 110: 1–7.

7. Fox K. A. A., Steg P. G., Eagle K. A., Goodman S. G., et al. Decline in rates of death and heart failure in acute coronary syndromes, 1999–2006 // JAMA, 2007; 297: 1892–1900.
8. Hamm C. W., Bassand J. P., Agewall S., et al. ESC Guidelines for the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation // www.escardio.org/guidelines-surveys/esc-guidelines/Pages/ACS-non-ST-elevation.aspx (accessed 17.11.2011).
9. Hoekstra J. W., Roe M. T., Peterson E. D., et al. Early glycoprotein IIb/IIIa inhibitor use for non-ST-segment elevation acute coronary syndrome: patient selection and associated treatment patterns // Acad Emerg Med, 2005; 12: 431–438.
10. Lim M. J., Spencer F. A., Gore J. M., et al. Impact of combined pharmacologic treatment with clopidogrel and a statin on outcomes of patients with non-ST-segment elevation acute coronary syndromes: perspectives from a large multinational registry // European Heart Journal, 2005; 26: 1063–1069.
11. Mandelzweig L., Battler A., Boyko V., et al. The second Euro Heart Survey on acute coronary syndromes: characteristics, treatment, and outcome of patients with ACS in Europe and Mediterranean Basin in 2004 // Eur Heart J, 2006; 27: 2285–2293.
12. Mehta S. R., Cannon C. P., Fox K. A. A., et al. Routine vs. selective invasive strategies in patients with acute coronary syndromes: a collaborative meta-analysis of randomized trials // JAMA, 2005; 293: 2908–2917.
13. Mehta S. R., Granger C. B., Boden W. E., et al. Early versus delayed invasive intervention in acute coronary syndrome // N Engl J Med, 2009; 360: 2165–2175.
14. Neumann F. J., Kastrati A., Pogatsa-Murray G., et al. Evaluation of prolonged antithrombotic pretreatment ("cooling off" strategy) before intervention in patients with unstable coronary syndromes: a randomized controlled trial // JAMA, 2003; 290: 1593–1599.
15. Peterson E. D., Pollack C. V., Roe M. T., et al. Early use of glycoprotein IIb/IIIa inhibitors in non-ST-elevation acute myocardial infarction: observations from the National Registry of Myocardial Infarction 4 // J Am Coll Cardiol, 2003; 42: 45–53.
16. Peterson E. D., Roe M. T., Mulgund J., et al. Association between hospital process performance and outcomes among patients with acute coronary syndromes // JAMA, 2006; 295: 1912–1920.
17. Riezebos R. K., Ronner E., Ter Bals E., et al. Immediate versus deferred coronary angioplasty in non-ST-elevation acute coronary syndromes // Heart, 2009; 95: 761.
18. Rogers W. J., Canto J. G., Lambrew C. T., et al. Temporal trends in the treatment of over 1.5 million patients with myocardial infarction in the U.S. from 1990 through 1999: the National Registry of Myocardial Infarction 1, 2, and 3 // J Am Coll Cardiol, 2000; 36: 2056–2063.
19. Topol E., Califf R. on behalf of *The PURSUIT Trial Investigators.* Inhibition of platelet glycoprotein IIb/IIIa with eptifibatide in patients with acute coronary syndromes // N Engl J Med, 1998; 339: 436–443.

Role of Native Microscopy in the Detection of Abnormal Vaginal Flora in Pregnancy

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Abstract

Abnormal vaginal flora is linked to preterm birth and can cause neonatal infections.

The aim of the study is to identify the relations between native microscopy and vaginal cultures results at the first antenatal visit of pregnancy.

A cross sectional, observational study of pregnant women receiving antenatal care in the five outpatient clinics in Rīga from August, 2010 until April, 2011 was conducted. 50 pregnant women with increased vaginal pH and next 50 ones with normal vaginal pH were included in the study. Upper vaginal wall specimens for native microscopy and swabs for aerobic, facultative anaerobic and genital mycoplasma cultures were taken. Abnormal vaginal flora was classified according to Donders. Vaginal culture results were compared between normal and abnormal vaginal microflora group.

91% of the participants were culture positive. There were 19 microorganisms found in the vagina, most often coagulase negative *Staphylococcus*, *Ureaplasma species*, *Escherichia coli*, *Candida* species and *Mycoplasma hominis*. 43 out of 50 participants with elevated vaginal pH and six of 50 pregnant women with normal vaginal acidity had abnormal vaginal flora on microscopy. Genital mycoplasmas and *Escherichia coli* were isolated more in the abnormal vaginal flora group ($p < 0.05$). *Escherichia coli* was more often associated with heavy or mild leucocytosis, comparing with no leucocytes ($p < 0.05$) on wet mount.

Genital mycoplasmas and *Escherichia coli* were more often isolated microorganisms in cases of abnormal vaginal microflora on native microscopy. Wet mount can be used as a "bed-side" examination method done by gynecologist for detection of abnormal vaginal flora and recognition of adverse pregnancy outcome risk group.

Keywords: abnormal vaginal flora, vaginal cultures, native microscopy, pregnancy.

Introduction

Preterm deliveries and neonatal infectious complications are one of the most challenging problems in perinatology. The etiology of preterm labor is multifactorial, but infections may account approximately in 40% of preterm deliveries [Klein, 2004]. The most common pathway to cause preterm labor

is ascending of vaginal endogenous flora [Romero, 2002]. Chorioamnionitis is correlated with preterm delivery [Hillier, 1988]. Most microorganisms found in the amniotic fluid and placenta are thought to come from vagina [Romero, 2006; Hillier, 1988]. Bacterial infections continue to be an important cause of morbidity and mortality in newborn infants. Although the incidence of perinatally acquired bacterial infection is low [Garcia-Prats, 2000], the consequences of infection may be very severe. Mortality may be as high as 50% among untreated infants [Bellig, 2002; Leitich, 2003].

Abnormal vaginal flora is linked to preterm birth in the most cohort studies [Hay, 1994; Romero, 2004]. Since Gardner and Dukes [Gardner&Dukes, 1955] discovered new genus of bacteria presented in vaginal smears in case of absent *lactobacillus*, condition called bacterial vaginosis is known. Bacterial vaginosis (BV) is caused by an overgrowth of *Gardnerella vaginalis*, anaerobes, *Mycoplasma hominis* (*M. hominis*) [Hill, 1993]. Besides anaerobic BV, another type of vaginal flora disturbances are recovered, called aerobic vaginitis (AV) [Donders, 2002]. Donders analyzed abnormal vaginal microflora, which were neither normal, nor could be defined as BV, and AV in his study was diagnosed if wet mount smears were deficient in lactobacilli, positive for cocci or coarse bacilli, parabasal epithelial cells, vaginal leukocytes. AV was associated with growth of group B streptococci (GBS), *Escherichia coli* (*E. coli*), *Staphylococcus aureus*. The association of AV and preterm deliveries is found [Donders, 2008].

There are different methods to evaluate vaginal flora. Beside Gram stain microscopy and cultivation methods, advanced molecular- based techniques have been developed to better understand compositions of microbial communities of vagina and pathogens, but they are expensive, not widely available and suitable for everyday practice, although identification of abnormal flora might be crucial during the first antenatal visit.

Wet mount is one of the most rapid diagnostic methods for vaginal microflora assessment. Native microscopy allows better validation of lactobacillary flora grades, probably because of lactobacilli loss during Gram fixation and coloring process [Donders, 2000] even dried-rehydrated samples are reliable [Donders, 1996]. Other authors have results comparing native microscopy with Gram stains and bacterial cultures [Donders, 2000], but the usefulness of native microscopy in daily gynecological practice is not extensively studied. At the same time early recognition of pathological vaginal flora with quick, bed-side, cheap diagnostic method done by gynecologist in pregnancy might be very important, especially in such low income country as Latvia is.

The aim

The aim of the study is to identify the relations between native microscopy and vaginal cultures results at the first antenatal visit of pregnancy.

Material and methods

A cross sectional, observational study of pregnant women receiving antenatal care in the five outpatient clinics in Rīga from August, 2010 until April, 2011 was conducted.

During the gynecological examination vaginal pH was measured on the glass slides smears from the upper vaginal wall using Machery Nagel pH strips with a pH range of 3.1–7.0. Vaginal pH ≥ 4.5 was considered abnormal. Those 50 pregnant women with increased vaginal pH and next 50 ones with normal vaginal pH were included in the study.

Exclusion criteria of the study were less than 18 years old, twin pregnancy, less than six and more than 14 weeks of gestation and severe extragenital diseases or present Chlamydia, gonorrhea and / or syphilis infections. The study was approved by the Ethical Committee of Rīga Stradiņš University; all patients signed an informed consent.

From all the included participants, upper vaginal wall specimens for native microscopy and swabs for cultures were taken. Vaginal discharge specimens for microscopy were spread on the glass slide, air-dried and then transported to the one investigator for later microscopy after rehydration of the smear

with a droplet of saline [Larsson, 1990]. A Leica DM1000 microscope (Warburg, Germany) was used with phase contrast at 400 times magnification. Microscopic examination included lactobacillar grades (LBG) and number of leucocytes (less than 10 per high power field (hpf), no leucocytosis, more than 10 per hpf, but less than 10 per epithelial cell, mild leukocytosis, and 10 or more per epithelial cell, heavy leucocytosis) according to Donders' modification of *Schröders'* classification [Donders, 1999]. LBG grade I consisted of predominant presence of *Lactobacillus* morphotypes, with very few coccoid bacteria presented, grade IIa (intermediate, mixed flora) of lactobacilli outnumbering other microorganisms, grade IIb of other microorganisms outnumbering lactobacillary morphotypes, and grade III (completely disturbed flora) had no lactobacilli present. LBG III was further divided in three subgroups: BV, AV and a mixed AV-BV flora. Diagnosis of bacterial vaginosis was performed according to the extension of the typical anaerobe granular microflora in the sample. Aerobic vaginitis flora was defined as the presence of small bacilli and/or cocci in pairs or chains and scored according to the severity, taking into account the lactobacillary grade, pattern of the backgraound, number of leucocytes, presence of toxic leucocytes and presence of parabasal cells [Donders, 2002]. Patterns with decreased or absent lactobacillus morphotypes (LBG IIb and all LBG III) were considered as abnormal vaginal flora.

Two cotton-tipped swabs were taken from upper vaginal wall and immediately placed in Amies medium and during 24 hours transported to the laboratory of Infectology Center of Latvia. Then the samples were inoculated to the appropriate media for the investigation of such microorganisms as *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Staphylococcus aureus*, Viridans group streptococci, enterococci, *Candida* spiecies *spp.*, pathogenic enteric bacteria, *Haemophilus spp.*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and genital mycoplasmas (*Ureaplasma urealyticum* and *Mycoplasma hominis*). More than 10^5 CFU/ml of *U. urealyticum* or *M. hominis* was considered as high numbers.

Vaginal culture results were compared between normal and abnormal vaginal microflora group and no, mild and heavy leucocytosis group based on native microscopy results.

Prevalence of the variables was assessed using 2 × 2 or r × c frequency tables. Statistical significance was tested using Pearson chi-square (χ^2) test or Fisher's exact test. The level of statistical significance was chosen at 5% ($p < 0.05$). Statistical analysis was performed using SPSS version 18.0 (PASW).

Results

From the included 100 patients 50% had normal (LBG I had 39%, (Figure 1), LBG IIa had 11% pregnant women) and 50% abnormal vaginal flora patterns (LBG IIb had 16% (Figure 2), LBG III BV had 8% (Figure 3), LBG III AV had 5% (Figure 4), and LBG III mixed AV-BV flora had 21% of the participants). 44 out of 50 participants with elevated vaginal pH and seven of 50 pregnant women with normal vaginal acidity had abnormal vaginal flora on microscopy.

91% of the participants were culture positive. There were 19 microorganisms found in the vagina, most often coagulase negative (CN) *Staphylococcus* (53 out of 100), *U. urealyticum* (34 out of 100), *E. coli* (18 out of 100), *Candida* species (16 out of 100) and *M. hominis* (15 out of 100), other results are represented in Table 1.

M. hominis (especially in high numbers), *U. urealyticum* and *E. coli* were isolated more in the abnormal vaginal flora group ($p < 0.05$) (Table 1).

All cases of high numbers of *M. hominis* were associated with abnormal flora (LBG III mixed BV-AV flora in eight cases and LBG III BV in one case), but *E. coli* was cultured mostly in the case of decreased amount of lactobacillus morphotypes (LBG IIb in six cases, LBG III aerobic vaginitis in four cases and LBG III mixed flora in three cases) (Table 2).

Heavy leucocytosis was identified in 13%, mild leucocytosis in 44% and no leucocytosis in 43%. *E. coli* was more often associated with heavy and mild leucocytosis, comparing with no leucocytosis ($p < 0.05$) on wet mount.

Figure 1. LBG I: predominant presence of *Lactobacillus* morphotypes

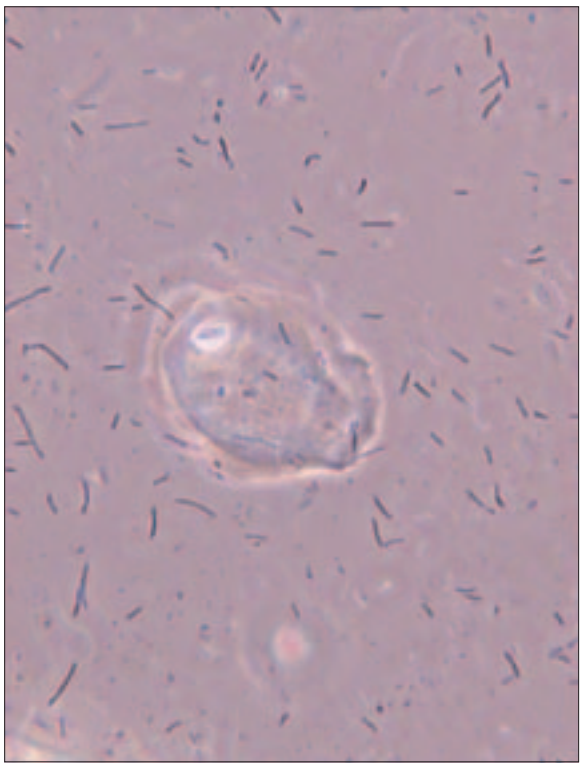

Figure 2. LBG IIb: other microorganisms outnumber lactobacillary morphotypes

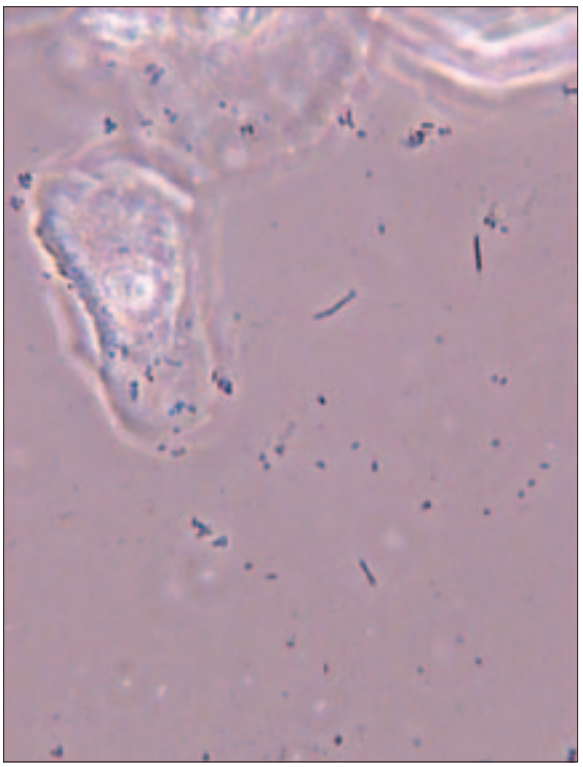

Figure 3. LBG III BV: bacterial vaginosis: anaerobe granular microflora, clue cells

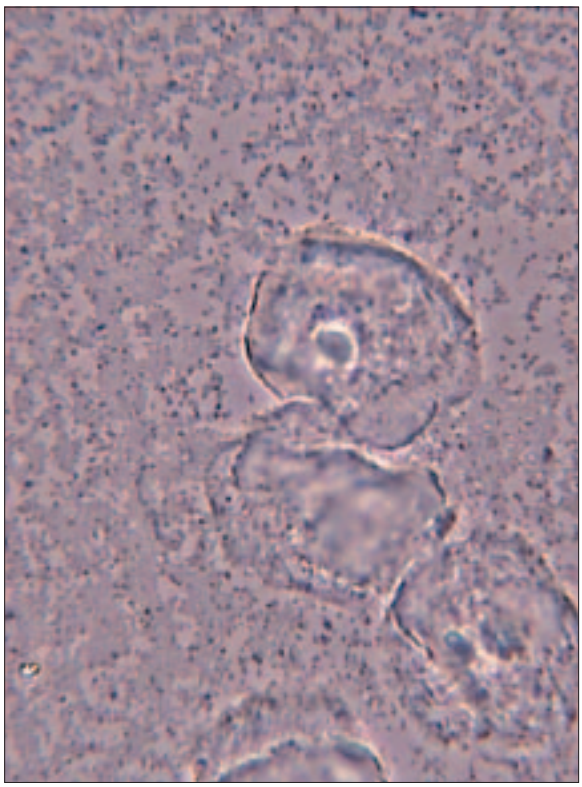

Figure 4. LBG III: aerobic vaginitis: presence of small bacilli and / or cocci

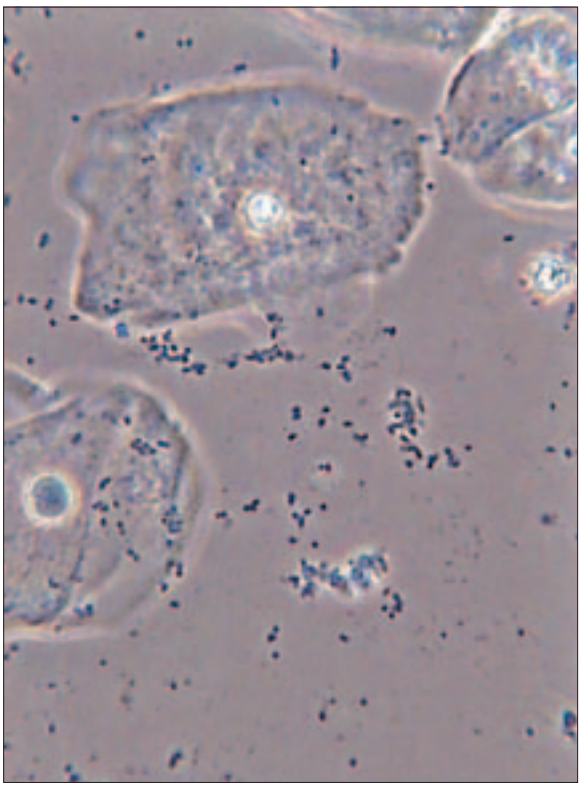

Table 1. Vaginal culture results in normal and abnormal vaginal flora groups

Cultured micro-organisms	Total number of cultures	Normal vaginal flora group (n = 50)	Abnormal vaginal flora group (n = 50)	p value
M. hominis	15	1	14	< 0.001
M. hominis (high numbers)	9	0	9	0.003
U. urealyticum	34	12	22	0.050
S. agalactiae	6	3	3	1.000
S. aureus	4	1	3	0.617
CN *Staphylococcus*	53	25	28	0.548
S. saprophyticus	3	3	0	0.242
S. viridans	5	5	0	0.056
S. oralis	2	1	1	1.000
Peptosteptococcus	2	2	0	0.153
S. vestibularis	1	1	0	1.000
S. salivarius	1	1	0	0.315
S. sanguinalis	1	1	0	1.000
Enterococcus faecalis	5	2	3	1.000
E. coli	18	4	14	0.017
Enterobacter	1	0	1	1.000
Acinetobacter jonsonii	2	1	1	1.000
Acinetobacter lwoffii	2	0	2	1.000
Klebsiella pneumonia	1	0	1	0.315
Candida spp.	16	7	9	0.401

Table 2. Vaginal culture results and lactobacillary grades

Cultured micro-organisms	LBG I (n = 39)	LBG IIa (n = 11)	LBG IIb (n = 16)	BV (n = 8)	AV (n = 5)	BV-AV (n = 21)
M. hominis	0	1	0	3	2	9
M. hominis (high numbers)	0	0	0	1	0	8
U. urealyticum	6	6	2	5	3	12
S. agalactiae	2	1	3	0	0	0
S. aureus	1	0	1	0	1	1
CN *Staphylococcus*	15	10	6	4	6	12
S. saprophyticus	3	0	0	0	0	0
S. viridans	3	2	0	0	0	0
S. oralis	1	0	0	0	0	1
Peptosteptococcus	2	0	0	0	0	0
S. vestibularis	0	1	0	0	0	0
S. salivarius	0	1	0	0	0	0
S. sanguinalis	1	0	0	0	0	0
Enterococcus faecalis	2	0	1	2	0	0
E. coli	1	3	6	1	4	3
Enterobacter	0	0	1	0	0	0
Acinetobacter jonsonii	1	0	0	0	0	1
Acinetobacter lwoffii	0	0	1	0	0	1
Klebsiella pneumonia	0	0	0	0	1	0
Candida spp.	7	0	4	1	2	2

Discussion

The study clearly demonstrated a good correlation between lactobacillary grades, heavy and mild leucocytosis on native microscopy done by gynecologist and potentially pathological bacterial colonization in the vagina.

It is a well recognized association between genital mycoplasmas and such adverse pregnancy outcomes like late miscarriages, preterm birth, low birth weight [Hay, 1994; Taylor-Robinson, 2007]. *M. hominis* and ureaplasmas can cause neonatal respiratory diseases [Steytler, 1970; Taylor-Robinson, 2007]. Rosenstein [Rosenstein, 1996] found that women with BV who are colonized by *M. hominis* organisms usually have much larger numbers than women who do not have BV. Particularly large numbers of *M. hominis* is an important factor for the development of preterm labor [Lamont, 1987]. In our study *M. hominis* in high numbers was also found only in the case of abnormal flora and *U. urealyticum* less frequently was cultured from vagina of pregnant women with normal flora patterns on native microscopy.

Our findings are similar to Donders' [Donders, 2000], which confirmed difference between bacterial vaginosis and abnormal vaginal flora type – aerobic vaginitis. We observed correlation between *E. coli* and heavy/mild leucocytosis, aerobic and mixed aerobic–anaerobic abnormal flora and intermediate flora and these associations were not typical for normal or only BV flora. Although the study did not prove similar correlations with other aerobic bacteria like GBS and *Enterococci*, it was probably because due to the limited number of cases. Of interest, incidence of *E. coli* colonization in the pregnant women vagina was more than GBS. Others have recognized not only GBS, but also increasing role of *E. coli* in the development of early neonatal disease and sepsis, especially in the preterm babies [Stoll, 2011; Lin, 2011]. Recognition of the potential risk group for infectious complications during the first antenatal visit by native microscopy examination seems to be feasible.

It is interesting to admit that *Candida* species colonization were found with the same frequency in the both normal and abnormal vaginal flora groups and was not statistically significant associated with heavy leucocytosis, which demonstrates candidas can exist in the different vaginal environment and can be as a part of co-infection. It is important to recognize the reasons for heavy leucocytosis, as it seems to be associated with aerobic bacteria overgrowth, which is an important risk factor for adverse pregnancy outcomes unlike *Candida* species.

Conclusions

Genital mycoplasmas and *E. coli* were more often isolated microorganisms in cases of abnormal vaginal microflora on native microscopy. Wet mount done by gynecologist can be used as a "bedside" examination method for detection of adverse pregnancy outcome risk group as the abnormal findings are mostly associated with vaginal colonization of potential pathogens. If sexually transmitted diseases are excluded, heavy and mild leucocytosis is more common associated with abnormal, aerobic vaginal flora.

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References

1. Donders G. G. G., Vereecken A., Bosmans E., et al. Aerobic vaginitis: abnormal vaginal flora entity that is distinct from bacterial vaginosis // British Journal of Obsterics and Gynecology, 2002; 109: 1–10.
2. Donders G. G. G., Spitz B., Vereecken A., van Bulck B. The ecology of the vaginal flora at first prenatal visit is associated with preterm delivery and low birth weight // The Open Infectious Diseases Journal, 2008; 2: 45–51.
3. Donders G. G. G., Vereecken A., Salembier G., et al. Assessment of lactobacillary flora in wet mount and fresh or delayed Gram's stain // Infectious Diseases in Obstetrics and Gynecology, 1996; 4: 2–6.
4. Donders G. G. G., Vereecken A., Dekeersmaecker A., et al. Wet mount reflects functional vaginal lactobacillary flora better than Gram stain // Journal of Clinical Pathology, 2000; 53: 308–314.
5. Donders G. G. G. Microscopy of bacterial flora on fresh vaginal smears // Infectious Diseases in Obstetrics and Gynecology, 1999; 7: 126–127.
6. Donders G. G. G., Vereecken A., Bosmans E., et al. Pathogenesis of abnormal vaginal bacterial flora // American Journal of Obstetrics and Gynecology, 2000; 182: 872–878.
7. Garcia-Prats J. A., Cooper T. R., Schneider V. F., et al. Rapid detection of microorganisms in blood cultures of newborn infants utilizing an automated blood culture system // Pediatrics, 2000; 105(3): 523–527.
8. Gardner H. L., Dukes C. D. Haemophilus vaginalis vaginitis. A newly defined specific infection previously classified "nonspecific" vaginitis // American Journal of Obstetrics and Gynecology, 1955; 69: 962–976.
9. Hay P. E., Lamont R. F., Taylor-Robinson D., et al. Abnormal bacterial colonisation of the genital tract and subsequent preterm delivery and late miscarriage // BMJ, 1994; 308 (6924): 295–298.
10. Hill, G. B. The microbiology of bacterial vaginosis // American Journal of Obstetrics and Gynecology, 1993; 169: 450–454.
11. Hillier S. L., Martius J., Krohn M., et al. A case-controlled study of chorioamnionitic infection and histological chorioamnionitis in prematurity // New England Journal of Medicine, 1988; 319: 972–978.
12. Lamont R. F., Taylor-Robinson D., Wigglesworth J. S., et al. The role of mycoplasmas, ureaplasmas and chlamydiae in the genital tract of women presenting in spontaneous early preterm labour // Journal of Medical Microbiology, 1987; 24: 253–257.
13. Larsson P. G., Platz-Christensen J. J. Enumeration of clue cells in rehydrated air-dried vaginal wet smears for the diagnosis of bacterial vaginosis // Obstetrics and Gynecology, 1990; 76: 727–730.
14. Leitich H., Brunbauer M., Bodner-Adler B., et al. Antibiotic treatment of bacterial vaginosis in pregnancy: a meta-analysis // American Journal of Obstetrics and Gynecology, 2003; 188(3): 752–758.
15. Lin C. Y., Hsu C. H., Huang F. Y., et al. The changing face of early-onset neonatal sepsis after the implementation of a maternal group B Streptococcus screening and intrapartum prophylaxis policy – a study in one medical center // Pediatrics & Neonatalogy, 2011; 52(2): 78–84.
16. Klein L. L., Gibbs R. S. Use of microbial cultures and antibiotics in the prevention of infection-associated preterm birth // American Journal of Obstetrics and Gynecology, 2004; 190(6): 1493–1502.
17. Romero R., Espinoza J., Gotsch F., et al. The preterm parturition syndrome // BJOG, 2006; 113(3): 17–42.
18. Romero R., Chaiworapongsa T., Kuivaniemi H., Tromp G. Bacterial vaginosis, the inflammatory response and the risk of preterm birth: a role for genetic epidemiology in the prevention of preterm birth // American Journal of Obstetrics and Gynecology, 2004; 190(6): 1509–1519.
19. Romero R., Espinoza J., Kalache K., et al. Infection and prematurity and the role of preventive strategies // Seminars in Neonatology, 2002; 7(4): 259–274.
20. Rosenstein I. J., Morgan D. J., Sheehan M., et al. Bacterial vaginosis in pregnancy: distribution of bacterial species in different gram-stain categories of the vaginal flora // Journal of Medical Microbiology, 1996; 45: 120–126.
21. Steytler J. G. Studies on endogenous infection by vaginal mycoplasma based on positive cord-blood cultures // South African Journal of Obstetrics and Gynaecology, 1970; 8: 14–22.
22. Stoll B. J., Hansen N. I., Sánchez P. J., et al. Early onset neonatal sepsis: the burden of group B streptococcal and E. coli disease continues // Pediatrics, 2011; 127(5): 817–826.
23. Taylor-Robinson D. The role of mycoplasmas in pregnancy outcome // Best Practice & Research Clinical Obstetrics & Gynaecology, 2007; 21: 425–438.

The Differences of Postoperative Period in Association with Beta-Herpesvirus Infection Activation Depending on the Anaesthesia Method Applied for Prolonged Reconstructive Surgeries

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Abstract

The aim of this study was to investigate the differences of postoperative period in association with β-herpesvirus infection and anaesthesia method applied in patients undergoing long lasting reconstructive microsurgical procedures.

25 patients after major reconstructive microsurgical procedures were enrolled in this investigation. For 11 patients general anaesthesia (GA) and for 14 patients regional anaesthesia (RA) was used. The markers of latent and active HHV-6 and HHV-7 infection were detected. The immune-competent CD4+, CD8+, CD38+, and CD16+ positive cells were detected. Duration of postoperative period, treatment in the ICU, numbers of repeated surgeries, therapy outcome were assessed during postoperative period.

After the surgery in GA group active viral infection was detected in 7 patients, 3 of them had active viral infection before the surgery and infection remained active after the surgery, but in 4 patients – reactivation of the viruses was detected. After the surgery in RA group the reactivation of β-herpesviruses was found in one patient (HHV-7). In GA group 5 cases of unfavourable surgery results were observed. In 3 cases these were infectious complications whereas in 2 cases – flap ischemia. In RA group no cases of unfavourable surgery results were observed. The number of repeated surgeries following prolonged reconstructive surgery until second blood sample was 2.33 ± 0.9 in patients of GA group and 0.84 ± 0.3 in RA group. In GA group the duration of postoperative period was 26.25 ± 0.3 days, in RA group 11.76 ± 1.5 days. Patients from GA group after a long lasting reconstructive surgery spent on average 5.25 ± 3.3 days also in ICU. For the patients of RA group there was no need to treat patients in the ICU.

Our study suggests that in patients undergoing prolonged surgery, general anaesthesia in comparison with regional anaesthesia is associated with a more frequent activation of β-herpesvirus infection and a longer and more complicated postoperative period with a worse clinical outcome.

Despite the limited number of patients, data of our study displayed a possible association between active β-herpesviruses infection, immunosupression and a significantly worse clinical outcome.

Keywords: anaesthesia, β-herpesviruses, postoperative period.

Introduction

Patients who have suffered severe trauma (e.g. bone and overlying tissue loss) tissue defects can be repaired using long lasting microsurgical procedures (e.g. local flaps or free flaps). Usually these patients are in the working age without significant co-morbidities.

Regardless of the young age and adequate previous medical condition of patients, considerable differences of the postoperative period, e.g., surgical outcome, complication rate and duration, have been observed. The above mentioned differences stimulated research on potential causes.

Nowadays there is plenty of information concerning human herpesviruses-6 and -7 and their role in the pathogenesis of various pathologies.

Human herpesvirus-6 and -7 (HHV-6 and HHV-7) are lymphotropic ubiquitous immunomodulating β-herpesviruses. More than 50.0% of adults have serological evidence of previous infection of these viruses. The viruses remain latent in the host after primary infection and persist lifelong in the organism. They can be reactivated in immunosuppressed states resulting in the development of direct pathological lesions. It is also possible that these viruses may indirectly contribute to the activation of other infectious processes by inhibiting the immune system.

Anaesthesia proves to be an essential factor suppressing the immune system during the perioperative period. This can provoke postoperative complications, e.g., wound-healing disturbances and infections, leading to sepsis, followed by multiple organ failure and potential lethal outcome [Homburger, 2006].

The data of our previous studies displayed the significant presence of latent/persistent β-herpesvirus infection preoperatively. HHV-6 infection was detected in 12 out of 25 (48.0%) and latent/persistent HHV-7 infection was detected in 23 out of 25 patients (92.0%). Also the activation of β-herpesvirus infection after long lasting surgical procedures applying different anaesthesia methods was reported in previous publications [Chapenko, 2004; Mamaja, 2007; Mamaja, 2008].

The aim

The aim of this study was to investigate the differences of postoperative period in association with β-herpesvirus infection and anaesthesia method applied in patients undergoing long lasting microsurgical procedures

Material and methods

A retrospective analysis was done for 25 patients (aged 10–65) who underwent long lasting (5–9 h) major reconstructive microsurgical procedures in Latvian Centre of Reconstructive and Microsurgery. Cohort was established with the approval of the Ethics Committee of Rīga Stradiņš University and all participants gave their informed consent prior to the examination. Patients were split into two study groups according to anaesthesia method applied – general anaesthesia (GA) group and regional anaesthesia (RA) group.

For 11 patients general anaesthesia (GA) and for 14 patients regional anaesthesia (RA) was used.

All patients had complicated previous history, 3–15 surgical procedures under GA or RA from initial trauma to long lasting reconstructive surgery.

GA was performed with propofol, fentanyl and inhalation agents (sevoflurane, isoflurane).

Surgical procedures performed are summarized in Table 1.

RA was performed with two methods simultaneously (spinal anaesthesia and brachial plexus block). For brachial plexus block ropivacaine or bupivacaine with lidocaine and with nerve stimulation technique was performed. For spinal anaesthesia bupivacaine spinal at L2-L3 was used.

Ethylenediaminetetraacetic acid (EDTA) anti-coagulated peripheral blood samples from patients were collected before and 10 days after the anaesthesia and surgery. After centrifugation plasma samples were stored at –70 °C.

Table 1. Surgical procedures performed

Type of flap	Number of surgeries provided, n
Local flaps:	
TRAM	2
Free flaps:	
Muscular	4
Fasciculocutaneus	11
Osteocutaneus	4
Toe transplantation	3

Immunological status. Absolute count of peripheral blood CD4+, CD8+, CD38+, and CD16+ positive cells were detected by laser flow cytofluorometer (FACS, Calibur, USA, Becton Dickinson) using corresponding monoclonal antibodies. Patients with CD4+ / CD8+ ratio below 1.0 were considered as having immunologic deficit.

Nested polymerase chain reaction (nPCR) was used for the detection of HHV-6 and HHV-7 sequences in peripheral blood leukocytes (PBL) and plasma DNAs. The presence of viral sequences in PBL DNAs was a marker of latent/persistent viral infection and in plasma DNAs – of active viral infection (plasma viremia).

Duration of postoperative period, treatment in the ICU, number of repeated surgeries, therapy outcome in relation to the anaesthesia method and activation of β-herpesviruses were assessed.

Results

Frequency of latent/persistent and active HHV-6 and HHV-7 infection before and after anaesthesia and surgery are summarized in Table 2.

Latent/persistent HHV-6 / HHV-7 viral infection (the presence of viral genomic sequences in PBL DNA) before the surgery was revealed in 18 patients in GA group and in17 patients in RA group.

Active HHV-6 / HHV-7 viral infection (the presence of viral genomic sequences in plasma DNA) before the surgery was revealed in 3 patients in GA group and in 3 patients in RA group. All 6 patients who had active viral infection before the surgery also had complicated previous history before the reconstructive surgery in comparison with patients without active viral infection.

After long lasting reconstructive surgery in GA group active HHV-6 / HHV-7 viral infection was detected in 7 patients, 3 of them had active viral infection before the surgery and infection remained active after the surgery, but in 4 patients – reactivation of the viruses had been detected after long lasting reconstructive surgery.

After long lasting reconstructive surgery in RA group the reactivation of HHV-6 / HHV-7 viral infection was found only in one patient (HHV-7). It should be stated that active HHV-7 infection which was revealed in one patient before the surgery was not detected after it. In RA group in two patients who had active HHV-6 infection before the surgery viral infection remains active also after the surgery.

Simultaneous activation of two viruses (HHV-6 + HHV-7) was revealed in one patient (GA group) after the surgery whereas in RA group simultaneous activation of two viruses (HHV-6 + HHV-7) was not revealed.

The main characteristics of postoperative period are summarized in Table 3.

In GA group 5 cases of unfavourable surgery results were observed. In 3 cases these were infectious complications whereas in 2 cases – flap ischemia.

Patients with CD4+ / CD8+ ratio below 1.0 were considered as having immunologic deficit. Patients with immunologic deficit are summarized in Table 4.

In two patients of GA group, to whom infectious complications were observed, immunologic deficiency was observed both prior and after the surgery.

Table 2. Frequency of latent / persistent and active HHV-6 and HHV-7 infection before and after anaesthesia and surgery

Anaesthesia		Active/latent viral infection	
		HHV-6	HHV-7
Before surgery	General (n = 11)	1 / 8	2 / 10
	Regional (n = 14)	0 / 4	3 / 13
After surgery	General (n = 11)	2 / 8	5 / 10
	Regional (n = 14)	0 / 4	3 / 13

Table 3. The main characteristics of postoperative period

Characteristics	General anaesthesia (n = 12)	Regional anaesthesia (n = 13)
Number of unfavourable surgeries, n:	5	0
Infectious complications	3	0
Flap ischemia	2	0
Number of repeated surgeries until second blood sample, n	2.33 ± 0.9	0.84 ± 0.3
Time spent in ICU, days	5.25 ± 3.3	0
Duration of postoperative period, days	26.25 ± 0.3	11.76 ± 1.5

Table 4. Patients with immunologic deficit

Patients	General anaesthesia (n = 11)	Regional anaesthesia (n = 14)
Number of patients with immunologic deficiency preoperatively, n	2	0
Number of patients with immunologic deficiency postoperatively, n	2	0

Furthermore, one patient with immunologic deficiency state and postoperative infectious complications had active β-herpesvirus infection already before surgery. In patients of the RA group no cases of unfavourable surgery results were observed.

The number of repeated surgeries following prolonged reconstructive surgery until second blood sample was 2.33 ± 0.9 in patients of GA group and 0.84 ± 0.3 in RA group.

In GA group the postoperative period was significantly longer (26.25 ± 0.3 days) in comparison with RA group (11.76 ± 1.5 days). Patients from GA group after a long lasting reconstructive surgery spent on average 5.25 ± 3.3 days also in ICU. For patients of RA group there was no need to treat patients in the ICU.

Discussion

Currently, there are no clinical studies evaluating the influence of surgery and the method of anaesthesia applied to the reactivation of β-herpesviruses. General or regional anaesthesia alone, without operation, has periodical and minor effect on the immunological system.

However, this situation can change in the course of operation [Schneemilch, 2005; Schneemilch, 2001]. It has been stated that different anaesthesia procedures during one and the same operation cause various alterations in the cytokine level in serum [Dermitzaki, 2009].

General anaesthesia does not suppress the surgical stress response, thus exacerbating postoperative immunosuppression [Stevenson, 1990; Lundy, 1978]. Spinal anaesthesia results in less immunosuppression, i.e. it maintains the number of Th1 cells, thus stimulating cell immunity. The effects are most pronounced in high risk patients undergoing procedures below the umbilicus [Liu, 1995]. Serious disorder of the immunological system may cause complications, as there are disorders in wound healing, increased number of infections, inadequate response to stress, multiorganic suppression and increased incidence of metastases [Rosen, 1992].

Much attention has been focused on identifying the role of β-herpesvirus infection in immunocompromised patients. The rationale is that all other herpesviruses cause significant diseases in this group of patients. HHV-6B and HHV-7 are ubiquitous throughout the adult population, periodically reactivating and causing several manifestations in immunocompromised hosts [Miyake, 2006]. In the immunocompetent setting, these re-activations are often asymptomatic, but in immunosuppressed individuals there can be serious complications. HHV-6 re-activation causes a severe disease in transplant recipients and can lead to graft rejection. HHV-6 has been reported in multiple sclerosis patients and has been implicated as a co-factor in several other diseases, including chronic fatigue syndrome, fibromyalgia, AIDS, and temporal lobe epilepsy but no definitive link has been established. Results of other investigations showed the activation of herpesviruses in early period after drug-induced immunosuppression [Chapenko, 2000; Osman, 1996].

Our study leads to an assumption that there could be a possible link between imunosupression due to anaesthesia and a possible reactivation of β-herpesviruses. Our aim was to investigate the association between the applied types of anaesthesia and activation of β-herpesviruses. In the study we have found that reactivation of β-herpesviruses after long lasting reconstructive surgery was significantly higher in patients with GA than with RA.

Also comparing the absolute count of CD38+ and NK cells in patients without activation of viral infection in general and regional anaesthesia groups in postoperative period we found: in the regional anaesthesia group after the surgery immune response showed statistically significant increase of activated lymphocytes CD38+ ($p < 0.05$) and tendency of increase in number of NK cells. In general anaesthesia group immune response was significantly weaker [Vilks, 2010].

Thus, these findings indicate a possible correlation between GA, immunosuppression and reactivation of β-herpesviruses. In real life it is difficult to distinguish between the contribution of surgical stress, anaesthetics, and analgesic agents to a patient's immune system.

Numerous studies [Kurosawa, 2008] have shown that recently, alongside with immune suppression caused by surgical stress, anaesthetics and analgesic agents commonly used in surgery and in intensive care may directly affect the functions of immune-competent cells. In comparison to surgical stress, anaesthetics probably have a minor effect on the immune system in patients undergoing surgery, because surgery by itself is reported to cause a three- to fourfold increase in the retention of tumour metastases when compared to findings in groups in which the effects of anaesthesia and surgery were combined [Vallejo, 2003]. Therefore GA could be considered as one of the reasons of reactivation of β-herpesvirus infection in postoperative period. In addition to surgical trauma, patient related factors such as malignancy, steroid medication, nutrition, age, sex and any neural impulse that could result in immune suppression should be considered to reach a more convincing conclusion regarding the immunologic effects of anaesthesia [Moudgil, 1986].

Conclusion

Our study suggests that in patients, undergoing prolonged surgery, general anaesthesia in comparison with regional anaesthesia is associated with a more frequent activation of β-herpesvirus infection and a longer and more complicated postoperative period with a worse clinical outcome.

Despite the limited number of patients, data of our study displayed a possible association between active β-herpesviruses infection, immunosupression and a significantly worse clinical outcome.

References

1. Chapenko S., Folkmane I., Tomsone V., et al. Co-infection of two beta-herpesviruses (CMV and HHV-7) as an increased risk factor for "CMV disease" in patients undergoing renal transplantation // Clin Transplant, 2000; 14(5): 486–492.
2. Chapenko S., Mamaja B., Donina S., et al. Activation of beta-herpes viruses by immunosuppression associated with general anesthesia and major reconstructive surgery // RSU Scientific Papers, 2004. – Pp. 171–174.
3. Dermitzaki E. A randomised study of maternal serum cytokine levels following caesarean section under general or neuraxial anaesthesia // International Journal of Obstetric Anesthesia, 2009; 18: 33–37.
4. Homburger J. A., Meiler S. E., Anaesthesia drugs, immunity, and long-term outcome // Current Opinion in Anaesthesiology, 2006; 19(4): 423–428.
5. Kurosawa S., Kato M. Anesthetics, immune cells, and immune responses // Journal of Anesthesia, 2008; 22: 263–277.
6. Liu S., Carpenter R. L., Neal J. M. Epidural anesthesia and analgesia. Their role in postoperative outcome // Anesthesiology, 1995; 82: 1474–1506.
7. Lundy J., Lovett E. J. I., Conran P. Halothane, surgery, immunosupression and artificial pulmonary metastases // Cancer, 1978; 41: 827–830.
8. Mamaja B., Chapenko S., Donina S., et al. Effect of general and regional anaesthesia on beta-herpes viruses activation // Thesis of RSU Scientific conference, 2007; 136.
9. Mamaja B., Chapenko S., Donina S., et al. Effect of general and regional anaesthesia upon major reconstructive surgery on beta-herpesviruses activation // RSU Scientific Papers, 2008; 42–45.
10. Mamaja B., Chapenko S., Donina S., et al. Effect of anaesthesia upon major reconstructive surgery on beta-herpesviruses activation // European J Anaesthesiol, 2008; 25(Suppl. 44): 123.
11. Miyake F., Yoshikawa T., Sun H., et al. Latent infection of human herpesvirus 7 in CD4+ T lymphocytes // Journal of Medical Virology, 2006; 78(1): 112–116.
12. Moudgil G. C. Update on anaesthesia and the immune response // Can Anaesth Soc J, 1986; (33): 54–60.
13. Osman H. K. E., Peiris J. S. H., Taylor C. E., et al. Cytomegalovirus disease in renal allograft recipients: is human herpesvirus 7 a co-factor for disease progression // Journal of Medical Virology, 1996; 48: 295–301.
14. Rosen C. B., Nagorney D. M., Taswell H. F., et al. Perioperative blood transfusion and determinants of survival after liver resection for metastatic colorectal carcinoma // Ann Surg, 1992; 216: 493–504.
15. Schneemilch C. E., et al. Effect of 2 anaesthetic techniques on the postoperative proinflammatory and anti-inflammatory cytokine response and cellular immune function to minor surgery // Journal of Clinical Anaesthesia, 2005; 17: 517–527.
16. Schneemilch C. E., Bank U. Release of pro- and anti-inflammatory cytokines during different anaesthesia procedures // Anaesthesiol Reanim, 2001; 26(1): 4–10.
17. Stevenson G. W., Hall S. C., Rudnick S., Seleny F. L., Stevenson H. C. The effect of anaesthetic agents on the human immune response // Anesthesiology, 1990; 72: 542–552.
18. Vallejo R., Hord D. E., Barna S. A., et al. Perioperative immunosuppression in cancer patients // Journal of Environmental Pathology, Toxicology and Oncology, 2003; 22(2): 139–146.
19. Vilks A., Donina S., Murovska M., et al. The effect of regional and general anaesthesia on activation of beta-herpesviruses and immune response // RSU Scientific Papers, 2010. – Pp. 95–99.

Timely and Late Recognition of the Coarctation of the Aorta in Neonates and Small Infants

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Abstract

The aim of the study is to explore the epidemiology of the coarctation of the aorta in newborn infants in Latvia, to analyze all the cases of aortic coarctations diagnosed and operated in our hospital within the first two months of human's life in the period from January 1, 2005 to December 31, 2010, and to indicate the time of diagnosis and its relation to the condition of the patient at the moment of the admission, the course of the disease and the outcomes.

Retrospective analysis of the case histories of all the neonates and infants in the age group up to two months (n = 45) diagnosed the coarctation of the aorta and undergone surgical correction of the coarctation.

The prevalence of the coarctation of the aorta in the period of 2005–2010 was 0.41 ± 0.11 per 1000 live-born infants. We studied 45 babies, 14 newborns (31%) and 31 infant (69%). Our data show that 64% (n = 29) of the patients were sent by maternity hospitals but 36% (n = 16) after the discharge from the maternity hospital. The diagnoses of the referral were the coarctation or congenital heart disease suspected in 73% (n = 33), but other diagnoses (sepsis, pneumonia, feeding disturbances) suspected in 27% (n = 12) of the cases. In the study group there were 51% (n = 23) patients with isolated aortic coarctation, 29% (n = 13) in combination with VSD but 20% (n = 9) cases with complex coarctation. There was correlation between concomitant intracardiac pathology and antenatal diagnosis observed. The admission after the discharge from maternity hospital correlates with older age at the time of the operation, slightly higher rates of the need for inotropes and assisted ventilation but lower overall mortality rates in the group of delayed diagnosis although no statistically significant. The infants who left hospital undiagnosed had a much greater proportion of serious acidosis at the time of diagnosis (45%) than those diagnosed before the discharge from the maternity hospital (7%; p = 0.031).

The substantial number of unrecognized cases in prehospital stage are indicative of the need for further education for paediatricians and general practitioners working with neonates and small infants, because delayed diagnosis may carry worsened surgical outcomes and increased length of hospital stay.

Keywords: diagnosis, congenital heart disease, coarctation.

Introduction

Congenital heart diseases (CHD) affect 8–12 per 1000 live-born infants and it is one of the most common and serious congenital anomalies. Approximately one quarter of these children will have critical congenital heart disease which requires surgery or catheter intervention in the first year of life [1, 2].

The incidence of the coarctation of the aorta is approximately 36 (29–49)/100 000 infants. Aortic coarctation accounts for 6–10% of all the congenital heart diseases [1, 3, 4, and 5]. According to the data from European surveillance of congenital anomalies, the prevalence of aortic coarctation excluding chromosomal anomalies during years from 2005 to 2009 in summary from all the registries ranged 2.49–3.01 per 10 000 live births (it ranked from 0.78 per 10 000 live births in Spain hospital network registry to 7.81 per 10 000 live births in Finland) [6]. Ductal dependent coarctation may require early surgical intervention. If not diagnosed early in life, it can result in severe morbidity and mortality. In approximately 64% of the infants with aortic coarctation it manifests as a leading congenital heart disease soon after birth [7]. Nevertheless, many infants born with congenital heart disease are discharged from the hospital with their condition undiagnosed, even with newer diagnostic techniques congenital heart diseases in many children are likely missed in the newborn period [1, 8]. There are still high infant mortality rates in Latvia which were 7.8/1000 in year 2005 and 8.42/1000 in year 2010 (the number of deaths of infants under one year old in a given year per 1000 live births in the same year) [9].Congenital malformations are one of the leading causes of infant death in developed countries. Timely recognition of CHD could improve outcomes, because in case of critical CHD, intervention is typically performed in the first weeks of life to optimize hemodynamics and prevent end-organ injury associated with delayed diagnosis [8, 11].

The aim

The aim of the study is to explore the epidemiology of the coarctation of the aorta in newborn infants in Latvia over the period of 2000–2010, and compare the data from our population to the data from literature. To analyze all the cases of aortic coarctations diagnosed and operated in our clinics within the first two months of life over the period of 2005–2010, to find out the time of diagnosis and its relation to the condition of the patient at the moment of the admission, the course of the disease and the outcomes. The age group was chosen due to the ductus dependent coarctation as a potentially life threatening disease in neonates and small infants manifesting with the severe progressing heart insufficiency and carrying serious morbidity and high mortality rates if left unrecognized and untreated. The more recent period of time was chosen because of better diagnostics and more often uses of prostaglandin E1 in perinatal centres in Latvia, since 2005, as well as much better prenatal diagnostics (PgE1 was used in only 1 case in the period of January 1, 2000–December 31, 2004 vs. 27 cases in the period of January 1, 2005–December 31, 2010).

Material and methods

The study was approved by the Ethics Committee of Children's Clinical University Hospital. Retrospective analysis of the case histories of all the neonates and infants in the age group up to two months diagnosed the coarctation of the aorta and undergone surgical correction of the coarctation in our institution between January 1, 2005 and December 31, 2010 was carried out. To analyze the epidemiology of the coarctation of the aorta in newborn infants in Latvia the analysis and the comparison of the data of all the neonates and infants up to the age of 12 months treated in our institution with the diagnosis of aortic coarctation in the period of January 1, 2000–December 31, 2004 and the period of January 1, 2005–December 31, 2010 in correlation to the birth rates in our country within these years was carried out. There is no national registry of congenital heart diseases in Latvia but since 2006 the registration of the newly diagnosed heart diseases in our institution has been started. For data storing and processing the Microsoft Office Excel 2003 program was used. Statistical analysis was performed with SPSS 16. The anthropometric and clinical characteristics were summarized as mean and standard deviations and as a percentage of the group for categorical variables. Pearson's chi-square (χ^2) test and Fishers exact tests were used to compare the groups of patients (the patients referred by the maternity hospital or admitted after the discharge home, the patients with and without antenatal diagnosis and the patients with poor outcome versus the discharged home after the operation). P value < 0.05 was considered statistically significant. Correlations were calculated by Spearman's rank correlation coefficient.

Results

Our institution – the Clinic for Paediatric Cardiology and Cardiac Surgery of Children's Clinical University Hospital in Rīga is the only institution in our country where congenital heart diseases in paediatric patients are treated, thus the obtained data represent the overall data of the population of our country. There are more than 6000 echocardiographies performed in our clinics annually and all the serious congenital heart diseases in our country are diagnosed, treated and followed up in our clinics. The birth rates in our country within the years 2000–2010 were 21.197 ± 1.212 live-born infants per year [9], the prevalence of the coarctation of the aorta was 0.343 ± 0.12 per 1000 live-born infants (in the period of 2000–2004 it was 0.256 ± 0.086, but in the period of 2005–2010 – 0.41 ± 0.11 per 1000 live-born infants. More detailed data about the incidence and prevalence of the disease are shown in Table 1.

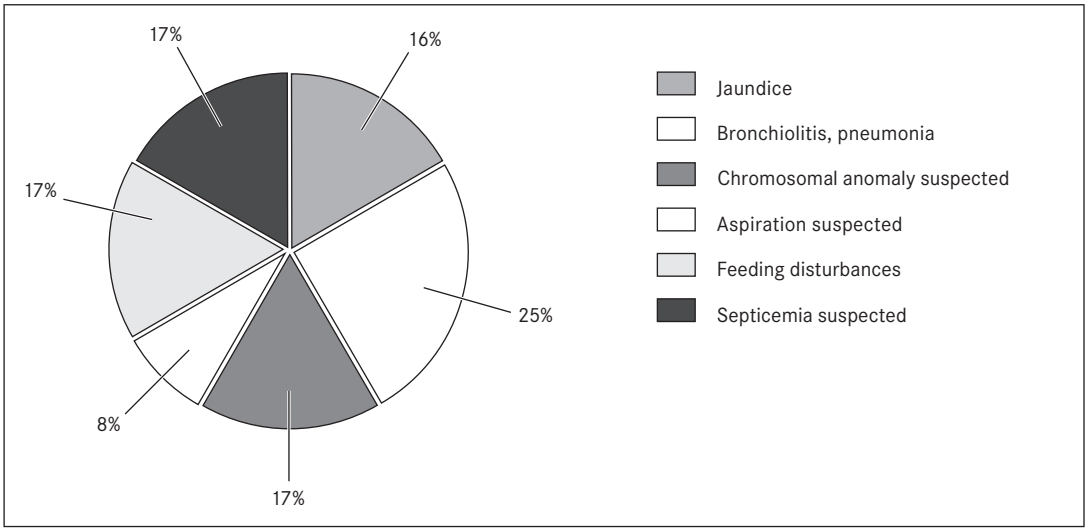
There were 50 patients in the age group up to 2 months old within the period of January1, 2005 to December 31, 2010 treated in our clinic with the diagnosis the coarctation of the aorta. 5 patients were excluded from the further analysis, they were 2 patients which had complex aortic coarctation (in combination with double inlet left ventricle and double outlet right ventricle and died without the operation due to severe heart insufficiency on the first and second days of life), one patient with severe aortic arch hypoplasia, who died on the first day of life despite the therapy, and one patient with borderline hypoplastic left heart syndrome, who did not suffer the operation due to severe left ventricle insufficiency, one patient with the transposition of the great arteries and ventricular septal defect (VSD), severe hypoxemia, acidosis and very late diagnosis at the age of 30 days, who did not suffer the reconstruction of the aortic arch simultaneously with the arterial switch operation and the closure of VSD. The study of 45 babies was carried out, the group consisted of 14 newborns (31%) and 31 infants (69%). There were 44% girls (n = 20) and 56% boys (n = 25) in the study group, the relationship boys to girls was 1/1.25. The data show that 64% (n = 29) of the patients were sent by maternity hospitals but 36% (n = 16) were sent to the hospital after the discharge from the maternity hospital by general practitioner or transferred to our tertiary level university hospital by the department of the emergency medicine or paediatrics of primary or secondary level hospital. The diagnoses of the referral were the coarctation or congenital heart disease with cardiovascular insufficiency suspected in 73% (n = 33) of the cases but other diagnoses (sepsis, pneumonia, feeding disturbances) suspected in 27% (n = 12) of the cases (Figure1).

Table 1. Epidemiology of aortic coarctation in Latvia

Year	Live-born infants in Latvia per year, n*	Incidence of AoCo per 100 000 newborn, %	AoCo operated in newborns and infants per year, n	Died without operation or during combined repair of intra-cardiac lesion, n	AoCo in newborn and infants per year, n	AoCo prevalence in newborn per year, %	AoCo prevalence per 10 000 live-born infants, %
2000	20 248	4.9	1	–	1	0.005	0.48
2001	19 664	40.7	7	–	7	0.04	4.06
2002	20 044	24.94	5	–	5	0.024	2.49
2003	21 006	28.56	5	1	6	0.028	2.85
2004	20 334	29.5	5	1	6	0.029	2.95
2000–2004	20 259 ± 328	25.71 ± 8.6	4.6 ± 1.4	–	5 ± 1.6	0.025	2.56 ± 0.86
2005	21 497	27.91	5	1	6	0.028	2.8
2006	22 264	49.4	11	–	11	0.049	4.9
2007	23 273	60.1	14	–	14	0.06	6.0
2008	23 948	50.1	11	1	12	0.05	5.0
2009	21 677	41.5	7	2	9	0.042	4.2
2010	19 220	20.8	3	1	4	0.02	2.0
2005–2010	21 979 ± 1 181	41.63 ± 11.6	8.5 ± 3.5	–	9.3 ± 3	0.042	4.1 ± 1.1
2000–2010	21 197 ± 1 212	34.4 ± 12.7	6.72 ± 2.9	–	7.36 ± 3	0.035	3.43 ± 1.2

* Source: www.csp.gov.lv/statistika-temas/iedzivotaji-galvenie-raditaji, accessed 04.02.2011. (Central Statistical Bureau of Latvia).

Figure 1. Other referral diagnoses suspected instead of the coarctation



The age of the patients at the time of diagnosis was 16.18 ± 19.8 days (minimum 1, maximum 60, mean 5), the age of the patients at the time of the operation was 23.47 ± 19.8 (minimum 2, maximum 61, mean 13), the weight of the patients was 3.45 ± 0.79 kg (minimum 2, maximum 5, mean 3.4, mode 3). There was antenatal diagnosis suspected in 27% (n = 12) of the cases. In the study group there were 51% (n = 23) patients with isolated aortic coarctation, 29% (n = 13) in combination with VSD, and 20% (n = 9) cases with complex coarctation (the coarctation of the aorta in combination with such intracardiac anomalies as mitral stenosis, aortic stenosis (valvular, subvalvular, supravalvular), double inlet left ventricle with VSD). There was a correlation between concomitant intracardiac pathology and antenatal diagnosis observed (Spearman's correlation coefficient 0.407, p = 0.006, n = 45) (Table 2).

The coarctation was infantile with isthmus hypoplasia in 87% (n = 39), juxtaductal in 9% (n = 4) and postductal in 2% (n = 1). The hypoplasia of the aortic arch was observed in 18% (n = 8). Prostaglandin E1 was used in 100% of antenatally suspected, but 48% postnatally detected cases. Many more cases in combination with VSD or the complex coarctations were transferred directly from the maternity hospital, but 75% (n = 12) of the cases referred only after the discharge from the maternity hospital were infants with isolated coarctations (p = 0.05). Acidosis in capillary blood (medium ph 7.147 ± 0.08) was observed in 45% of infants hospitalised after the discharge from the maternity hospital versus 7% in those infants transferred from the maternity hospital (p = 0.031). The admission after the discharge from maternity hospital correlates with older age at the time of the operation: 13.6 ± 13.8 days versus 41.1 ± 16.08 days (Spearman's correlation coefficient 0.7, p < 0.01, n = 45). There were slightly higher rates of the need for inotropes and assisted ventilation but lower overall mortality rates in the group of delayed diagnosis although not statistically significant. The differences between two groups are represented in Table 3.

There were 6 fatal cases in the postoperative period (within 30 days following surgery) (3 cases of severe heart insufficiency, 2 cases of septicaemia and 1 case of renal insufficiency) and 5 late deaths in the further follow up period in this group (2 cases of fibroelastosis, one case of sudden death at home, one case of death during repair of complex intracardiac pathology later, one case of severe metabolic acidosis, possible septicaemia and cardiac insufficiency (no detailed information, autopsy not performed). There were more fatal cases between patients operated in the neonatal period (p = 0.01), referred from maternity hospital (p = 0.035), the patients with concomitant intracardiac lesions (p = 0.01) and hypoplastic aortic arches (p = 0.04). They were more frequently in need of assisted ventilation (p = 0.028), ProstaglandinE1 infusions (p = 0.003) and more often had elevated levels of blood urea (p = 0.028, median levels 9.82 ± 2.21 mkmol/L, normal ranges 2.5–6.4 mkmol/L). These data in detail are represented in Table 4.

Table 2. Antenatal diagnosis of the coarctation

Group of patients	Antenatal diagnosis		p value (Fisher's exact test)
	Yes	No	
Isolated coarctation	25% (n = 3)	61% (n = 20)	0.008
Coarctation + VSD	25% (n = 3)	30% (n = 10)	
Complex coarctation	50% (n = 6)	9% (n = 3)	

Table 3. Comparison of patients transferred from the maternity hospital and referred after the discharge from the maternity hospital

Factors		Transferred by maternity hospital	Referred after the discharge	p value*
Concomitant intracardiac pathology	Isolated AoCo AoCO+VSD Complex AoCo	38% (11) 34% (10) 28% (8)	75% (12) 19% (3) 6% (1)	0.05
Acidosis in capillary blood	Normal Ph Ph↓	93% (27) 7% (2)	69% (11) 31% (5)	0.031
Inotropic stimulation	Yes No	17% (5) 83% (24)	25% (4) 75% (12)	NS
Assisted ventilation	Yes No	14% (4) 86% (25)	19% (3) 81% (13)	NS
Elevated blood urea	Yes No	86% (25) 14% (4)	81% (13) 19% (3)	NS
Elevated blood creatinin	Yes No	90% (26) 10% (3)	81% (13) 19% (3)	NS
Mortality	Discharged home Postoperative death	n = 24 n = 5	n = 15 n = 1	NS

* Tests of significance were chi-square (χ^2) test and Fisher's exact test. NS – not significant, $p > 0.05$.

Table 4. Mortality risk factors

Factors		Alive	Early or late death	p value*
Antenatal diagnosis	Yes No	24% (8) 76% (26)	36% (4) 64% (7)	NS
Referred by maternity hospital	Yes No	56% (19) 44% (15)	91% (10) 9% (1)	0.035
Age at the time of operation	Neonate Infant	59% (20) 41% (14)	100% (11)	0.01
Concomitant intracardiac pathology	Isolated AoCo AoCo+VSD Complex	62% (21) 23% (8) 15% (5)	19% (2) 45% (5) 36% (4)	0.01
Hypoplasia of aortic arch	Yes No	9% (3) 91% (31)	55% (6) 45% (5)	0.04
PgE1	Used No	50% (17) 50% (17)	100% (11)	0.003
Inotropic stimulation	Yes No	15% (5) 85% (29)	36% (4) 64% (7)	NS
Assisted ventilation	Yes No	9% (3) 91% (31)	36% (4) 64% (7)	0.028
Blood urea	Normal Elevated	91% (31) 9% (3)	64% (7) 36% (4)	0.028
Blood creatinin	Normal Elevated	88% (30) 12% (4)	82% (9) 18% (2)	NS

* Tests of significance were chi-square (χ^2) test and Fisher's exact test. NS – not significant, $p > 0.05$.

Discussion

The obtained data reveal that the prevalence of the coarctation of the aorta does not significantly differ from the data of literature although there are differences between more recent data and the data from the first half of the last decade. As birth rates do not significantly differ, it might witness better diagnostics of congenital heart diseases in newborn and small infants within the last five years with less congenital heart diseases being not diagnosed at all. The data from literature show that infantile aortic coarctation as well as hypoplastic left heart syndrome both constitute three thirds in the group of the missed congenital heart deseases as a cause of death in infants [1]. The data about delayed recognition of congenital heart diseases by Massin and Desy in 2006 showed aortic coarctation as the most frequent diagnosis in the group of delayed diagnosis of acyanotic congenital heart diseases manifesting with the cardiogenic shock preoperatively in half of the cases [13]. Wren, et al. found that 54% of babies leaving hospital with undiagnosed heart disease had coarctation of the aorta and 44% had an interrupted aortic arch [16]. Diagnosis of the congenital heart disease in the first days of life might be a difficult task and furthermore the trends towards early maternity hospital discharge and home deliveries might make it even more challenging. Our data show that still one third or 36% of the babies are referred only after the discharge from the maternity hospital. Early referral correlates with other concomitant intracardiac lesions which allow diagnosing the congenital heart disease. Furthermore, our data demonstrated that the infants who left hospital undiagnosed had a much greater proportion of serious acidosis at the time of diagnosis (45%) than those diagnosed before the discharge from the maternity hospital (7%; $p = 0.031$) which is the sign of potential end organ damage.

Early postnatal diagnosis may improve preoperative condition which influences the postoperative outcome and the length of hospital stay. Missed or lately diagnosed congenital heart diseases limit the timely surgical correction of the disease before severe cardio-vascular deterioration. About one third of newborns with life-threatening congenital heart diseases leave nurseries without the problem being recognized, and risk of death or serious damage from circulatory collapse [9]. In the past few years, researchers have suggested pulse oximetry as an aid to clinical examination for the detection of some forms of congenital heart diseases although it is not sensitive enough to serve as an independent tool. The cost of a universal echocardiographic screening programme has been estimated to be too high. Pulse oximetry can detect just over 50% of newborn infants with coarctation [8, 14, 15, 16, 17, 18], screening pulse oximetry may be the best off-the-shelf techniques available with the combination of physical examination with the cardiological examination for those screened positive. During the last years reduced lengths of stay in maternity units have been practiced but also postnatal examinations have altered, and many units now carry out less neonatal physical examinations. This might be an important factor in the reduced detection of duct dependent circulation. Some maternity units have stopped examining femoral pulses routinely but since substantial number of the babies with duct dependent circulation have poor or absent femoral pulses as a major alerting sign, the omission of palpation of femoral pulses is likely to reduce the detection of duct dependent circulation on clinical examination. The Academy of Paediatrics recommends that every newborn infant should be evaluated within 3–5 days of birth and within 48–72 hours after discharge home, careful cardio-vascular examination should be performed during this visit to detect possible missed congenital heart disease [10].

Conclusions

There is still a very high number of delayed diagnoses of aortic coarctation in neonates and small infants. Most antenataly detected cases of the coarctation are combined with other intracardiac lesions, therefore carry higher mortality rates. Postnatal, after the discharge from the maternity hospital detected, cases correlate with older age, more frequent acidosis in capillary blood observed, slightly although not statistically significantly higher rates of need for inotropes and assisted ventilation but lower mortality rates. The substantial number of unrecognized cases in prehospital stage are indicative of the need for further education for paediatricians and general practitioners working with neonates and small infants, because delayed diagnosis may carry worsened surgical outcomes and increased length hospital stay.

References

1. Chang R. K., Gurvitz M., Rodriguez S. Missed diagnosis of critical congenital heart disease // Arch Pediatr Adolesc Med, 2008; 162: 969–974.
2. Hoffman J. I. E., Kaplan S. The incidence of congenital heart disease // J Am Coll Cardiol, 2002; 39: 1890–1900.
3. Myung K. Park, Troxler G. Pathophysiology of obstructive and valvular regurgitant lesions. Coarctation of the aorta // Pediatric cardiology for practitioners. – 5th ed. – Philadelphia: Mosby Elsevier, 2008. – Pp. 135–136.
4. Marek J., Fenton M., Khambadkone S. Aortic arch anomalies: coarctation of the aorta // Echocardiography in paediatric and congenital heart disease: from fetus to adult. – Wiley-Blackwell, 2009. – Pp. 339–350.
5. Lindinger A., Schwedler G., Hense H. W. Results of PAN study: congenital heart defects in newborns in Germany – prevalence and association with demographic, genetic and peripartal parameters // Cardiology in the Young, 2010; 20(Supp S2): S3.
6. European surveillance of congenital anomalies // www.eurocat_network.eu/prevdata/resultsPdf.aspx?title=A5&allanom=false&allregf=true&allrega=true&anomalies=32&winx=1416&winy=770 (accessed 26.04.2011).
7. Samanek M., Voriskova M. Congenital heart disease among 815 569 children born between 1980–1990 and their 15-year survival: A prospective Bohemia survival study // Pediatr Cardiol, 1999; 20: 411–441.
8. Mahle W. T., Newburger J. W., Matherne P., et al. Role of pulse oximetry in examining newborns for congenital heart disease: a scientific statement from the AHA and AAP // Paediatrics, 2009; 124(2): 823–836.
9. de-Wahl Granelli A., Mellander M., Sandberg K., Sunnegardh J., Östman-Smith. Screening for duct-dependent congenital heart disease with pulse oximetry: a critical evaluation of strategies to maximize sensitivity // Acta Paediatrica, 2005; 94: 1590–1596.
10. Committee on Practice and Ambulatory Medicine; Bright Futures Steering Committee. Recommendations for preventive pediatric health care // Paediatrics, 2007; 120(6): 1376.
11. Central Statistical Bureau of Latvia // www.csp.gov.lv/statistika-temas/iedzivotaji-galvenie-raditaji (accessed 04.02.2011).
12. Freedom R. M., Yoo Sh., Mikailian H., Williams W. G. The natural and modified history of congenital heart disease. – Wiley-Blackwell, 2003. – P. 251.
13. Massin M. M., Dessy H. Delayed recognition of congenital heart disease // Postgrad Med J, 2006; 82(969): 468–470.
14. Chang R. K. How many infants with critical congenital heart disease are missed? // Circulation, 2007; 116: II–614.
15. Rosati E., Chitano G., Dipaola L., et al. Indications and limitations for a neonatal pulse oximetry screening of critical congenital heart disease // J Perinat Med, 2005; 33: 455–457.
16. Mahle W. Physical examination and pulse oximetry in newborn infants: out with the old, in with the new? // The Journal of Pediatrics, 2008; 152(6): 747–748.
17. Wren C., Reinhardt Z., Khawaja K. Twenty-year trends in diagnosis of life-threatening neonatal cardiovascular malformations // Arch Dis Child Fetal Neonatal Ed, 2008; 93: F33–35.
18. De-Wahl Granelli A., Wennergren M., Sandberg K., et al. Impact of pulse oximetry screening on the detection of duct dependent congenital heart disease: a Swedish prospective screening study in 39 821 newborns // BMJ, 2008; 337a3037doi:10.1136/bmj a3037.

Correcting Induced Age-related Hyperopia and Hyperopic Astigmatism by Conductive Keratoplasty – a Single Centre Experience

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Abstract

The immense popularity of surgical vision correction as a by-product has resulted in the emergence of a significant patient group suffering from secondary hyperopia induced by previous refractive surgery; intraocular surgery or traumata playing a much lesser part. Though, LASIC is considered to be the treatment of choice for such patients, conductive keratoplasty (CK) may become a valuable alternative.

CK has been used in the Eye Microsurgery Centre since 2007 (Near Vision REFRACTEC ViewPoint TM CK System produced by Refractec, USA). 17 patients with induced hyperopia were treated in 2007–2009, 8 patients (12 eyes) were subsequently followed-up for more than a year; their detailed analysis is presented. 7 patients were 4–8 years after LASIK, 1 had radial keratotomy in 1989 and LASIK in 2007. The initial refraction was between 0.00 ± 1.0D, astigmatism up to 2.0D.

Refraction immediately after CK was as planned, except the patient after radial keratotomy + LASIK, for whom the result was achieved in 1 month. All patients developed a 0.5–0.75D hyperopic shift within a year, no additional correction was necessary. Mild astigmatism disappeared after CK without subsequent regression. Patients acquired an ability to read newspaper texts without glasses. Far vision improved in most patients, double vision disappeared in patients with astigmatism. All patients had a minimal corneal syndrome, the symptoms resolved within 2–3 days. No other complications have been observed.

Thus, CK is effective for correcting mild induced hyperopia (up to + 1.5D) and medium (up to + 2.0D) hyperopic astigmatism. CK specifics make it suitable for anatomically changed eyes. The results allow evaluating CK as a promising method for correcting induced hyperopia and hyperopic astigmatism.

Keywords: conductive keratoplasty, induced hyperopia, refractive surgery.

Introduction

Induced age-related hyperopia is a widespread by-product of refractive surgery; its prevalence is on the rise due to the latter's enduring popularity. Other causes like traumata and intraocular surgery (surgical treatment of cataract being the most common) exist, though they are much less frequent. Thus, a significant cohort of patients with indications for secondary correction is being formed.

LASIC has been considered a method of choice for these patients; still, its use has practical limitations [1, 2]. The use of implants for secondary correction is technically problematical and not free of complications [3]. Recent studies [4] demonstrated that conductive keratoplasty (CK) may turn out to be a simple and safe method for secondary correction.

The CK technology is based on a controlled impact of radio waves on paracentral corneal stroma that makes the peripheral corneal layer to contract, increasing the cornea's curvature and, consequently, refraction [5, 6]. The method was approved by FDA for hyperopia and presbyopia in 2002; the Federal Aviation Administration approved its use for airline pilots in 2005. CK appeared in Europe in 2004, but still remains fairly uncommon there.

The Eye Microsurgery Centre (EMC) in Rīga has been using both LASIC and CK for primary hyperopia since 2007, thus providing additional flexibility and a more individualised approach to correction. Both techniques are also employed for secondary correction of previously surgically treated eyes. The paper presents our 2007–2009 data on the use of CK for secondary correction.

Material and methods

17 patients (25 eyes) who wished to correct far-sightedness that had developed after previous refractive surgery were treated by CK in 2007–2009. The main criteria for preferring CK to excimer laser correction available in the Centre (Allegretto Wave Eye-Q system, approved by FDA for correcting hyperopia up to +6.0D and astigmatism up to +4.0D) were: thin cornea (below 480 μ at the flap site), fast postoperative rehabilitation required, somatic diseases or a rather common reluctance of patients to undergo laser surgery.

All patients were routinely checked on days 1 and 7 after the surgery. Due to very good subjective condition, patients as a rule preferred to limit their follow-up, so only 8 patients (12 eyes) have been followed for a year or longer and were included in the study.

The age of the patients was between 48 and 64.3 were men and 5 women. 7 patients had had LASIK correction 4–8 years previously, 1 had underwent radial keratotomy in 1989 and LASIK in 2007. The initial refraction in the analyzed group varied from 0.00 to +1.0D with astigmatism up to 2.0D (Table 1).

The CK procedure was performed using Near Vision REFRACTEC ViewPoint TM CK System (produced by Refractec, USA) and surgical microscope Leica M841 EBS.

The correction in these cases was aimed at the attainment of the most comfortable achievable glass-free reading or glasses simplification in cases of hyperopic astigmatism. So each planned result had been calculated individually; for example, in case of "monovision" – with the accent on the dominant eye. Both eyes were corrected simultaneously for hyperopy, while the procedure was performed separately with interval of 1–4 weeks for hyperopic astigmatism.

The evaluations of correction outcome and follow-ups were performed measuring near and far visual acuity, refractometry and corneal topography (automatic sign projector Nidek CP-690, autorefractometer Nidek ARK 510A, topographic system ALLEGRO Topolyzer).

Results

Results of refraction correction. The results of CK correction and their dynamic changes are shown in Table 1.

Table 1. Average values for refraction and keratometry in relation to initial refraction

Group	Number of eyes	Average refraction				Average keratometry		
		Before CK	After 24 hours	After a month	After a year	Before CK	After 24 hours	After a year
1	5	0.00	−1.25	−0.90	−0.80	42.90	43.71	43.30
2	4	+1.0	−1.75	−0.92	−0.89	42.82	45.86	45.40
3	2	Astigmatism +0.75	−0.50	0.00	0.00	42.55	43.10	42.71
4*	1	Astigmatism +2.0	0.00	0.25	0.75	40.70	42.10	41.20

* The single patient after radial keratotomy + LASIK.

In all cases the refraction immediately after CK was as planned, except the patient after radial keratotomy + LASIK, for whom the planned result was achieved in a month after the procedure.

Refractometry of all patients performed within a year after CK revealed a 0.5–0.75D hyperopic shift with a corresponding change of keratometry. No additional correction was necessary.

In both patients with mild astigmatism (group 3) control check-ups showed its stable disappearance after CK.

Vision acuity. The fully corrected vision acuity in all patients remained unchanged after CK. All patients from groups 1 and 2 acquired an ability to read newspaper texts without glasses.

Far vision acuity after CK varied between 20/40 and 20/20. 4 out of 5 first group patients did not complain about worsened far vision, in spite of the induced myopia (see Table 1). One patient lost two lines of uncorrected far vision. 3 out of 4 patients of the second group observed an improvement of far vision (though they had not used glasses for far vision before CK).

Both patients from group 3 noted improved far vision and disappearance of text letters' shadows (double vision).

The last patient observed an improvement of far and near vision without glasses.

Postoperative period. All patients had a minimal corneal syndrome, possibly due to decreased sensitivity of the previously surgically treated cornea. They were allowed to work next day after the procedure; all limitations were raised upon complete resolving of corneal symptoms (usually in 2–3 days).

Anti-inflammatory and antibacterial drops were administered for 1 week, and artificial tears were used on demand.

No other postoperative complications have been observed.

Discussion

Some of the induced ametropias are not prone to the "ideal" correction; still, the need for secondary correction is steadily increasing, for many patients who have difficulties with the choice of glasses benefit from vision improvement due to simplified refraction.

The presented patients demonstrate that CK correction could be successfully employed for correcting mild induced hyperopia and hyperopic astigmatism, that is well in accordance with the literature data [6, 7, 8]. The procedure was very well tolerated, created only minimal postoperative discomfort and had no risk of complications.

According to our experience, CK produced mild and quickly resolving primary hypereffect that allows for fast postoperative rehabilitation.

The correction effect remained stable during the first year after CK; we have not observed the significant regression described by several users [9]. A longer follow-up will be necessary for more definite conclusions.

Thus, the use of CK offers a simpler alternative to laser correction for patients with mild induced ametropy. On the other hand, being a more "gentle" approach in comparison to LASIC, CK offers additional possibilities for surgical correction in cases when anatomical changes after previous surgical treatment make LASIK potentially unsafe. Thus, the availability of two alternative methods provides a refractive surgeon with a considerable advantage.

Conclusions

Our experience with CK demonstrates that the method is suitable and effective for correcting mild induced hyperopia (up to +1.5D) and medium (up to +2.0D) hyperopic astigmatism.

The procedure is technically simple, precise and, according to our experience, produces stable effect. Its main advantages include minimal intervention, fast rehabilitation and minimal postoperative restrictions.

The patients observed decreased dependence on glasses and in general a more comfortable vision.

CK specifics make it suitable for correcting anatomically changed eyes, like after previous surgery or trauma, as well as in case of keratoconus.

The results allow evaluating CK as a promising method for correcting induced hyperopia and hyperopic astigmatism.

References

1. Gressel M. G., Belsole V. L. Laser in situ keratomileusis flap tear during lifting for enhancement in the presence of post-photorefractive keratectomy corneal haze // J Cataract Refract Surg, 2004; 30(3): 706–708.
2. Francesconi C. M., Nosé R. A., Nosé W. Hyperopic laser-assisted in situ keratomileusis for radial keratotomy induced hyperopia // Ophthalmology, 2002; 109(3): 602–605.
3. Devgan U. The surgical correction of hyperopia after RK // Cat & Refr Surg, 2008; 71–73.
4. Karpecki P. M. Does CK do OK after LASIK? // Rev of Optometry, Apr 2006.
5. Gatinel D. Presbyopia surgery // Rev Prat, 2008; 58(10): 1049–1054.
6. Stahl J. E. Conductive keratoplasty for presbyopia: 3-year results // J Refract Surg, 2007; 23(9): 905–910.
7. Ardjomand N., Wohlfart C., McAlister J. C., El-Shabrawi Y., Vidic B. Conductive keratoplasty for asymmetric corneal astigmatism // J Cataract Refract Surg, 2008; 34(5): 874–875.
8. Du T. T., Fan V. C., Asbell P. A. conductive keratoplasty // Curr Opin Ophthalmol, 2007; 18(4): 334–337.
9. Esquenazi S., He J., Kim D. B., Bazan N. G., Bui V., Bazan H. E. Wound-healing response and refractive regression after conductive keratoplasty // J Cataract Refract Surg, 2006; 32(3): 480–486.

Influence of Interleukin-10 Gene Polymorphisms on the Activity of Rheumatoid Arthritis in the Population of Latvia

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Abstract

A central feature of rheumatoid arthritis (RA) is a relative imbalance of cytokine production with a relative excess of proinflammatory molecules including tumor necrosis factor, interleukin-1 (IL-1), IL-6 compared with antiinflammatory mediators such as IL-10. IL-10 polymorphisms were studied for association with RA in multiple populations, but have not been tested so far in the population of Latvia.

The purpose of our study was to determine whether polymorphisms of IL-10 gene in position -592 (rs1800872), -819 (rs1800871), -1082 (rs1800896) are associated with RA and its characteristics in the population of Latvia.

The study included 105 patients with RA diagnosed according to the criteria of the ACR. The following data was obtained from RA patients: age of disease onset, number of tender and swollen joints, visual analogue scale of pain and disease activity, C-reactive protein, ESR, antibody status, RTG stage, DAS28 was calculated. DNA from healthy control population was obtained form Genome Database of Latvian Population. Genotypes were obtained by direct sequencing. SNPs were studied and frequencies of alleles and genotypes were compared between patients and controls. A p value less than 0.05 was accepted as statistically significant.

Statistically significant dependency between rs1800872 C/A, rs1800871 C/T genotypes and disease activity parameters such as TEN28 ($p = 0.009$), physician assessment of disease activity ($p = 0.025$), ESR ($p = 0.049$), DAS28 ESR ($p = 0.018$) and association between these genotypes and DAS28 CRP (0.063) was identified. IL-10 rs1800872 AA, CA and IL-10 rs1800871 TT, CT genotypes were found with more active disease compared with CC genotypes in both positions. There were no differences found between these genotypes distribution and antibody status (RF, antiCCP), RTG stages. We found no differences in rs1800896 A/G genotypes distribution and disease parameters.

The study suggests that the genetic polymorphisms of IL-10 gene have an influence on rheumatoid arthritis activity in the population of Latvia.

Keywords: rheumatoid arthritis, IL-10, genetics, susceptibility.

Introduction

Rheumatoid arthritis (RA) is the systemic inflammatory disease, characterized by chronic synovitis and progressive joint destruction. The etiology of RA is multifactorial and includes a significant genetic component. Cytokines act as mediators of immune and inflammatory responses and play an important

role in the pathophysiology of joint inflammation and destruction [Feldman, et al., 1996]. A central feature of RA is a relative imbalance of cytokine production with a relative excess of proinflammatory molecules including tumor necrosis factor α (TNFα), interleukin-1 (IL-1), IL-6 compared with anti-inflammatory mediators such as IL-10 and IL-4 [Feldman et al., 1996]. IL-10 is produced by variety of cell types, including monocytes and B lymphocytes. Variation in IL-10 secretion is genetically determined and the differences in secretion have been associated with various chronic inflammatory and infectious diseases [Westendorp, et al., 1997]. IL-10 has anti-inflammatory capabilities, it can directly down-regulate production of TNFα, IL-1, IL-8 and interferon-γ [Katsikis, et al., 1994]. The IL-10 gene maps to the junction of 1q31–q32 [Eskdale, et al., 1997]. Eight single nucleotide polymorphisms (SNPs) have been identified in the promoter region of this gene [D'Alfonso, et al., 2000], three of which have been studied in some detail: -1082 G/A, -819 C/T and -592 C/A [Eskdale, et al., 1999]. Increased IL-10 secretion has been described with the common GCC haplotype and reduced IL-10 secretion with the least common ATA haplotype [Turner, et al., 1997].

The aim

In the present study we examined IL-10 gene -1082, -819, -592 promoter polymorphisms in relation to the RA susceptibility, activity and severity.

Material and methods

A total of 105 patients with RA (women and men) according to the revised criteria of the American College of Rheumatology (ACR) for RA [Arnett, et al., 1988] were consecutively recruited into the study during a 1-year period. Patients with RA were recruited from the outpatients and inpatients' populations from Rheumatology department clinic "Linezers", Rīga Eastern Clinical University Hospital, Latvia. Informed consent was obtained from all patients.

The evaluation of the patients included a physical examination with particular focus on the pattern of joint involvement and laboratory analyses. The following data were obtained from the RA patients: age of disease onset, number of tender (TEN) and swollen (SW) joints, visual analogue scale (VAS) of pain and disease activity, physician assessment of disease activity, presence and value of rheumatoid factor (RF) and anti-cyclic-citrullinated peptides (antiCCP) antibodies, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), roentgenological (RTG) stage. The modified disease activity score DAS28 was calculated as described [Prevoo, et al., 1995]. Simple x-rays of the hands and feet were analyzed using the Steinbrocker method [Pincus, et al., 1997].

Within the RA group, 8 patients were without presence of joint erosions on the radiograph (stage I: nonerosive RA) and 97 patients with presence of erosions (stage II–IV: erosive RA; 19 – stage II, 75 – stage III, and 3 – stage IV). Demographic, clinical, and laboratory characteristics of RA patients are summarized in Table 1.

A total of 242 healthy subjects with similar age and sex distribution were available for the study from Genome database of Latvian population (median 53, range 17–84 years, female 195 (81.3%), male 45 (18.8%)).

Genomic DNA was extracted from peripheral blood sample using standard phenol-chloroform extraction method. Region of interest was PCR amplified using specific primers, amplicons were purified using Sap-ExoI protocol and the sequences were obtained by direct sequencing of amplified products on 3100 ABI prism DNA sequencer (Applied Biosystems). Alleles were called on the positive strand of DNA. Primers for amplification and sequencing see in Table 2. Following PCR mix and conditions were used. PCR mix for 1 sample contained 15 mkl of 2 × PCR Master Mix (Fermentas life sciences, Lithuania), forward and reverse primers (1 mM) and 28 ng of dried genomic DNA. The cycling conditions of PCR were as follows: 5 minutes of initial denaturation at 95 °C, following 32 cycles of 15 seconds at 95 °C, 30 seconds at 56 °C, 30 seconds at 72 °C, and final extension – 10 minutes at 72 °C.

Table 1. Demographic, clinical and laboratory characteristics of the patients with rheumatoid arthritis

Characteristics	Value*	Number of patients
Age, years	58 (26–83)	105
Disease duration, years	4.7 (0.1–49.4)	105
Female, %	89 (84.8)	105
Male, %	16 (15.2)	105
Swollen joint count (28)	6 (0–20)	105
Tender joint count (28)	8 (0–26)	105
VAS pain, mm	49 (1–97)	105
VAS disease activity, mm	50 (2–93)	105
VAS physician's assessment, mm	34 (4–89)	105
ESR, mm/h	26 (2–76)	105
CRP, mg/l	6.90 (0.00–113.4)	104
DAS28(ESR)	5.04 (0.93–7.77)	105
DAS28(CRP)	4.5 (1.84–7.6)	104

* Values given as medians (range) or as number (%).

Table 2. Primers used in our study

Primer	Sequence	Amplicon size
IL-10-PCR-F	5'-TTCCCCAGGTAGAGCAACAC-3'	–
IL-10-PCR-R	5'-ATCCTCAAAGTTCCCAAGCA-3'	685bp
IL-10-1082rsseq	5'-GATGGGGTGGAAGAAGTTGA-3'	–
IL-10-819, 592seq	5'-TCTAAGGCCAATTTAATCCAAGG-3'	–

The chi-square (χ^2) test was used for the calculation of the deviation from Hardy-Weinberg equilibrium. Fisher's exact test and chi-square (χ^2) test was used to examine allele/genotype association with presence of RA and other qualitative variables (positive RF, antiCCP and others). Dichotomized variables were created for RF and antiCCP levels (for RF positivity ≥ 14 IU/ml; for antiCCP positivity > 5.0 units/ml). ANOVA tests were used to compare quantitative variables (age at onset of RA, VAS, DAS28) between genotype-stratified subgroups of RA patients. A p value less than 0.05 was considered statistically significant. Odds ratio (OR) was calculated with 95% confidence interval (CI). All statistics were done with the software package SPSS, version 13.0.

Results

All genotyping results fit Hardy-Weinberg equilibrium. SNP markers used in the study are shown in Table 3.

Table 3. SNP markers used in the study

SNP gene	Common allele	Rare allele	MAF* controls	MAF cases	p value	OR** (95% CI)
rs1800872 IL-10-592	C	A	0.26	0.27	0.707	1.06 (0.82–1.36)
rs1800871 IL-10-819	C	T	0.26	0.27	0.851	1.03 (0.80–1.33)
rs1800896 IL-10-1082	A	G	0.44	0.40	0.277	0.88 (0.70–1.10)

* MAF – minor allele frequency; ** OR – odds ratio.

Figure 1. Association of IL-10 rs1800872 with tender joint count (TEN28)

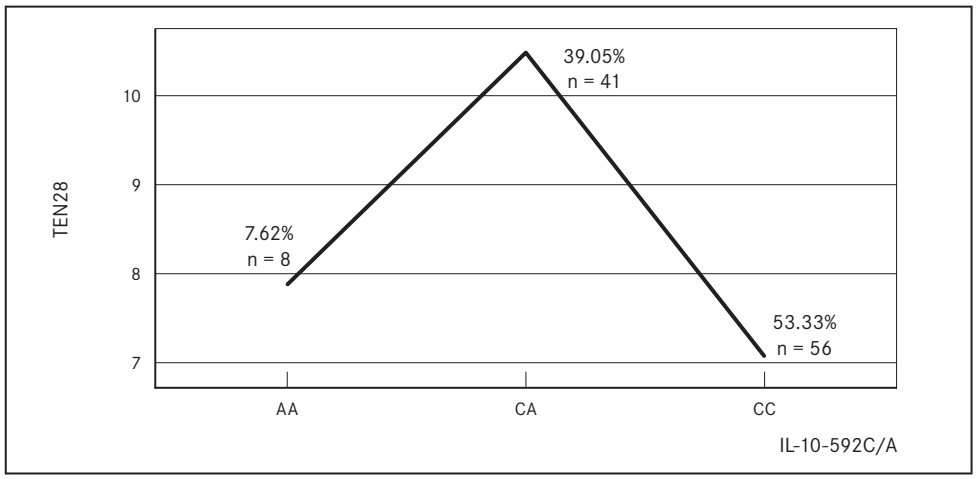


Figure 2. Association of IL-10 rs1800872 with VAS physician assessment of disease activity

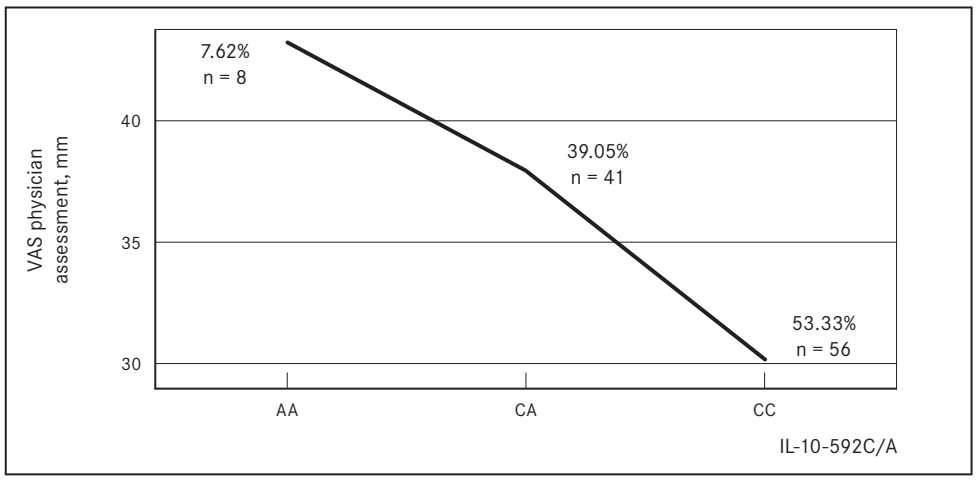


Figure 3. Association of IL-10 rs1800872 with ESR

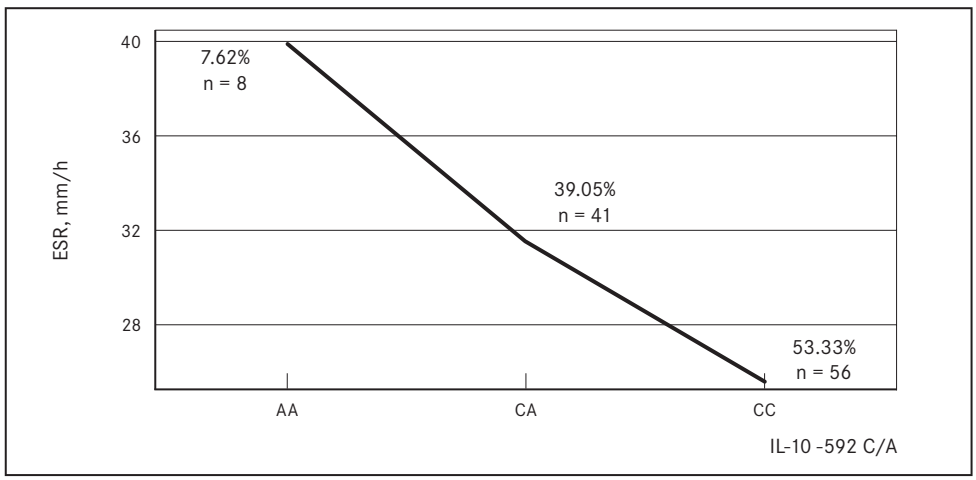


Figure 4. Association of IL-10 rs1800872 with DAS28 (CRP)

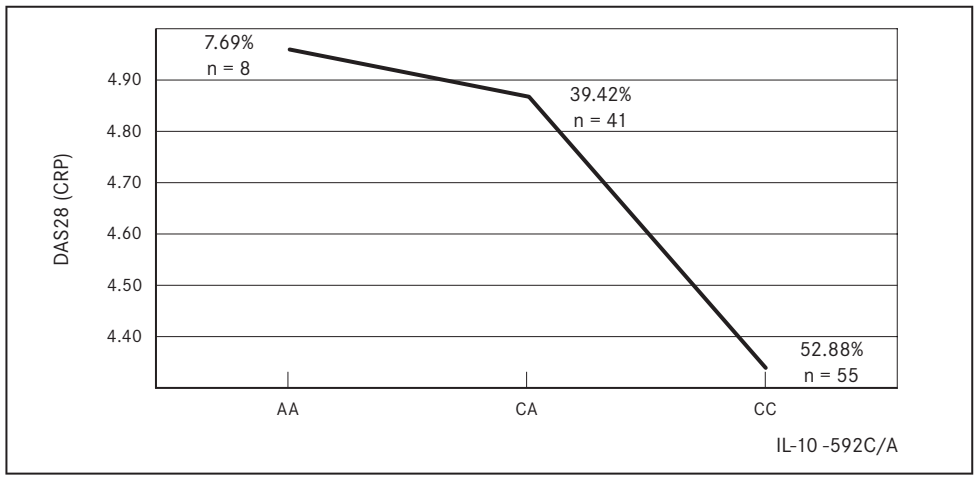
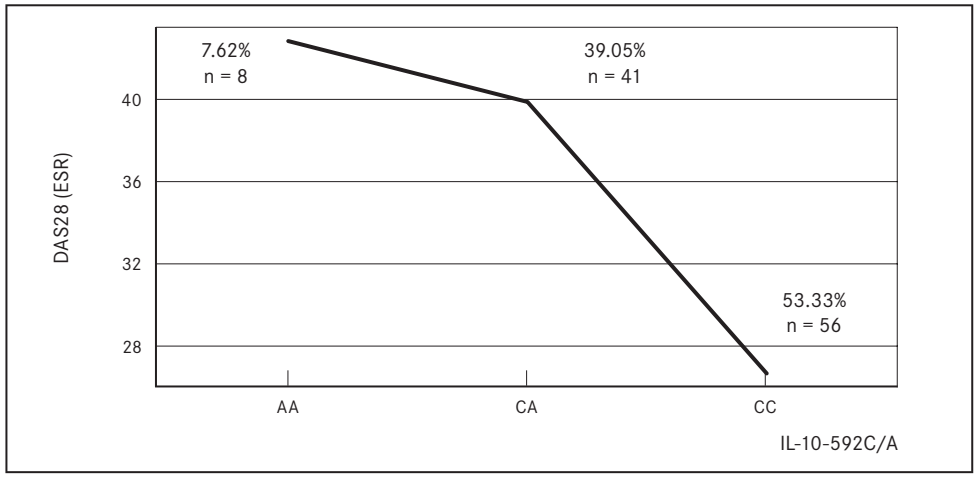


Figure 5. Association of IL-10 rs1800872 with DAS28 (ESR)



There are no differences in IL-10 -592C/A, -819C/T and -1082A/G alleles distribution between RA patients and control. There were no differences found in the distribution of the IL-10 genotypes between RA patients and controls (data not shown).

But we found statistically significant dependency between rs1800872 C/A, rs1800871 C/T genotypes and disease activity parameters such as TEN28 ($p = 0.009$), physician assessment of disease activity ($p = 0.025$), ESR ($p = 0.049$), DAS28 ESR ($p = 0.018$) and association between these genotypes and DAS28 CRP (0.063) (Figures 1–5). IL-10 rs1800872 AA, CA and IL-10 rs1800871 TT, CT genotypes were found with more active disease compared with CC genotypes in both positions.

There were no differences found between studied genotypes distribution and antibody status (RF, antiCCP). We found no differences in rs1800896 A/G genotypes distribution and disease parameters.

Discussion

In the present study we examined SNPs at the -1082, -819, -592 positions of the IL-10 gene in the patients with RA in relation to disease susceptibility, activity and severity and found statistically significant dependency between IL-10 -592, -819 genotypes and disease activity parameters. Genotypes -592 AA, CA and -819 TT, CT were found with more active disease.

The IL-10 promoter polymorphism has been studied in relation to the susceptibility and/or severity to RA, but direct comparisons between these studies are difficult as they have employed different experimental protocols. Hee, et al. observed a significant difference in allele frequencies (-824CT/TT, -597CA/AA) between 84 RA patients and 95 healthy volunteers and showed that the -1087A/-824T/-597A (ATA) haplotype, which comprises all mutant alleles, was associated with lower IL-10 production when compared with the other haplotypes [Hee, et al., 2007]. Padyukov, et al. showed association of -1087 genotypes in women with RA [Padyukov et al., 2004]. Marinou, et al. concluded that IL-10 -592C was associated with more severe radiographic damage only in antiCCP and RF-negative patients [Marinou, et al., 2007]. However, Pawlik, et al. found no correlation between IL-10 polymorphism and disease activity parameters such as ESR, CRP and number of swollen and tender joints [Pawlik, et al., 2005]. Moreno, et al. also concluded that IL-10 promoter polymorphisms were not important for the development or severity of rheumatoid arthritis in Colombian population [Moreno, et al., 2007].

RA is a heterogenic disorder and can be divided into two major subsets based on the presence or absence of antibodies to citrullinated peptide antigens (ACPA). The recent genome-wide association study conducted by Padyukov et al. [Padyukov, et al., 2011] showed significant differences in genetic associations between ACPA-positive and ACPA-negative RA. Therefore, further research is needed with a possibly larger patient group taking into account the last consideration.

Conclusion

The data from the present study indicate that the IL-10 promoter polymorphism has an influence on RA disease activity.

References

1. Arnett F. C., Epworth S. M., Bloch D. A., et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis // Arthritis Rheum, 1988; 31: 315–324.
2. D'Alfonso S., Rampi M., Rolando V., et al. New polymorphisms in the IL-10 promoter region // Genes Immun, 2000; 1: 231–233.
3. Eskdale J., Keijsers V., Huizinga T., Gallagher G. Microsatellite alleles and single nucleotide polymorphisms (snp) combined to form four major haplotype families at the human interleukin-10 (IL-10) locus // Genes Immun, 1999; 1: 151–155.
4. Eskdale J., Kube D., Tesch H., Gallagher G. Mapping of the human IL-10 gene and further characterization of the 5' flanking sequence // Immunogenetics, 1997; 46: 120–128.
5. Feldman M., Brennan F. M., Maini R. N. Role of cytokines in rheumatoid arthritis // Annu Rev Immunol, 1996; 14: 397–440.
6. Hee C. S., Gun S. C., Naidu R., et al. Comparison of single nucleotide polymorphisms in the human interleukin-10 gene promoter between rheumatoid arthritis patients and normal subjects in Malaysia // Mod Rheumatol, 2007; 17: 429–435.
7. Katsikis P. D., Chu C. Q., Brennan F. M., et al. Immunoregulatory role of interleukin-10 in rheumatoid arthritis // J Exp Med, 1994; 179: 1517–1527.
8. Marinou I., Healy J., Mewar D., et al. Association of interleukin-6 and interleukin-10 genotypes with radiographic damage in rheumatoid arthritis is dependent on autoantibody status // Arthritis Rheum, 2007; 56: 2549–2556.
9. Moreno O. M., Gonzalez C. I., Saaibi D. L., et al. Polymorphisms of IL-10 gene promoter and rheumatoid arthritis in Colombian population // Biomedica, 2007; 27: 56–65.
10. Padyukov L., Hytonen A. M., Smolnikova M., et al. Polymorphism in promoter region of IL-10 gene is associated with rheumatoid arthritis in women // J Rheumatol, 2004; 31: 422–425.
11. Padyukov L., Seielstad M., Ong R., et al. A genome-wide association study suggest contrasting associations in ACPA-positive versus ACPA-negative rheumatoid arthritis // Ann Rheum Dis, 2011; 70: 259–265.
12. Pawlik A., Kurzawski M., Szklarz B. G., et al. Interleukin-10 promoter polymorphism in patients with rheumatoid arthritis // Clin Rheumatol, 2005; 24: 480–484.

13. Pincus T., Larsen A., Brooks R. H., et al. Comparison of 3 quantitative measures of hand radiographs in patients with rheumatoid arthritis: Steinbrocker stage, Kaye modified sharp score, and Larsen score // J Rheumatol, 1997; 24: 2106–2112.
14. Prevoo M. L., van't Hof M. A., Kuper H. H., et al. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis // Arthritis Rheum, 1995; 38: 44–48.
15. Turner D. M., Williams D. M., Sankaran D., et al. An investigation of polymorphism in the interleukin-10 gene promoter // Eur J Immunogenet, 1997; 24: 1–8.
16. Westendorp R. G., Langermans J. A., Huizinga T. W., et al. Genetic influence on cytokine production and fatal meningococcal disease // Lancet, 1997; 349: 170–173.

Three Novel Mutations in the Phenylalanine Hydroxylase Gene (PAH) Observed in Latvian Patients with Phenylketonuria

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Abstract

Phenylketonuria (PKU) is an inborn, metabolic disease affecting the enzyme phenylalanine hydroxylase. It is transmitted in an autosomal recessive pattern and is highly heterogeneous at the molecular level. More than 560 different mutations in the phenylalanine hydroxylase (*PAH*) gene have been reported associated with different geographical and ethnic groups.

In the present study we report on the identification of three novel mutations in the *PAH* gene of Latvian patients with phenylketonuria: P292T, K371E and IVS12-1G>A. These mutations were detected during the characterisation of the *PAH* genotypes of 70 patients with PKU. The results obtained let us assume that mutations P292T and IVS12-1G>A possibly are disease-causing, but mutation K371E rather cause mild hyperphenylalaninemia (HPA).

Keywords: phenylalanine hydroxylase (PAH), phenylalanine hydroxylase gene (*PAH*), hyperphenylalaninemia (HPA), phenylketonuria, PKU, mutation analysis.

Introduction

Phenylketonuria (PKU; OMIM #261600) is an autosomal recessive disorder caused by a deficiency of phenylalanine hydroxylase (PAH, EC 1.14.16.1). Phenylalanine hydroxylase is an iron- and tetrahydropterin-dependent hepatic enzyme that catalyses the conversion of phenylalanine (Phe) to tyrosine (Tyr). Dysfunction of the PAH enzyme results in an elevated serum level of phenylalanine and mental retardation [Scriver, 2001].

Phenylketonuric infants appear normal at birth. Retardation of development may not be evident of months. Vomiting, irritability, an eczematoid rash, and an unusual odour may also be observed very early in life. The "mousy" odour of the phenylketonuric patient is that of phenylacetic acid [Nyhan, 2005].

PAH deficiency is diagnosed upon routine screening of newborns and is based on the detection of an elevated plasma Phe concentration. On the basis of blood pre-treatment Phe concentrations, PAH deficiency can be classified into classic PKU (Phe > 1200 µmol/L), mild PKU (Phe = 600–1200 µmol/L) and mild HPA, where blood Phe is elevated above upper reference limit, but < 600 µmol/L [Williams, 2008]. Sometimes a moderate classification is included for concentrations of 900–1200 µmol/L [Blau, 2010]. Nevertheless, PKU classification may differ by country according to each country guidelines or clinician experience.

The prevalence of phenylketonuria in Europe is about one case per 10 000 livebirths, and 1 : 8000 live-births in Latvia [Blau, 2010; Purina, 1995].

The restriction of dietary phenylalanine remains the mainstay of phenylketonuria management, and begins immediately after confirmation of hyperphenylalaninaemia in a neonate [Blau, 2010].

PKU is highly heterogeneous at the molecular level. To date more than 560 different mutations in the phenylalanine hydroxylase (*PAH*) gene have been described associated with different geographical and ethnic groups (http://www.pahdb.mcgill.ca/cgi-bin/pahdb/association_query.cgi).

Previous studies have revealed a very high genotypic homogeneity in Latvian PKU patients, as 73% of the mutant alleles carry the R408W mutation typical for Eastern Europe. More than 50% of patients were homozygous for R408W [Pronina, 2007; Pronina, 2003].

Material and methods

A total of 70 PKU families (184 individuals, 74 of them being patients and 110 relatives) were investigated. The patients were identified by a neonatal screening program and were considered to have PKU when the phenylalanine levels were above 240 µmol/L.

DNA from each individual was extracted from peripheral blood leukocytes by Genomic DNA Purification Kit ("Fermentas", Lithuania). Diagnostic identification of mutation R408W was based on the fact that it creates new restriction enzyme *Sty*I site in the exon 12. Thirty-four (49%) patients were compound heterozygous with mutation R408W mutation in one allele. These patients were screened for mutation in other allele by PCR amplification of the entire *PAH* gene, including all 13 exons and splice regions, using DGGE and sequence analysis.

To exclude the common population specific polymorphisms for novel single nucleotide changes (P292T, K371E and IVS12-1G>A), we tested control samples of 100 volunteered individuals without PKU. To predict the possible impact of an amino acid substitution on the structure and function of a human protein, the PolyPhen (Polymorphism Phenotyping) software was used.

This prediction was based on straightforward empirical rules which were applied to the sequence, phylogenetic and structural information characterising the substitution (http://genetics.bwh.harvard.edu/pph/).

Minihaplotypes based on short tandem repeats and variable number of tandem repeats (STR and VNTR) were determined as described by Goltsov et al. (1992) and Zschocke et al. (1994) [Goltsov, 1992; Zschocke, 1994].

The study was conducted in accordance with the Helsinki Convention and approved by the Central Medical Ethics Committee.

Results

In this study we detected three novel single nucleotide changes for four Latvian PKU patients (Table 1). Two of them, resulted in missense mutations (P292T and K371E), were found in coding region. The third one was splice site mutation IVS12-1G > A on the border between intron 12 and exon 13 that was found in two unrelated chromosomes. None of these novel mutations had been observed in the 100 normal subjects tested.

Using PolyPhen software, no evidence for damaging effect was seen for mutation K371E and substitution was considered benign (http://genetics.bwh.harvard.edu/ggi/pph/7a5342f2e2a2a0c5e4db58a57d60ee753540c860/2607880.html). Conversely, mutation P292T was predicted to be probably damaging for PAH protein structure and function (http://genetics.bwh.harvard.edu/ggi/pph/7a5342f2e2a2a0c5e4db58a57d60ee753540c860/2605042.html) (Table 1).

No other disease-causing mutations were found by sequencing analysis of all 13 *PAH* gene exons for these four patients.

All four patients were found to be compound heterozygous. Three of them had mutation R408W in homologous allele and one patient had another splice site mutation IVS10-11G>A (Table 2). Three patients had severe PKU according to their pre-treatment Phe level and Phe tolerance, but only two of them were on treatment. Patient with genotype K371E/R408W had normal development without low phenylalanine dietary treatment.

Table 1. Novel nucleotide changes identified in Latvian PKU patients

Patient	Mutation name	Systematic name	Exon / Intron	Minihaplotype VNTR/STR	PSIC * score difference	PolyPhen prediction
1	P292T	c.874C > A	Ex8	8/226	2.858	Probably damaging
2	K371E	c.1111A > >G	Ex11	3/238	0.302	Benign
3	IVS12-1G > A	c.1316-1G > A	I12	7/242	–	–
4	IVS12-1G > A	c.1316-1G > A	I12	7/242	–	–

* PSIC software (Position-Specific Independent Counts) – to calculate the logarithmic ratios of the likelihood of the given amino acid occurring at a particular position to the likelihood of this amino acid occurring at any position (background frequency).

Table 2. Characteristic of patients presented the novel *PAH* gene mutations

Patient	Genotype	Pre-treatment Phe level	PKU clinical form	Treatment
1	P292T / R408W	2 454 μmol/L	Severe	Strong diet
2	K371E / R408W	162 μmol/L	HPA	Normal development without diet
3	IVS12-1G > A/R408W	1 836 μmol/L	Severe	Strong diet
4	IVS12-1G>A / IVS10-11G > A	N/A *	Severe	Refused from the diet

* Pre-treatment Phe level for this patient was not available, patient was born in 1991, Phe was tested by qualitative method and measured by putting crosses ("+" to "++++"), this patient had "+++".

Discussion

In total, three novel mutations were found in the *PAH* gene: two missense mutations and one splice site mutation. Both missense mutations were found only once and corresponded to a frequency of 0.7% each. Splice site mutation was observed in 2 mutant chromosomes – 1.4%.

In one family a PKU patient presented the P292T mutation that was a 874C>A substitution in the *PAH* gene in exon 8 at amino acid 292, resulting in a missense mutation – hydrophobic proline is substituted by hydrophilic threonine. This mutation was not observed in 100 control samples from healthy individuals. Mutation was linked to minihaplotype 8/226. The patient had a neonatal diagnosis, with phenylalanine level indicating severe PKU, and received diet therapy soon after. Mutation P292T was found in heterozygosity with R408W mutation that is a null mutation associated with < 0.3% of normal activity and a severe PKU phenotype [Tighe, 2003]. Considering that PKU clinical form depends on the combination of mutant alleles inherited, we suggest that mutation P292T has to be associated with low residual activity of PAH enzyme that in its turn corresponds with prediction analysis results by PolyPhen tool.

In this family only one of the two mutations of a PKU patient was confirmed in the parents: mutation R408W was inherited from patient's mother but the paternal chromosomes did not carry either of the PKU mutations identified in the child. Since the results of paternity testing were compatible with paternity, the second mutation has to be arisen *de novo* and the risk for another child with PKU in this family is low.

These findings demonstrate two important points: the necessity of screening the whole coding region of the *PAH* gene for diagnostic purposes on the one hand, and second the usefulness of confirming inheritance of mutations from both parents when possible. Otherwise the prediction of the expected phenotype or the calculation of risk for another child with PKU may be incorrect.

The 1111A>G substitution in exon 11 of the *PAH* gene at amino acid 371 results in a missense mutation – lysine is substituted by glutamic acid. This substitution was found in a PKU patient and his father and was associated with minihaplotype 3/238. The patient was diagnosed through the neonatal screening and had slightly elevated phenylalanine level that did not require treatment. Mutation K371E also was found in heterozygosity with R408W mutation. This finding suggests that K371E is mild mutation with enough PAH residual activity for normal clinical phenotype that matches with PolyPhen analysis results. Genotype K371E/R408W could be defined as functionally hemizygous. The normal individuals tested for this mutation did not present it.

The c.1316-1G>A substitution in intron 12 of the *PAH* gene results in a mutation. This substitution was found in two unrelated patients with severe PKU. The mutation IVS12-1G>A is located at the boundary of IVS12 and exon 13 and affects the conserved dinucleotide at the 3' splice site. According to its location this mutation results in a truncated protein lacking the C-terminal 52 residues (residues 401–452).

In one patient this mutation was found in association with mutation R408W but in another one in association with another known splice site mutation IVS10-11G>A. Both patients were diagnosed through the neonatal screening. Patient with genotype IVS12-1G>A/R408W received diet therapy soon after and is currently asymptomatic. Patient with genotype IVS12-1G>A/IVS10-11G>A has severe mental retardation because of parents' refusal to maintain the adequate dietary treatment. In both cases mutation IVS12-1G>A was linked to minihaplotype 7/242.

Conclusions

Considering all available information about the three novel mutations in Latvian mutant chromosomes associated with PKU, we assume that these variants are functionally relevant although this need to be confirmed by additional in vitro expression analysis. Reporting novel mutations is of extreme importance, in order to increase the known mutational spectrum of the *PAH* gene and enable a better understanding of mutant alleles from different geographic regions, which in turn will improve the mutation detection, prediction of phenotype severity, as well as help to choose the appropriate treatment strategy.

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References

1. Blau N., van Spronsen F. J., Levy H. L. Phenylketonuria // Lancet, 2010; 376: 1417–27.
2. Goltsov A. A., Eisensmith R. C., Konecki D. S., Lichter-Konecki U., Woo S. L. C. Association between mutations and a VNTR in the human phenylalanine hydroxylase gene // American Journal of Human Genetics, 1992; 51: 627–636.
3. Nyhan W. L., Barshop B. A., Ozand P. T. Atlas of metabolic diseases. – 2nd ed. – Berlin: Hodder Arnold, 2005. – Pp. 127–136.
4. PAH: Phenylalanine hydroxylase locus specific database web site // http://www.pahdb.mcgill.ca/cgi-bin/pahdb/association_query.cgi
5. PolyPhen: prediction of functional effect of human nsSNPs online software // http://genetics.bwh.harvard.edu/pph/
6. PolyPhen analysis results for mutation K371E // http://genetics.bwh.harvard.edu/ggi/pph/7a5342f2e2a2a0c5e4db58a57d60ee753540c860/2607880.html

7. PolyPhen analysis results for mutation P292T // http://genetics.bwh.harvard.edu/ggi/pph/7a5342f2e2a2a0c5e4db58a57d60ee753540c860/2605042.html
8. Pronina N., Giannattasio S., Lattanzio P., Lugovska R., et al. The molecular basis of phenylketonuria in Latvia // Human Mutatation, 2003; 21(4): 398–399.
9. Pronina N., Lugovska R., Vevere P. Mutational spectrum in Latvian patients with phenylalanine hydroxylase deficiency // RSU Collection of Scientific Papers, 2007; 76–79.
10. Purina G., Lugovska R., Sokolova L., et al. Medical genetical service in Latvia: developmental trends // Proceedings of the Latvian Academy of Sciences, 1995; 5/6: 105–108.
11. Scriver C. R., Kaufman S. The hyperphenylalaninemias // The Metabolic and molecular bases of inherited disease / Ed. by Scriver C. R., Beaudet A. L., Sly S. W., Valle D., Childs B., Kinzler K. W., Vogelstein B. – 8th ed. – New York: McGraw-Hill, 2001 (Revised—April 2008). – Pp. 1–6.
12. Tighe O., Dunican D., O'Neill C., Bertorelle G., et al. Genetic diversity within the R408W phenylketonuria mutation lineages in Europe // Human Mutation 2003; 21: 387–393.
13. Williams R. A., Mamotte C. D. S., Burnett J. R. Phenylketonuria: an inborn error of phenylalanine metabolism // Clinical Biochemistry, 2008; 29: 31–41.
14. Zschocke J., Graham C. A., McKnight J. J., Nevin N. C. The STR system in the human phenylalanine hydroxylase gene: true fragment length obtained with fluorescent labelled PCR primers // Acta Paediatrica, 1994; 83(407): 41–42.

Correlation between Urinary Incontinence and Severity of Neurological Lesion Caused by Stroke

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Abstract

Stroke is one of the most common illnesses which affect elderly people. Urinary incontinence is one medical problem which may develop as a result of stroke, and, according to several authors, its occurrence frequency may reach up to 80%. Clinical symptoms of urinary incontinence are considered to be among symptoms which, along with the severity of stroke and the level of functional limitations, allow developing reliable predictions and make targeted use of resources.

The aim of the study is to explore whether post-stroke urinary incontinence correlates with the localization of lesion and clinical symptoms.

The study included patients, who had received treatment in acute stroke unit, in after-stroke period. Patients were asked questions about the state of their health and functional changes after stroke; clinical symptoms and functioning problems caused by stroke were examined, paying particular attention to symptoms of urinary inconsistence. The examination took place at the hospital a few days after stroke had occurred. Data were collected and processed with SPSS 15.0 version.

There were 180 participants to the study, who were patients after their first stroke – 102 men and 78 women.

Symptoms of urinary incontinence were reported for 70.6% of study participants. The most common types of urinary incontinence in the acute period were urge and mixed incontinence (functional and urge incontinence). In 46% cases a combined incontinence occurred (urinary and fecal incontinence). A comparison of patient groups with and without symptoms of urinary incontinence showed that patients older than 75 years showed more symptoms of urinary incontinence ($p = 0.013$), and the same can be said about patients with low indicators of Barthel ($p = 0.001$) and patients with cognitive disorders ($p = 0.001$). Severity of paresis, aphasia and sensory disorders shows a reliable correlation with the type of urinary incontinence.

Urinary incontinence develops more frequently among patients with front of the brain circulatory disorders and subcortical brain lesion. There is a reliable correlation between severity of neurological lesion and urinary incontinence.

Keywords: stroke, rehabilitation, urinary incontinence.

Introduction

Urinary incontinence is a serious health disorder that develops after stroke, and it occurs within the first days or weeks after affection in between 37% and 79% cases [1].

Urinary incontinence is not seen as a specific early symptom of stroke with characteristic brain lesion localization; however, several authors view it as an indicator of potential occurrence of death, disability and re-hospitalization. Lesion of various brain structures, movement and speech or language disorders, characteristic to post-stroke patients, may result in loss of bladder signal and loss of or limitedness of toilet skills. The improvement of the ability to focus is an important task in the process of rehabilitation. Training of attention may stimulate a renewal of bladder control for patients who recognize their own urinary incontinence [2, 3].

Although the information on urinary retention or urgent and frequent need to urinate is widely available, little is known of its original causes and their role in the prediction of recovery results. If bladder control is lost as a result of nerve impulse pathway damage, in most cases not only will the perception be bad, but there will also be poor understanding and perception of the surroundings [4, 5]. The factor of nerve growth level, necessary for the growth and regeneration of the nerve, correlates with the severity of neurological lesion of patients with circulatory problems in the brain, and not with symptoms of urinary incontinence and urodynamic indicators [6]. Lesion of white matter is an important factor in all cases of urinary incontinence, because there is a general disorder in the matters, which are linked to the ability to perceive and recognize bodily signals. Time should be devoted to reduction of these disorders during the process of rehabilitation [7, 8].

There is an array of factors, which may cause the development of urinary incontinence after cerebral infarction (stroke), for instance, the patient is older than 65 years, and there is a heightened irritability of detrusor sphincter, which is clinically manifested as an urgent need to urinate or urinary incontinence [9]. Other authors have specified such factors of urinary incontinence as micturitions in the damage control mechanism, resulting in a hyper-reflexive bladder, a hypotonic bladder and a “brain shock” for patients, who take anticholinergic medication, post-stroke structural problems in the tract and mobility, communication and perception disorders caused by stroke [10].

To this date, there is little evidence-based research on clinical types of post-stroke urinary incontinence. Several studies of urodynamics show that a hyperactive detrusor is the most common dysfunction; however, it has been proved that changes may occur in the urodynamic after stroke [11, 12]. Literature describes three basic types of urinary incontinence, most frequently occurring among post-stroke patients during the rehabilitation period: urge urinary incontinence, functional urinary incontinence and stress incontinence. In case of urge urinary incontinence, patients complain about urinary leakage in combination with a feeling of urge, which usually is a result of brain lesion or edema causing disorders of micturition control. Functional urinary incontinence is a disability to contain urine due to functional disorders such as movement and communication deficit, which is often combined with limited access to the environment. In case of stress urinary incontinence, patients complain about urinary leakage provoked by coughing or sneezing [13], which often is observed prior to the stroke, especially if the cough is linked to aspiration and swallowing disorders [14]. A large proportion of patients with movement and cognitive disorders have a variety of reasons for urinary incontinence and various types of urinary incontinence, which may lessen or disappear, once the functional and general health improves [9, 4, 12].

Yet the incidence and prevalence of the variety of types of urinary incontinence have not been sufficiently studied, which interferes with the development of guidelines for routine monitoring of patients, treatment and the prediction of results [12, 13].

The aim

The aim of the study is to clarify whether post-stroke urinary incontinence and its clinical types are linked to the localization of brain lesion and severity of neurological lesion.

Material and methods

There were 180 participants to the study, who were patients after their first stroke – 102 men and 78 women, who had received treatment at Stroke Unit of Rīga Eastern Clinical University Hospital in the time period between February 1st and June 30th, 2009. All patients were examined, and diagnosis was

set in accordance with the definition of World Health Organization: "A stroke is a local or general disorder of brain functions, which occurs suddenly and is observed for 24 hours or more and may cause death; it may be explained by vascular causes, yet it cannot be linked to any demonstrable cause". Brain tomography was performed for all patients within 24 hours after hospitalization.

Urine incontinence was defined as urinary leakage more than once a day and no less than for two days. According to the guidelines of the International Continence Society, urinary incontinence is classified as a stress urinary incontinence, urge urinary incontinence and functional urinary incontinence, as well as bladder emptying problems – urinary retention. Urinary analysis was tested for all patients in the acute period in order to exclude urinary tract infections. Residual urine was determined for patients; residual urine of more than 150 ml was considered urinary retention.

Paresis, sensory disorders and aphasia were assessed by the National Institute of Health Stroke Scale in order to determine their levels of severity (0 – no damage, 1 – slight damage, 2 – moderate damage, 3 – severe damage).

Participants of the study were asked questions on their functioning prior to the stroke; concomitant illnesses, neurological lesion and functional disorders caused by the stroke were assessed; symptoms of urinary incontinence, disability (Barthel index) and cognitive processes (Mini-Mental state examination) were assessed. Patients were examined within 10 days after hospitalization.

The obtained data were processed with SPSS (version 15.0), by applying descriptive statistics, including frequency, median indicators and standard deviation for quantitative indicators (age, gender etc.). The nonparametric test (correlation coefficient) was used to assess correlation between urinary incontinence and neurological symptoms.

Results

The study included 180 patients (78 women, 102 men). The average age of participants was 68.0 years (min 36, max 92). The average age of women was 70.8 (SD ± 9.94), the average age of men was 65.9 (SD ±13.1). By testing the statistical hypothesis of the average age of men and women by an independent samples t-test, it was found that the average age of participants show statistically reliable differences ($t = 2{,}740$; $p = 0.007$). The study included 149 patients with cerebral infarct [68 women (87.2%), 81 men (79.4%)] and 31 patients with spontaneous intracerebral hemorrhage [10 women (12.8%), 21 men (20.6%)]. By testing the statistical hypothesis dividing men and women according to the type of stroke by using the chi-square test, no statistically significant differences were found ($\chi^2 = 1{,}871$; $df = 1$; $p = 0.171$).

Short description of patients included in the study is reflected in Table 1.

The frequency of urinary incontinence in the acute period was 127 (70.6%). 16 patients developed urinary retention in the acute period (over 150 ml residual urine), of which 11 patients had suffered a cerebral infarct and 5 patients had suffered a spontaneous intracerebral hemorrhage. Of the 149 patients with cerebral infarct, 95 developed urinary incontinence, divided into types of urinary incontinence: 30 cases of urge urinary incontinence, 3 cases of stress urinary incontinence, 62 cases of functional urinary incontinence. Of the 31 patients with spontaneous intracerebral hemorrhage, 22 developed urinary incontinence, divided into types of urinary incontinence: 4 cases of urge urinary incontinence, 3 cases of stress urinary incontinence, 15 cases of functional urinary incontinence. By testing the statistical hypothesis of types of urinary incontinence according to the type of stroke with the chi-square test, it was found that there are no statistically significant differences in these divisions ($\chi^2 = 6.121$; $df = 6$; $p = 0.410$). The analysis showed that out of 78 women included in the study, 59 women developed urinary incontinence: 23 cases of urge urinary incontinence, 3 cases of stress urinary incontinence and 33 cases of functional urinary incontinence. Of 102 men included in the study, 68 developed urinary incontinence: 11 cases of urge urinary incontinence, 3 cases of stress urinary incontinence, 54 cases of functional urinary incontinence. Statistical hypothesis of urinary incontinence types according to gender was tested by a chi-square test and revealed no reliable statistical differences ($\chi^2 = 8.944$; $df = 6$; $p = 0.177$).

Table 1. Descriptive statistics of the studied population

Indicators	Number of patients, n (%)
Gender:	
Male	102 (56.7)
Female	78 (43.3)
Concomitant illnesses:	
Arterial hypertension	157 (88.2)
Diabetes	30 (16.9)
Heart rhythm disorders	31 (30.4)
Smoking	52 (28.2)
Use of alcohol	16 (9.0)
Obesity	39 (38.2)
Reoccurring stroke	13 (7.2)
Family history:	
Lives alone	44 (21.5)
Lives together with family	136 (78.4)
Localization of lesion:	
Right cerebral hemisphere	88 (46.1)
Left cerebral hemisphere	86 (45.1)
Vertebrobasilar basin	16 (8.8)

Depth of paresis was linked to post-stroke urinary incontinence. 49 patients did not develop urinary incontinence, of which in 40 (82%) cases there was no development of paresis, in 5 (10%) cases a slight paresis, in 2 (4%) cases a moderate paresis and in 2 (4%) cases a deep paresis. 28 patients developed urge urinary incontinence: in 19 (68%) cases with no subsequent paresis, in 5 (18%) cases a slight paresis, in 2 (7%) cases a moderate paresis, and in 2 (7%) cases a deep paresis. 3 patients developed stress urinary incontinence: in 2 (68%) cases without a paresis and in 1 (32%) case a slight paresis. 63 patients developed functional urinary incontinence: in 36 (43%) cases without a paresis, in 6 (10%) cases a slight paresis, in 18 (26%) cases a moderate paresis and in 17 (24%) a deep paresis. 16 patients developed urinary retention, of which 8 (50%) cases without paresis, in 4 (25%) cases a slight paresis, in 3 (19%) moderate paresis and 1 (6%) case a deep paresis. Division of patients according to depth of paresis and types of urinary incontinence shows statistical reliability ($\chi^2 = 38.725$; df = 18; p = 0.003). There is a statistically significant correlation between urinary incontinence and depth of paresis (r = 0.337; p = 0.001).

Severity of aphasia was linked to types of post-stroke urinary incontinence. 49 patients did not develop urinary incontinence, of which 6 (12%) did not develop aphasia, 2 (4%) developed a moderate aphasia, 41 (84%) developed severe aphasia. 28 patients developed urge urinary incontinence: 2 (7%) cases of slight language disorders, 5 (18%) cases of moderate language disorders, 21 (75%) cases of severe language disorders. 3 patients were diagnosed with stress urinary incontinence: 1 (33%) without language disorders, 2 (67%) patients with slight aphasia. 77 patients were diagnosed with functional urinary incontinence: 39 (53%) patients were diagnosed with slight aphasia, 22 (32%) patients were diagnosed with moderate aphasia, 16 (15%) developed severe aphasia. Division of patients according to severity of language disorders and type of urinary incontinence shows statistically reliable differences ($\chi^2 = 70.147$; df = 12; p = 0.001). There is a statistically reliable correlation between urinary incontinence and severity of language disorders (r = 0.425; p = 0.001).

100 patients developed sensory disorders of varying severity. 77 patients did not develop sensory disorders, 3 patients did not develop urinary incontinence, 2 patients developed urge urinary incontinence, 2 patients developed stress urinary incontinence, 57 patients developed functional urinary

incontinence, 14 patients developed urinary retention. Of 58 patients with sensory disorders of moderate severity, 11 patients did not develop urinary incontinence, 22 patients developed urge urinary incontinence, 2 patients developed stress urinary incontinence, 18 patients developed functional urinary incontinence, 2 patients developed urinary retention. Of 42 patients with severe sensory disorders, 35 did not develop urinary incontinence, 4 patients developed urge urinary incontinence, 3 patients developed functional urinary disorders. Division of patients according to sensory disorder severity and urinary incontinence shows statistically reliable differences ($\chi^2 = 146.685$; df = 12; p = 0.001). There is a statistically reliable correlation between type of urinary incontinence and severity of sensory disorders (r = 0.378; p = 0.001).

The above-mentioned results are reflected in Table 2, which shows the division of number of patients according to the type or urinary incontinence and neurological lesion.

Table 2. The division of the number of patients according to the type or urinary incontinence

Severity of neurological lesion	Type of urinary incontinence					p
	None	Stress	Urge	Functional	Urinary retention	
Paresis:						
None	40	2	23	36	8	0.003
Slight	5	1	5	6	4	
Moderate	2	0	2	14	3	
Severe	2	0	2	17	1	
Aphasia:						
Slight	6	0	2	39	9	0.001
Moderate	2	1	5	22	4	
Severe	41	2	25	16	3	
Sensory disorders:						
Slight	3	1	2	57	14	0.001
Moderate	11	2	25	18	2	
Severe	35	0	5	2	0	

Discussion

Routine monitoring of urinary incontinence is not a widespread approach in the treatment of post-stroke patients either in hospital or in further rehabilitation environment. This health problem is often considered to be of secondary importance, requiring responsibility from the care personnel. Urinary incontinence should be explored not only as a medical problem, but also as a social problem, and a multi-professional team must engage in finding a solution for it. Early prevention of urinary incontinence should allow a timely identification of the risk of medical and social problems. Patel, et al. (2001) [15], Tilling, et al. (2001) [16], van Kuijk, et al. (2001) [1] and co-authors concluded that urinary incontinence is a negative predictive indicator of functional recovery of post-stroke patients. In 2003, Vanags, et al. developed a predictive model of death rate in the first year after the stroke to be used in clinical practice. This model contained eight characteristics. Urinary incontinence was inserted on top of the list with the highest indicator – 9, followed by dysphagia – 7, other indicators being 4 or less. A summary of this data shows that urinary incontinence is considered to be a reliable predictive factor, which shows a negative functional result after stroke. The results of our study lead to the conclusion that functional recovery is worse among patients with functional urinary incontinence, which is linked to movement and cognitive disorders.

Conclusion

There is no link between gender or type of stroke and post-stroke urinary incontinence in the acute post-stroke period ($p > 0.05$). There is a statistically significant correlation between severity of paresis and urinary incontinence ($p = 0.003$). There is a statistically significant correlation between severity of aphasia and the type of urinary incontinence ($p = 0.001$). There is a statistically significant correlation between sensory disorders and urinary incontinence ($p = 0.001$).

References

1. van Kuijk A. A., van der Linde H., van Limbeek. J. Urinary incontinence in stroke patient after admission to a post acute inpatient rehabilitation program // Arch Phys Med Rehabil, 2001; 82: 1407–1411.
2. Pettersen R., Wyller T. B. Prognostic significance of micturition disturbances after acute stroke // J Am Geriatr Soc, 2006; 54: 1878–1884.
3. Pettersen R., Saxby B. K., Wyller T. B. Post-stroke urinary incontinence: one-year outcome and relationships with measures of attentiveness // J Am Geriatr Soc, 2007; 55(10): 1571–1577.
4. Pettersen R., Haig Y., Nakstad P. H., Wyller T. B. Subtypes of urinary incontinence after stroke: relation to size and location of cerebrovascular damage // Age Ageing, 2008; 37(3): 324–327 // doi: 10.1093/ageing/afm196
5. Turhan N., Atalay A., Atabek H. K. Impact of stroke etiology, lesion location and aging on post-stroke urinary incontinence as a predictor of functional recovery // Intern Jour of Rehab Research, 2006; 29(4): 335–338.
6. Liu H. T., Liu A. B., Chancellor M. B., Kuo H. C. Urinary nerve growth factor level is correlated with the severity of neurological impairment in patients with cerebrovascular accident // BJU Int, 2009; 104(8): 1158–1162. Epub 2009 Mar 30.
7. Olsen-Vertland P. Urinary incontinence after a cerebro-vascular accident // Nursing Standards, 2003; 17: 37–41.
8. Pettersen R., Stien R., Wyller T. B. Post-stroke urinary incontinence with impaired awareness of the need to void: clinical and urodynamic features // BJU Intern, 2007; 99: 1073–1077 // doi:10.1111/j.1464-410X.2007.06754.x
9. Barret J. A. Bladder and bowel problems after stroke // Reviews in Clinical Gerontology, 2001; 12: 253–267.
10. Abrams P., Blaivas J. G., Stanton S. L., Andersen J. T. Standardization of terminology of urinary tract function // Neurol Urodyn, 1998; 7: 403–427.
11. Ween J. E., Alexander M., D'Esposito M., Roberts M. Incontinence after stroke in a rehabilitation setting: outcomes association and predictive factors // Neurology 1996; 47: 659–663.
12. Gelber D. A., Good D. C., Laven L. J., Verhulst S. J. Causes of urinary incontinence after acute hemispheric stroke // Stroke, 1993; 24: 378–382.
13. Gupta A., Taly A. B., Srivastava A., Thyloth M. Urodynamics post-stroke in patients with urinary incontinence: Is there correlation between bladder type and site of lesion? // Ann Indian Acad Neurol, 2009; 12(2): 104–107.
14. Abrams P., Cordozo L., Fall M., Griffiths D., et al. The standardization of terminology of urinary tract function: report from the standardization subcommittee of International Continence Society // Neurol Urodyn, 2002; 21: 167–178.
15. Patel M., Coshale C., Rudd A. G., Wolfe Ch. D. A. Natural history and effects on 2-year outcomes of urinary incontinence after stroke // Stroke, 2001; 32: 122–127 // doi: 10.1161/01.STR.32.1.122
16. Tilling K., Sterne J., Rudd A. G., Glass T. A., et al. A new method for predicting recovery after stroke // Stroke, 2001; 32: 2867–2873 // doi: 10.1161/hs1201.099413

Acute and Transient Psychotic Disorders in Latvia: a 6-year Follow-up Study

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Abstract

Acute and transient psychotic disorders (ATPD; F23, ICD-10) have been described as acute psychosis with brief onset, polymorphous symptomatology and rapid resolution. Studies with standardized methods of the ATPD are rare and unsystematic.

Retrospective chart review study of all first time hospitalized patients fulfilling ICD-10 criteria for ATPD (WHO, 1993) treated at Rīga Centre of Psychiatry and Addiction Disorders, Latvia during a 3-year period was carried out. During an average of a 6.1-year follow-up period, patients were assessed using standardized instruments.

Within the same time period 294 patients were first time hospitalized with ATPD diagnosis, 54% (159) of them were females. Over an average of a 6.1-year follow-up period 51% (150) of the patients were not re-hospitalized. Later diagnosis was changed to schizophrenia in 105 (73%) patients. During the follow-up period ATPD relapses we found total in 22 (15%) patients. During the index episode in 73 (25%) cases a typical polymorphic symptomatology was found. Stressful life events before the first episode were found in 44% of the patients (129).

Half of the patients were hospitalized only once during the follow-up period. In a subgroup of re-hospitalized patients, the most common diagnostic change was to schizophrenia. Stressful life events before the first episode were prevalent in all patients. Detailed study of nosologic units of this diagnosis showed a significant difference within the group itself, which might play a significant role in the development of amendments to the new classification. Possible provoking factors could help to predict further development of the disease. Further prospective research on the topic is warranted to understand the evaluation of the diagnosis in ICD-10 and possible changes in ICD-11.

Keywords: transient psychotic disorder, clinical features, stressful life events.

Introduction

Acute and transient psychotic disorder (ATPD) as a separate nosological unit was recognized in International Classification of Diseases (ICD-10; World Health Organization) only in 1992.

The disorder is characterized by brief onset (within 2 weeks in all the cases), and has clinical features like typical schizophrenic symptoms, polymorphic, rapidly changing states and fast resolution. Unlike schizophrenia, this illness has a good prognosis and can achieve complete remission within 2–3 months. ATPD is included in classification as four-digit code:

1) acute and transient polymorphic psychotic disorder without symptoms of schizophrenia (F23.0);
2) acute polymorphic psychotic disorder with symptoms of schizophrenia (F23.1);

3) acute schizophrenia-like psychotic disorder (F23.2);
4) other acute polymorphic psychotic disorder (F23.3).

All these subtypes differ one from other with the associated symptoms and the duration of the episode, and if the acute episode of psychosis has an asossiation with stress, this is separatly indicated with number 1 in the end of diagnosis [1].

Systematic epidemiological studies with standardized methods of the ATPD mostly are rare.

Results in all trials were similar and show that the incidence of this disorder is ten times greater in the developing countries than in industrialized ones [2].

Some cohort studies found that the incidence of ATPD is greater in those patients who have mood disorders in the family [3, 4].

In Danish cohort study, scientists obtained the data that disorder prevalence is more frequent in women than in men and occur more occasionally in adult (30–40 year old) patients [5].

Stressful life events have been long time associated with an increased risk of mental disorder.

K. Jaspers found a strong link between psychotic episode and stress and named it "reactive psychosis" [6]. Nowadays there are a lot of new researches on this topic. For example, a research from London postulated that stressful life events would cluster before the first onset of psychosis. The study showed that stressful events, especially before 3 months, may trigger many cases of first episode psychosis [7]. The impact of these events on brain anatomy remains poorly understood, but there are some studies that show that stressful life events are associated with changes in grey matter volume in brain regions. The decrease in grey matter volume has been observed in the *anterior cingulate*, *hippocampus*, and *parahippocampal gyrus* [8]. Some authors try to understand these changes with the help of genetics and investigate functional polymorphism in the catechol-O-methyltransferase (COMT Val158Met) and find moderation of stress-induced psychosis [9]. One study showed that stressful life events occur more frequently in six month prior to the onset of the ATPD episode than to the onset manic episode [10].

Consequently, there is little known about the epidemiology of the disease, clinical aspects and prognosis, as well as etiological and pathological mechanisms of this disorder. The incidence of this disorder is very variable. ATPD comprise 8–9% of all psychotic disorders. It has been described in a range 1–2 per 100 000 population [11, 12]. In Latvia, in year 2000, the incidence was 12.5 per 100 000 population, while in 2009 from 518 first time diagnosed cases of psychosis (F 20–29), more than 148 cases were diagnosed with ATPD, which suggests that the prevalence of diagnosis in Latvia is high [13].

The aim

The aim of the study was to describe stressful life events before the first episode, clinical features at the index episode and to analyze longitudinal changes of ATPD diagnosis and associated sociodemographic characteristics of patients in Latvia.

Material and methods

In retrospective chart review study we identified all consecutive cases of first time hospitalized patients (n = 314; 165 females, 149 males) with acute and transient psychotic disorder diagnosis (ATPD, F23, ICD-10, WHO, 1993) treated at Rīga Centre of Psychiatry and Addiction Disorders, Latvia during a 3-year period (01.01.2004–31.12.2006). The hospital is situated in Rīga, the capital of Latvia, and is the largest hospital in the country. It takes care of approximately 50% of all inhabitants of Latvia (catchment area of 1 million). ATPD is very acute, serious illness and it always needs treatment in hospital. Therefore, the sample of patients from Rīga Centre of Psychiatry and Addiction Disorder can be regarded as a representative sample of clinical inpatient population with ATPD.

Clinical diagnosis was re-evaluated independently by two of the authors based on the chart review. Twenty patients (6 females and 14 males) were excluded because they did not fulfill ICD-10 criteria for ATPD.

During a 3-year period 294 patients were included (159 females and 135 males). Patients were followed up for an average of 6.1 years after the index episode.

Patients were divided into three groups. In the first "pure ATPD" patient group we included all not re-hospitalized patients and patients whose later re-hospitalization diagnosis was ATPD. In the second group of patients we included those whose ATPD diagnosis later was changed to schizophrenia (F20, ICD-10, WHO, 1993). In the third group we included all ATPD patients whose diagnosis during 6.1 years was changed to other diagnosis. The primary intention was to compare first and second group of patients for clinical features during first episode of disease and precipitating stressful life events.

Assessment of demographic and clinical features during the index episode was similar to the principles used in HASBAP study (Marneros, Pillmann, 2004) methodology [12]. Stressful life events, with broader definition about six month before index episode, were assessed using methods of Marneros too, which is in accordance with the criteria generally used in life-event research [12]. Relapse was defined as the occurrence of a major affective syndrome or of psychotic symptoms leading to hospitalization.

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS), version 19.0. In addition, the two-tailed chi-square (χ^2) tests or the two-tailed Fisher's exact test were performed where appropriate. Statistically significant was considered $p < 0.05$. The study protocol was approved by the local ethics committee.

Results

During a 3-year period 294 patients were first time hospitalized with ATPD diagnosis, 54% (159) of them were females. Over an average of a 6.1-year follow-up period 51% (150) of patients were not re-hospitalized. During the follow-up period ATPD relapses we found in 22 (15%) patients, 16 (73%) females. Later diagnosis was changed to schizophrenia in 105 (73%) patients. ATPD change to other diagnosis was seldom, only in 17 (12%) patients. In details demographic characteristics are described in Table 1.

Comparing ATPD subgroups during first episode psychosis, it was discovered that the percentage of patients with acute polymorphic psychotic disorder without symptoms of schizophrenia (F23.0) were 18% (54), with acute polymorphic psychotic disorder with symptoms of schizophrenia (F23.1) were 59% (173). It was also indicated that ATPD F23.0 diagnosis was more frequent for "pure" ATPD patients 23% (40) than for ATPD, which later developed into schizophrenia 9% (10), $p < 0.003$ (Table 2).

Table 1. Demographics and longitudinal changes of diagnosis of the study group

Demographic variables	Total, n (%)	Males, n (%)	Females, n (%)	Statistics
Number	294 (100%)	135 (46%)*	159 (54%)*	p = 0.05*
Not re-hospitalized	150 (51%)	73 (48%)*	77 (52%)*	p = 0.35*
Re-hospitalized	144 (49%)	62 (43%)*	82 (57%)*	p = 0.35*
ATPD changed to schizophrenia	105 (73%)	55 (52%)*	50 (48%)*	p < 0.001*
ATPD relapses	22 (15%)	6 (27%)*	16 (73%)*	p < 0.01*
ATPD changed to other diagnosis	17 (12%)	1 (6%)*	16 (94%)*	p < 0.0001*

* Statistical analysis by Fisher's exact test, two-tailed.

Table 2. ICD-10 codes of diagnosis in the study groups

ICD-10 codes of diagnosis	ATPD – "pure" (n = 172), n (%)	ATPD – develops to schizophrenia (n = 105), n (%)	ATPD – develops to other diagnosis (n = 17), n (%)	Total (n = 294), n (%)	Statistics
F 23.0	40 (23%)*	10 (9%)*	4 (23%)	54 (18%)	p = 0.003*
F23.1	94 (55%)*	70 (67%)*	9 (54%)	173 (59%)	p = 0.05*
F23.2	38 (22%)*	25 (24%)*	4 (23%)	67 (23%)	p = 0.7*

* Statistical analysis by Fisher's exact test, two-tailed.

During the index episode abrupt onset (within 48 hours) was found in 64 (22%) patients and acute onset (less than two weeks) in 230 (78%) patients. An abrupt ATPD onset was higher for "pure" ATPD and found in 32% patients than for ATPD which later developed into schizophrenia, 7%, $\chi^2 = 24.041$; $p < 0.0001$.

In 73 (25%) cases a typical polymorphic symptomatology was found, 34% in "pure" ATPD and only 8% in ATPD which later developed into schizophrenia, $\chi^2 = 24.475$; $p < 0.0001$.

Clinical features like hallucinations were found in total in 93 (32%) patients, but in 44% of the patients who later developed schizophrenia, and only in 26% with "pure" ATPD, $\chi^2 = 9.876$; $p < 0.01$. Affective disturbance was found in 101 (34%), 37% in "pure" ATPD and 27% in ATPD which later developed into schizophrenia, $\chi^2 = 3.267$; $p = 0.08$). In 171 (58%) cases anxiety was found: 65% in "pure" ATPD and 51% in ATPD which later developed into schizophrenia, $\chi^2 = 4.650$; $p = 0.03$.

Stressful life events before the first episode were found in 44% of the patients (129), in 43% (56) females and 57% (73) males, $\chi^2 = 4.481$; $p = 0.03$. Most common stressful life events like death of significant other were present in 13%, separation/divorce in 16% of the patients. Moreover, change of job or school in 29%, serious illness/operation in 16%. We found "moving house" in 16% of the patients (23% of males and only 5% of females; $p < 0.001$). Serious problems in family 20% (29% of females and 14% of males; $p = 0.04$). Stressful life events and gender differences are described in details in Table 3.

Table 3. Stressful life events 6 months before first-time psychosis

Stressful life events	Males (n = 73), n (%)	Females (n = 56), n (%)	Total (n = 129), n (%)	Statistics
Death of significant other	6 (8%)*	11 (20%)*	17 (13%)	p = 0.06*
Separation / divorce	6 (8%)*	15 (27%)*	21 (16%)	p = 0.007*
Change of job or school	19 (26%)*	19 (34%)*	38 (29%)	p = 0.33*
Moving house	17 (23%)*	3 (5%)*	20 (16%)	p = 0.006*
Serious illness / operation	7 (10%)*	13 (23%)	20 (16%)	p = 0.04*
Serious problems in family	10 (14%)*	16 (29%)*	26 (20%)	p = 0.04*
Serious problems at work	8 (11%)*	14 (25%)*	22 (17%)	p = 0.05*
Major journey	9 (12%)*	12 (21%)*	21 (16%)	p = 0.22*
Unemployment	34 (25%) n = 135	55 (35%)* n = 159	89 (30%) n = 294	p = 0.09*

* Statistical analysis by Fisher's exact test, two-tailed.

Discussion

There are a few studies in the world about acute and transient psychotic disorder, but in the last years this topic has become more and more important, especially in the light of discussions regarding changes in the new ICD-11 classification.

Study data do not show statistically significant gender differences between the patients, unlike other authors [12]. It was an interesting observation that half of the patients were hospitalized only once during the follow-up period. This could indicate a much better prognosis of ATPD than, for example, in schizophrenia. If diagnosis was changed, most frequent was schizophrenia diagnosis; this data was similar to other researchers [5, 12].

Detailed study of nosologic units of this diagnosis showed a significant difference within the group itself. It was indicated that first subtype of diagnosis was "closer" to the "pure" ATPD, but other conceptually to the schizophrenia. This should play a significant role in the development of amendments to the new classification.

According to the study, statistically significant differences were found between clinical features in first time episode psychosis of two patient groups ("pure" ATPD and ATPD which transfers to schizophrenia). Patients with ATPD witch develops to schizophrenia presented higher rates of hallucinations, but "pure" ATPD presented more typical polymorphic symptomatology and abrupt onset.

Recent scientific developments are giving opportunity to visualise changes in brain cells after stress and find possible target gene regions, which may be the response for human reaction on stress. In psychiatry we have some theories considered as stress exacerbates personality characteristics (personality regress to psychotic levels of functioning) [6]. But unfortunately we have no studies yet which can prove the strong link between stress and psychosis.

There are published data that show correlation of the ATPD with stressful life events before first time psychotic episode [10]. In this study it was found that a rather large part of our patients had stressful life events for six month before psychosis, thus it can be concluded that sometimes it could be an important factor which can facilitate the development of the disease. Taking into account that our study included one of the largest numbers of patients among other published studies, we were able to observe some gender differences of type of stressful life events associated with initiations of psychotic episode. The current study is ongoing and there is a plan to include more patients in the study, which will increase statistical power to detect some meaningful differences. The most important factor that has limited our research was the retrospective nature of the study. No structured life events scale was used and life event data might have been influenced by recall bias. This should be taken into account drawing any conclusions. A prospective study aimed to evaluate first episode patients with ATPD is currently underway.

Conclusions

Prevalence of ATPD has no gender difference in Latvia. Half of the patients were hospitalized only once during the follow-up period. In subgroup of re-hospitalized patients the most common diagnostic change was to schizophrenia. Patients with ATPD which develops to schizophrenia presented higher rates of hallucinations, but "pure" ATPD presented more typical polymorphic symptomatology, abrupt onset, anxiety and trend for affective disturbance. Stressful life events before the first episode were presented in 44% of ATPD patients, with higher rates for males. Females during the index episode presented higher rates in serious problems in family, at work, but males "moving house". Clinical features of the disease at the first episode and possible provoking factors could help to predict further development of the disease. Further prospective research on the topic is warranted to understand the evaluation of the diagnosis in ICD-10 and possible changes in ICD-11.

References

1. The ICD-10 Classification of Mental and Behavioural Disorders. – Geneva; Oxford University Press, 1992.
2. Malhotra S. Acute and transient psychosis: a paradigmatic approach // Indian J Psychiatry, 2007; 49(4): 233–243.
3. Das S. Family study of acute and transient psychotic disorders: comparison with schizophrenia // Social Psychiatry and Psychiatric Epidemiology, 1999; 34: 328–332.
4. Sajith S. Acute polymorphic psychotic disorder: diagnostic stability over 3 years // Acta Psychiatrica Scandinavica, 2002; 105: 104–109.
5. Jorgensen P. Acute and transistent psychotic disorder; a 1-year follow-up study // Acta Psychiatrica Scandinavica, 1997; 96: 150–154.
6. Stromgren E. The development of the concept of reactive psychoses // Psychopathology, 1987; 20: 62–67.
7. Raune D., Kuipers E. Stressful and intrusive life events preceding first episode psychosis // Epidemiol Psichiatr Soc, 2009, 18(3): 221–228.
8. Papagni S., Benetti S., Arulanantham E. Effects of stressful life events on human brain structure: a longitudinal voxel-based morphometry study // Stress, 2011, 14(2): 227–232.
9. Stefanis N., Henquet C., Avramopoulos D. COMT Val158Met moderation of stress-induced psychosis // Psychol Med, 2007, 37(11): 1651–1656.
10. Chakraborty R., et al. Life events in acute and transient psychosis – a comparison with mania // German Journal of Psychiatry, 2007; 10: 36–40.
11. Kaplan G., Sadocks B. Synopsis of Psychiatry / Ed. by Sadock B. J., Sadock V. A. – 10th ed. – Philadelphia: Williams & Wilkins, 2007. – Pp. 336–347.
12. Marneros A., Pillmann F. Acute and transient psychoses. – Cambridge University Press, 2004.
13. Mental health care in Latvia // Statistics Yearbook. – 10th ed. – Rīga: Ministry of Health of the Republic of Latvia, Centre of Health Economy, 2010.

Basal CK19 Expression in Triple-negative Breast Cancer

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Abstract

The triple-negative breast cancer is tumour subtype that is negative for expression of the estrogen progesterone receptors and HER2 protein. The basal-like breast cancer (BLC) is characterised by poor prognosis and lack of specific targeted therapies. BLC is characterised by positive expression of basal cytokeratins. The aim of the study was to evaluate CK19 expression and its prognostic significance in triple-negative breast cancer cases.

26 triple-negative breast cancer cases were studied for carrying out the research. All triple-negative breast cancer cases were immunohistochemically stained for CK19 (*Clone* RCK, DAKO).

CK19 immunohistochemistry showed strong and moderate membranous staining in breast carcinoma cells, as well as, strong expression in normal breast tissues CK19 negative results were in 3 cases (11.5%), but positive results in 23 cases (88.5%). Positive CK19 expression in GII breast carcinoma cases was in 80%, but in GIII carcinoma cases – 90.5%.

Basal-like carcinoma features according to CK19 expression in our study were in 23 cases (88.5%). Cytokeratins immunohistochemistry could be the additional marker in the triple-negative breast cancer. CK19 negativity has failed to be a predictive marker of poor outcome in the triple-negative breast cancer.

Keywords: triple-negative breast cancer, basal-like breast cancer, CK19.

Introduction

Breast cancer is a heterogeneous disease with various microscopic appearances, molecular profile and clinical behaviour. Four breast cancer subgroups have emerged based on gene expression profiling, two of which are estrogen positive tumours and two of them are estrogen receptor negative tumours (basal-like and HER2 positive). Basal-like breast tumours are almost estrogen, progesterone, HER2 negative, so called triple-negative phenotype [Kassam F., et al., 2009]. The triple-negative breast cancer is tumour subtype that is negative for expression of the estrogen progesterone receptors and HER2 protein.

The basal-like breast cancer (BLC) is characterised by poor prognosis and lack of specific targeted therapies. BLC is characterised by positive expression of basal cytokeratins [Rakha E. A., et al., 2009]. The "gold standard" for BLC investigation is gene expression profile, but in some studies basal cytokeratin immunohistochemistry is used to define BLC [Potemski P., et al., 2005].

The biological significance of the differential expression of cytokeratins in breast carcinomas is unclear [Malzahn K., et al., 1998]. Keratin phenotype in breast carcinoma is associated with short overall and disease-free survival, and ER negativity. All these tumours express simple cytokeratins (CK7, CK8, CK18, CK19) [Gusterson B. A., et al., 2005].

According to some studies, CK19 negativity in basal-like carcinoma could be a predictor of poor local, distant and overall survival [Parikh R. R., et al., 2008].

CK19 is keratin with the lowest molecular weight in the basal layer of the squamous epithelium and may be seen in epidermal basal cells. CK19 is one of the main keratins expressed in epithelium in various carcinomas including breast cancer [Chu P. G., et al., 2002]. CK19 positive are human breast stem cells [Gudjonsson T., et al., 2002].

The aim

The aim of the study was to evaluate CK19 expression and its prognostic significance in triple-negative breast cancer cases.

Material and methods

26 triple-negative breast cancer cases were studied for carrying out the research. In all cases IHC *Herceptest* (DAKO), estrogen and progesterone receptor (DAKO) detection was done. For the interpretation of the *Herceptest* results, semi-quantitative scoring criteria were used. Estrogen and progesterone receptor status was reported as a percentage in 2 separate high power fields. Then triple-negative breast cancer cases were immunohistochemically stained for CK19 (*Clone* RCK, DAKO). CK19 was scored positive if any (weak or strong) cytoplasmatic or membranous staining was observed in carcinoma cells. Histological type and grade was determined according to Scarff-Bloom-Richardson system.

Statistical analysis was performed using SPSS 12.0.

Results

26 breast cancer cases were investigated, where ER, PR receptors and HER2 protein expression were negative (triple-negative breast carcinoma). In most cases tumour cells were with eosinophilic cytoplasm and enlarged nuclei. The majority of tumours were with solid areas and without tubule formation (Figure 1). In some cases central necrosis were surrounded by a zone of poorly differentiated carcinoma cells (Figure 2).

In the majority of cases tumour was T2 stage (58%). The patients' age was between 30–83 (the median age 51.08). Majority of the tumours were invasive ductal carcinomas GIII (80.8%) (Table 1).

CK19 immunohistochemistry showed strong and moderate membranous staining in breast carcinoma cells (Figures 3 and 4) as well as strong expression in normal breast tissues (Figure 5). CK19 negative results were in 3 cases (11.5%), but positive results in 23 cases (88.5%). Positive CK19 expression in GII breast carcinoms cases was in 80%, but in GIII carcinoma cases – 90.5%.

Figure 1. Poorly differentiated carcinoma with solid areas, H & E, × 100

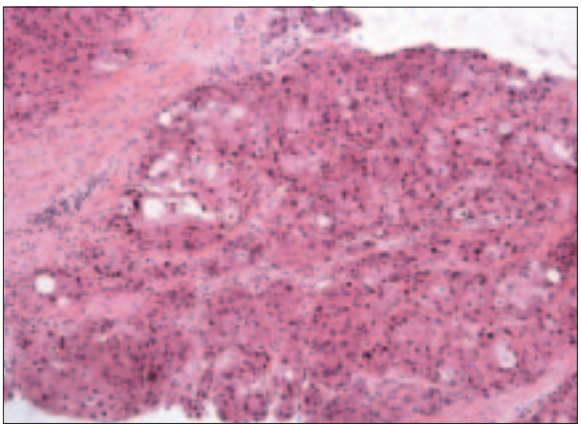


Figure 2. Poorly differentiated carcinoma with necrosis, H & E, × 100

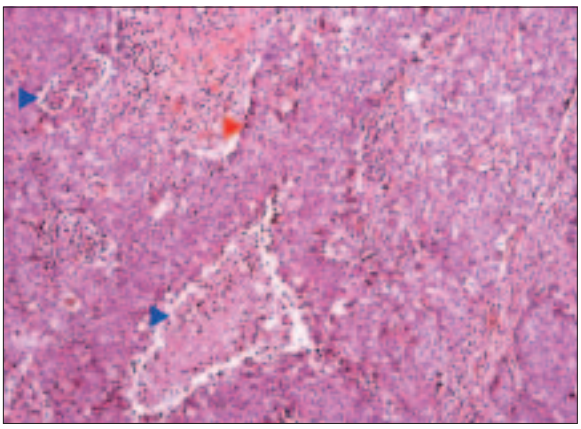


Table 1. Clinical and morphological features in 26 triple-negative breast cancer patients

Feature	Patients, n	Rate, %
Tumour stage:		
T1	11	42.0
T2	15	58.0
Tumour grade:		
GII	5	19.2
GIII	21	80.8
Lymph node status:		
Negative	21	80.8
Positive	5	19.2
Age:		
Premenopausal	13	50.0
Postmenopausal	13	50.0
CK9 expression:		
Negative	3	11.5
Positive	23	88.0

Figure 3. CK19 expression in triple-negative breast cancer – strong membranous staining, CK19, *Clone* RCK, DAKO, × 200

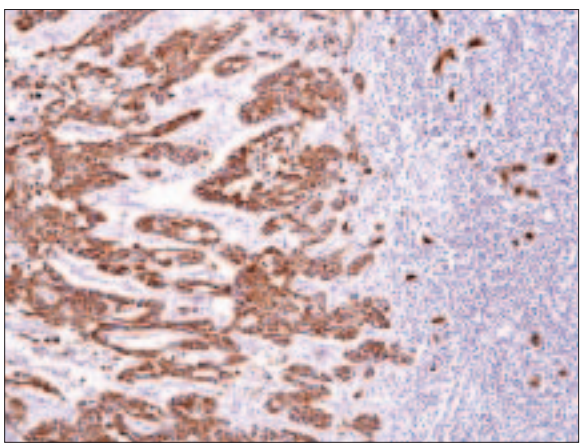


Figure 4. CK19 expression in carcinoma tissues (right side) and in normal breast tissues (left side), CK19, *Clone* RCK, DAKO, × 100

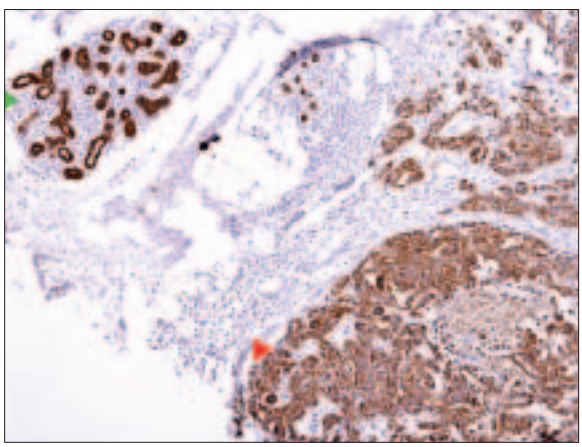
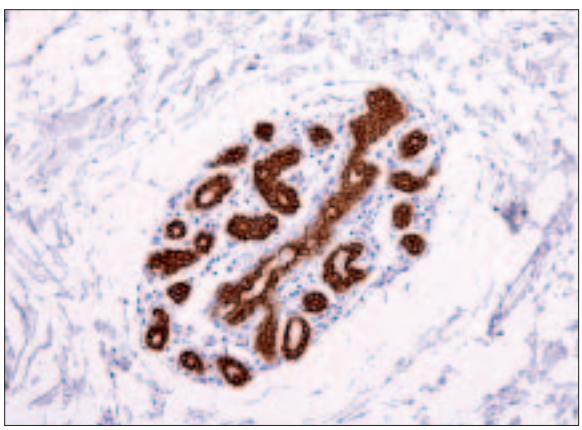


Figure 5. CK19 expression in terminal duct-lobular unit, CK19, *Clone* RCK, DAKO, × 200



The evaluation of survival in time period from 9 to 57 months was done. Carcinomas dissemination was observed in 4 cases (17.4%), 3 of them died. In all these cases CK19 expression was strong positive. There were no data available about the time of progression (TTP) for all patients.

Discussion

There are no internationally accepted definitions and criteria for basal-like carcinomas routine diagnosis. The basic method for the identification of BLCs is gene expression profiling, but immunohistochemical staining could be a surrogate of gene expression analysis [Rakha E. A., et al., 2009]. In Nielsen study immunohistochemical surrogate markers were ER and HER2 negativity, CK5/6 and/or EGFR positivity [Nielsen T. O., et al., 2004]. But in some issues basal CKs alone were used to define the BLC [Laakso M., et al., 2005; Potemski P., et al., 2005]. In our study we used basal CK19 to define basal-like carcinoma subtype. From all triple-negative invasive breast cancer cases, positive CK19 expressions was in 23 cases (88.5%).

CK19 negativity in basal-like carcinoma was a predictor of poor local, distant and overall survival [Parikh R. R., et al., 2008]. The study evaluated survival in the period from 9 to 57 months. Three patients died. In all these cases CK19 expression was positive.

Besides, the examination of CK expression in surrounding (normal) breast tissues was done. The positivity was strong in normal terminal duct-lobular unit. But in *Hsiao* issue, CK19 positivity was heterogeneous in normal breast tissues.

There are some issues about basal-like carcinoma morphologic features [Fulford L. G., et al., 2006; Livasy C. A., et al., 2006]. In Fulford's study all tumours were grade III with pushing margins and pushing growth pattern, with necrosis and prominent lymphocytic infiltrate. In Livasy's research tumours also were with pushing margins, solid architecture with no tubule formation. Our study presented similar morphological features – solid growth pattern, necrosis and no tubule formation.

Conclusions

1. In our study, according to CK19 expression, the basal-like carcinoma features were in 23 cases (88.5%).
2. Cytokeratins immunohistochemistry could be the additional marker in the triple-negative breast cancer.
3. CK19 negativity has failed to be a predictive marker of poor outcome in the triple-negative breast cancer.

References

1. Chu P. G., Weiss L. M. Keratin expression in human tissues and neoplasms // Histopathology, 2002; 40: 403–439.
2. Fulford L. G., Easton D. F., Reis-Filho J. S., et al. Specific morphological features predictive for the basal phenotype in grade 3 invasive ductal carcinoma of breast // Histopathology, 2006; 49: 22–34.
3. Gudjonsson R., Villadsen R., Nielsen H. L., Ronnov-Jensen L., et al. Isolation, immortalization, and characterization of a human breast epithelial line with stem cell properties // Genes Dev, 2002; 16: 693–706.
4. Gusterson B. A., Ross D. T., Heath V. I., Stein T. Basal cytokeratins and their relationship to the cellular origin and functional classification of breast cancer // Breast Cancer Research, 2005; 7: 143–148.
5. Hsiao Y. H., Chou M. C., Fowler C., Mason J. T., Man Y. G. Breast cancer heterogeneity: mechanisms, proofs, and implications // J Cancer, 2010; 1: 6–13.
6. Kassam F., Enright K., Dent R., Dranitsaris G., et al. Survival outcomes for patients with metastatic triple-negative breast cancer: implications for clinical practice and trial design // Clinical Breast Cancer, 2009; 9: 29–33.
7. Laakso M., Loman W., Borg A., Isola J. Cytokeratin 5/14-positive breast cancer: true basal phenotype confined to BRCA1 tumors // Modern Pathology, 2005; 18(10): 1321–1328.

8. Livasy C. A., Karaca G., Nanda R., Tretiakova M. S., et al. Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma // Modern Pathology, 2006; 19: 264–271.
9. Malzahn K., Mitze M., Thoenes M., Moll R. Biological and prognostic significance of stratified epithelial cytokeratins in infiltrating ductal breast carcinomas // Virchows Arch, 1998; 433: 119–129.
10. Nielsen T. O., Hsu F. D., Jensen K., Cheang M., et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma // Clin Cancer Res, 2004; 10(16): 5367–5374.
11. Parikh R. R., Yang Q., Higgins S. A., Haffty B. G. Outcomes in young women with breast cancer of triple-negative phenotype: the prognostic significance of CK19 expression // Int J Radiat Oncol Biol Phys, 2008; 1(70): 35–42.
12. Potemski P., Kusinska R., Watala C., Pluciennik E., et al. Prognostic relevance of basal cytokeratin expression in operable breast cancer // Oncology, 2005; 69(6): 478–485.
13. Rakha E. A., El-Sayed M. E., Reis-Filho J., Ellis J. O. Patho-biological aspects of basal-like breast cancer // Breast Cancer Res Treat, 2009; 113: 411–422.

Neuropeptides in the Nasal and Nasopharyngeal Mucosa in Patients with Postnasal Drip Syndrome

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Abstract

The postnasal drip is very common symptom of sinusitis, allergic rhinosinusopathy, gastroesophageal reflux disease, but there are some patients who have a postnasal drip sensation of foreign body in the nasopharynx and a non-specific irritant cough with no other symptoms or signs of sinus inflammations or allergy. In the posterior rhinoscopy mucus discharge can be observed. Ethiology and pathogenesis of this syndrome evolution is still unclear.

The aim of the study was to identify neuropeptide appearance and distribution in nasal and nasopharyngeal mucosa in patients with isolated postnasal drip syndrome.

We investigated biopsies of nasal and nasopharyngeal mucosa from 5 adult patients who had isolated postnasal drip syndrome by conventional light microscopy and immunohistological techniques for protein gene product 9.5 (PGP), neuropeptide Y (NPY), serotonin, substance P (SP), vasoactive intestinal peptide (VIP), calcitonin gene related peptide (CGRP) and chromogranin A (CgA).

The conventional light microscopy showed very thick basal membrane, sclerosis of small blood vessels, hyperplasia of mucosal glands – neurogenic inflammation, mostly in nasopharyngeal mucosa. Abundance of PGP-containing nerve fibres were found around glands, sclerotic arterioles in almost all cases. The main neuropeptides that were found in the mucosa of patients were VIP, NPY, CgA, mostly in the nasopharyngeal mucosa.

From mucosal tissue, the nasopharyngeal one is mainly affected by neuropeptides-containing innervation in postnasal drip patients. The main neuropeptides that are found in nasal and nasopharyngeal mucosa samples in present study were vasoactive intestinal peptide (VIP), neuropeptide Y (NPY) and chromogranin (CgA). The main histological changes were thickened basal membrane, hyperplasia of basal cells, pronounced hyperplasia of mucosal glands, sclerosis of small arterioles.

Keywords: postnasal drip syndrome, neurogenic inflammation, neuropeptides.

Introduction

Postnasal drip is usually a symptom of rhinosinusitis, allergic rhinosinusitis, gastroesophageal reflux disease. There is a group of patients who have no other symptoms of sinus inflammation, allergy or reflux, but still they have mucus discharge in nasoparynx, sensation of foreign body in nasopharynx and irritant cough – so called postnasal drip syndrome [1].

There is an increasing evidence of great role of the neuroendocrine regulation and neuropeptides in respiratory tract mucosa [1, 2].

The nasal mucosa is richly innervated by sensory, sympathetic and parasympathetic nerve fibres which secrete, when become activated, a variety of transmitter molecules such as noradrenalin (NA), acetylcholine (ACh) or neuropeptides. Neuropeptides are proteins that are synthesized in sensory nerve cell nuclei and transported to the vesicle from axons. The source of neuropeptides may be nerve cells and neurons associated cells, like glyocytes and fibroblasts. C and Aδ fiber stimulation causes the release of neuropeptides. Release of neuropeptides by sensory nerve endings produces vasodilatation and increased vascular permeability, phenomena primarily described in rodents that have been collectively termed neurogenic inflammation [3]. Normal human respiratory tract (nasal and bronchial) mucosa epithelium and glandular cells as well as lung lymphocytes and macrophages produce neuropeptide. Substance P (SP), serotonin, calcitonin gene related peptide (CGRP), gastrine releasing peptide, neuropeptide Y (NPY), vasoactive intestinal peptide (VIP), chromogranin are found in nasal mucosa. These neuropeptides cause vasodilatation and upper airways oedema, characterized by nasal obstruction, and bronchial constrictions, increased mucus formation.

Concentration of neuropeptides in the bronchial and nasal secretions is relatively low for asymptomatic patients, but it is highly elevated in patients with allergic respiratory diseases, including nasal polyps. In case of chronic upper and lower respiratory tract illnesses, respiratory mucosa shows an increased quantity of pro-inflammatory sensory neuropeptides. Neuropeptide concentrations correlate with patients' symptom intensity [4, 5].

There are still conflicting views about just how exactly neurogenic inflammation is mediated in the human body [6]. The neurogenic inflammatory of nasal mucosa has so far been studied mostly in animals and *in vitro* [7, 8].

Since neuroendocrine cells in airway mucosa are discovered, increasing interest is attached to the yet unexplored neurogenic inflammation in the case of chronic airway inflammation, especially if it is characterized by increased airway reactivity. Existing literature highlights suggest that nowadays a chronic inflammatory process of respiratory mucosa may no longer be viewed *per se*, but always in conjunction with the respiratory neural and neurohumoral regulation of neurogenic inflammation, which is not yet fully explored [12].

The aim

The aim of the study was to identify neuropeptide appearance and distribution in nasal and nasopharyngeal mucosa in patients with isolated postnasal drip syndrome.

Material and methods

Inferior nasal turbinate and nasopharyngeal mucosa specimens were investigated, obtained from 5 adult patients (age 18–50) with isolated postnasal drip syndrome.

All patients were examined by otolaryngologist to exclude rhinosinusopathy – normal pneumatisation of all sinuses at CT scan, allergy – no changes in skin prick tests, IgE range (total and specific) and gastroesophageal reflux disease (clinically and by performing endofibrogastroscopy).

All patients had symptoms of postnasal drip for more than 6 months, and had no reaction on common therapy – topical steroid (fluticasone), antihistamine (loratidin), antireflux therapy (diet and omeprasole).

Tissue pieces (1–2 mm²) from the middle part of lower nasal turbinate and the middle part of nasopharyngeal arch area were taken under control of the endoscope, under local anaesthesia (submucosal administration of 1–2 ml of 1% lidocaine solution). For immediate fixation previously prepared saturated picric acid solution (formaldehyde 2%, 0.2%o Picric acid, 1 M phosphate buffered, pH 7.2) was used. Tissue slices were stained with haematoxylin and eosin, and by use of immunohistological technique for protein gene product 9.5 (PGP), substance P (SP), neuropeptide Y (NPY), vasoactive intestinal peptide (VIP), serotonin, calcitonin gene related peptide (CGRP) and chromogranin A (CgA) (Table 1).

The samples were examined under Leica microscope for conventional histological picture. The results of immunohistochemistry were listed by semi-quantitative counting method (Tobin, et al., 1990; Pilmane, 1997) (Table 2).

Table 1. Data on antibodies applied in immunohistochemistry

Samples	Obtained from	Working dilution	Manufacturer	Code
PGP	Rabbit	1 : 600	DAKO (Denmark)	Z5116
Serotonin	Mouse	1 : 10	DAKO (Denmark)	M758
NPY	Rabbit	1 : 10	DAKO (Denmark)	B48–100
VIP	Rabbit	1 : 400	Abcam (UK)	Ab22736
SP	Mouse	1 : 1000	Abcam (UK)	Ab14184
CGRP	Rabbit	1 : 20	Quartet (Germany)	281328
CgA	Rabbit	1 : 400	DAKO (Denmark)	A0430

Table 2. Semi-quantitative analysis of the immunohistochemically determined structures

Applied markings	Semi-quantitative explanation
–	No positive structures seen in the visual field
0 / +	Rare positive structures seen in the visual field
+	Few positive structures seen in the visual field
+ / ++	Few to moderate number of positive structures seen in the visual field
++	Moderate number of positive structures seen in the visual field
++ / +++	Moderate to numerous positive structures seen in the visual field
+++	Numerous positive structures seen in the visual field
+++ / ++++	Abundance of positive structures in the visual field

Results

Nasal mucosa demonstrated pronounced partially patchy thickened basal membrane. Epithelium was dysplased, basal cell hyperplasia and intraepithelial infiltration with lymphocytes, marked hyperplasia of glandulocytes were seen along the epithelial lining (Figure 1). One patient showed metaplasia of epithelium (stratified squamous epithelium instead of pseudostratified ciliated epithelium). *Lamina propria* also demonstrated infiltration with lymphocytes, hypertrophy of glands and sclerosis of small arterioles (Figure 2). One patient showed granulation tissue of subepithelium and marked infiltration of lymphocytes, and one other patient had lymphatic nodule of the subepithelium.

Numerous PGP-containing nerve fibres were observed mainly around the secretory parts of seromucosal glands and sclerotic arterioles (Figure 3). The patient with lymphatic nodules in the submucosa showed less PGP-containing nerve fibres. The patient who had granulations and methaplasia of nasal epithelium, showed almost negative PGP structures in all mucosa samples (0/+) (Table 3).

Nasopharyngeal mucosa showed even more expressed changes in comparison to nasal mucosa – very thickened basal membrane, hyperplasia of basal cells, pronounced hyperplasia of mucosal glands, sclerosis of small arterioles, some patients had infiltrations of lymphocytes in the submucosa. Also one patient, the same with granulations in the nasal mucosa, showed granulation tissue in the nasopharyngeal submucosa too. Abundance of PGP-containing nerve fibres were found around glands, sclerotic arterioles in almost all samples, except the patient with granulations in submucosa; he had only few PGP-containing fibres and only next to some seromucous glands (Figure 4).

All nasal and nasopharyngeal mucosa samples were serotonin negative (Table 3).

Figure 1. Hyperplasia of mucosal glands, infiltration of lymphocytes, sclerosed blood vessels in nasal mucosa. H & E, × 200

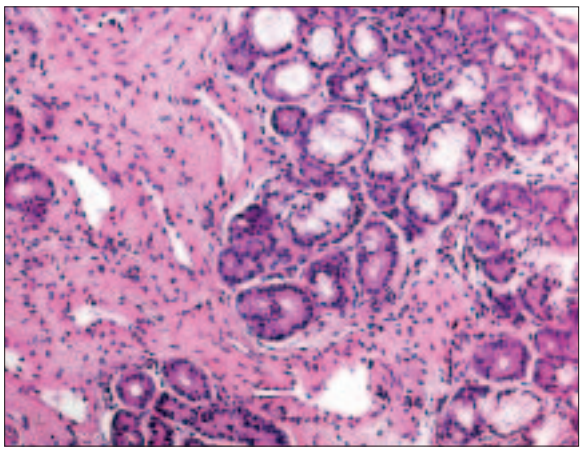


Figure 2. Infiltration of lymphocytes, thic basal membrane in nasopharyngeal mucosa. H & E, × 200

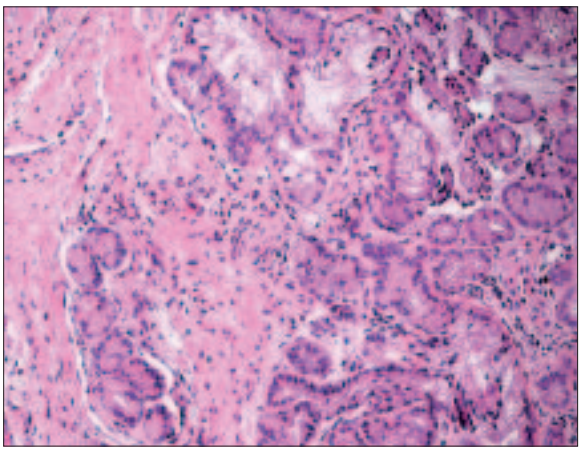


Figure 3. Abundance of PGP-containing fibers around sclerosed blood vessels and seromucosal glands in nasal mucosa. PGP, × 250

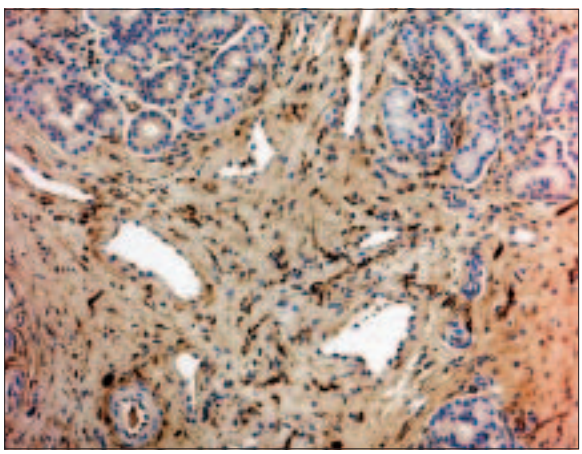


Figure 4. Few PGP-containing structures next to seromucosal glands in nasal mucosa, patient with granulation in submucosa. PGP, × 250

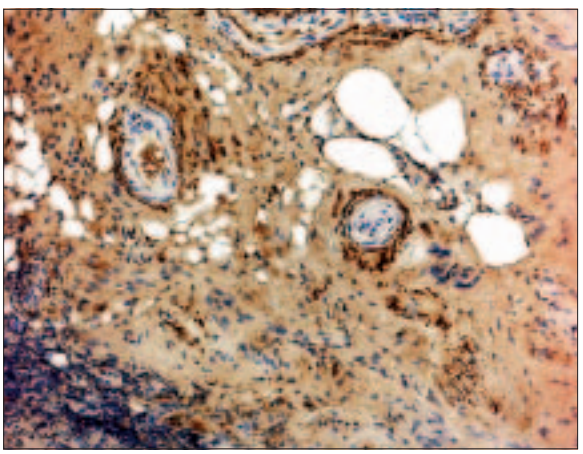


Figure 5. Numerous VIP positive structures next to blood vessels in nasopharyngeal mucosa. VIP, × 250

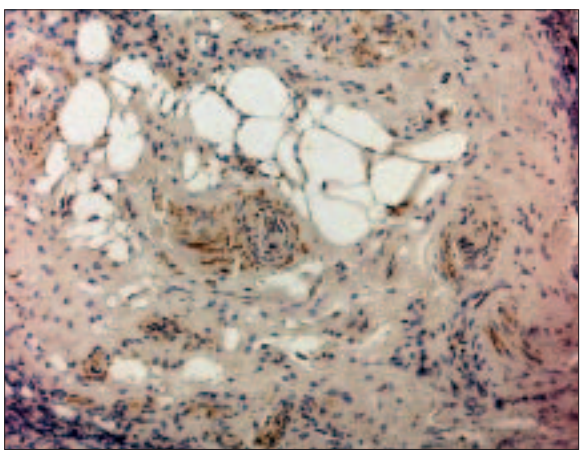
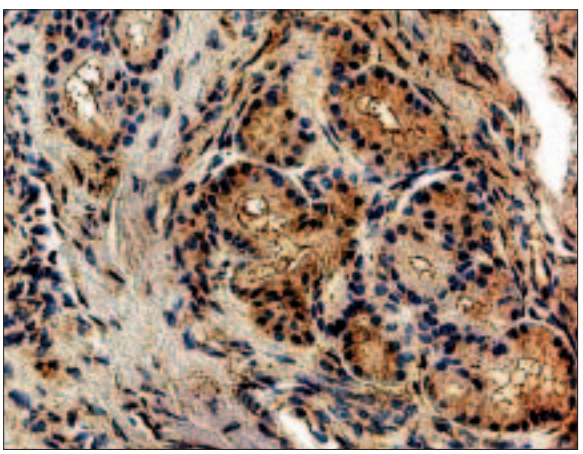


Figure 6. Numerous CgA containing granules in cells next to mucosal glands in nasal mucosa. CgA, × 250



Most nasal and nasopharyngeal mucosa samples showed rare NPY-containing nerve fibres (0/+), some showed few NPY positive structures (+), mostly around small sclerotic arterioles in the nasopharyngeal mucosa. The exception was observed in mucosal samples from the patient with lymphatic nodules in the submucosa, in this case more positive structures in comparison to other patients' mucosa samples around arterioles in nasopharyngeal mucosa (++) were defined (Table 3).

Nasal mucosa showed moderate to numerous number of VIP-containing nerve fibres (++/+++) around submucosal glands and small sclerotic arterioles, but in the nasopharyngeal mucosa samples, VIP-containing nerve fibres were even more, especially around sclerotic arterioles (++/+++) (Table 3, Figure 5).

In all nasal and nasopharyngeal mucosa samples rare (0/+) or negative substance P containing structures were found (Table 3).

A few CGRP positive structures (+) in the visual field were seen in the nasal and nasopharyngeal mucosa, mainly in nasopharyngeal mucosa next to blood vessels and around seromucosal glands (Table 3).

Almost in all nasal and nasopharyngeal mucosa samples, moderate to numerous (++/+++) CgA-containing cells were found, mainly in seromucosal glands and basal layer of epithelium (Figure 6).

Table 3. Results of immunohistochemical finding in nasal and nasopharyngeal mucosa

Mucosa	PGP	NPY	VIP	SP	CGRP	Serotonin	CgA
Glands and nasal mucosa	From ++–**+++** to +++/++++	**0/+**–+	+–++	0/+	0/+	–	++/+++–+++
Glands and nasopharyngeal mucosa	+++–+++/++++	**0/+**–+	From +–**++** to +++	0/+	0/+	–	++/+++–**+++**
Blood vessels and nasal mucosa	From ++–**+++** to +++/++++	**0/+**–+	+–++	0/+	0/+	–	**++**–++/+++
Blood vessels and nasopharyngeal mucosa	++–+++/++++	From **0/+**–+ to ++	From +–**++** to +++	0/+	0/+	–	From ++–**++/+++** to +++

(–) No positive structure seen in the visual field, (0/+) rare positive structures seen in the visual field, (+) a few positive structures seen in the visual field, (+/++) a few to moderate number of positive structures seen in the visual field, (++) moderate number of positive structures seen in the visual field, (++/+++) moderate to numerous positive structures seen in the visual field, (+++) numerous positive structures seen in the visual field, (+++/++++) abundance of positive structures in the visual field.

Marked with bold tipe is most common appearance of positive structures.

Discussion

This study shows infiltrations of lymphocytes, thick basal membrane and sclerotic small arterioles and hyperplasia of seromucosal glands in nasal and nasopharyngeal mucosa of patients with postnasal drip syndrome. These findings are very common in the case of allergic rhinosinusitis and hyperreflectoric rhinitis [13, 14]. Similar results describe also Fisher et al. by histological examination of nasal mucosa in case of allergic rhinitis [15]. As our patients excluded clinical symptoms of allergy, we cannot exclude some impact of local hyperactive mucosa due to the innervation changes.

In the nasopharyngeal mucosa PGP-containing neural fibres were even more observed than in the nasal mucosa, probably due to the density of innervations, which is more pronounced in the mucosa of nasopharynx compared with the mucosa of lower nasal turbinate [17].

Our study showed moderate to numerous number of VIP-containing nerve fibres around seromucosal glands and small sclerotic arterioles, especially in the nasopharyngeal mucosa samples, it shows that VIP could implicate in pathogenesis of mucus discharge in the nasopharynx and probably also stimulate the scleratisation. This indirectly is proved by fact that postganglionic fibres contain neuropeptides such as vasoactive intestinal peptide (VIP), which has many functions such as vasodilatation and glandular discharge [18].

In our study just rare NPY positive structures were found in nasal and nasopharyngeal mucosa. Neuropetide Y is co-localized with norepinephrine in a population of sympathetic neurons in the walls of human nasal mucosal arterioles [20]. Sympathetic stimulation induces vasoconstriction and increased nasal airway potency. There is also evidence that sympathetic activity can induce airway glandular secretion through stimulation of serous cells through β receptors [18, 19]. We can predict that patients with post nasal drip syndrome have reduced NPY production, which can lead to airway obstruction and production of the mucus. Tai and Baraniuk reported that NPY is more effective than oxymetazoline, an α-adrenergic agonist vasoconstrictor that is used clinically as a topical nasal decongestant, at reducing symptoms of nasal obstruction and the weight of mucus secretions after nasal allergen challenge [21].

The study also included the investigation of substance P appearance in nasal and nasopharyngeal mucosa. The results showed rare or negative (0/+) SP containing structures. Sensory nerve neuropeptides include the tachykinins (i.e., substance P and neurokinin A), the calcitonin gene-related peptide [23]. Effects of these neuropeptides include glandular activation, leukocyte recruitment, differentiation and activation of various immune cells, including lymphocytes, eosinophils, mast cells and macrophages [24, 25]. Non-olfactory sensory nerves consist of both myelinated and unmyelinated (Aδ, C) fibres. C fibres dendrites can be antidromically stimulated by action potentials that originate at different terminals of the same neuron. Antidromic stimulation is also known as the axon reflex [22]. The aim of antidromic stimulation is the fact that its end result is the release of inflammatory neuropeptides from peripheral neurosecretory varicosities. According to these known effects, it is unusual that substance P was not found in nasal and nasopharyngeal mucosa samples, acquired results may suggest disturbances in SP production and possible axonal reflex problems. Additionally, as reported by Fajac, et al., one of the effects of substance P is increasing allergen-induced eosinophil accumulation [25].

Serotonin effects are smooth muscle contraction, vasodilatation, and increased vascular permeability. Our results showed the absence of serotonin in all nasal and nasopharyngeal mucosal samples. It is uncommon because serotonin as well as histamine, leukotrienes, prostaglandins and tryptase are released and account for the immediate airway allergy symptoms, mainly these mechanisms lead to anaphylaxis, rhino conjunctivitis and urticaria, and it is often found in case of hyperreflectoric rhinitis [28].

Calcitonin gene-related peptide is one of the neuropeptides that is released in human nasal mucosa after trigeminal nerve stimulation. The main effects of this neuropeptide are vasodilatation, mucus secretion, plasma extravasations; CGRP exerted also a significant dose-dependent stimulation on ciliary's beat frequency [30]. According to these known effects, inexplicable are negative CGRP-containing nerve fibres in the present study. In studies described in literature, CGRP is usually found in allergic and hyperreflectoric rhinitis [16, 20, 30, 31]. That seemingly suggests differences between allergic and neurogenic inflammation.

The obtained results showed moderate to numerous CgA immunopositive cells in nasal and nasopharyngeal mucosa in the case of isolated postnasal drip syndrome. Chromogranin A belongs to the granin family of uniquely acidic secretory proteins co-stored and co-secreted with other hormones and peptides in elements of the diffuse neuroendocrine system. The granins secretions detected by different genes and are characterized by numerous sites for post-translational cleavage into shorter peptides with postulated regulatory properties. In nasal mucosa chromogranin mediates as marker of neuropeptides, neuroendocrine cells. CgA peptides take part in regulation of calcium and glucose metabolism, cardiovascular functions, gastrointestinal motility and nociception, tissue repair, inflammatory responses and as host defence peptides in the first phase of microbial invasions [29].

Conclusions

The main neuropeptides that are found in nasal and nasopharyngeal mucosa samples are vasoactive intestinal peptide, neuropeptide Y and chromogranin in postnasal drip syndrome patients. The main histological changes are thickened basal membrane, hyperplasia of basal cells, pronounced hyperplasia of mucosal glands, sclerosis of small arterioles. That suggests the significant role of inflammation and also neuropeptides in evolution of isolated postnasal drip syndrome. Notable decrease of SP-nerves among the sympathetic and parasympathetic nerves indicate the possible involvement of some other tachykinins into the pathogenesis of postnasal drip syndrome.

References

1. Dykewicz M. S., Hamilos D. L. Rhinitis and sinusitis // Journal of Allergy and Clinical Immunology. 2010; 125(2): 3–23.
2. Sanu A., Eccels R. Postnasal drip syndrome. Two hundred years of controversy between UK and USA // Rhinology, 2008; 46(2): 86–91.
3. McDonald D. Neurogenic inflammation in the rat trachea. Changes in venules, leucocytes and epithelial cells // J Neurocytol, 1988; 17: 583–603.
4. Baraniuk J. N. Neural regulation of mucosal function // Pulm Pharmacol Ther, 2008; 21(3): 442–448.
5. Fisher A., McGregor G. P., Saria A., Philippin B., Kummer W. Induction of tachykinin gene and peptide expression in guinea pig nodose primary afferent neurons by allergic airway inflammation // J Clin Invest, 1996; 262: 646–653.
6. Myers A., Kajeka R., Undem B. Allergic inflammation-induced neuropeptide production tin rapidly adapting afferent nerves in guinea pig airways // Am J Physiol Lung Cell Mol Physiol, 2002; 282: L775–781.
7. Mullol J., Rieves R. D., Baraniuk J. N., et al. The effects of neuropeptides on mucosus glycoprotein secretion from human nasal mucosa in vitro // Neuropeptides, 1992; 21 4): 231–238.
8. Mizutani N., Nabe T., Ohtani Y., et al. Polycyclic aromatic hydrocarbons aggravate antigen-induced nasal blockage in experimental allergic rhinitis // J Pharmacol Sci, 2007; 105(3): 291–297.
9. Ricco M. M., Myers A. C., Undem B. J. Immunomodulation of afferent neurons in guinea pig isolated airway // J Physiol, 1996; 491: 499–509.
10. Lacroix J. S., Landis B. N. Neurogenic inflammation of the upper airway mucosa // Rhinology, 2008; 46: 163–165.
11. Chan K. H., et al. Chronic rhinosinusitis in young children differs from adults: a histopathology study // J Pediatr, 2004; 144(2): 206–212.
12. Heppt W., Peiser C., Cryer A. Innervation of human nasal mucosa in environmentally triggered hyperreflectoric rhinitis // Journal of Occupational and Environmental Medicine, 2002; 44: 924–929.
13. Fischer A., Wussow A., Cryer A. Neuronal plasticity in persistent perennial allergic rhinitis // Journal of Occupational and Environmental Medicine, 2005; 47(1): 20–25.
14. Jornot L., Lacroix J. S., Rochat T. Neuroendocrine cells of nasal mucosa are a cellular source of brain-derived neurotrophic factor // The European Respiratory Journal, 2008; 32(3): 769–774.
15. Jornot L., Grouzmann E., Lacroix J. S., Rochat T. DNF and DPP-IV in polyps and middle turbinates epithelial cells // Rhinology, 2007; 45: 129–133.
16. Sarin S., Undem B., Sanico A., Togias A. The role of the nervous system in rhinitis // Journal of Allergy and Clinical Immunology, 2006; 118(5): 999–1014.
17. Baraniuk J. Sensory, parasympathetic, and sympathetic neural influences in the nasal mucosa // J Allergy Clin Immunol, 1992; 90: 1045–1050.
18. Hook V., Toneff T., Baylon S., Sei C. Differential activation of enkephalin, galanin, somatostatin, NPY, and VIP neuropeptide production by stimulators of protein kinases A and C in neuroendocrine chromaffin cells // Neuropeptides, 2008; 42: 503–511.
19. Baraniuk J., Castellino S., Lundgren J., Goff J., et al. Neuropeptide Y (NPY) in human nasal mucosa // Am J Respir Cell Mol Biol, 1990; 3: 165–173.
20. Tai C., Baraniuk J. Upper airway neurogenic mechanisms // Curr Opin Allergy Clin Immunol, 2002; 2: 11–19.
21. Boulais N., Misery L. Merkel cells // Journal of the American Academy of Dermatology, 2007; 57(1): 147–165.
22. Lisney S., Bharali L. The axon reflex: an outdated idea or a valid hypothesis? // NIPS Proc, 1989; 4: 45–48.
23. Baraniuk J., Saria A., Goff J. et al. Gastrin-releasing peptide in human nasal mucosa // J Clin Invest, 1990; 85: 998–1005.
24. O'Connor T. M., O'Connell J., O'Brien D. I., et al. The role of substance P in inflammatory disease // J Cell Physiol, 2004; 201: 167–180.
25. Korsgen M., Erjafalt J., Hinterholzl J., et al. Neural expression and increased lavage fluid levels of secretoneurin in sesonal allergic rhinitis // J Respir Crit Care Med, 2003; 167: 1504–1508.
26. Hanf G. Substance P induced histamine release from nasal mucosa of subjects with and without allergic rhinitis // Inflamm Res, 2000; 49(10): 520–523.
27. Konno A. Role of substance P in the vascular response of nasal mucosa in nasal allergy // Ann Otol Rhinol Laryngol, 1996; 105(8): 648–653.
28. Bubnoff D., Geiger E., Bieber T. Antigen-presenting cells in allergy // Journal of Allergy and Clinical Immunology, 2001; 108(3): 329–339.
29. Helle K. B. The endocrine role for chromogranin A: a prohormone for peptides with regulatory properties // Cell Mol Life Sci, 2007; 64(22): 2863–2886.
30. Schuil P. J. Calcitonin gene-related peptide in vitro stimulation of ciliary beat in human upper respiratory cilia // Eur Arch Otorhinolaryngol, 1995; 252(8): 462–464.
31. Undem B. J., Kajekar R., Hunter D. D., et al. Neural integration and allergic disease // Journal of Allergy and Clinical Immunology, 2000; 106(5): 213–220.

Pediatric Acute Myeloid Leukemia in Latvia: BFM-based Treatment Efficacy (1993–2010)

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Abstract

BFM-based treatment for acute myeloid leukemia (AML) has been used in Latvia since 1993, first as AML-BFM 93 protocol elements, later as complete AML-BFM-93 and AML-BFM 98 protocols, since 2005 augmented by hematologic stem cell transplantation (HSCT). More recent BFM-AML 2004 and 2010 protocols have not been implemented because of technical and diagnostic problems. In comparison to pre-BFM period, introducing BFM elements in 1993–2004 produced dramatic increase in long-term survival (5-year OS and EFS from zero to 0.36, p = 0.001 and 0.003, respectively). Employment of complete BFM with HSCT in 2005–2010 did not affect EFS, but further rose 5-year OS to 0.72 (p = 0.061). In comparison to international data, the study revealed several problematic issues, namely: very high early death rate (17% to 7% in the original AML BFM-93, higher incidence of poor response to therapy (13% to 10%), lower EFS (0.36 at 5 years to 0.51 due to more frequent relapses) and shorter post-relapse survival (0.11 at 5 years to 0.4–0.6). Both early death rate and poor response were addressed in 2005–2010, though former not sufficiently; HSCT availability sharply increased post-relapse survival (from zero to 0.35 at 5 years, p = 0.006); relapse rate remained unaffected. Improvements in supportive care, employment of diagnostic options necessary for the correct patient stratification and follow-up as well as implementation of new drugs and targeted therapy should become priorities in order to upgrade paediatric AML treatment to an internationally acceptable level.

Keywords: acute myeloid leukemia, childhood, BFM.

Introduction

Acute myeloid leukemia (AML) is a tumour of immature myeloid cells (blasts) that proliferate in bone marrow with subsequent spillover into peripheral blood and further colonization. AML is a rare malignancy in children, constituting from 5% to 20% of paediatric leukemias worldwide [1, 2] and 19.8% in Latvia [3]. AML morbidity in the USA, Scandinavia and Eastern Europe is about 5/1 000 000 children [1]; the rate in Latvia is slightly higher – 6.9/1 000 000 children in 1988–2009 [3]. Worldwide epidemiology studies showed no gender predisposition and slightly higher incidence in infants and adolescents; children with Down syndrome have a 10–15-fold higher risk of developing AML [4, 5].

Aggressive prolonged chemotherapy, sometimes augmented by hemopoetic stem cell transplantation (HSCT) has been in use for AML patients for at least 30 years. Still, the treatment of childhood AML remains significantly less effective than that of acute lymphoid leukemia or lymphoma [6, 7]. The main factors are high early mortality due to bleeding, infections and toxicity, and higher relapse rate. Though treatment results have been gradually improving worldwide, AML remains a challenge to paediatric oncohematology.

BFM (Berlin-Frankfurt-Münster) protocol originated as far as in 1978 and, with its multiple national and institutional modifications and subvariants, it has become the most used AML treatment approach in Western Europe [8–11]. The main principle of BFM approach is an aggressive Ara-C and Anthracycline-based chemotherapy divided into blocks of remission induction and several consolidations followed by maintenance. There have been 6 generations of AML-BFM in 1978–2004 [10, 11]. AML-BFM 78 introduced intensive chemotherapy with seven drugs, CNS irradiation and 2-year maintenance, raising 5-year OS to 40%; AML-BFM 83 intensified induction and addressed supportive care; AML-BFM 87 demonstrated the benefit of CNS irradiation in preventing CNS/systemic relapses; AML-BFM 93 introduced Idarubicin at first induction followed by intensification with HAM; AML-BFM 98 modified approach to consolidation and standardized stem cell transplantation indications; AML-BFM 2004 employed stratification by genotype and response on day 15, introduced L-DNR during induction and 2-CDA during HR consolidation and improved treatment of patients with relapse or nonresponse. Finally, AML-BFM 2010 is being currently prepared for release. Along with the protocol changes, patient survival gradually increased due to lower relapse rate, better relapse treatment and decrease of early toxic mortality [10, 11].

Before BFM-based protocols were introduced in Latvia, prognosis for children with AML treated symptomatically or by "5 + 2" chemotherapy courses had been extremely poor. Since 1993, with the help of one of the BFM key persons at the time Professor Gunther Schellong, a modified AML-BFM 93 was introduced, including remission induction (ADE block), 6 weeks remission consolidation with low-dose Ara-C blocks, CNS irradiation 18 Gy and maintenance with 6-TG and Ara-C (Table 1). The idea of applying reduced protocol was to evaluate the hospital and the department's resources for aggressive treatment and to acquire experience of aplasia-inducing protocols, implanted catheters, infectious complications, including fungal infections, etc.

Table 1. Outline of the used AML-BFM protocols

<table>
<tr><th>Protocols</th><th>Induction (ADE)</th><th>Consolidation</th><th>Maintenance / CNS irradiation</th></tr>
<tr><td>AML-BFM 93 before 2000</td><td rowspan="2">ADE block:
Ara-C 100 mg/m^2, d 1–8
DNR 30 mg/m^2, d 3–5
VP-16 150 mg/m^2, d 6–8
Ara-C 20–40 mg intrathecal, d 1, 8</td><td>Phase 1:
Prednisolon 40 mg/m^2, d 1–28
6-TG 60 mg/m^2, d 1–28
VCR 1,5 mg/m^2, d 1, 8, 15, 22
ADR 30 mg/m^2, d 1, 8, 15, 22
Ara-C 75 mg/m^2, d 3, 4, 5, 6 / 10, 11, 12,13/17, 18, 19, 20/24, 25, 26, 27
Ara-C 20-40 mg intrathecal, d 1, 15
Phase 2:
6-TG 60 mg/m^2, d 29–43
Ara-C 75 mg/m^2, d 31, 32, 33, 34/38, 39, 40, 41
Cph 500 mg/m^2, d 29, 43
Ara-C 20–40 mg intrathecal, d 29, 43</td><td rowspan="3">For 1 year:
6-TG 40 mg/m^2 every day
Ara-C 40 mg/m^2 every 4 week for 4 days
CNS irradiation 18 Gy
Ara-C 20–40 mg intrathecal 4 × once a week, along with CNS irradiation</td></tr>
<tr><td rowspan="2">AML-BFM 93 / AML-BFM 98 after 2000</td><td rowspan="2">AI block:
Ara-C 500 mg/m^2, d 1–4
IDA 7 mg/m^2, d 3, 5
Ara-C 20–40 mg intrathecal, d 1, 6
HAM block:
Ara-C 2 g/m^2, d 1–3
MTO 10 mg/m^2, d 3, 4
Ara-C 20–40 mg intrathecal, d 1, 6
HAE block (if HSCT not planned):
Ara-C 6 g/m^2, d 1–3
VP-16 125mg/m^2, d 3–5
Ara-C 20–40 mg intrathecal, d 1</td></tr>
<tr><td>HAM block
(for insufficient ADE responders):
Ara-C 6 g/m^2, d 1–3
MTO 10 mg/m^2, d 3, 4
Ara-C 20–40 mg intrathecal, d 1</td></tr>
</table>

Ara-C – Cytarabine, DNR – Daunorubicin, VP-16 – Etoposide phosphate, 6-TG – Thioguanine, VCR – Vincristine, ADR – Adriamycin, Cph – Cyclophosphamide, MTO – Mitoxantrone, IDA – Idarubicine.

Problems with toxicity and with availability of adequate supportive treatment persisted up to 2000 when the application of the complete AML-BFM-93 became possible, including all intensification blocks for high-risk patients with insufficient response. As the next step, AML-BFM-98 and aggressive relapse treatment were introduced in 2002–2004.

Hemopoetic stem cell transplantation (HSCT) has become available for Latvian children with AML since 2005, since 2006 – on regular basis, the main indication being the second remission with a compatible non-related donor available.

Thus, all components of AML-BFM 98 were in use by 2005, ensuring the most up-to-date treatment options for paediatric AML. On the other hand, the subsequent AML-BFM 2004 has not been implemented because of insufficient level of available cytogenetics, molecular genetics and flow cytometry that had been mandatory for correct stratification. The emerging AML-BFM 2010 protocol seems to be even more unattainable due to the same genetics issue together with high-priced experimental medications proposed.

Latvian Association of Paediatric Hematooncology has an observer status at the BFM working group.

The aim

The results of BFM treatment of Latvian paediatric AML patients have never been published. The aim of the study was to analyze the treated patients' data in order to evaluate the efficacy of the adapted to local conditions BFM treatment and to define problematic issues.

Material and methods

Treatment and follow-up data from 61 consecutive paediatric AML patients, who had undergone BFM treatment from July, 1993 till December, 2010, were analyzed; retrospectively. 2 patients had Down syndrome and AML, 1 was excluded because of an unclear GATA-1 status; 1 GATA-1 negative patient was included in the survival analysis. 2 patients suffered from acute promyelocytic leukemia (FAB-M3), they were both included since M3 treatment is BFM-based.

Survival was calculated for 60 patients (44 boys and 16 girls, M: F = 2.75:1; age from 2 months to 17 years).

27 AML patients with available earlier hospital records (before BFM became available, 1988–1993, 16 boys and 11 girls, Down syndrome excluded) who had been treated by symptomatic therapy or non-standardized chemotherapy were used for comparison.

AML diagnosis was confirmed by cytology and cytochemistry (Myeloperoxidase, PAS, α-naphthol-esterases) for all patients; since 2000 bone marrow histology and immunohistochemistry and since 2006 – flow cytometry became available for diagnostics and follow-up.

The statistical analysis was carried out in June, 2011, 6 months after the study end-point. SPSS v.17 statistical analysis software was used for survival analysis, Kaplan-Meyer and log rank (Mantel-Cox) were employed.

Results

All Latvian children with AML have been traditionally treated at a single institution – Children's Clinical University Hospital, Hematooncology Department in Rīga. Treatment compliance in 1993–2010 was good; only 2 patients were lost to follow-up (one of them transferred to adult Hematology 1 month after diagnosis). Survival rates are summarized in Table 2.

Of the 60 BFM-treated patients analyzed, 24 (40%) were alive by the time of analysis (19 in remission and 5 in relapse); 34 (57%) died, 2 (3%) were lost to follow-up 1 and 5 months after the diagnosis.

10 patients (17%) died before treatment commencement or within 7 weeks after diagnosis during intensive treatment, 8 patients (13%) did not respond to treatment and died within a year from disease progression, 15 patients (25%) died in relapse, 1 patient (2%) died in remission. 71% deaths occurred within the first year after the diagnosis, 91% – within 2 years.

Comparison of 1993–2004 and 2005–2010 periods demonstrated major decrease of early death rate (from 18.2% to 12.5%). There has not been a single case of death due to refractory disease in 2005–2010 (22.7% in 1993–2004) (Figure 1).

Boys and children older than 12 years had better survival, though the difference was not statistically different (Table 2).

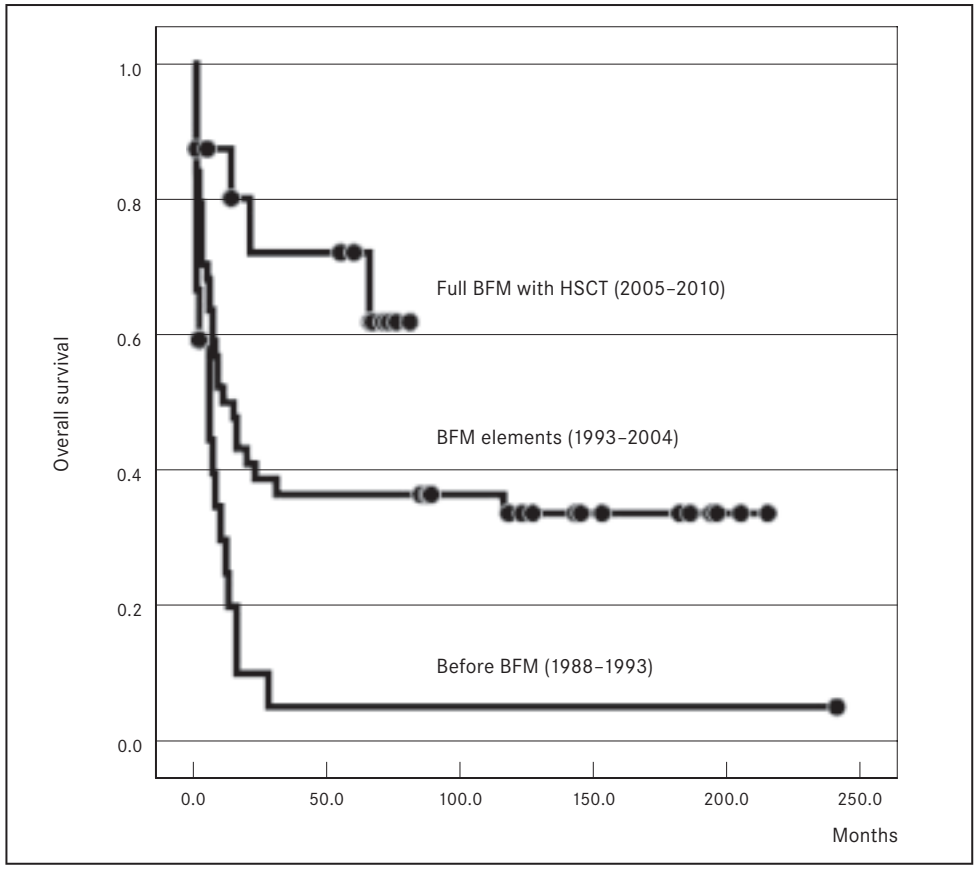
Of the 3 documented patients with Down syndrome and AML, 2 died early and one (proven to be GATA1-negative) achieved a stable remission. One patient with AML-M3 died before treatment was commenced, the other one remains in remission.

Table 2. Survival of pediatric AML patients

Protocols		OS rate				EFS rate			
	N	1 yr	5 yrs	10 yrs	p (log rank)	1 yr	5 yrs	10 yrs	p (log rank)
Before BFM	27	0.25	0.05	0.05	< 0.001	0.25	0.05	0.05	< 0.001
Total BFM (07.1993–2010)	60	0.60	0.45	0.40		0.52	0.37	0.34	
Boys	49	0.66	0.55	0.44	NS	0.60	0.43	0.38	NS
Girls	38	0.52	0.33	0.33		0.44	0.30	0.30	
Age 0–11	40	0.49	0.42	0.33	NS	0.44	0.31	0.27	NS
Age 12–17	20	0.80	0.51	0.51		0.68	0.47	0.47	

OS – overall survival, EFS – event-free survival, NS – non-significant.

Figure 1. OS during BFM elements implementation (1993–2005) and using complete BFM program (2005–2010); in comparison to pre-BFM treatment



19 patients relapsed in a proven remission (31.6% of total or 45.3% of responders). 53% relapses (10 patients) occurred within a year from diagnosis, 9 patients died, one is alive in second remission and is currently being prepared for HSCT. Early relapse rate was similar in 1993–2004 and 2005–2010 (16% and 19%, correspondingly). 47% (9 patients) relapsed more than a year after the diagnosis, 6 of them died (one after HSCT and second relapse), 3 were alive at the time of analysis. All 3 relapse survivors have had HSCT; not a single relapsed patient survived in 1993–2004, before HSCT became available.

The most common (89%) relapse site was bone marrow (17 patients, one of them combined bone marrow and CNS). One patient had an extramedullary relapse (lymph nodes) and one relapsed 3 times in different sites (testicular followed by CNS and bone marrow).

Discussion

OS and EFS before BFM were near zero; BFM produced highly significant survival improvement ($p < 0.001$; Table 2). Still, the survival was visibly lower in comparison to the original AML-BFM 93 data (5-year OS 0.60 and 5-year EFS 0.51 [10], Latvian data being only 0.36 for both). Difference in early deaths, refractory disease and relapses caused the disparity with the international results.

Early death rate in Latvian patients was evidently higher than the original AML-BFM 93 figure of 7.3% and AML-BFM 98 of 3.2% [10, 11], and, even having visibly decreased in 2005–2010, still remained at 12.5% level. Three principal causes could be responsible: poor clinical condition at presentation due to delayed diagnosis, excessive toxicity because of an overly aggressive regimen selected and supportive therapy issues during the induction phase. Special concern has been given at the Hematooncology Department to the enhancement of nursing care and supportive treatment at highly toxic periods, the mentioned decrease of early death rate clearly mirrors these efforts, still a considerable potential for further improvement remains.

The overall non-responders' rate of 13% was slightly higher than 10% of the original AML-BFM 93, the most probable reason being an incomplete protocol, particularly for high-risk patients and poor responders in 1993–2004. The issue was successfully addressed in 2005–2010 by implementing the second induction (Table 1).

There were two main issues of the relapse-related mortality: higher relapse rate and poorer post-relapse survival. Relapse incidence in primary responders for the whole 1993–2010 period was 45.2% as compared to 27.8% in BFM 93, the rate did not improve with time (56.3% in 2005–2010).

Extremely short survival of relapsed patients improve neither with introduction of BFM elements, nor with applying intensive chemotherapy in 2000–2004 (Table 3, Figure 2). A breakthrough has been achieved only by combining remission-aimed therapy followed by HSCT; 5-year survival increased from zero in 1988–1993 and 1993–2004 to 0.35 in 2005–2010, the difference being statistically highly significant (Table 4) and well corresponds with the international data [12]. Consistently better survival of relapsed patients could be the main factor that increased long-term OS at the time to 0.72.

A slightly better prognosis for boys was unexpected, since international data indicate no gender-related survival discrepancy. Better survival of adolescents is a well-recognized fact [1, 2].

Thus, retrospectively, even limited BFM in 1993–2004 by itself caused 7-fold increase in long-term survival ($p = 0.001$ for OS and $p = 0.003$ for EFS; Table 3, Figure 3). Introduction of all BFM components, switch to AML-BFM 98 and HSCT availability have minimally affected EFS; effect on OS was substantial (though, statistically non-significant ($p = 0.061$): 5-year OS doubled and reached 0.72, which is even better than AML-BFM 98 results [10], but is still behind latest treatment regimen [6]. Relapsed patients benefited most from the improved treatment.

Paediatric AML treatment in Latvia still faces considerable problems, highlighted by high rate of early deaths and relapses. Supportive care improvement depends on personnel quantity and training, enhanced infection control, interdisciplinary cooperation and financial means. Technical support, like cytapheresis independent of patient's weight, and diagnostic means like the already mentioned cytogenetic and molecular genetic testing, is still lacking. Finally, alternative treatment strategies for distinct patient groups are currently being implemented worldwide [6] but remain unavailable for children in Latvia.

Table 3. Overall survival rate of relapsed patients

Protocols	N	1 yr	5 yrs	10 yrs	p (log rank)
Before BFM 07.1993–2010	11 19	0 0.36	0 0.11	0 –	0.006
Before BFM 07.1993–2005 2005–2010	11 11 8	0 0.17 0.70	0 0 0.35	0 0 –	NS 0.003

Figure 2. Survival of relapsed patients – impact of HSCT

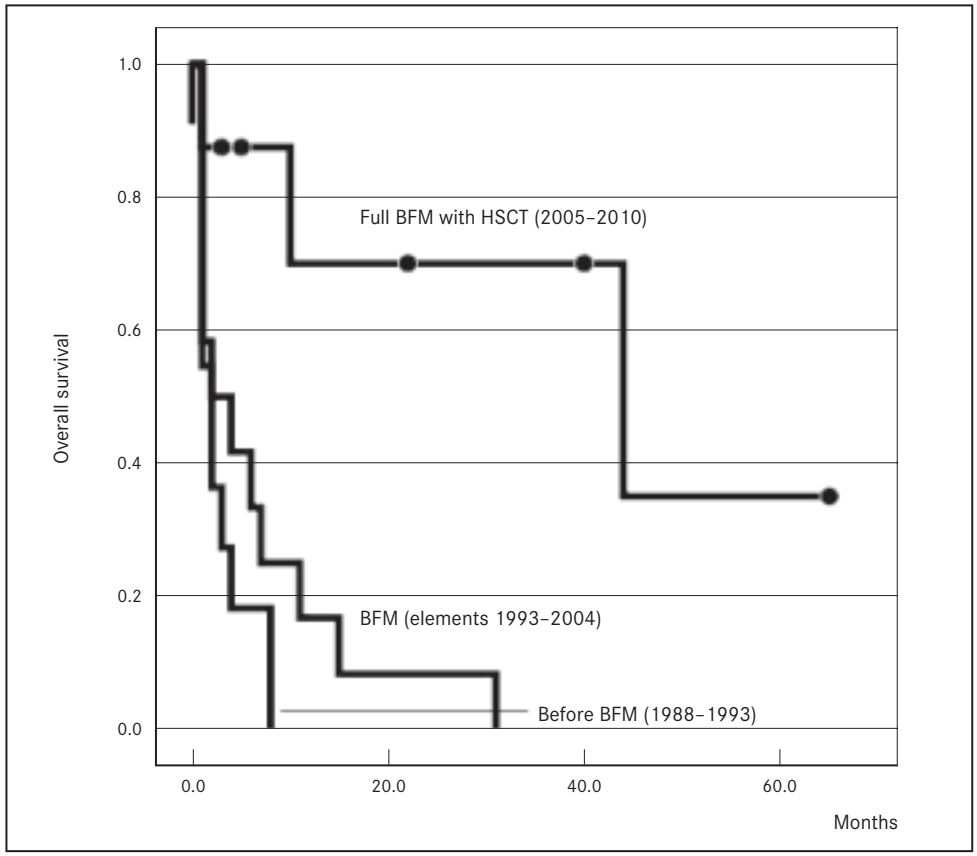
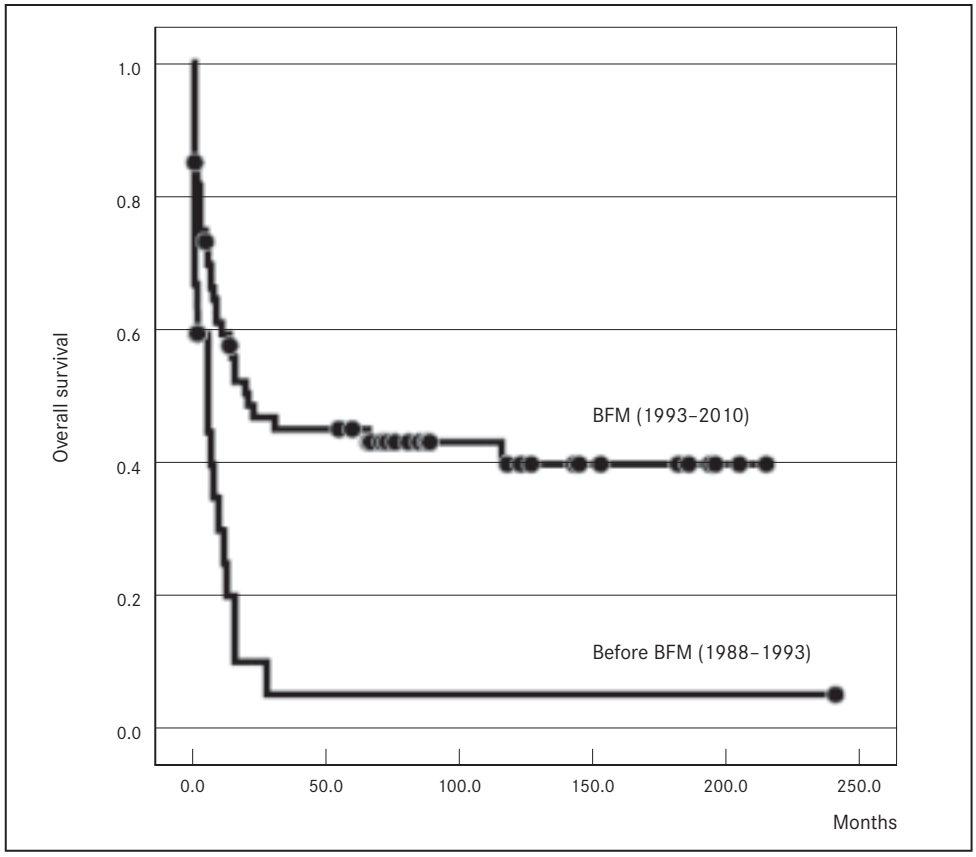


Table 4. Impact of introducing BFM elements (1993–2004) and full BFM implementation (2005–2010) on survival (from Creutzig et al., 2006 [10])

Protocols		OS rate				EFS rate			
	N	1 yr	5 yrs	10 yrs	p (log rank)	1 yr	5 yrs	10 yrs	p (log rank)
Before BFM	27	0.25	0.05	0.05	0.005	0.25	0.05	0.05	0.001
07.1993–2004 (BFM 93 data)*	44	0.50	0.36 **(0.58)**	0.34	NS (0.061)	0.46	0.36 **(0.51)**	0.34	NS
2005-2010 (BFM 98 data)*	16	0.88	0.72 **(0.62)**	–		0.73	0.37 **(0.50)**	–	

OS – overall survival, EFS – event-free survival, NS – non-significant.

Figure 3. BFM treatment impact on overall survival



Conclusions

BFM-based treatment introduction for children in Latvia with AML resulted in a dramatic increase of long-term survival. Even incomplete BFM protocols increased 10-year OS from 0.00 to 0.34, providing patients with a fair recovery chance in spite of high toxicity and high early death incidence.

Implementation of the complete BMT program with HSCT for relapses according to AML-BFM 98 protocol decreased early mortality and therapy resistance as well as significantly improved post-relapse survival, resulting in further doubling 5-year survival to 0.72.

Several critical problematic issues have been defined: decreasing but still unacceptably high early death rate; high relapse rate; technical and financial impossibility to keep in line with modern treatment programs.

Treatment protocol, originating in late 1990s, is still being used. Improvements in supportive care, employment of diagnostic options necessary for the correct patient stratification and follow-up as well as implementation of new currently available therapies should ensure modernisation of the treatment program.

Further follow-up to evaluate late effects, particularly in transplanted patients, is indicated.

References

1. Bhatia S., Neglia J. P. Epidemiology of childhood acute myelogenous leukemia // J Pediatr Hematol Oncol, 1995; 17: 94–100.
2. Linet M. S., Wacholder S., Zahm S. H. Interpreting epidemiologic research: lessons from studies of childhood cancer // Pediatrics, 2003; 112(1): 218–232.
3. Kovalova Z., Sergejeva J., Vishnevska-Preciniece Z., et al. Uneven demographic and geographic distribution of childhood leukemias in Latvia // RSU Research articles in medicine & pharmacy, 2010; 19–28.
4. Zipursky A., Brown E., Christensen H., et al. Leukemia and/or myeloproliferative syndrome in neonates with Down syndrome // Semin Perinatol, 1997; 21: 97–101.
5. Mundschau G., Gurbuxani S., Gamis A. S., et al. Mutagenesis of GATA1 is an initiating event in Down syndrome leukemogenesis // Blood, 2003; 30: 30.
6. Abrahamsson J., Forestier E., Heldrup J., et al. Response-guided induction therapy in pediatric acute myeloid leukemia with excellent remission rate // J Clin Oncol, 2010; 29: 310–315.
7. Kaspers G. J. L., Zwaan C. M. Pediatric acute myeloid leukemia: towards high-quality cure of all patients // Haematologica, 2007; 92: 1519–1532.
8. Creutzig U., Berthold F., Boos J., et al. Improved treatment results in children with AML: results of study AML-BFM 93 // Klin Padiatr, 2001; 213: 175–185.
9. Creutzig U., Zimmermann M., Reinhardt D., et al. Early deaths and treatment-related mortality in children undergoing therapy for acute myeloid leukemia: analysis of the multicenter clinical trials AML-BFM 93 and AML-BFM 98 // J Clin Oncol, 2004; 22: 4384–4393.
10. Creutzig U., Zimmermann M., Lehrnbecher T., et al. Less toxicity by optimizing chemotherapy, but not by addition of granulocyte colony-stimulating factor in children and adolescents with acute myeloid leukemia: results of AML-BFM 98 // J Clin Oncol, 2006; 24: 4499–4506.
11. Sander A., Zimmermann M., Dworzak M., et al. Consequent and intensified relapse therapy improved survival in pediatric AML: results of relapse treatment in 379 patients of three consecutive AML-BFM trials // Leukemia, 2010; 24: 1422–1428.
12. Tallman M. S., Dewald G. W., Gandham S., et al. Impact of cytogenetics on outcome of matched unrelated donor hematopoietic stem cell transplantation for acute myeloid leukemia in first or second complete remission // Blood, 2007; 110(1): 409–417.

Hemopoetic Stem Cell Transplantation for Pediatric Hematological Diseases in Latvia: Preliminary Results

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Abstract

Allogeneic hemopoetic stem cell transplantation (HSCT) is being used as a life-saving option for many hematological diseases. HSCT has been regularly performed abroad for Latvian children since 2005, using the form E112 procedure; the results are presented. 17 patients were selected for the HSCT in 2005–2010, 16 were actually transplanted: 5 ALL, 4 AML, 2 MDS, 2 SAA, 1 CML, 1 NHL and 1 WAS. 9 patients were alive by the end of 2010, 8 in CR, 6 patients relapsed. 3 patients died in early post-transplant period and 4 after relapse. 2 patients developed severe chronic GvHD. OS at 36 months for the whole group was 0.54 and EFS 0.42, demonstrating a benefit for the cohort. Overall, results were worse for ALL patients, though the group is too small for precise conclusions. Further monitoring for relapse and late complications will be necessary.

Keywords: hemopoetic stem cell transplantation, leukemia, childhood.

Introduction

Allogeneic hemopoetic stem cell transplantation (HSCT) is a major treatment option for both clonal and non-clonal hematological diseases that has dramatically changed the outcome of several previously fatal disorders. There are two main approaches to patient selection for HSCT: first, diseases that are otherwise incurable and for whom HSCT, according to previous experience, offers a fighting chance. And second, when other therapeutic options are exhausted and HSCT could still be helpful.

HSCT has been regularly performed for Latvian children since 2005. Latvia does not possess its own facilities for pediatric HSCT. The program was commenced after joining the European Union and drawing on the form E112 procedure for donor search and the procedure itself.

The aim

The aim of the study was to perform a preliminary evaluation of the results of pediatric HSCT performed in foreign institutions in 2005–2010 when the program was operational, in order to make a tentative estimation of its benefit to the patients' cohort, its efficacy and rationality.

Material and methods

17 patients had been approved for HSCT since 2005; there were 10 boys and 7 girls, patients' age ranged from 1 to 12 years.

16 patients were transplanted: 5 patients with acute lymphoblastic leukemia (ALL); 4 with acute myeloid leukemia (AML); 2 with myelodysplastic syndrome – MDS (1 refractory cytopenia with multilineage dysplasia – MDS-RCMD, 1 refractory anemia with excess of blasts type I – MDS-RAEB I); 2 severe aplastic anemia (SAA, one of them had developed secondary MDS), 1 chronic myeloid leukemia, BCR/ABL + (CML); 1 B-cell non-Hodgkin lymphoma (B-NHL) and 1 Wiscott-Aldrich syndrome (WAS) (Table 1). 1 patient (N13) with high-risk ALL died before transplantation.

HSCT was planned for 5 patients from the start of treatment (2 MDS, 2 high-risk T-cell ALL and 1 WAS) as the only life-saving option. 12 remaining patients were either refractory to therapy (2 cases of AML and 1 CML) or relapsed.

First, 3 transplantations were performed in Giessen University Clinic, Freiburg University Clinic and Karolinska University Clinic. Later partnership with St. László Hospital in Budapest was established (6 transplantations in 2006–2007) followed by cooperation with Charité Hospital in Berlin (7 transplantations in 2007–2009 and ongoing).

Clinical information on pre-transplant history and post-transplant follow-up was obtained from the archives of Departments of Pediatrics (WAS) and Pediatric Hematooncology (neoplasms and SAA) of Children's Clinical University Hospital. Details of HSCT procedure and the initial post-transplant period were provided by the transplanting institution.

SPSS v.17.0 statistical software was used for Kaplan-Meyer survival analysis.

Results

Procedure. There were only 2 HSCT from siblings, in 14 cases an unrelated donor was found by a search in international databases (Table 1). 14 donors (including both related HSCT's) were completely matched; one mismatched (9/10, patient No 16), no data on donor match could be obtained for one unrelated HSCT (patient No 4).

Donor bone marrow cells and peripheral blood stem cells (PSC) were used as the transplant source with equal frequency.

Outcome. 9 out of 16 transplanted patients were alive by the end of 2010, 8 of them currently in complete remission (CR). 6 patients relapsed, 4 of them died within 3 months, one is alive at 10 months (No 12); one patient (No 3) had a molecular relapse 8 months after HSCT; donor lymphocyte infusion (DLI) was performed and second CR achieved. The patient was the only one who had donor cell reinfusion, no patients had been re-transplanted.

3 patients died in early post-transplant period from the procedure-induced toxicity, 4 patients died soon after relapse (Table 1).

Overall survival (OS) at 12 months for the whole group was 0.69 and event-free survival (EFS) 0.63, both curves stabilized at 27 and 26 months at 0.54 and 0.42, correspondingly.

Post-transplant period. Late post-transplantation period for most patients was uncomplicated, with the standard immunosuppressive therapy (IST) required. 1 patient (No 3) had IST prematurely terminated due to emergence of incomplete chimerism.

IST was administered in Latvia, controls at host institutions were performed when necessary.

Post-transplant complications included generalized Varicella-Zoster infection in 2 patients.

One patient (No 1) had an extensive pelvic adhesive disease and amenorrhea as a complication of TBI at conditioning.

1 patient developed psychosomatic deviations after HSCT.

Graft-versus-host disease (GvHD) was a grave problem for 2 patients. Patient No 2 developed severe bowel lesion that had to be controlled for 4 years by the second-line immune suppression. Fortunately, the problem gradually resolved. Patient No 4 had severe skin scleroderma-like damage extending to joints and tendons that was poorly controlled by second line IST; PUVA was applied (the effect is still to be seen).

Table 1. Paediatric HSCT in 2005–2009: overview of patients and outcomes

No	Diagnosis	Age Dg/HSCT	Gender	HSCT year, institution	Donor, source	Events	Late complications	IST
1	B-NHL, relapse, II CR	5 yrs / 6 yrs	F	2005, Justus-Liebig-University, Gießen, Germany	MRD (sister), BM	No at 72 months	Secondary amenorrhea, pelvic adhesive disease after TBI	Standard
2	MDS-RCMD	7 yrs / 7 yrs	F	2005, Universitats klinikum Freiburg, Germany	MUD, PSC	No at 72 months	Severe GvHD (bowels), delayed growth, osteoporosis	4 years, II line IST
3	AML, poor response	6 yrs / 8 yrs	M	2006, Karolinska University Clinic, Sweden	MUD, PSC	Molecular relapse at 8 months, DLI, alive at 62 months, currently CR	–	Standard, interrupted at 8 m due to increased donor chimerism
4	ALL II relapse, III CR	2 yrs / 4 yrs	F	2006, St. László Hospital, Budapest, Hungary	Unrelated donor, no match data, PSC	No at 55 months	Severe GvHD (skin, joints), growth delay	4 years, II line IST ineffective, PUVA in process
5	T-ALL, high-risk, I CR	12 yrs / 12 yrs	M	2007, St. László Hospital, Budapest, Hungary	MUD, PSC	Death within 1 month (toxic death)	–	–
6	MDS-RAEB I	4 yrs / 5 yrs	F	2007, St. László Hospital, Budapest, Hungary	MUD, PSC	Death within 1 month (toxic death)	–	–
7	CML accelerated phase, post Imatinib	1 yr / 8 yrs	M	2007, St. László Hospital, Budapest, Hungary	MRD (brother), PSC	No at 43 months	–	Standard
8	WAS	7 months / 1 yr	M	2007, St. László Hospital, Budapest, Hungary	MUD, BM	No at 44 months	–	Standard
9	ALL relapse, II CR	6 yrs / 8 yrs	M	2007, St. László Hospital, Budapest, Hungary	MUD, PSC	Relapse at 26 months, death at 27 months (disease progression)	Generalised VZ infection	Standard

10	AML, relapse, II CR	9 yrs / 11 yrs	F	2008, Charité Klinikum, Berlin, Germany	MUD, BM	No at 34 months	–	Standard
11	ALL, relapse, II CR	3 yrs / 8 yrs	M	2008, Charité Klinikum, Berlin, Germany	MUD, BM	Relapse at 16 months, death at 21 month	Generalised VZ infection psychosomatic deviations	Standard
12	ALL, relapse, II CR	1 yr / 6 yrs	M	2008, Charité Klinikum, Berlin, Germany	MUD, BM	Relapse at 15 months, alive at 25 months	–	Standard
13	T-ALL, high risk, poor response	3 yrs	M	Planned 02.2009, Charité Klinikum, Berlin, Germany	MUD (planned)	Died 01.2009, before HSCT (disease progression)	–	–
14	AML, relapse, II CR	8 yrs / 9 yrs	M	2009, Charité Klinikum, Berlin, Germany	MUD, BM	Relapse at 4 months, death at 6 months (disease progression)	–	Standard
15	AML, poor response	5 yrs / 5 yrs	F	2009, Charité Klinikum, Berlin, Germany	MUD, BM	Relapse at 6 months, death at 7 months	–	Standard
16	SAA, secondary MDS	9 monts / 11 months	M	2010, Charité Klinikum, Berlin, Germany	MMUD, BM	Death at 3 months, CNS bleeding	–	–
17	SAA	6 months / 8 months	F	2010, Charité Klinikum, Berlin, Germany	MUD, PCS	No at 10 months	–	Standard

Abbreviations: ALL – acute lymphoblastic leukemia; AML – acute myeloid leukemia; MDS-RCMD – myelodysplastic syndrome – refractory cytopenia with multilineage dysplasia, MDS-RAEB I – myelodysplastic syndrome – refractory anemia with excess of blasts type I, CML – chronic myeloid leukemia; B-NHL – B-cell non-Hodgkin lymphoma, WAS – Wiscott-Aldrich syndrome, SAA – severe aplastic anemia, CR – complete remission, MRD – matched related donor, MUD – matched unrelated donor, MMUD – mismatched unrelated donor, IST – immunosuppressive therapy BM – bone marrow, PSC – peripheral stem cells, TBI – total body irradiation, GvHD – graft-versus-host disease, VZ – Varicella Zoster, PUVA – Psoralen + long-wave ultraviolet radiation treatment.

Discussion

The transplanted patients suffered either from otherwise incurable diseases (WAS, MDS, imatinib-refractory CML, refractory or relapsed AML, immune suppression-resistant SAA) or from diseases where their chances with chemotherapy treatment were unpromising (below 30% in relapsed ALL [3, 5]). Survival analysis showed 54% OS at 3 years, with 40% patients remaining disease-free; that should be considered an evident benefit.

The selection of patients was carried out according to international standards [1–3]; 13 transplanted patients had indications that are currently recognized worldwide as life-saving: ALL in 2nd remission [4, 5], lymphoma in 2nd remission [6], CML, MDS, refractory or relapsed AML, [1–3, 7], SAA after 2 unsuccessful courses of immune suppression [1–3] inherited immunodeficiency [1–3]. Moreover, for these conditions availability of a donor is considered to be a major prognosis-influencing factor [8, 9]. It has been shown that very high risk ALL patients do benefit from HSCT [3, 9], so selection of patients No 5 and No 13 was reasonable in spite of the outcome.

Small number of available sibling donors should be mentioned as a notable limiting factor due to financial and logistic issues (donor search), to a less clear benefit in comparison to chemotherapy for several patient groups (the earlier mentioned very high risk ALL is a well-known example [5]) and particularly due to a high transplant-associated lethality [5]. Nevertheless, it is evident that because of the Latvian demographic situation the program in the future will be increasingly aimed at unrelated HSCTs, with corresponding indications.

The pre-transplant logistics, including the form E112 acquirement, donor search and making arrangements with the host institution, could be evaluated as sufficiently fast. One patient (No 13) selected for HSCT did not survive until procedure, but it should be noted that in that case the date for HSCT had been fixed at 6 months from the initial diagnosis, that is faster than European average of 9 months [2].

Overall outcome after HSCT did not significantly differ from the European data. Myeloid leukemias (AML and CML) did rather well with 3 out of 5 patients remaining disease-free. ALL patients did much worse: one patient died before HSCT, 1 died in early post-transplant period, 3 patients relapsed; the remaining patient, though disease-free, suffers from severe chronic GvHD. It contrasts rather unflatteringly with the accepted post-transplant EFS rate of 40–60% [1, 3, 8], that is significantly higher than for chemotherapy [5]. There is no clear explanation besides a too small number of cases and a possible coincidence, but the fact is disturbing and should be further looked into.

Patients with other diagnoses are too few for separate analysis.

Mortality rate in the early post-transplant period was as expected (0/2 for related donor HSCT, 3/14 for unrelated HSCT) for international shows that a third or more of patients are prone to regimen-related lethality after unrelated HSCT [5].

Further therapeutic options should be carefully considered for the relapsed patients. If a complete remission is achieved by chemotherapy, re-transplantation remains a possibility, though it is performed on more or less experimental basis [9, 10].

A comparison between bone marrow and blood stem cells as cell sources HSCT would not be quite correct [1, 2, 11], for procedures had been performed in different institutions and for different diseases. Still, the existing data show no dramatic difference between the groups, so there seems to be no reason to select a host institution by its preferred transplant type.

Frequency of post-transplant complications was not excessive. It should be noted, though, that only medical complications were reported, while the extensive study of Bieri, et al. [12] revealed in patients after HSCT, besides poorer physical well-being, a significant deterioration of social and financial status. The issue should be addressed.

The usual late HSCT effects (ocular effects including cataract and secondary malignancies) were not observed in the study, most probably due to the short follow-up period. Longer evaluation will be necessary to assess them as well as to have additional time for survival analysis. It is reasonable that HSCT survivors should be carefully monitored in the future to ensure a timely medical and social assistance, when needed.

The 2005–2010 experience shows that there are sufficient local facilities for post-transplant follow-up and therapy, including drugs for the second line immune suppression.

Current indications for pediatric HSCT in Latvia do not comprise other traditionally transplantable diseases like Fanconi anemia and some ALL patients with a documented benefit from transplantation. But these disorders are rare, so it could be concluded that the requirements for pediatric BMT are more or less covered by the current program.

Conclusions

There were indications for HSCT for 17 pediatric patients during the 2005–2010 period, returning the average number of about 3 patients per year. A related donor was available in two cases only.

Preliminary results of the pediatric HSCT program are tentatively encouraging, with more than a half patients alive at 3 years. Still, longer follow-up and larger patients' groups will be necessary for accurate evaluation of survival and patients' benefit from the treatment.

ALL patients did worse than other groups and considerably worse in relation to international data.

The time between diagnosis and performed HSCT was sufficiently short to ensure timely transplantation.

A reliable post-transplant follow-up for the transplanted children was ensured.

Though occasional additional Latvian pediatric patients (like some inherited syndromes and severe autoimmune disorders) could benefit from HSCT, it could be concluded that the requirement for the procedure is mostly satisfied.

References

1. Sudbury, Mass., Jones and Bartlett Pediatric Stem Cell Transplantation. / Edited by Paulette Meh., illustrated, 2004, P. 486.
2. Copelan E. A. Hematopoietic stem-cell transplantation // N Engl J Med, 2006; 354(17): 1813–1826.
3. Lanzkowsky P. Manual of pediatric hematology and oncology. – 4th ed. – Academic Press, 2005. – Pp. 126–141.
4. Satwani P., Sather H., Ozkaynak F., et al. Allogeneic bone marrow transplantation in first remission for children with ultra-high-risk features of acute lymphoblastic leukemia: a children's oncology group study report // Biol Blood Marrow Transplant, 2007; 13(2): 218–227.
5. Krance R. Transplantation for children with acute lymphoblastic leukemia // Bone Marrow Transplantation, 2008; 42: S25–S27.
6. Bradley M. B., Cairo M. S. Stem cell transplantation for pediatric lymphoma: past, present and future // Bone Marrow Transplantation, 2008; 41: 149–158.
7. Shenoy S., Smith F. Hematopoietic stem cell transplantation for childhood malignancies of myeloid origin. // Bone Marrow Transplantation, 2008; 41: 141–148.
8. Nguyen K., Devidas M., Cheng S. C., et al. Children's Oncology Group. Factors influencing survival after relapse from acute lymphoblastic leukemia: a children's oncology group study // Leukemia, 2008; 22(12): 2142–2150.
9. Balduzzi A., Valsecchi M. G., Uderzo C., et al: Chemotherapy versus allogeneic transplantation in childhood very high risk acute lymphoblastic leukemia in first complete remission: a comparison by genetic randomization in an international prospective study // Lancet, 2005; 366: 635–642.
10. Mehta P., Davies S. Allogeneic transplantation for childhood ALL. // Bone Marrow Transplantation, 2008; 41: 133–139.
11. Wall D. A. Allogeneic peripheral blood versus bone marrow transplantation: children are small adults and more // Journal of Clinical Oncology, 2004; 24(22): 4865–4866.
12. Bieri S., Roosnek E., Helg C., et al. Quality of life and social integration after allogeneic hematopoietic SCT // Bone Marrow Transplantation, 2008; 42: 819–827.

Accuracy of Self-estimated Body Weight-height Relationship in Students

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Abstract

Inadequate body weight – too low or too high – for the height is associated with increased risk of metabolic, cardiovascular, joint diseases, and increases risk of various diseases. One of primary measures is the visual self-evaluation. Yet it does not always correspond to the objective reality and thus can stimulate inadequate corrective activity or inactivity.

The aim of this study is to evaluate the adequacy of self-estimation of body weight-height relationship to objectively measured parameters of body size and composition.

The current investigation involved 512 (103 male and 409 female) students of Rīga Stradiņš University in 19–49 age range. Students selected appropriate body silhouette from the modified Stunkard scale. Afterwards height and weight were measured and body mass index (BMI) was calculated. Body fat percent with Tanita MC-180 bioimpedance analyser was determined. The fluid and food intake, as well as physical activity before the test was reglamented.

Male students evaluated their body more precisely than females: 54% of males and only 34% of females estimated their BMI correctly, 31% of males and 61% of females overestimated it, but the weight of 15% and 5% respectively was underestimated. The ability to estimate weight-height relationship was related to the person's actual BMI – persons with the higher BMI more underestimated their weight-height ratio while persons with the lower BMI overestimated it. This tendency was observed also in case when students were divided due to their fat percent.

Precision of self-estimation of body weight-height relationships is low, especially in women. Women more overestimated their weight-height ratio while men had more tendency to underestimate it. Persons with the higher BMI and fat percent more underestimated their BMI but persons with lower BMI overestimated it.

Keywords: self-estimation, BMI, fat percent.

Introduction

Increased body weight and obesity is quite widespread and in some countries has reached even epidemic rates. Generally, the incidence of adiposity has increased in all age groups, but especially high increase is observed in young adults. The adiposity in this age increases diabetes melitus, hypertension, vascular disease, degenarative joint disease and other pathology risks in further life [1]. Nowadays the composition and availability of food is changed and physical activity level is markedly decreased. In such conditions the natural body weight regulation mechanisms sometimes cannot provide effective body weight control which increases the importance of conscious activities to maintain normal body

size [2]. The correct self-evaluation of body size is of high importance when life-style, eating habits or physical activity interventions are introduced. More precise self-evaluation would help in selecting propper goal directed diet or physical activity. Besides that, especially in teenagers and young adults, self-perception of body size can affect person's self-confidence and psychological wellbeing. Low self-esteem, depression and eating disturbances are more frequently observed in people who are not satisfied with their body image [3].

The ability to evaluate person's body weight-height relationship correctly would be a fast and inexpensive tool that could signal if or what intervention is needed to maintain normal body weight.

This study investigates how precisely people determine their body size from silhouette pictures and whether the ability to determine their body weight-height relationships depend on their actual body mass index (BMI) and fat percentage.

The aim

The aim of this study is to evaluate the adequacy of self-estimation of body weight-height relationship to objectively measure parameters of body size and composition.

Material and methods

All the students, who are residents of Latvia and who attended physiology course at Rīga Stradiņš University in the time period 2009–2011, were invited to participate in the investigation. The study involved 512 (103 male and 409 female) students in 19–49 age range without contrindications to bioimpedance measurements. Persons were involved voluntarily and the investigation was conducted according to the requirements of the Ethical Committee of Rīga Stradiņš University.

At the start of the research, all the students were asked to identify their body image with one of the body silhouettes from self-administrated modified Stunkard scale [4, 5]. Every silhouette in this scale is drawn for the person with definite BMI, numbers of which were not shown to the participants.

After students had evaluated their body image, the body height with standard wall height measuring device with the precision to 0.1 cm was measured. Body weight with the precision to 0.05 kg and fat percentage with the precision to 0.1% were measured with a multi-frequency body composition analyzer MC-180MA (Tanita, Japan). To obtain more precise results, fluid and food intake and physical activity level in participants before the test were limited. Students with metal implants and pregnant female students were excluded from the investigation. Body mass index was calculated and evaluated according to the International Classification of adult underweight, overweight and obesity according to BMI [1]. According to the BMI, all the participants were divided into four groups – underweight, normal weight, overweight and obese. Body fat percentage was evaluated according to the predicted values [6], and all the students were divided into four groups – underfat, healthy, overfat and obese.

The obtained data were analysed using SPSS software version 17.0 (SPSS Inc., Chicago). Alpha level $p < 0.05$ was considered as statistically significant. Differences of variables between both genders were evaluated using independent t-test.

Results

There were more female students in the study population. Males among the study population had higher BMI and lower fat percentage than females (Table 1). Nevertheless, the BMI showed normal weight on average for both genders.

The participants of the research were asked to evaluate their body image according to the modified Stunkard scale. More than half of the male participants estimated their body image appropriately to the BMI that was calculated from the objective measurements of body weight and height. However, 31% overestimated and 15% underestimated it (Figure 1).

Table 1. Characterisation of study population according to the body mass index (BMI) and fat percentage

Parameter	Mean ± SD		Significance of gender difference
	Males (n = 106)	Females (n = 413)	
BMI, kg/m^2	23.0 ± 2.9	21.7 ± 3.4	$p < 0.01$
Fat percentage	15.0 ± 5.8	24.8 ± 5.6	$p < 0.01$

Figure 1. Division of males according to the self-evaluation of body mass index

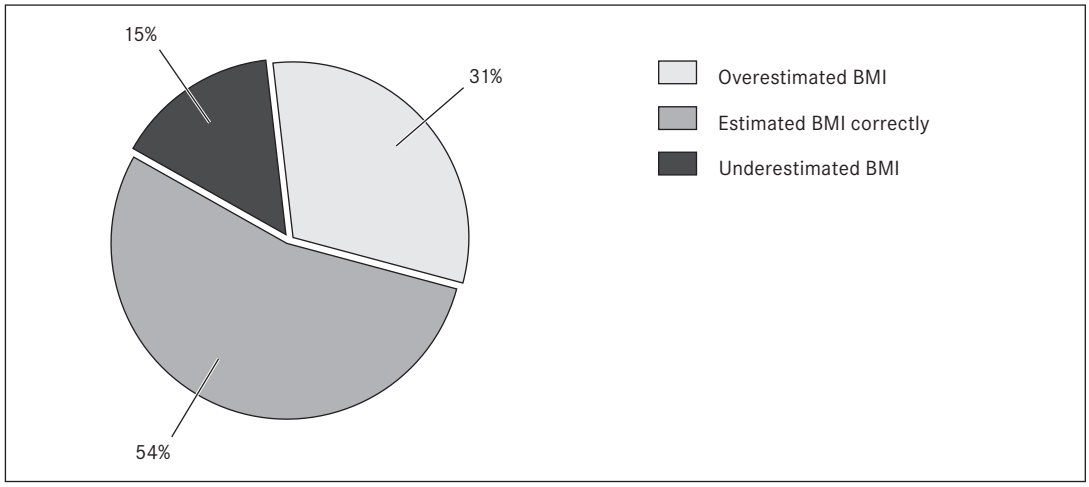
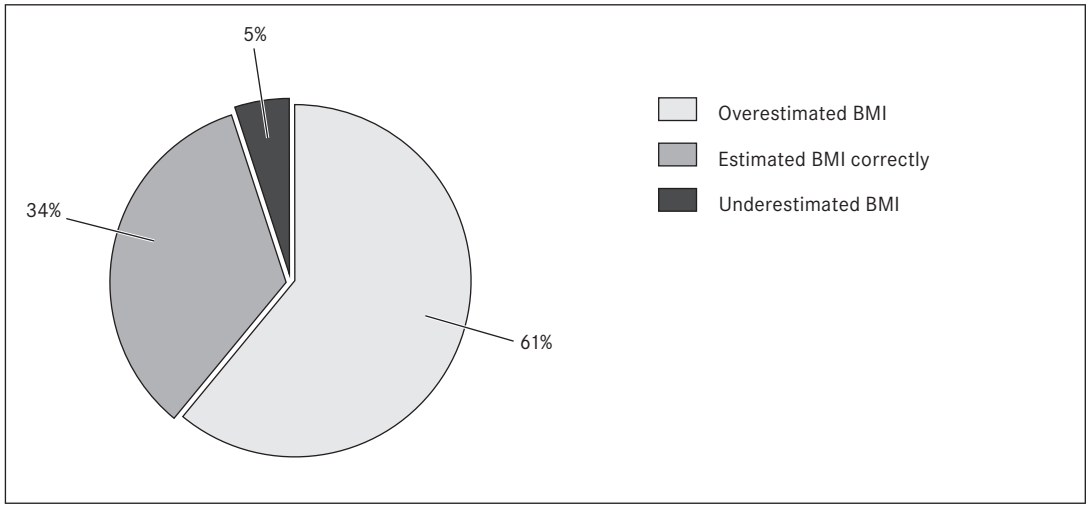


Figure 2. Division of females according to the self-evaluation of body mass index



Females were less precise in the evaluation of their body image. More than half of them overestimated their BMI and only 34% of women evaluated their BMI correctly. Only a small portion – 5% of females underestimated their BMI (Figure 2).

All the participants were divided into groups according to the objectively determined BMI, and the ability to estimate their BMI from images was analysed.

For males the ability to determine BMI correctly decreased with the increase of BMI (Figure 3). All obese and 24% of overweight students underestimated their BMI, while in normal weight group only 12% of students perceived themselves under real weight. The best ability to determine their weight correctly was shown by underweight students from whom 67% were precise, the rest of these students

perceived themselves heavier than objectively measured. From normal weight students 55% perceived their weight correctly but one third of the students thought that their weight was less than the really determined one.

In females the precision of weight-height ratio estimation increases with the increase of BMI (Figure 4). From underweight students only 27% estimated their weight correctly, but majority – 71% – overestimated their BMI. In the normal weight group picture is similar to underweight – only 34% of these females could estimate their weight correctly, but most of these students underestimated their BMI. The accuracy of estimation of BMI was slightly better in groups of overweight and obese students. In overweight group, 38% of students estimated their BMI correctly but still more than one third of female students overestimated their BMI. In obese group, the correct evaluation of BMI was the most frequent – 43%. The same proportion of obese students underestimated their BMI, but only 14% thought that they are heavier than they really were.

Figure 3. Precision of body mass index (BMI) estimation for males with differently calculated BMI

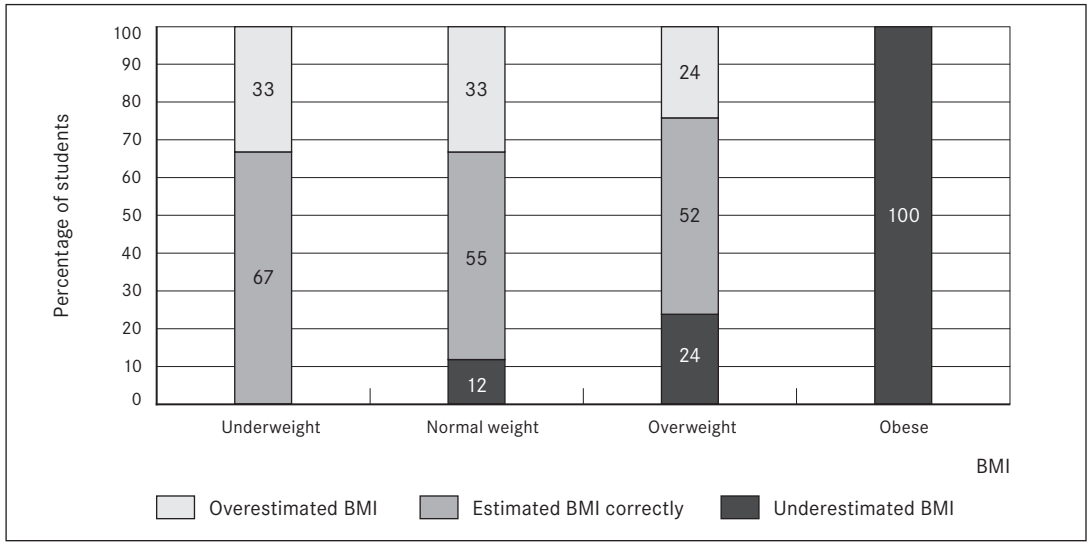
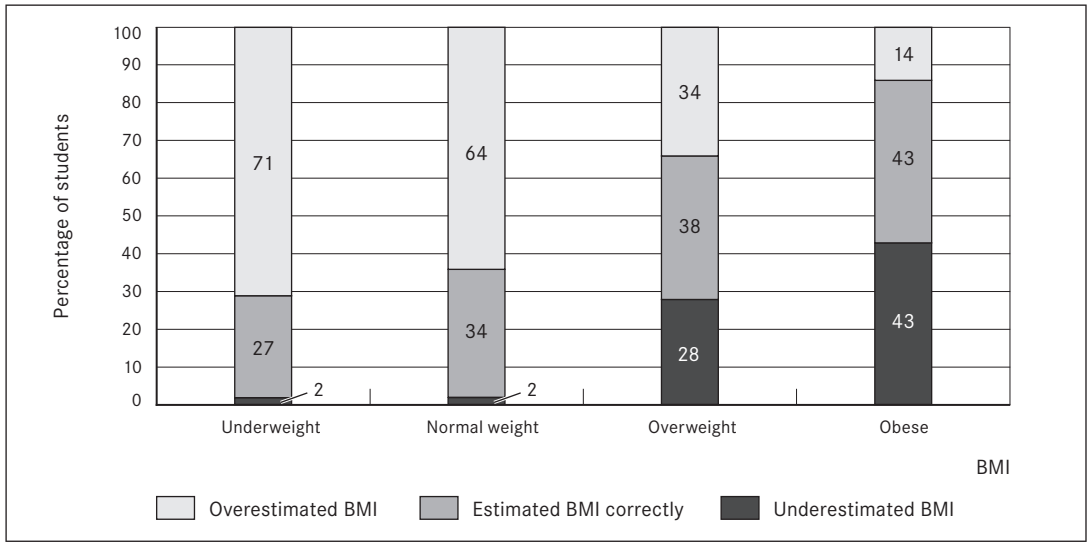


Figure 4. Precision of body mass index (BMI) estimation for females with differently calculated BMI



When students were divided into groups according to the fat percentage, the situation was similar. Males showed a better ability to evaluate their weight-height relationships according to the modified Stunkard scale (Figure 5). In the group of underfat males 80% of the students estimated their BMI correctly, and in healthy and increased fat percent groups still 50% or more students evaluated their body image correctly. In the healthy and overfat groups, according to the fat percentage, 30% and more students overestimated their BMI but more than 30% of obese students regarding fat percentage underestimated their weight-height relationships.

Females with lower fat percentage estimated their BMI less precisely than those with higher fat percentage (Figure 6). More than 60% of female students with underfat and healthy fat percentage overestimated their weight-height ratio while roughly 30% of females in these groups estimated it correctly. Despite the precision of evaluation of their body image increased with the increase of fat percentage, 27% of overfat and 22% of obese women underestimated and 31% and 11%, respectively, overestimated their BMI.

Figure 5. Precision of body mass index estimation for males with differently measured fat percentage

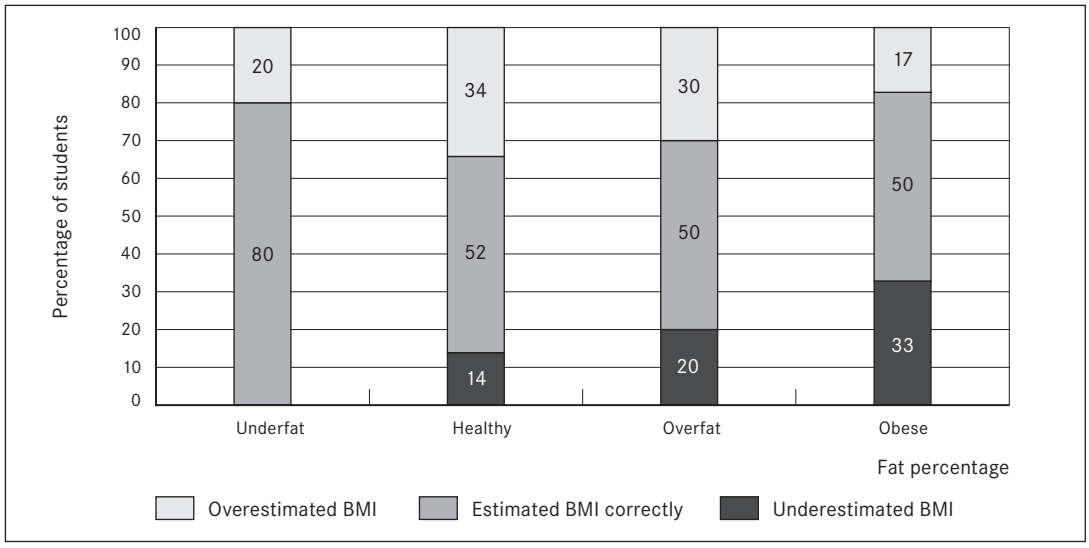
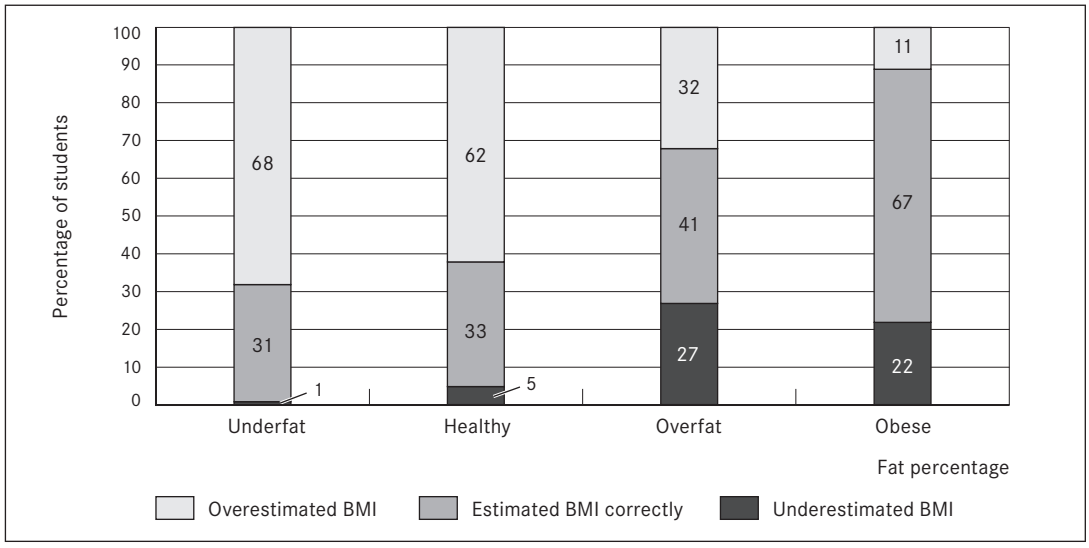


Figure 6. Precision of body mass index estimation for females with differently measured fat percentage



Discussion

On average, BMI in female and male group indicates healthy weight. Investigation of students' BMI in other 16 European countries does not show essential differences [7]. The data obtained in our study are more alike in the countries where students report higher BMI. However, in other universities self-reported body weight and height was used for the calculation of BMI. The studies which have compared self-reported and objectively measured height and weight values found that people tend to underestimate their weight and overestimate their height, thus leading to substantial underestimation of BMI [8, 9].

Body image is an important element of the intricate mechanism of one's own identity. It is the mental picture we have of our body's measures, contours and shape, our feelings related to these characteristics and to our body parts [10]. There is a great role of sociocultural factors and media in selection of ideal and evaluation of one's own body image [11].

Generally, precision of self-estimation of BMI in studies across the world as well as in our study is quite low. Males are more tended to underestimate their weight [13, 14]. This could be related to lesser concern about the body image in the society and inability to recognise which compartment mainly gives increase or decrease of body size – fat or fat free mass. Slight increase of body weight might cause body size changes that are not obvious without specific measurements. Thus, it can make wrong impression that body size is increased due to muscle mass increase and further decrease awareness of overweight and even obesity problem. The accuracy of body weight perception is less precise in overweight and obese group [13, 14]. This misperception is associated with less likelihood of interest in or attempts at weight loss and less physical activity [12].

Historically, a greater importance in the society is placed on body ideals for women than for men. Females tend to exaggerate the problem of overweight and obesity and overestimate their body image [7, 13, 14]. Young women are pursuing absurdly thin ideal body weights and risking their mental and physical health. They more frequently perceive themselves overweight and engage in different weight control activities that are irrational and even hazardous [15, 16]. Body size overestimation is common not only in between underweight people with eating disorders, but also in individuals with normal weight. The initiation of dieting is associated with large discrepancy between the actual and ideal body size [17, 18].

Female students with the low fat percentage estimated their BMI almost as inaccurately as the students with low BMI. Many women associate thinness with beauty, rather than health so they may tend to concentrate on losing weight regardless their body composition. They cannot estimate accurately their fatness which may result in loss of fat free mass due to wrong interventions [19, 20]. Females with higher fat percentage are more precise in the estimation of BMI. These females are more aware of their obesity and most of them might have already participated in some weight decreasing activities [21].

Student self-selection for the participation in this study might give an influence on study results. However, we do not have any reliable information to confirm or deny this hypothesis.

Conclusions

Precision of self-estimation of body weight-height relationships is low, especially in women. Women overestimated their weight-height ratio more while men had a tendency to underestimate it. Persons with the higher BMI and fat percentage more underestimated their BMI, but persons with lower BMI over-estimated it.

References

1. WHO. Obesity: preventing and managing the global epidemic / Report of a WHO Consultation // WHO Technical Report Series 894, 2000.
2. Lowe M. R. Self-regulation of energy intake in the prevention and treatment of obesity: is it feasible? // J Obesity Research, 2003; 11: 44S–59S.

3. Friedman K. E., Reichmann S. K., Costanzo P. R., Mustante G. J. Body image partially mediates the relationship between obesity and psychological distress // J Obesity Research, 2001; 10: 33–41.
4. Bulik C. M., Wade T. D., Heath A. C., et al. Relating body mass index to figural stimuli: population-based normative data for Caucasians // Int J of Obesity, 2001; 25: 1517–1524.
5. Pulvers K. M., Lee R. E., Kaur H., et al. Development of a culturally relevant body image instrument among urban African Americans // J Obesity Research, 2004; 12: 1641–1651.
6. Gallagher D., Heymsfield S. B., Heo M. et al. Healthy percentage body fat ranges: an approach for developing guidelines based on body mass index // Am J Clin Nutr, 2000; 72: 694–701.
7. Wardle J., Haase A. M., Steptoe A. Body image and weight control in young adults: international comparison in university students from 22 countries // Int J of Obesity, 2006; 30: 644–651.
8. Health Survey for England // http://www.dh.gov.uk/en/Publicationsandstatistics/PublishedSurvey/HealthSurvey ForEngland/index.htm
9. Tehard B., Van Liere M., Com Nougue C., Clavel-Chapelon F. Anthropometric measurements and body silhouette of women: validy and perception // J Am Diet Assoc, 2002; 102(12): 1779–1784.
10. Lynch E., Liu K., Wei G. S. et al. The relation betweeen body size perception and change in body mass index over 13 years // Am J Epidemiol, 2009; 169(7): 857–866.
11. Martin J. B. The development of ideal body image perceptions in the United States // Nutrition Today, 2010; 45(3): 98–110.
12. Duncan D. T., Wolin K. Y., Scharoun-Lee M., et al. Does perception equal reality? Weight misperception in relation to weight-related attitudes and behaviours among overweight and obese US adults // Int J Behav Nutr Phys Act, 2011; 8: 20.
13. Madrigal H., Sanchez-Villegas A., Martinez-Gonzalez M. A., et al. Underestimation of body mass index through perceived body image as compared to self-reported body mass index in the European Union // Public health, 2000; 114: 468–473.
14. Bhuiyan A. R., Gustat J., Srinivasan S. R., Berenson G. S. Differences in body shape representations among young adults from a biracial (black-white), semirural community // Am J Epidemiol, 2003; 158 (8): 792–797.
15. Crawford D., Owen N. The behavioral epidemiology of weight control // Austr J Publ Health, 1994; 18: 143–148.
16. Patton G. C., Carlin J. B., Shao Q., et al. Adolescent dieting: healthy weight control or borderline eating disorder? // J Child Psychol Psychiatry, 1997; 38: 863–880.
17. Thompson J. K. Body image, eating disorders, and obesity: an integrative guide for assessment and treatment // American Psychological Association, 1996; Pp.1–20.
18. Smeets M. A. M., Smit F., Panhuysen G. E. M., et al. The influence of methodological differences on the outcome of body size estimation studies in anorexia nervosa // Br J Clin Psychol, 1997; 36: 263–267.
19. Tanaka S., Itoh Y., Hattori K. Relationship of body composition to body-fatness estimation in Japanese university students // J Obesity Research, 2001; 10(7): 590–596.
20. Tanaka S., Hattori K., Tobe H., et al. Change of body composition in about eight years among Japanese university students // J Nutr Health Aging, 1999; 3: 165–168.
21. Stewart T. M., Bachand A. R., Han H., et al. Body image changes associated with participation in an intensive lifestyle weight loss intervention // Obesity (Silver Spring), 2011; 19(6): 1290–1295.

Human Leukocyte Antigen Class II Alleles in Latvian Patients with *Borrelia burgdorferi* Infection

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Abstract

In this study we sought to identify HLA class II alleles that might be associated with *Lyme borreliosis* in patients from Latvia. Case patients and control subjects were similar in age, sex, and ethnic heritage and differed only in the presence of *Borrelia burgdorferi* infection. The frequency of HLA-DRB1*15 (OR 5.21; $p < 0.132$) and HLA-DRB1*17(03) (OR 3.77; $p < 0.248$) were significantly increased in the Lyme disease patients compared with the control groups. The HLA-DQA1*0201, -DQA1*0501 and DQB1*0201 were also increased in patients of the respective groups, but these differences were quite modest. The allele DRB1*13 (OR 0.27; $p < 0.233$) was smaller in *Borreliosis* patients and significantly higher in controls.

Keywords: Lyme borreliosis, HLA alleles, PCR.

Introduction

Lyme borreliosis is a tick-borne multisystem disease that affects primarily the skin, nervous system, heart and joints. At least three species of *Borrelia burgdorferi sensu lato*, namely *Borrelia burgdorferi sensu stricto*, *Borrelia garinii*, and *Borrelia afzelii*, can cause the disease [1, 2].

Lyme disease is the most common vector-borne disease in the United States [3, 4]; the incidence is high in Latvia as well. During last 10 years (2001–2010), tick-borne encephalitis morbidity in Latvia varies from 6.2 to 22.3 per 100 000 inhabitants (142 to 494 people per year). *Lyme borreliosis* morbidity in Latvia during the same time period was between 14 and 36.9 per 100 000 inhabitants (328 to 829 people per year). The largest number of cases of *Lyme borreliosis* was recorded in 2010 – 829 [5]. Latvia is considered to be an endemic territory; the maximum incidence rates in patients were observed in the age group 60–69 [6].

The illness may evolve in stages, beginning with erythema migrans and progressing through a stage of dissemination during which arthritic, neurological, and cardiac complications may occur [7]. Some patients with Lyme arthritis experience recurrent episodes of joint inflammation for months or even years [8, 9]. Although the pathogenesis of this condition is unclear, there are some similarities between the bacterial agents and HLA molecules due to the development of one or another immune response to infection.

There are many hypotheses about the direct role of HLA molecules in the pathogenesis of infection [10]. In this study we sought associations between human leukocyte antigen class II (DRB1 and DQB1) markers and Lyme disease [10, 11]. The clarification of the polymorphism of HLA immunogenetic molecular markers in identifying regularities in the development and pathology to develop a new approach to treating this disease was attempted.

The aim

The aim of the study was to determine HLA-DR, -DQ molecules in patients with clinical, epidemiological and laboratory approved *Lyme borreliosis* diagnosis.

Material and methods

The study included 17 patients (4 male, 13 female; between 35 and 74 years old) with clinical stage – *erythema migrans* and 20 control (healthy) persons (9 male, 11 female; between 21 and 57 years old). The clinical diagnosis was confirmed at Infectology Center of Latvia. Immunogenetic examinations were performed at Rīga Stradiņš University Immunogenetic and Clinical Immunology Laboratory.

Genomic DNA was extracted from proteinase-K-treated peripheral blood leukocytes using the routine "salting-out" method [11, 12]. The DNA was stored in TE buffer (10 ml Tris–HCl, pH 7.5, and 2 ml 0.5 M Na_2 EDTA per liter of distilled water). The DNA concentration, around 100–200 µg/ml was determined by fluorescence with a DNA fluorimeter.

HLA- typing. HLA-DR genotyping by PCR low-resolution for DRB1* 01 to 18; HLA-DQA1 typing for DQA1* 0101, 0102, 0103, 0201, 0301, 0501, 0401/601; HLA-DQB1 typing for DQB1* 0201-0602/608 was performed by PCR with sequence-specific primers (PCR–SSP) [12, 13]. The reaction mixture (15 µl) included 1.0 µl DNA, 1.5 µl PCR buffer [50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3)], 0.6 µl dNTPs (25 mmol/l), 1.0 µl specific primers (0.2 mmol/l), and 0.5 U of the *Taq* DNA polymerase (Promega). The reaction mixture was subjected to 35 amplification cycles, each consisting of one denaturation cycle at 94 °C (60 s), seven annealing cycles at 94 °C (40 s) and 67 °C (15 s), and final 28 extension cycles at 93 °C (10 s) and 65 °C (9 s). PCR products were visualized by agarose-gel electrophoresis [12]. After addition of 2M loading buffer, the PCR reaction mixtures were loaded in agarose gels prestained with ethidium bromide (0.5 µk/ml gel). Gels were run for 15 min at 10 V/cm gel in 0.5m M TBE (0.89 M Tris, 0.89 M Boric acid and 0.02 M EDTA in aqueous solution) buffer and then examined under UV illumination and recorded [12].

Statistical analysis. The significance of differences in individual subtypes between patients and controls was assessed by Mantel-Haenszel test and Fisher exact correction for small numbers [12, 13]. Odds ratio (OR) and 95% confidence intervals (CI) were computed by standard methods [13].

Results

Typing of all sixteen alleles DRB1 were investigated (Table 1). The frequency of HLA-DRB1*15 (OR 5.21; $p < 0.132$) and HLA-DRB1*17(03) (OR 3.77; $p < 0.248$) were significantly increased in the Lyme disease patients compared with the control groups (Table 1). For the HLA-DRB1*18(03) allele, the evidence is controversial. Although DRB1 *18(03) allele's presence in our healthy persons and among *Borreliosis* patients suggests that is not associated with the disease, this should be interpreted carefully because of the small number of studied individuals.

Typing of all sixteen -DQ alleles were also studied (Table 2). The HLA-DQA1*0201, -DQA1*0501 and DQB1*0201 were shown to be considerably increased in patients, although the difference was no longer significant when the p value was not corrected for the number of alleles.

Allele DRB1*13 (OR 0.27; $p < 0.233$) was smaller in *Borreliosis* patients and significantly higher in controls.

Table 1. The frequency of DRB1* alleles studied in patients and healthy controls from Latvia

Allele DRB1	Pacients (n = 17) 34 alleles	Controls (n = 20) 40 alleles	OR (95% CI)	p value
*01	0	1	ND	ND
*02	0	1	ND	ND
*03	3	4	0.87	0.806
*04	4	0	ND	ND
*07	3	5	0.68	0.451
*08	1	3	0.37	0.371
*09	2	3	0.77	0.577
*10	1	1	1.18	0.711
*11	6	9	0.74	0.607
*12	3	4	0.87	0.591
*13	1	4	0.27	0.233
*14	0	2	ND	ND
*15	4	1	5.2	0.132
*16	2	0	ND	ND
*17(03)	3	1	3.77	0.248
*18(03)	1	1	1.18	0.711

Abbreviations: ND – not defined; OR – odds ratio, p – probability, significant associations for $p < 0.05$.

Table 2. The frequency of DQA1*, DQB1* alleles studied in-patients and healthy controls from Latvia

Allele DQA1	Pacients (n = 17) 34 alleles	Controls (n = 20) 40 alleles	OR (95% CI)	p value	Allele DQB1	Pacients (n = 17) 34 alleles	Controls (n = 20) 40 alleles	OR (95% CI)	p value
*0101	5	8	0.69	0.553	*0201	9	6	**2.04**	0.224
*0102	3	2	1.84	0.516	*0301	2	4	0.56	0.520
*0103	2	5	0.44	0.335	*0302	9	10	1.08	0.886
*0201	4	2	**2.53**	0.291	*0303	2	3	0.77	0.783
*0301	7	10	0,78	0.655	*0304	1	2	0.58	0.656
*0501	8	5	**2.15**	0.217	*0305	2	4	0.56	0.520
*0401 / 0601	5	8	0.69	0.553	*0501	1	3	0.37	0.390
–	–	–	–	–	*0601	1	3	0.37	0.390
–	–	–	–	–	*0602/8	7	5	1.81	0.350

Abbreviations: ND – not defined; OR – odds ratio, p – probability, significant associations for $p < 0.05$.

Discussion

Most diseases with autoimmune features have HLA associations, particularly with class II MHC alleles [14, 15]. In our study *HLA* genes have been identified as key genetic factors contributing to the development of chronic Lyme arthritis, disease characterized by autoimmune features. The role of *HLA* encoded molecules in the pathogenesis of this disease is unresolved.

About 80 percent of the patients with Lyme disease experience some joint pain, which may include arthritis. For a small percentage of patients (about 10%), Lyme arthritis does not respond to antibiotics and remains chronic [16]. To determine why some patients recover and others develop chronic Lyme arthritis, the antigens of the major histocompatibility complex were examined in Latvian patients with *Borrelia burgdoferi* infection.

In our HLA study, an association was shown between *Lyme borreliosis* and the HLA-DRB1*15 (is part of the older HLA-DR2 and DR5 serotype group), and a secondary association was noted with HLA-DRB1 *17(03) (part of the older HLA-DR3). The distribution of alleles in the patients included in this

study follows the world tendency: DRB1*17(03) was the most frequent allele in Caucasian population [16]. Thereafter, we began to determine HLA-DQ alleles in patients from Latvia with Lyme arthritis. A second association was found between HLA-DQA1*0201, -DQA1*0501 and DQB1*0201 and Lyme disease, although it was not as strong as for HLA-DRB1*15. The distribution of any HLA-DQ alleles (Table 2.) did not differ between case patients and control subjects by test.

Conclusions

HLA predisposition to *Lyme borreliosis* appears not to be limited to HLA-DR or -DQ, but some alleles also have a significant influence. In particular, HLA-DRB1*15 contributes definitely to a genetic predisposition to *Borrelia burgdorferi* infection in Latvian population, which may have implications in our understanding of pathogenesis of this disease. To receive more reliable data on the prevalence of HLA alleles in Latvian population and their possible relationship with *Borreliosis*, it is necessary to continue the investigation and extend the range of persons included in the research. The definite conclusion of the disease-associated subtypes requires different ethnic group studies. It is a further step towards improving our understanding of the role of HLA molecules in this severe infectious disease.

The development of an effective vaccine against Lyme disease and the improved understanding of the long-term outcome of Lyme disease can be considered the most important advances in the field over the past several years.

References

1. Steere A. C. Lyme disease // N Engl J Med, 2001; 345: 115–125.
2. Dattwyler R. J., Halperin J. J., Volkman D. J., Luft B. J. Treatment of late Lyme borreliosis – randomized comparison of ceftriaxone and penicillin // Lancet, 1988; 1: 1191–1194.
3. Steere A. C., Levin R. E., Molloy P. J., et al. Treatment of Lyme arthritis // Arthritis Rheum, 1994; 37: 878–888.
4. Dattwyler R. J., Wormser G. P., Rush T. J., et al. A comparison of two treatment regimens of ceftriaxone in late Lyme disease // Wien Klin Wochenschr, 2005; 117: 393–397.
5. Statistical data on incidence of infectious diseases in Latvia // www.lic.gov.lv
6. Arša F., Bormane A., Krūmiņa A., et al. Tick-borne encephalitis and Lyme borreliosis in Latvia: epidemiological situation in 2007–2009 // Abstract book ESCAIDE 2010; (Poster No 20100123), 173.
7. Chen J., Field J. A., Glickstein L., et al. Association of antibiotic treatment-resistant Lyme arthritis with T cell responses to dominant epitopes of outer-surface protein A (OspA) of *Borrelia burgdorferi* // Arthritis Rheum, 1999; 42: 1813–1822.
8. Wormser G. P., Nadelman R. B., Dattwyler R. J., et al. Practice guidelines for the treatment of Lyme disease. The Infectious Diseases Society of America // Clin Infect Dis, 2000; 31: S1–14.
9. Steere A. C., Dwyer E., Winchester R. Association of chronic Lyme arthritis with HLA-DR4 and HLA-DR2 alleles // N Engl J Med, 1990; 323: 219–223.
10. Nepom B. S., Glass D. N. Juvenile rheumatoid arthritis and HLA: report of the Park City III workshop // J Rheumatol Suppl, 1992; 33: 70–74.
11. Klitz W., Maiers M., Spellman S., et al. New HLA haplotype frequency reference standards: high-resolution and large sample typing of HLA DR-DQ haplotypes in a sample of European Americans // Tissue Antigens, 2003; 62: 296–307.
12. Olerup O., Aldener A., Fogdell A. HLA-DQB1 and -DQA1 typing by PCR amplification with sequence-specific primers (PCR-SSP) // Tissue Antigens, 1993; 41(3): 119–134.
13. Categorical data analysis using the SAS system // www.google.com/books?hl=lv&lr=&id=JcOcTfRYCn4C&oi=fnd&pg=PA1&dq=assessed+by+Mantel+%E2%80%93+Haenszel+test+and+Fisher+exact+correction
14. Gregersen P. K., Silver J., Winchester R. J. The shared epitope hypothesis: an approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis // Arthritis Rheum, 1987; 30: 1205–1213.
15. Weyand C. M., Hicok K. C., Conn D. L., Goronzy J. J. The influence of HLA-DRB1 genes on disease severity in rheumatoid arthritis // Ann Intern Med, 1992; 117: 801–806.
16. Lengl-Janssen B., Strauss A. F., Steere A. C., Kamradt T. The T helper cell response in Lyme arthritis: differential recognition of Borrelia burgdorferi outer surface protein A (OspA) in patients with treatment-resistant or treatment-responsive Lyme arthritis // J Exp Med, 1994; 180: 2069–2078.

Relation of Adipokine Profile to Insulin Resistance among Overweight and Obese Adults with and without Metabolic Syndrome

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Abstract

Obesity and the metabolic syndrome (MetS) have emerged as clinical and public health crises in many populations, but not all obese patients have the syndrome. The aim of the study was to evaluate changes in serum levels of interleukin-6 (IL-6), tumour necrosis factor α (TNFα), adiponectin, leptin and resistins and their association with insulin resistance (IR) in MetS patients who were categorized as having III BMI (body mass index) category obesity. Additionally, we compared these results with results obtained from obese subjects I-II BMI category, overweight or normal weight subjects without MetS.

57 male patients were categorized into four groups: 10 obese MetS patients within the III BMI category (4), 23 obese subjects within the I–II BMI category (3) and 12 overweight subjects (2). 12 normal weight subjects were selected as controls (1). IR was assessed by the HOMA-IR method, but serum IL-6, TNFα, adiponectin, leptin and resistins levels were measured by xMAP technology.

Serum levels of IL-6, TNFα, leptin and resistin in groups 2, 3 and 4 were increased in comparison with group 1 ($p < 0.001$), except for IL-6 and TNFα in group 2, besides leptin, resistin, and adiponectin concentrations were correlated with HOMA-IR indexes ($r = 0.51$, $p < 0.001$ for leptin and $r = 0.57$ and $r = -0.66$, $p < 0.0001$ for resistin and adiponectin, respectively). Serum adiponectin level was significantly lower in groups 2, 3 and 4 when compared to subjects in group 1 ($p < 0.0001$). In addition, serum level of IL-6 was positively correlated with TNFα ($r = 0.33$, $p < 0.05$).

Obese MetS patients have a more pronounced increase of IL-6, TNFα, leptin, resistin and HOMA-IR and more decreased adiponectin levels than other subjects without MetS.

Keywords: MetS, IR, obesity, IL-6, TNFα, leptin, resistin, adiponectin

Introduction

IR is defined as a failure of target tissues (adipose, liver, skeletal and cardiac muscle) to respond normally to insulin [Saltiel, 2001; Luca and Olfesky, 2008]. IR plays a major role in the development of type 2 diabetes (T2D) [Olefsky and Glasss, 2010]. Furthermore, the consequences of IR manifest at many levels and in many metabolic processes, producing a cluster of homeostatic abnormalities including glucose intolerance, overt hyperglycemia, hyperinsulinemia and atherogenic dyslipidemia, collectively referred to as MetS [Koster, et al., 2010; Meas, et al., 2010; Al-Hamodi, et al., 2011]. Weight gain and obesity are major risk factors for conditions and diseases ranging from IR and T2D to atherosclerosis and

MetS [Gnacinska, et al., 2010; Koster, et al., 2010; Samuel, et al., 2010; Conroy, et al., 2011]. In recent years, much interest has been focused on this association between obesity, chronic inflammation and IR. It is estimated that the number of those affected by the disease will double by the year 2030 [Gnacinska, et al., 2010]. Consequently, obesity is epidemic, which is going to become the biggest health problem of our century.

Obesity and IR are associated with low-grade systemic inflammatory response characterised by altered adipokines production and activation of inflammatory signalling pathways [Wellen, et al., 2003; Savage, et al., 2005; Fuentes, et al., 2010]. Adipose tissue consists of a variety of cell types, including adipocytes, immune cells (macrophages and lymphocytes), preadipocytes, and endothelial cells [Olefsky and Glass, 2010; Vykoukal, et al., 2011]. Among these cell types, adipocytes and macrophages release cytokines and chemokines such as IL-6 [Bastard, et al., 2000], plasminogen activator inhibitor (PAI-1) [Ma, et al., 2004], TNFα [Moller, 2000], IL-6 [Koster, et al., 2010], resistin [Steppan and Lazar, 2002] that promote inflammation. In addition, adipocytes are the unique source of adipokines such as leptin [Mlinar, et al., 2007; Hasan-Ali, et al., 2011; Margoni, et al., 2011] and adiponectin [Almeda-Valde, et al., 2010; Hasan-Ali, et al., 2011; Margoni, et al., 2011], which can promote insulin sensitivity [Mlinar, et al., 2007].

Leptin, adiponectin and TNFα levels are different in T2D and obese individuals compared with nondiabetic, nonobese individuals. Morbid rates of leptin and adiponectin may provide a link between obesity, TD2, MetS and increased cardiovascular risk. The protein leptin, a satiety hormone, regulates appetite, energy homeostasis and glucose/lipid metabolism [Steppan and Lazar, 2002; Mlinar, et al., 2007]. Obesity is characterized by hyperleptinemia [Considine, et al., 1996; Ravussin, et al., 2002; Drevon, 2005], whereas leptin levels decrease considerably during weight loss [Anderlova, et al., 2006]. Adiponectin exerts profound antidiabetic, antiatherogenic and antiinflammatory roles [Ouchi and Walsh, 2007; Li, et al., 2010; Hasan-Ali, et al., 2011]. Obesity is characterized by hypoadiponectinemia, because adiponectin is inversely correlated with BMI [Oda, et al., 2008; Hasan-Ali, et al., 2011] and low adiponectin are an independent predictor of incident T2D and MetS [Weyer, et al., 2001; Almeda-Valdes, et al., 2010] and cardiovascular disease [DeFronzo, 2009; Tretjakovs, et al., 2009]. Both hormones improve insulin resistance [Silha, et al., 2003], although their blood concentrations may be contradictory, depending on adipocyte deposition [Oda, et al., 2008; Fuentes, et al., 2010; Conroy, et al., 2011]. TNFα, a cytokine secreted by adipocytes, influences energy balance and glucose homeostasis. TNFα causes IR and plays a major role in the pathogenesis of obesity-linked T2D [Gnacinska, et al., 2010; Margoni, et al., 2011]. Furthermore, TNFα levels are also increased in obesity and MetS [Steppan, et al., 2002]. Associations between the expression of TNFα in adipose tissue and obesity and IR were reported in both humans and animals because it is over expressed in adipose tissue from obese animals and humans, and obese mice lacking either TNFα or its receptor showing protection against developing IR [Hotamisligil, et al., 1995; Nieto-Vazquez, et al., 2008]. In large epidemiological studies, IL-6 and resistin has been strongly and consistently associated with MetS [Steppan and Lazar, 2002; Koster, et al., 2010; Figler, 2011] and identified as a predictive marker for myocardial infarction or MetS [Mlinar, et al., 2007; Vykoukal, et al., 2011]. In longitudinal data, higher circulating resistin is associated with proatherogenic inflammatory markers, MetS and increased cardiovascular risk [Mlinar, et al., 2007; Vykoukal, et al., 2011]. Studies with IL-6 have suggested its strong implication in IR, since plasma concentrations and adipose tissue expression, and polymorphisms of IL-6, correlate well with obesity and IR. IL-6 was shown to interfere with insulin signalling [Senn, et al., 2003], to inhibit adipogenesis and secretion of adiponectin [Kershaw and Flier, 2003].

The aim

The aim of the study was to evaluate changes in serum levels of IL-6, TNFα, adiponectin, leptin and resistin and their association with IR in MetS patients who were categorized as having III BMI category obesity. Additionally, we compared these results with results obtained from obese subjects I–II BMI category, overweight or normal weight subjects without MetS.

Material and methods

57 male patients, whose mean age was 44 ± 8 (mean ± SD) were recruited and categorized into four groups: 10 obese patients with MetS (BMI > 40 kg/m^2, obese class III) (group 4), 23 obese patients without MetS (BMI 30–39.9 kg/m^2, obese class I and III) (group 3), 12 overweight patients without MetS (BMI 28.5–29.9 kg/m^2) (group 2), 12 healthy subjects with normal weight were selected as controls (BMI 18.5–24.9 kg/m^2) (group 1). MetS was diagnosed according to the International Diabetes Foundation (IDF) criteria with specific reference to European population [Alberti, et al., 2005]. MetS was based on the existence of a waist circumference ≥ 94 cm in men and more of the following components:

1) a fasting triglyceride concentration ≥ 1.7 mmol/l;
2) an HDL-C concentration < 1.03 mmol/l;
3) blood pressure ≥ 130 mm Hg (systolic) or ≥ 85 mm Hg (diastolic);
4) fasting plasma glucose ≥ 5.6 mmol/l.

MetS patients were not included if their systolic blood pressure was ≥ 160 mm Hg or diastolic ≥ 95 mm Hg, and if they were treated with antihypertensive drugs other than angiotensin-converting enzyme inhibitors.

Exclusion factors were acute inflammatory condition or chronic inflammatory states such as rheumatoid arthritis, systemic lupus eryhematosus, vasculitis, inflammatory bowel disease, and other diseases, which are known to be associated with significant changes of cytokines, including surgery or trauma within the preceding 30 days. Malignancy and alcoholism were also exclusion criteria. We did not include patients who were taking COX-2 inhibitors, nonsteroidal antiinflammatory agents or corticosteroids, or had been taking them within the preceding 30 days. All subjects gave their informed consent to the protocol, which was approved by the local Medical Ethics Committee of the University of Latvia for Biomedical Research.

Blood samples (5 ml) for IL-6, TNFα, adiponectin, leptin and resistin determination were collected after 12-hour fast in the supine position. Samples for the determination of chemokines were collected without anticoagulant and were allowed to coagulate for 20 to 30 min at room temperature. After that, samples were centrifuged at 4 °C for 20 min at 1600 × g. All specimens were immediately aliquoted, frozen, and stored at – 80 °C. IL-6, TNFα, adiponectin, leptin and resistin concentrations were measured by xMAP multiplex immunobead assay technology (Luminex 200 analyzer, Luminex Corp., Austin, TX, USA) [Kofoed, et al., 2006]. Regarding IR, we used the homeostasis model assessment (HOMA-IR) to quantify IR (fasting glucose × fasting insulin/22.5) [Matthews, et al., 1995; Bonora, et al., 2000]. The HOMA-IR values have been shown to correlate well with values obtained using the "gold standard" clamp technique [Bonora, et al., 2000]. Fasting concentrations of lipids, insulin, and glucose were analyzed by standard methods [Rask-Madsen and King, 2007].

After testing the normality of data distribution, statistical differences between four groups were assessed by one-way ANOVA using Fisher's multiple comparison test. Data were recorded as means ± SD and two-tailed values of $p < 0.05$ were considered to be significant. Correlation analyses were performed using one-factor linear regression analysis. All analyses were performed using STATISTICA 6.0 software (StatSoft Inc, USA).

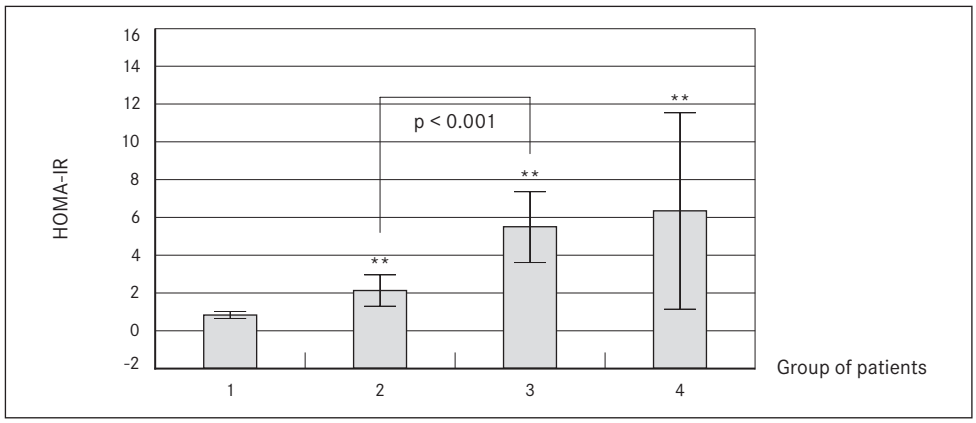
Results

All three groups of patients had significantly higher values for HOMA-IR than normal weight control group. Comparison of HOMA-IR among the groups showed that obese subjects within the I–II BMI category had significantly higher insulin resistance than overweight subjects (Group 2: 2.16 ± 0.83 vs. group 3: 5.51 ± 1.85, $p < 0.001$), but did not differ from group of obese MetS patients (Figure 1).

Serum level of IL-6, TNFα, leptin and resistin were significantly higher in overweight subjects, obese subjects within the I–II BMI category and obese MetS patients ($p < 0.001$) than in normal weight control group (Figures 2, 3, 4 and 5), except for IL-6 and TNFα in overweight subjects group (Figures 2 and 5). Serum adiponectin level was significantly lower in overweight subjects, obese subjects

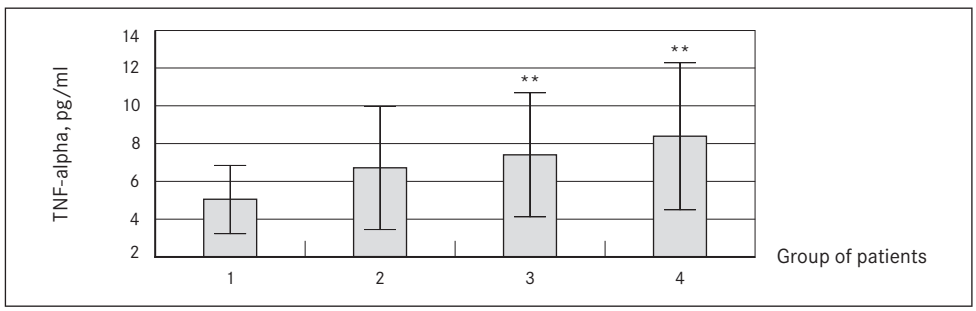
within the I–II BMI category and obese MetS patients when compare to subjects with normal weight ($p < 0.0001$). Furthermore, adiponectin level was significantly lower in the obese subjects I–II BMI category when compared to overweight subjects ($p < 0.001$) (Figure 6).

Figure 1. Insulin resistance (HOMA-IR) in normal weight control subjects (1), overweight subjects (2), obese subjects within the I–II BMI category (3), and obese MetS patients within the III BMI category (4)*



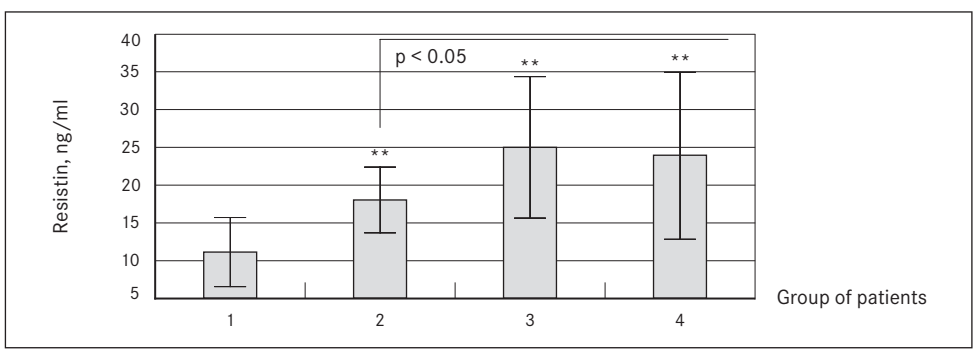
* Data are expressed as mean ± SD.
** $p < 0.001$ vs. controls.

Figure 2. TNFα in normal weight control subjects (1), overweight subjects (2), obese subjects within the I–II BMI category (3), and obese MetS patients within the III BMI category (4)*



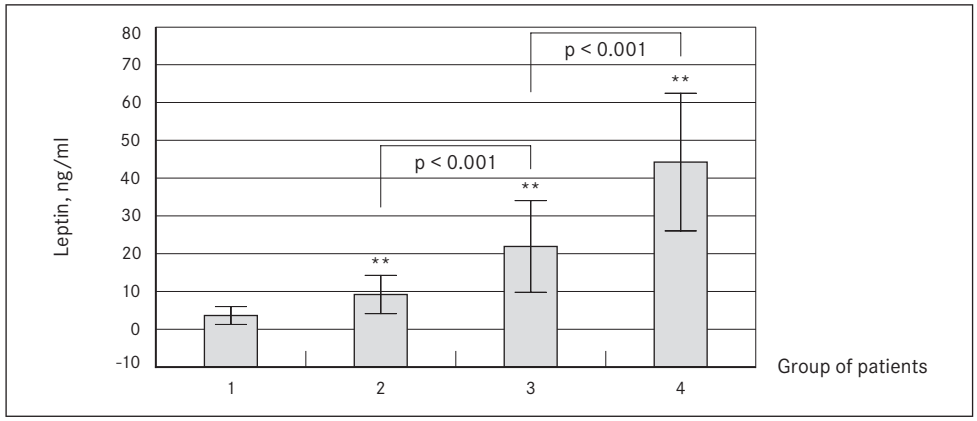
* Data are expressed as mean ± SD.
** $p < 0.05$ vs. controls.

Figure 3. Resistin in normal weight control subjects (1), overweight subjects (2), obese subjects within the I–II BMI category (3), and obese MetS patients within the III BMI category (4)*



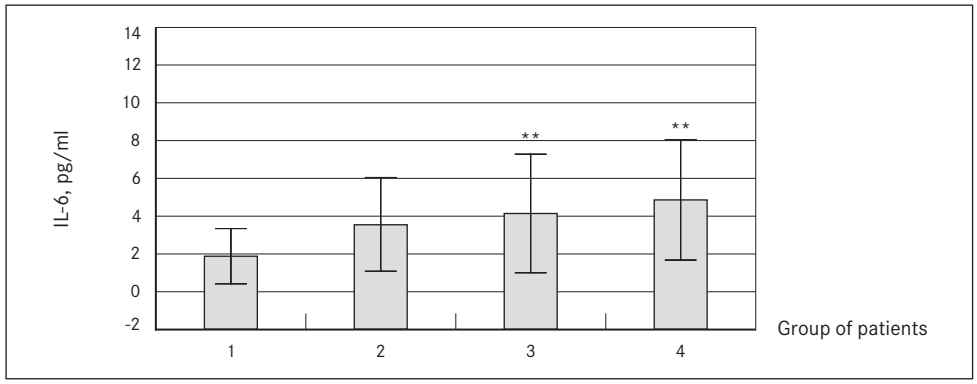
* Data are expressed as mean ± SD.
** $p < 0.001$ vs. controls.

Figure 4. Leptin in normal weight control subjects (1), overweight subjects (2), obese subjects within the I–II BMI category (3), and obese MetS patients within the III BMI category (4)*



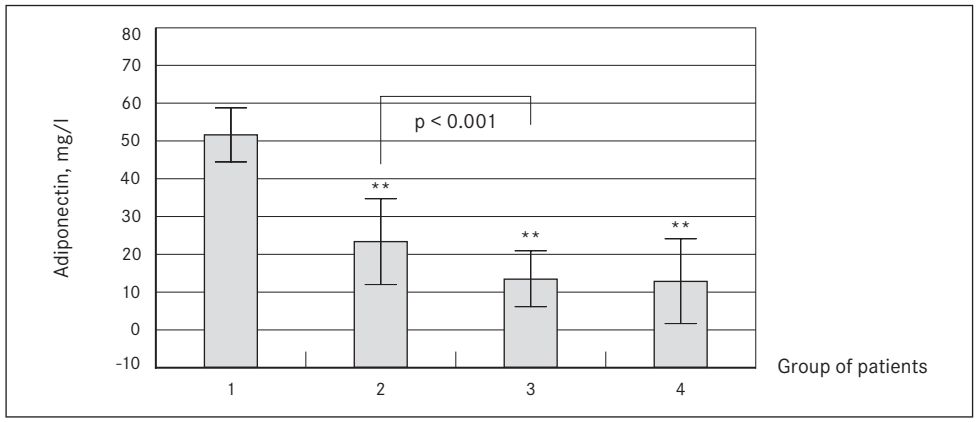
* Data are expressed as mean ± SD.
** p < 0.0001 vs. controls.

Figure 5. IL-6 in normal weight control subjects (1), overweight subjects (2), obese subjects within the I–II BMI category (3), and obese MetS patients within the III BMI category (4)*



* Data are expressed as mean ± SD.
** p < 0.0001 vs. controls

Figure 6. Adiponectin in normal weight control subjects (1), overweight subjects (2), obese subjects within the I–II BMI category (3), and obese MetS patients within the III BMI category (4)*



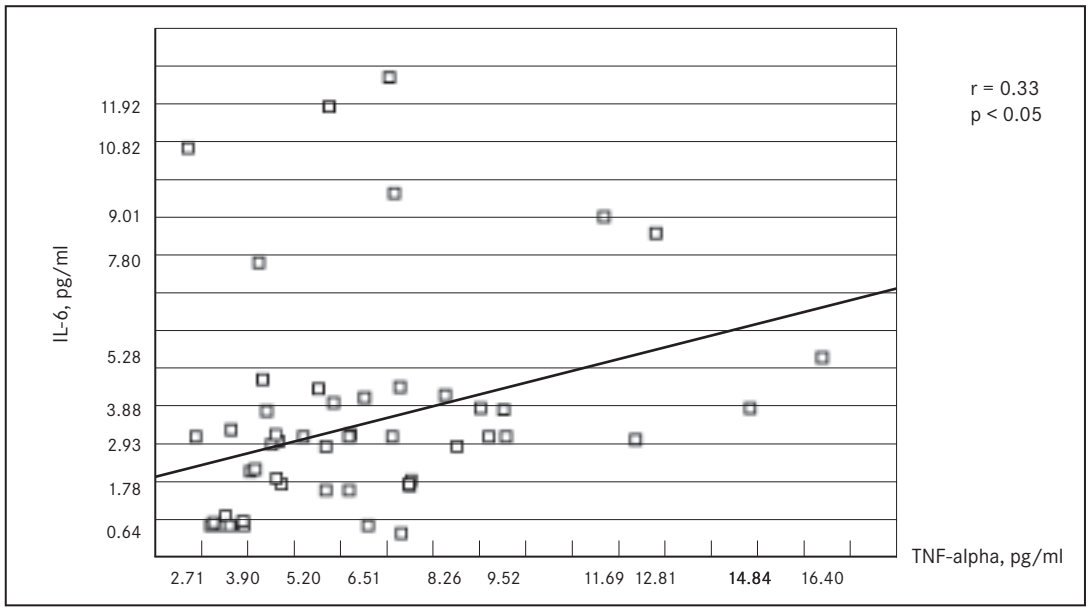
* Data are expressed as mean ± SD.
** p < 0.0001 vs. controls.

HOMA-IR indexes were significantly correlated with leptin, resistin, and adiponectin levels ($p < 0.001$ for leptin, $p < 0.0001$ for resistin and adiponectin) (Table 1). In addition, serum level of IL-6 was positively correlated with TNFα ($r = 0.33$, $p < 0.05$) (Figure 7).

Table 1. Correlations between study biomarkers in total clinical material

Insulin resistance	Leptin, ng/ml		Resistin, ng/ml		Adiponectin, mg/l	
	r	p	r	p	r	p
HOMA-IR	0.51	< 0.001	0.57	< 0.0001	– 0.66	< 0.0001

Figure 7. Correlation of TNFα and IL-6 in total study clinical material



Discussion

The results of our study suggest hat obese MetS patients, in comparison with obese subjects within the I–II BMI category, overweight subjects, and normal weight subjects (without MetS) may have higher serum levels of IL-6, TNFα, leptin and resistin and higher HOMA-IR, but lower adiponectin levels. The study confirmed correlations between HOMA-IR and circulating levels of leptin and resistin, which is consistent with the findings of previous research [Ahonen, et al., 2010], besides our results demonstrate an inverse correlation between adiponectin and HOMA-IR [Tretjakovs, et al., 2009].

IR as the putative cause of MetS raises the question as to whether it is possible to separate obesity from IR. IR exists to various degrees in all categories of BMI, suggesting the contribution of an independent, inheritable factor to at least some extent [Gnacinska, et al., 2010]. In some populations (South Asians) [Conroy, et al., 2011], IR exists in mildly overweight people and this is said to be primary IR. In this case, IR can be classified as a specific aetiological factor for MetS [Grundy, et al., 2004]. The increased HOMA-IR in overweight and obese subjects do not contradict with the Conroy, et al. findings [Conroy, et al., 2011] that IR exists to various degrees in all categories of BMI. Moreover, in our study HOMA-IR in obese subjects within the I–II BMI category was significantly higher than in overweight subjects.

It has previously been shown that the change in BMI predicts the development of MetS [Gnacinska, et al., 2010; Koster, et al., 2010]. MetS is strongly linked to obesity [Mlinar, et al., 2007], which is why the change in BMI has been speculated to be the central factor affecting the course of MetS. Our study suggests that significantly lower leptin and resistin levels are observed in overweight subjects than in obese subjects within the I–II BMI category. In contrast, adiponectin levels are lower in obese subjects

within the I–II BMI category than in overweight subjects. Furthermore, results suggest that a high TNFα and IL-6 levels depend on the change in BMI predict the resolution of MetS. There is no significant difference between IL-6 and TNFα levels in the normal weight group in comparison to the overweight group subjects. Because lower serum adiponectin levels and higher leptin, and IL-6 levels are associated with MetS [Kiess, et al., 1998; Reinehr, et al., 2005; Vykoukal, et al., 2011], our findings suggest that the higher risk of MetS among obese subjects within the I–II BMI category compared to overweight subjects.

The previous studies concerning the role of resistin in obesity and IR are ambiguous. Some studies presented decreased expression of mRNA for resistin in adipose tissue in obese animals. In addition, in several studies in obese humans there was no visible relation between obesity and resistin level [McTernan, et al., 2006]. Only a few authors indicate increased resistin concentration or expresion of mRNA for resistin in obesity [Steppan and Lazar, 2002; Vykoukal, et al., 2011]. Our results showed a similar pattern – resistin level significantly increased with the severity of obesity evaluated by BMI. A recent large study involving the Framingham offspring cohort found a significant relationship between IR and resistin; however, this relationship was considerably weaker than the relationship with adiponectin and was lost after adjustment for BMI [Hiver, et al., 2008].

It is unclear whether leptin and adiponectin are specific biomarkers for adipose tissue distribution; however, it appears that adiponectin concentrations are predominantly determined by visceral and leptin by subcutaneous adipose tissue [Park, et al., 2004]. Furthermore, plasma leptin levels correlate with body fat content [Mlinar, et al., 2007; Fuentes, et al., 2010]; the increase of fat cells in number and in size is coupled with an increase in leptin secretion [Ravussin, et al., 2002; Farnier, et al., 2003; Drevon, 2005].

Recent evidence shows that adipocyte hypertrophy correlates better with IR than any other measures of adiposity. Adipocyte hypertrophy may be indicative of diminished adipocyte proliferation and differentiation. The increased fat cell size may also be a consequence of impaired fat oxidation. In rodents, it has been shown that inhibition of fat oxidation leads to increased intracellular lipid and IR in vivo. In humans, decreased fasting fat oxidation predicts weight gain and is associated with IR [Jequier, 1998; Ravussin and Smith, 2002]. Fat oxidation in both muscle and adipose tissue is regulated by a number of factors, including endocrine factors secreted from the adipocyte, such as TNFα, adiponectin and leptin [Heilbronn, et al., 2003; Muller, et al., 2005].

Our study has several limitations that need to be considered: the cross-sectional study design, small number of patients in the groups, and the study included only men of Latvian population, therefore larger studies would be required. Moreover, future studies are needed that measure visceral, subcutaneous, and total adipose tissue among a different BMI category MetS patients and obese subjects, to clarify the association between adipokines and distribution of adipose tissue.

Conclusions

Our findings show that obese MetS patients have more pronounced increase in serum levels of IL-6, TNFα, leptin and resistin, simultaneously with both higher IR and lower adiponectin concentrations than obese and overweight subjects without MetS. Resistin level in serum is involved in the pathogenesis of the MetS and obesity.

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References

1. Ahonen T. M., Saltevo J. T., Kautiainen H. J., et al. The association of adiponectin and low-grade inflammation with the course of metabolic syndrome // Nutrition, Metabolism and Cardiovascular Diseases, 2010; in press.
2. Alberti K. G., Zimmet P., Shaw J. The metabolic syndrome – a new worldwide definition // Lancet, 2005; 366(9491): 1059–1062.

3. Al-Hamodi Z., Ismail I. S., Saif-Ali R., et al. Association of plasminogen activator inhibitor-1 and tissue plasminogen activator with type 2 diabetes and metabolic syndrome in Malaysian subjects // Cardiovascular Diabetology, 2011; 10(23): 1–9.
4. Almeda-Valdes P., Cuevas-Ramos D., Mehta R., et al. Total and high molecular weight adiponectin have similar utility for the identification of insulin resistance // Cardiovascular Diabetology, 2010; 9(26): 1–10.
5. Anderlova K., Kremen J., Dolezalova R., et al. The influence of very-low-calorie-diet on serum leptin, soluble leptin receptor, adiponectin and resistin levels in obese women // Physiological Research, 2006; 55(3): 277–283.
6. Bastard J. P., Jardel C., Bruckert E., et al. Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss // The Journal of Clinical Endocrinology and Metabolism, 2000; 85(9): 3338–3342.
7. Bonora E., Targher G., Alberiche M., et al. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity // Diabetes Care, 2000; 23(1): 57–63.
8. Conroy S. M., Chai W., Lim U., et al. Leptin, adiponectin, and obesity among Caucasian and Asian women // Mediators of Inflammation, 2011; in press.
9. Considine R. V., Sinha M. K., Heiman M. L., et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans // The New England Journal of Medicine, 1996; 334(5): 292–295.
10. DeFronzo R. A. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard lecture 2009 // Diabetologia, 2010; 53(7): 1270–1287.
11. Drevon C. A. Fatty acids and expression of adipokines // Biochimica et Biophysica Acta, 2005; 1740(2): 287–292.
12. Farnier C., Krief S., Blache M., et al. Adipocyte functions are modulated by cell size change: potential involvement of an integrin/ERK signalling pathway // International Journal of Obesity and Related Metabolic Disorders, 2003; 27(10): 1178–1186.
13. Figler R. A., Wang G., Srinivasan S. et al. Links between insulin resistance, adenosine A2B receptors, and inflammatory markers in mice and humans // Diabetes, 2011; 60(2): 669–679.
14. Fuentes L., Roszer T., Ricote M. Inflammatory mediators and insulin resistance in obesity: role of nuclear receptor signaling in macrophages // Mediators of Inflammation, 2010; in press.
15. Gnacińska M., Małgorzewicz S., Guzek M., et al. Adipose tissue activity in relation to overweight or obesity // Polish Journal of Endocrinology, 2010; 61(2): 160–168.
16. Grundy S. M., Brewer H. B., Cleeman J. L., et al. Definition of metabolic syndrome: Report of the National Heart, Lung and Blood Institute / American Heart Association conference on scientific issues related to definition // Circulation, 2004; 109: 433–438.
17. Hasan-Ali H., El-Mottaleb N. A., Hamed H. B., et al. Serum adiponectin and leptin as predictors of the presence and degree of coronary atherosclerosis // Diagnostic Methods, 2011; 22(4): 264–269.
18. Heilbronn L. K., Smith S. R., Ravussin E. The insulin-sensitizing role of the fat derived hormone adiponectin // Current Pharmaceutical Design, 2003; 9(17): 1411–1418.
19. Hivert M. F., Sullivan L. M., Fox C. S., et al. Associations of adiponectin, resistin, and tumor necrosis factor alpha with insulin resistance // Journal of Clinical Endocrinology and Metabolism, 2008; 93: 3165–3172.
20. Hotamisligil G. S., Arner P., Caro J. F., et al. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance // The Journal of Clinical Investigation, 1995; 95(5): 2409–2415.
21. Jequier E. Effect of lipid oxidation on glucose utilization in humans // The American Journal of Clinical Nutrition, 1998; 67(3): 527S–530S.
22. Kershaw E. E., Flier J. S. Adipose tissue as an endocrine organ // The Journal of Clinical Endocrinology and Metabolism, 2004; 89(6): 2548–2456.
23. Kiess W., Anil M., Blum W. F., et al. Serum leptin levels in children and adolescents with insulin-dependent diabetes mellitus in relation to metabolic control and body mass index // European Journal of Endocrinology, 1998; 138(5): 501–509.
24. Kofoed K., Schneider U. V., Scheel T., et al. Development and validation of a multiplex add-on assay for sepsis biomarkers using xMAP technology // Clinical Chemistry, 2006; 52(7): 1248–1293.
25. Koster A., Stenholm S., Alley D. E., et al. Body fat distribution and inflammation among obese older adults with and without metabolic syndrome // Obesity, 2010; 18: 2354–2361.
26. Li F. Y., Cheng K. K., Lam K. S., et al. Cross-talk between adipose tissue and vasculature: role of adiponectin // Acta Physiologica (Oxford, England), 2010; in press.
27. Luca C., Olefsky J. M. Inflammation and insulin resistance // FEBS Letter, 2008; 582(1): 97–105.
28. Ma L. J., Mao S. L., Taylor K. L., et al. Prevention of obesity and IR in mice lacking plasminogen activator inhibitor 1 // Diabetes, 2004; 53(2): 336–346.

29. Margoni A., Perrea D. N., Vlachos I., et al. Serum leptin, adiponectin and tumor necrosis factor-a in hyperlipidemic rats with/without concomitant diabetes mellitus // Molecular Medicine, 2011; 17(1–2): 36–40.
30. Matthews D. R., Hosker J. P., Rudenski A. S., et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man // Diabetologia, 1995; 28(7): 412–419.
31. McTernan P. G., Kusminski C. M., Kumar S., et al. Resistin // Curr Opin Lipidol, 2006; 17: 170–175.
32. Meas T., Deghmoun S., Chevenne D., et al. Plasminogen activator inhibitor type-1 is an independent marker of metabolic disorders in young adults born small for gestational age // Journal of Thrombosis and Haemostasis, 2010; 8(12): 2608–2613.
33. Mlinar B., Marc J., Janež A., et al. Molecular mechanisms of insulin resistance and associated diseases // Clinica Chimica Acta, 2007; 375(1–2): 20–30.
34. Moller D. E. Potential role of TNFα in the pathogenesis of insulin resistance and type 2 diabetes // Trends in Endocrinology and Metabolism, 2000; 11(6): 212–217.
35. Muller G., Ertl J., Gerl M., et al. Leptin impairs metabolic actions of insulin in isolated rat adipocytes // The Journal of Biological Chemistry, 1997; 272(16): 10585–10593.
36. Nieto-Vazquez I., Fernandez-Veledo S., Kramer D. K., et al. Insulin resistance associated to obesity: the link TNF-alpha // Archives of Physiology and Biochemistry, 2008; 114(3): 183–194.
37. Oda N., Imamura S., Fujita T., et al. The ratio of leptin to adiponectin can be used as an index of insulin resistance // Metabolism, 2008; 57(2): 268–273.
38. Olefsky J. M., Glass C. K. Macrophages, inflammation, and insulin resistance // Annual Review of Physiology, 2010; 72: 219–246.
39. Ouchi N., Walsh K. Adiponectin as an anti-inflammatory factor // Clinica Chimica Acta, 2007; 380(1–2): 24–30.
40. Park K. G., Park K. S., Kim M. J., et al. Relationship between serum adiponectin and leptin concentrations and body fat distribution // Diabetes Research and Clinical Practice, 2004; 63(2): 135–142.
41. Rask-Madsen C., King G. L. Mechanisms of disease: endothelial dysfunction in insulin resistance and diabetes // Nature Clinical Practice Endocrinology and Metabolism, 2007; 3(1): 46–56.
42. Ravussin E., Smith S. R. Increased fat intake, impaired fat oxidation, and failure of fat cell proliferation result in ectopic fat storage, insulin resistance, and type 2 diabetes mellitus // Annals of the New York Academy of Science, 2002; 967: 363–378.
43. Reinehr T., Kratzsch J., Kiess W., et al. Circulating soluble leptin receptor, leptin, and insulin resistance before and after weight loss in obese children // International Journal of Obesity, 2005; 29(10): 1230–1235.
44. Saltiel A. R. New perspectives into the molecular pathogenesis and treatment of type 2 diabetes // Cell, 2001; 104(4): 517–529.
45. Samuel V. T., Petersen K. F., Shulman G. I. Lipid-induced insulin resistance: unravelling the mechanism // Lancet, 2010; 375: 2267–2277.
46. Savage D. B., Petersen K. F., Shulman G. I. Mechanisms of insulin resistance in humans and possible links with inflammation // Hypertension, 2005; 45: 828–833.
47. Senn J. J., Klover P. J., Nowak I. A., et al. Suppressor of cytokines signalling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes // The Journal of Biological Chemistry, 2003; 278(16): 13740–13746.
48. Silha J. V., Krsek M., Skrha J. V., et al. Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance // European Journal of Endocrinology, 2003; 149(4): 331–335.
49. Steppan C. M., Lazar M. A. Resistin and obesity-associated insulin resistance // Trends in Endocrinology and Metabolism, 2002; 13(1): 18–23.
50. Tretjakovs P., Jurka A., Bormane I., et al. Relation of inflammatory chemokines to insulin resistance and hypoadiponectinemia in coronary artery disease patients // European Journal of International Medicine, 2009; 20(7): 712–717.
51. Vykoukal D., Davies M. G. Vascular biology of metabolic syndrome // Journal of Vascular Surgery, 2011; in press.
52. Wellen K. E., Hotamisligil G. S. Obesity-induced inflammatory changes in adipose tissue // The Journal of Clinical Investigation, 2003; 112(12): 1785–1788.
53. Weyer C., Funahashi T., Tanaka S., et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hiperinsulinemia // The Journal of Clinical Endocrinology and Metabolism, 2001; 86(5): 1930–1935.

The Acute Effects of Cigarette Smoking on Microvascular Responsiveness and Haemodynamic Measurements in Healthy Male Smokers

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Introduction

Cigarette smoking is an established risk factor for the development and progression of cardiovascular and peripheral vascular diseases. The mechanism of the increased cardiovascular risk of cigarette smoke is not well understood, but it is presumed to be related to endothelial dysfunction [Saijonmaa, et al., 2005]. It is established that long-term cigarette smoking is associated with impaired endothelium-dependent peripheral and coronary vasodilatation, regardless of the presence or absence of atherosclerotic wall thickening [Puranik and Celermajer, 2003]. Endothelial dysfunction results in abnormal regulation of blood vessel tone and loss of atheroprotective properties of normal endothelium [Yufu, et al., 2009]. Therefore, impaired endothelial function appears as an important pathogenic mechanism for atherosclerosis and may be an early manifestation of certain cardiovascular diseases [Yufu, et al., 2009]. Normal endothelial cells promote vasodilation and inhibit atherosclerosis and thrombosis in part because of the release of nitric oxide [Harrison, 1997]. Not only nicotine is found in smoke, other components of cigarettes lead to impaired nitric oxide (NO) production and increased damage of endothelium through increased oxidative stress as well [Ambrose and Barua, 2004]. Free radicals and aromatic compounds diminish the endothelial synthesis of NO, causing impaired endothelium-dependent relaxation of arteries, the earliest clinical sign of endothelial dysfunction [Powell, 1998]. Smoking alters the shear forces and rheology at the endothelial surface and these changes enhance the effects of products of tobacco combustion to up-regulate leucocyte adhesion molecules on the endothelial surface [Powell, 1998].

Cigarette smoke contains several thousands of chemical compounds that include toxic substances such as carbon monoxide and polycyclic aromatic hydrocarbons, as well as metals, many free radicals that can initiate oxidative damage [Zhang, et al., 2006; Benowitz and Gourlay, 1997]. It is not clear which components of cigarette smoke are responsible for endothelial damage and contribute to the pathogenesis of cardiovascular disease. The products of tobacco combustion are absorbed and metabolized into the systemic circulation and injure the arterial endothelium and promote atherogenesis [Powell, 1998].

Yamaguchi, et al. (2007) acutely exposed rats to the gas phase of cigarette smoke from which nicotine and tar had been removed, serum levels of oxidative stress markers rapidly increased. They suggested that stable oxidants in cigarette smoke extract can pass through the pulmonary alveolar wall into the blood and

induce systemic oxidative stress, which may facilitate endothelial dysfunction [Yamaguchi, et al., 2007]. This indicates that some other smoke components are expected to participate in the functional changes caused by smoking [Toda and Toda, 2010].

However, nicotine is the most widely studied compound of cigarette smoke. It has a variety of effects on vascular biology that may contribute to atherosclerosis [Saijonmaa, et al., 2005]. Clinical studies have shown that cigarette smoking acutely increases plasma levels of adrenaline and noradrenaline [Benowitz and Gourlay, 1997]. The inhalation of cigarette smoke results in rapid absorption of nicotine, with consequent systemic release of adrenaline and noradrenaline. These catecholamines bind to α1-adrenergic receptors on vascular smooth muscle cells to cause muscle contraction and vasoconstriction [Powell, 1998]. As well, the release of catecholamines has important effects on cardiac function, vascular tone and lipid metabolism [Powell, 1998].

Cigarette smoking increases heart rate (HR), blood pressure (BP) and plasma concentration of norepinephrine, as well as skin sympathetic nerve activity [Shinozaki et al., 2008]. These changes are attenuated markedly by α-adrenergic and β-adrenergic blockade, indicating that these haemodynamic effects of cigarette smoking are derived from sympathetic activation [Shinozaki, et al., 2008]. Also the increase in coronary vascular resistance due to cigarette smoking is inhibited by α-adrenoceptor blocking agents [Benowitz and Gourlay, 1997].

Cigarette smoking is associated with reduced endothelium-dependent vasodilatation, endothelial nitric oxide synthase (eNOS) activity and NO generation [Barua, et al., 2001]. Smoking and nicotine share many detrimental actions on blood flow regulation through NO [Toda and Toda, 2010].

Cigarette smoking could cause endothelial damage and accelerate atherosclerosis by a variety of mechanisms. Some of these are haemodynamic stress, oxidant injury, neutrophil activation, enhanced thrombosis, platelet activation and increased fibrinogen and blood viscosity [Benowitz and Gourlay, 1997; Shinozaki, et al., 2008]. Clinical and experimental studies indicate that either active or passive exposure promotes vasomotor dysfunction, atherogenesis and thrombosis in multiple vascular beds [Ambrose and Barua, 2004].

Assessment of microvascular function is of major importance in understanding the physiology of vasculature and in investigating the vascular effects of pathological conditions [Tesselaar and Sjöberg, 2010]. There are many clinical and experimental reports showing the acute sympathetic effects of cigarette smoking. There are several methods to evaluate endothelial function in humans. Studies on forearm blood flow changes in response to NO-releasing substances and physical stimuli are commonly used [Toda and Toda, 2010]. Measurements of forearm blood flow responses to endothelium-dependent vasodilators, shear stress, and eNOS inhibitors in humans is quite a useful tool for evaluating endothelial functioning under basal and active conditions not only at the brachial vascular area but also to assess the functional state of endothelial cells in other discrete vascular regions [Toda and Toda, 2010].

The aim

The aim of the study was to assess the effects of acute cigarette smoking (CS) on skin microvascular responsiveness by vasodilatory capacity and haemodynamic measurements in healthy habitual smokers.

Material and methods

Subjects. 20 healthy habitual male smokers were included in the study. They were matched by age: 10 young smokers (YS) and 10 adult smokers (AS). We did not include participants with acute inflammatory condition or chronic inflammatory state, hypertension and metabolic syndrome. None of them had used any medication for some months before the examination. The characteristics of study subjects are shown in Table 1. All subjects gave their informed consent to the protocol, which was approved by the local Medical Ethics Committee of the University of Latvia for Biomedical Research.

Table 1. The characteristics of the study subjects*

Category	Young smokers (YS) (n = 10)	Adult smokers (AS) (n = 10)	p value
Age, years	26 ± 6	44 ± 6	< 0.05
BMI, kg/m^2	24 ± 4	28.4 ± 4.6	< 0.05
Waist circum, cm	88.6 ± 12.1	98.6 ± 16	NS
Cigarettes/day	14 ± 5	16 ± 5	NS
Pack years**	8.4 ± 4	15.7 ± 7	< 0.05

* Data presented as mean ± SD, NS – not significant.

** Pack years were calculated by the formula: number of pack years = (number of cigarettes smoked per day × number of years smoked)/20

Cutaneous perfusion measurements. We used laser Doppler imaging (LDI) equipment (MoorLDI2, Moor Instruments Ltd., UK) for non-invasive and continuous measurement of perfusion changes during vascular provocations in the skin of the dorsum of the palm. The vasodilatory response to the local heating application of +44 °C and the iontophoretic administration of 1% acetylcholine (ACh) were studied before and after cigarette smoking. Laser Doppler flux was expressed in arbitrary perfusion units (PU). Coefficient of variation with LDI method was 20.7% [Millet, et al., 2011].

Local thermal hyperemia. The vasodilatory response of cutaneous microvessels to a step increase in local temperature is biphasic, with an initial peak occurring within minutes, followed by a nadir, and then a late phase with a progressive rise to a plateau in 20–30 min. [Golay, et al., 2004]. This maximal thermal vasodilatation corresponds to the maximal vasodilator capacity of the vessels. Local heating-evoked vasodilatation is mediated by at least two independent mechanisms. The initial rapid phase relies predominantly on local sensory nerves and is mediated by an axon reflex thought to be dependent on calcitonin-gene-related peptide (CGRP) and substance P [Minson, et al., 2001; Golay, et al., 2004]. A more slowly late phase largely is endothelium-dependent vasodilatation that relies on local production of NO, because it is suppressed by inhibitors of NO synthase and insensitive to local anesthesia [Minson et al., 2001] and prostanoids do not seem to have a role [Golay, et al., 2004].

Acetylcholine iontophoresis. Iontophoresis is based on the principle that a charged drug in solution migrate across the skin under the influence of a direct low-intensity electric current [Kalia, et al., 2004]. Endothelial function can be assessed in humans by studying vascular response to an agonist such as ACh, which is known to release NO and prostacyclin (PGI_2) from endothelial cells [Christen, et al., 2004]. The iontophoretic stimulation produces a stimuli-response plateau with maximal ACh-mediated vasodilation at the end of the measurement.

Haemodynamic measurements. Systolic and diastolic blood pressure and heart rate were determined with an automatic device (Omron M6), with accuracy of the measurement for blood pressure: ± 3 mmHg; for pulse: ± 5% of display reading. Measurements were performed before LDI scanning was started. The average of the three measurements during each period was calculated [Ijzerman, et al., 2003].

Study design. Measurements were conducted in a quiet, temperature-controlled room (22 ± 0.5 °C), with the subjects in supine position. All subjects were asked to refrain from caffeine and alcohol-containing drinks on the examination day, and to refrain from smoking for at least 3 hours before the examination. Microvascular and haemodynamic measurements were obtained after 15 minutes of acclimatization and were performed with the investigated hand at heart level. The basal blood flow was studied for 3 minutes and the microcirculatory measurements of local heating +44 °C (LDI-heating) – for 25 minutes. Iontophoretic application of 1% acethylcholine solution (LDI-Ach) was performed to the other hand. The basal blood flow also was calculated during the first 3 minutes and the microcirculatory response to Ach iontophoresis was measured for 13 minutes.

After the LDI-heating test each participant was asked to smoke a cigarette. After smoking, the local heating test was repeated. In addition, blood pressure and heart rate were measured 5 minutes after smoking. After the test participants were asked to smoke another cigarette and Ach iontophoresis test was repeated anew.

The acute effects of smoking on microvascular responsiveness and haemodynamic parameters were compared with the results before smoking in both study groups.

Statistical analysis. Data were analysed by STATISTICA 7.0 software (StatSoft Inc, USA). After testing normality, the data were expressed as mean ± SD and differences between groups were analysed using the Mann-Whitney U test (despite normal distribution of the data, we used nonparametric statistical method because of a small number of participants). A two-tailed value of $p < 0.05$ was considered to be significant.

Results

Microvascular responsiveness. There was no significant difference in basal blood flow of skin as compared before and after CS in both study groups.

The vasodilatory response to the local heating was slightly increased via an axon reflex-mediated vasodilatation (LDI-heating$_{1.max}$) (Figure 1), and obviously tended to decrease in endothelium-dependent vasodilatation (LDI-heating$_{2.max}$) (Figure 2) in both study groups due to CS. However, in response to Ach iontophoresis we observed significantly decreased endothelium-dependent vasodilatation (LDI-Ach$_{max}$; $p < 0.05$) in both YS and AS groups (Figure 3) after CS. Results are represented in Table 2.

Haemodynamic measurements. Only young smokers demonstrated significantly increased systolic ($p < 0.05$) and diastolic ($p < 0.05$) BP and HR ($p < 0.05$) due to CS. Adult smokers showed significantly increased systolic ($p < 0.05$) and slightly increased diastolic BP and HR after cigarette smoking. Measurements are represented in Table 2.

Furthermore, we found negative correlation of LDI-Ach$_{max}$ values with pack years ($p = 0.02$; $r = -0.52$) (Figure 4) and age ($p = 0.026$; $r = -0.50$). As well as, LDI-heating$_{1.max}$ values significantly correlated with pack years ($p = 0.05$; $r = -0.43$) and age ($p = 0.005$; $r = -0.60$). Results are represented in Table 3.

Figure 1. Local heating +44 °C induced vasodilatation (LDI-heating$_{1.max}$) in young smokers (YS) and adult smokers (AS) before and after cigarette smoking (Data are expressed as mean ± SD)

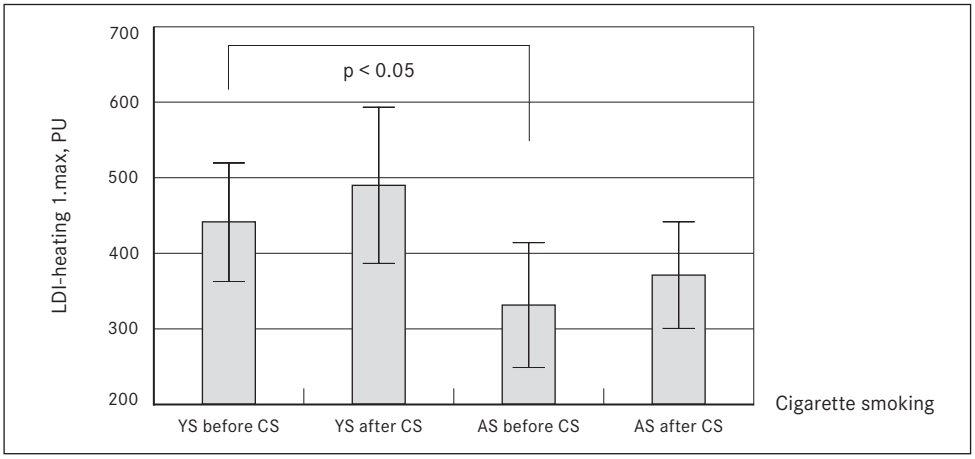

Figure 2. Local heating +44 °C induced vasodilatation (LDI-heating$_{2.max}$) in young smokers (YS) and adult smokers (AS) before and after cigarette smoking (Data are expressed as mean ± SD)

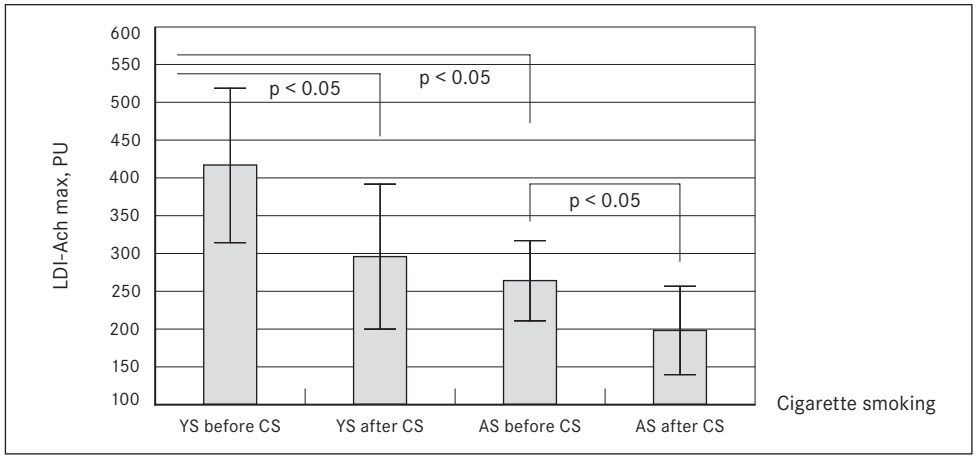

Figure 3. Acetylcholine induced vasodilatation in young smokers (YS) and adult smokers (AS) before and after cigarette smoking (Data are expressed as mean ± SD)

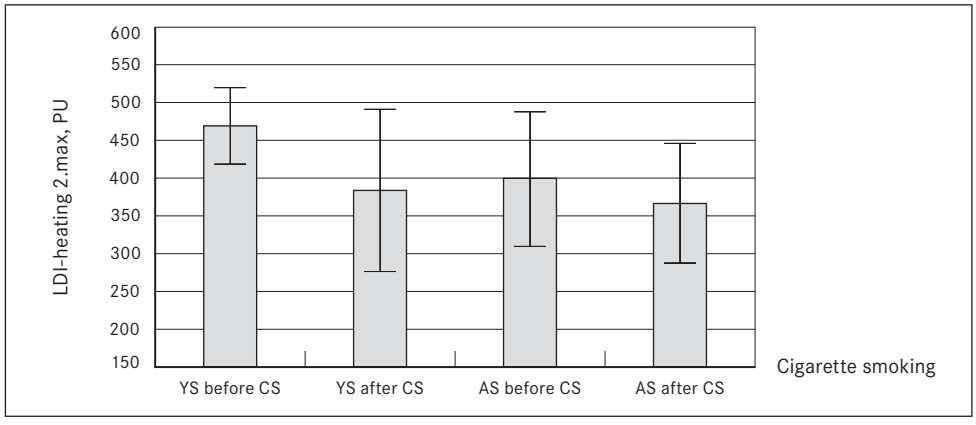

Table 2. Values of microvascular and haemodynamic measurements before and after cigarette smoking in young smokers (YS) and adult smokers (AS) (Values presented as mean ± SD)

Type of measurement	Younger smokers (YS) mean ± SD			Adult smokers (AS) mean ± SD		
	Before CS	After CS	p value	Before CS	After CS	p value
LDI-heating $_{basal}$, PU*	81.4 ± 23.9	87 ± 36.8	NS	94.3 ± 39.9	84.2 ± 40.5	NS**
LDI-heating $_{1.max}$, PU	440.5 ± 78.7	490 ± 108	NS	335.5 ± 78	375.2 ± 80	NS
LDI-heating $_{2.max}$, PU	465 ± 55	390 ± 108	NS	400 ± 88.4	365.4 ± 84	NS
LDI-Ach $_{basal}$, PU	68 ± 27.5	66.3 ± 31	NS	71.2 ± 31.5	60.8 ± 22	NS
LDI-Ach $_{max}$, PU	415.5 ± 97	300.5 ± 98	< 0.05	262.6 ± 58	199.8 ± 52	< 0.05
Systolic pres, mmHg	119.8 ± 8	128 ± 9	< 0.05	126 ± 10	136 ± 9.8	< 0.05
Diastolic pres, mmHg	76.6 ± 7.8	84 ± 7	< 0.05	81.2 ± 13	86.3 ± 12	NS
Heart rate, beat/min.	63.3 ± 5	71.4 ± 7.8	< 0.05	65.3 ± 7.8	68.5 ± 8	NS

* PU – perfusion units.
** NS – not significant.

Figure 4. Correlation of pack years and acethylcholine induced endothelium-dependent vasodilatation (LDI-Ach$_{max}$) in all study subjects

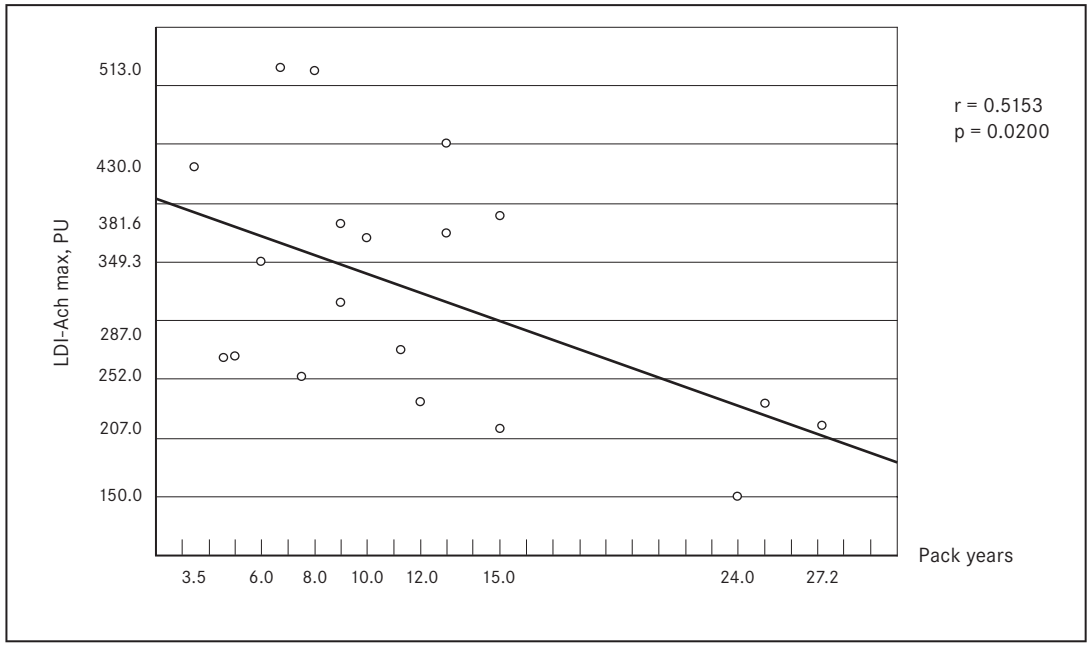

Table 3. Correlations of pack years and age with microvascular responsiveness

Category	LDI-heating $_{1.max}$		LDI-Ach $_{max}$	
	r	p	r	p
Pack years	−0.43	0.05	−0.52	0.02
Age	−0.60	0.005	−0.50	0.026

Discussion

Several studies have demonstrated that both active and passive cigarette smoke exposure were associated with impaired endothelium-dependent vasodilation in macrovascular beds such as coronary and brachial arteries and in microvascular beds [Ambrose and Barua, 2004]. In many experimental and clinical studies the role of nicotine and effect of acute cigarette smoking-induced decrease in vasodilatory function has been reported. Nicotine in cigarette smoke is probably the most studied component among all other chemical compounds. Although nicotine plays a major role in smoking-related increases in cardiac output, heart rate, and blood pressure [Ambrose and Barua, 2004; Ijzerman, et al., 2003], the precise mechanisms responsible for negative effect of nicotine on endothelial function remain unclear [Neunteufl, et al., 2002].

The findings in the study of Neunteufl, et al. demonstrated that nicotine causes acute endothelial dysfunction in long-term smokers and suggest that there may be other constituents of cigarette smoke that contribute to this adverse effect [Neunteufl, et al., 2002]. Cigarette smoke contains large amounts of free radicals, which may injure the endothelium [Ambrose and Barua, 2004; Ijzerman, et al., 2003; etc.].

We observed important increase in systolic and diastolic blood pressure and heart rate after acute cigarette smoking in our study subjects. These effects are in accordance with the results of other studies. The other studies also showed increased blood pressure and heart rate after smoking a cigarette [Ijzerman, et al., 2003; Shinozaki, et al., 2008].

The observed the increase in BP after smoking could be due to sympathetic activation. Increased stiffness of large vessels in cigarette smokers [Celermajer, et al., 1993; Yufu, et al., 2009], also impaired vasorelaxant capacity of the coronary circulation cause a rise of the total peripheral vascular resistance,

thus increasing the workload of the heart [Andersson, et al., 2003]. Decreased endothelium-dependent vasodilatation has been observed not only in microcirculation, but also in flow-mediated brachial arterial dilatation in smoking subjects [Celermajer, et al., 1993; Yufu, et al., 2009].

Moreover, in our study we found a tendency of increase in peripheral blood flow observed in initial phase in response to local heating due to acute cigarette smoking. This neurally regulated vasorelaxation was observed in young and adult smokers. Nevertheless, endothelium-dependent vasodilatation observed in late phase mediated by local thermal hyperemia was decreased in both study groups after smoking a cigarette. This attenuated vascular responsiveness did not reach statistical significance, most probably due to the small study groups. Sympathetic activation due to nicotine was observed in the initial peak phase in our results. In addition, the role of nicotine to inhibit the activity of nitric oxide synthase may explain decreased vasodilatory capacity in plateau phase of our study. In several studies acute smoking was associated with impaired microvascular endothelium-dependent vasodilatation [Ijzerman, et al., 2003], indicating the harmful effect of cigarette smoking on the microcirculation. Endothelium-dependent blood flow responses are indicative of stimulated nitric oxide production that has been observed as decreased in cigarette smokers [Guthikonda, et al., 2003], thus, our data showed that stimulated nitric oxide production decreased after cigarette smoking in both study groups.

According to Butler, et al. (2001a), cigarette smoking was associated with blunted basal NO bioactivity [Butler, et al., 2001]. In our study we did not find attenuated basal tone of skin microcirculation either younger or adult smokers due to acute cigarette exposure. Stimulated nitric oxide responses may be a more sensitive marker of endothelial dysfunction than basal nitric oxide [Guthikonda, et al., 2003].

Endothelial function can be assessed in humans by studying the vascular response to an agonist such as ACh, which is known to release both NO and PGI_2 from endothelium [Pellaton, et al., 2002]. It has been found in other studies – attenuated Ach-induced vasodilatation in cutaneous blood flow in long-term smokers [Toda and Toda, 2010; Butler, et al., 2001].

Our results also showed significantly diminished Ach-induced vasodilatation in both study groups due to acute cigarette smoking. Interestingly, we had observed significantly decreased vasodilatory response to acetylcholine iontophoresis and local thermal hyperemia in adult smokers compared with younger smokers before cigarette smoke exposure. This is in accordance with other several studies. Pellaton, et al. (2002) had reported that the endothelium-dependent vasodilatation in the skin microvasculature is impaired in subjects who have smoked cigarettes for many years [Pellaton, et al., 2002]. Similar observation had been found in macrocirculation where flow-mediated brachial arterial dilatation was impaired or absent in cigarette smokers. As well, a flow-mediated dilatation was inversely related to lifetime dose smoked [Toda and Toda, 2010]. Our results showed significant negative correlations between microcirculation function and pack years (Table 3).

Several recent large epidemiologic studies have shown a trend for more cardiovascular events in heavier active smokers. There has been found a significant dose-dependent correlation between cardiovascular risk and the number of cigarettes smoked or the pack years of exposure [Ambrose and Barua, 2004].

Conclusions

In this study we observed that acute cigarette smoking attenuates microvascular endothelium-dependent vasodilatation and contributes importantly to cardiovascular sympathetic activity in healthy men. Moreover, acute cigarette smoking increased neurally regulated – neuropeptides mediated vasodilatation due to local thermal hyperemia in both age groups.

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References

1. Ambrose J. A., Barua R. S. The pathophysiology of cigarette smoking and cardiovascular disease // J Am Coll Cardiol, 2004; 43: 1731–1737.
2. Andersson S. E., Edvinsson M. L., Edvinsson L. Cutaneous vascular reactivity is reduced in aging and in heart failure: association with inflammation // Clin Sci, 2003; 105(6): 699–707.
3. Barua R. S., Ambrose J. A., Eales-Reynolds L. J., et al. Dysfunctional endothelial nitric oxide biosynthesis in healthy smokers with impaired endothelium-dependent vasodilatation // Circulation, 2001; 104: 1905–1910.
4. Benowitz N. L., Gourlay S. G. Cardiovascular toxicity of nicotine: implications for nicotine replacement therapy // J Am Coll Cardiol, 1997; 29: 1422–1431.
5. Butler R., Morris A. D., Struthers A. D. Cigarette smoking in men and vascular responsiveness // Br J Clin Pharmacol, 2001; 52: 145–149.
6. Celermajer D. S., Sorensen K. E., Georgakopoutos D., et al. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults // Circulation, 1993; 88: 2149–2155.
7. Christen S., Delachaux A., Dischl S., et al. Dose-dependent vasodilatory effects of acetylcholine and local warming on skin microcirculation // J Cardiovasc Pharmacol, 2004; 44(6): 659–664.
8. Golay S., Haeberli C., Delachaux A., et al. Local heating of human skin causes hyperemia without mediation by muscarinic cholinergic receptors or prostanoids. J Appl Physiol, 2004; 97: 1781–1786.
9. Guthikonda S., Sinkey C., Barenz T., Haynes W. G. Xanthine oxidase inhibition reverses endothelial dysfunction in heavy smokers // Circulation, 2003; 107: 416–421.
10. Harrison D. Cellular and molecular mechanisms of endothelial dysfunction // J Clin Invest, 1997; 100: 2153–2157.
11. Ijzerman R. G., Serné E. H., van Weissenbruch M. M., et al. Cigarette smoking is associated with an acute impairment of microvascular function in humans // Clinical Science, 2003; 104: 247–252.
12. Kalia Y. N., Naik A., Garrison J., Guy R. H. Iontophoretic drug delivery // Adv Drug Deliv Rev, 2004; 56: 619–658.
13. Millet C., Roustit M., Blaise S., Cracowski J. L. Comparison between laser speckle contrast imaging and laser Doppler imaging to assess skin blood flow in humans // Microvascular Research, 2011; 82(2): 147–151.
14. Minson C. T., Berry L. T., Joyner M. J. Nitric oxide and neurally mediated regulation of skin blood flow during local heating // J Appl Physiol, 2001; 91: 1619–1626.
15. Neunteufl T., Heher S., Kostner K., et al. Contribution of nicotine to acute endothelial dysfunction in long-term smokers // J Am Coll Cardiol, 2002; 39: 251–256.
16. Pellaton C., Kubli S., Feihl F., Waeber B. Blunted vasodilatory responses in the cutaneous microcirculation of cigarette smokers // Am Heart J, 2002; 144(2): 269–274.
17. Powell J. T. Vascular damage from smoking: disease mechanisms at the arterial wall // Vascular Medicine, 1998; 3: 21–28.
18. Puranik R., Celermajer D. S. Smoking and endothelial function // Prog Cardiovasc Dis, 2003; 45(6): 443–458.
19. Saijonmaa O., Nyman T., Fyhrquist F. Regulation of angiotensin-converting enzyme production by nicotine in human endothelial cells // AJP – Heart, 2005; 289(5): 2000–2004.
20. Shinozaki N., Yuasa T., Takata S. Cigarette smoking augments sympathetic nerve activity in patients with coronary heart disease // Int Heart J, 2008; 49(3): 261–272.
21. Tesselaar E., Sjöberg F. Transdermal iontophoresis as an in-vivo technique for studying microvascular physiology // Microvasc Res, 2011; 81(1): 88–96.
22. Toda N., Toda H. Nitric oxide-mediated blood flow regulation as affected by smoking and nicotine // European Journal of Pharmacology, 2010; 649: 1–13.
23. Yamaguchi Y., Nasu F., Harada A., Kunitomo M. Oxidants in the gas phase of cigarette smoke pass through the lung alveolar wall and raise systemic oxidative stress // J Pharmacol Sci, 2007; 103: 275–282.
24. Yufu K., Takahashi N., Okada N., et al. Influence of systolic blood pressure and cigarette smoking on endothelial function in young healthy people // Circ J, 2009; 73: 174–178.
25. Zhang W. Z., Venardos K., Chin-Dusting J., Kaye D. M. Adverse effects of cigarette smoke on NO bioavailability: role of arginine metabolism and oxidative stress // Hypertension, 2006; 48: 278–285.

Use of Blood Erythrocytes for *in vivo* Estimation of Adrenoreceptors Functional State

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Abstract

The study included the investigation of the binding of n-toluene sulfonate4-(n-dimethylaminostyrene)-I-methylpyridinium (DSM) to human and animal erythrocyte membrane. The spectral characteristics and binding parameters of the probes in norm and in various pathologies, at extreme effects on the organism, at changing adrenoreactivity of the animal organism were found to differ. It is concluded that the probes can be used to assess the changes in the membrane properties and in the beta-adrenoreceptive function of both the erythrocytes and the organism as a whole.

Keywords: erythrocytes, beta-adrenoreceptors, symphatetic nervous system.

Introduction

Analysis of the structural–functional organization of cell membranes, specifically, the studies of the functional state of beta-adrenoreceptors is a topical problem in medicine and biology. Earlier researches have shown the unidirectivity of the changes in the adrenoreceptive properties of various tissues and organisms, red blood cells including, during the pharmacological action on the adrenergic mechanisms of regulation in an organism [Sominsky, Berdisheva, Bluma, Kalnina, 1989; Somisky, Anshelevich, Okun, 1990]. To a high degree the symphatetic nervous system status indirectly may be determined by catecholamine content in blood and urine. The direct detection of functional activity of adrenoreceptors is methodologically complicate and therefore not suitable for systematic determination. [Doule, Fraser, 1961]. Thus, red blood cells are a convenient test object which makes it possible to judge on the changes in the adrenoreactivity of an organism on the whole. Cell and membrane studies make extensive use of fluorescent probes [Lakowicz, 2006]. The membrane probe-cation DSM (n-toluene-sulfonate-4-(n-dimet hylaminostyrene)-I-methylpyridinium) possess such an advantageous combination of properties as low toxicity for cells, considerable increase of the fluorescence quantum yield during their binding to cell structures. Fluorescence of this probe is sensitive to the change in polarity, viscosity of the medium. There are also data in the literature on the polychromaticity of the properties of DSM [Dobrecov, et al., 1981]. In this context, the probe DSM in studies of the structural / functional properties of the human and animal erythrocyte membrane was used.

Material and methods

The research design used in this study was approved by the Ethics Committee of Rīga Stradiņš University (Latvia), and all patients enrolled in this investigation had provided their informed consent.

Three groups of patients were examined in this study: group 1 comprised 10 healthy subjects (at the age of 39 on the average, including 3 women); group 2 comprised 13 patients suffering cardiac infarction, during the first three days of their illness (at the age of 62 on the average, including 3 women); group 3 comprised 10 patients suffering unstable stenocardia (at the age of 59 on the average, including 4 women); To serve as controls 24 (average 32–68 year-old volunteers (of both sexes) were employed to ascertain normal levels of erythrocyte fluorescence intensity and other characteristics.

Blood collection. Blood samples from every patient were collected usually between 8–10 a.m. (for fasting values). Then blood from healthy volunteers was collected; this was also performed between 8–10 a.m. In all cases, the peripheral venous blood samples were collected into vacutainer tubes containing preservative – free heparin (30 IU per tube). A total volume of ~7 ml was routinely the target collection volume.

Changing the adrenoreactivity of the animal's organism. Tests of the change in the adrenoreactivity were performed on guinea pigs and rats (line Wistar, weight 150–250 g). Increased adrenoreactivity was due to intraperitoneal injection of guanethidine (30–50 mg/kg, 24 h) [Evans, Iweyama, Burnstock, 1973]. Decreased adrenoreactivity was caused by intraperitoneal injection of propranolol (5 mg/kg, 20 min) [Rasmussen, Lake, Allen, 1975]. The preparations were manufactured by the company VEB Arzneimittelwerk, Germany. For the purpose of control, animals subjected to intraperitoneal injection of physiological, salt solution was used. Blood was obtained by decapitation of animals.

Preparation of erythrocyte suspension. The erythrocytes were separated out from fresh blood by high-speed centrifugation at 1700 g, followed by three-times washing of the precipitate with isotonic phosphate buffer. Thereafter, 0.5 ml of the erythrocytes precipitate was suspended in 3.7 ml of the same buffer, thus obtaining a suspension featuring 10% hematocrit.

Optimal conditions of DSM binding to the erythrocytes membrane. The fluorescent probe (p-toluenesulfonate-4-(p-dimethylaminostyrene)-1-methylpiridinium DSM) was synthesized at the Latvian Institute of Organic Synthesis, Latvian Academy of Sciences [Dobrencov, et al., 1981]. The photostability factor (Δ) of DSM solution in phosphate buffer was determined by the change in fluorescence intensity at the action on a sample of a monochrome illumination of a wavelength corresponding to maximum in the emission spectra, where F_0 is the fluorescence intensity at the initial time t_0. F_t is the fluorescence intensity at time (t). Concentration of the probe corresponding to the detection limit *c min*) was calculated by the formula $c_{min} = \Delta F_{min} / a$, where a is the angwular coefficient of the calibration curve; $\Delta F_{min} = 3\ S$, where S is the root-mean-square deviation of the fluorescence intensity index for a blank sample (supernatant over erythrocytes without probe added). The kinetics of probe binding to erythrocyte membrane was followed by the amount of the probe bound to the membrane depending on the duration of their joint incubation. The amount of the erythrocyte-bound probe was determined as the difference between the total amount of the probe added and that of free probe. The content of free probe in the supernatant was calculated by the calibration curve of the dependence of fluorescence intensity on the concentration of the probe added to the supernatant.

Sample preparation and fluorescence measurements. The erythrocyte suspension was incubated with DSM (concentration in sample 8.05 μM) at room temperature (18–20 °C) for 5 minutes. The time interval (one hour) between the cell isolation and the measurement of fluorescence was constant for all samples. The fluorescence parameters were registered on a Spectrofluo JY 3 spectrofluorimeter (ISA Jobin Yvon Instruments, S. A., Longjumeau, France) at excitation wavelengths 470 nm and 495 nm and on emission wavelength of 520–700 nm. The amount of the erythrocyte-bound probe (expressed in μmol per l of erythrocyte suspension) was determined as the difference between the total amount of the probe added to 1 ml of erythrocyte suspension and that of free probe. The content of free probe in

the supernatant was calculated by the calibration curve of the dependence of fluorescence intensity on the concentration of the probe added to the supernatant. Because the quantum yield of luminescence of the bound probe was much higher than that of the free probe, the registered intensity of fluorescence (F) of the probe in $A_F = \frac{F_0 - F_1}{F_0} \times 100\%$ erythrocyte suspension is in fact determined by the probe immobilized on the membrane. To register the luminescence from the surface of its excitation, the sample was placed into l × 10 × 40 mm^3 cuvette fixed at an angle of 30' to the excitation light beam. To investigate the properties of cell membranes, the following parameters of probe binding: concentration of bound probe were determined; binding constant K; number of binding sites N; intensity in the maximum of the shortwave band of the probe fluorescence in erythrocyte suspension; the ratio of the intensities of the fluorescent probes in the maxima of the shortwave and long wave bands (560–565 nm and 600~610 nm).

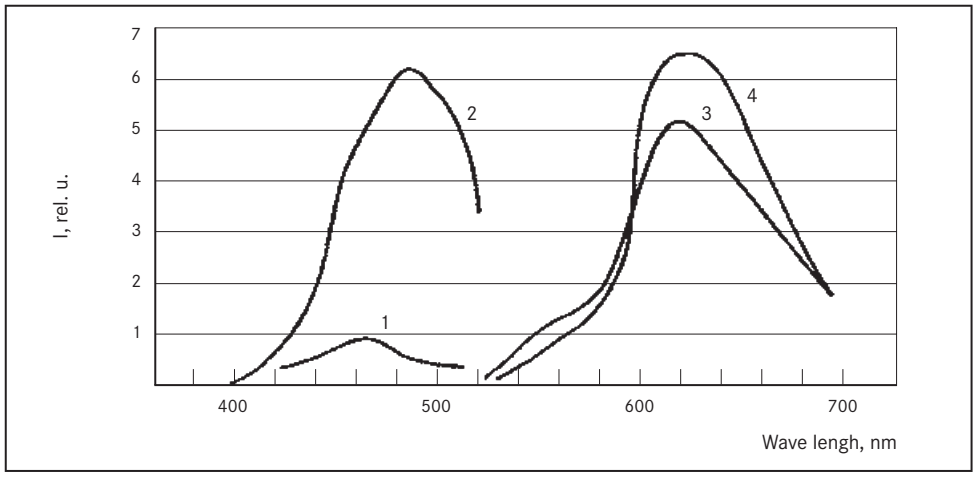
Determination of the DSM binding parameters. To investigate the properties of cell membranes, we determined the following parameters of probe binding: concentration of bound probe c; binding constant K; number of binding sites N; intensity of the probe fluorescence in the maximum of the shortwave and long wave bands in erythrocyte suspension; the ratio of intensities of the fluorescent probes in the maxima of short wave and long wave bands (560–565 nm and 610–620 nm). The amount of erythrocyte-bound probe (expressed in μmol per l of erythrocyte suspension) was determined as the difference between the total amount of the probe added to 1 ml of erythrocyte suspension and that of free probe. The content of free probe in the supernatant was calculated by the calibration curve of the dependence of fluorescence intensity on the concentration of the probe added to the supernatant. Parameters K (binding constant) and N (binding sites number) were determined by the graphic method of Klotz [Baulie, Raynod, 1970].

Statistical analysis. Statistical differences among groups having different spectral characteristics were determined using Students t-test and Mann-Whitney U-test. Correlative relationship between spectral characteristics of DSM and the measured binding parameters were determined as outlined by Currel and Dowman [Currel, Dowman, 2010]. Data are expressed as means $M \pm m$.

Results

Spectral characteristics of DSM. The maxima of the excitation spectrum for the probe in buffer (pH 7.0) were observed at 450 nm; these of the fluorescence emission at 620 nm. We revealed two bands of luminescence of the erythrocyte-bound probe in erythrocyte suspension with the maxima within 560–565 nm and 610–620 nm. Each of those bands had its excitation maximum (at 470 nm and 495 nm, respectively; see Figure 1).

Figure 1. Excitation (1, 2) and emission (3, 4) spectra of DSN fluorescence in erythrocyte suspension*



* The emission spectra were obtained at λ_{ex} 470 (3) and 495 (4) nm; the excitation spectra were recorded at λ_{cm} 560 (1) and 640 (2) nm. Concentration of the probe – 50 μM, hematocrit 10%, monochromator slits – 2.86 nm.

Such shape of the spectrum is due to two different chromophores formed during the sorption of the probe on the outer surface of the erythrocyte membrane and in the more hydrophobic region of the lipid bilayer. The fluorescence of the probe in the red region of the spectrum contributes to the high analytical sensitivity of the method.

Optimal conditions of probe binding to the erythrocyte membrane. The intensity of DSM decreased maximally (6.3%) in 20.0 ± 2.0 min. The photostability index makes it possible to assess the error due to the photodecomposition of the sample and, if necessary, apply a correction both during the single measurements in a given point of the spectrum and in studies of binding kinetics of a probe.

To select the working range of probe, concentration was established at the limit of which the supernatant over erythrocytes can still be found. The concentations of the DSM used (3–16 mkM) during the determination of the parameters of their binding to erythrocytes significantly exceeded the indices of determination limit and provided for the linear rise of the intensity of fluorescence in the Klotz plot.

These are conditions within which the use of probe DSM can be considered to be correct and most efficient.

Studies of the properties of cell membranes using fluorescent probe. To investigate the properties of cell membranes, we determined the binding parameters of the probes in the following cases:

1) at various diseases accompanied by disturbed states of cell membranes (myocardial infarction, neurocirculatory dystonia), by the hypertensive type, instable stenocardia (Table 1);
2) at changing adrenoreactivity of the animal organism (guinea pigs, rats) (Table 2).

The beta-adrenoreceptor function of erythrocyte membranes was assessed studies of the DSM binding during the changing adrenoreactivity of the organism. The studies were done on guinea pigs and rats. At the increased adrenoreactivity of the organism it was discovered that the values of binding sites number *N* go up and those of binding constant *K* down, while at the decreased adrenoreactivity there was a lowering of *N* and rise of *K* (Table 1) As judged by the changes of fluorescence intensity in the maximum of the long wave band (F, 610 nm) at the increased adrenoreactivity of the organism, the concentration of the probe bound to the animals erythrocytes increased, at decreased one, it goes down.

Studies of the pathologies also revealed a decrease of concentration of bound probe, binding constant *K* and increase of binding sites concentration *N* as compared with the initial data (Table 2). It should be noted that the values of obtained parameters for each group of cardio-vascular patients differ from the respective parameters for other groups (in relation of pronouncement of disease severity).

It should be noted the similarity in the character of the membrane response to changed adrenoreactivity of organism and in cardio-vascular diseases.

Table 1. Parameters of DSM binding to erythrocyte membrane from healthy subjects and patients ($M \pm m$)

No	Disease	Concentration of bound probe, μM	K, μM^{-1}	N, μM
1	Control (healthy subjects)	4.76 ± 0.12	2.00 ± 0.06	1.40 ± 0.05
2	Myocardial infarction	2.8 ± 0.22	0.25 ± 0.07	2.41 ± 0.06
3	Instable stenocardia	3.41 ± 0.27	0.75 ± 0.07	1.58 ± 0.07
$p < 0.05$ between groups		1–2; 1–3; 2–3	1–2; 1–3; 2–3	1–2; 2–3

Note: Concentration of the probe added, 8.05 μM.
K – binding constant.
N – number of binding sites.

Table 2. Changes in parameters of DSM binding to erythrocytes after injection of guanethidine and propranolol to animals

Variant	*R*, μM	*K*, μM^{-1}	*N*, μM	I_p (560), rel.u.	F_p (610), rel.u.
Guinea-pigs					
Control	–	0.072 ± 0.003*	29.4 ± 1.5*	5.0	6.0
Guanethidine	–	0.051 ± 0.003*	50.0 ± 1.7*	4.7	7.0
Propranolol	–	0.095 ± 0.005*	24.8 ± 1.1*	4.9	4.9
Rats					
Control	3.85 ± 0.14	0.084 ± 0.012*	10.96 ± 1.56**	ND	ND
Guanethidine	4.42 ± 0.15	0.050 ± 0.007*	20.17 ± 3.17**	ND	ND

* $p < 0.01$

** $p < 0.05$

Note: Increased adrenoreactivity was due to intraperitoneal injection of guanethidine (30–50 mg/kg, 24 h); decreased reactivity, by intraperitoneal injection of propranolol (5 mg/kg, 19 min).

ND – not defined.

K – binding constant.

N – number of binding sites.

F (560 nm), *F* (610 nm) – fluorescence intensity of probe in the maximum of the short wave and long wave band, correspondingly.

Discusion

It is well known that a complex of biochemical modifications, predetermining development of various pathologies (cardio-vascular disorders, including also stress), is first of all characterized by disturbances in lipid metabolism. In various pathologies and at extreme effect on the organism the system of the antioxidant protection of the lipids is enhanced, the share of cholesterol is increased, and the lipid set in the erythrocyte membrane is changed [Mozffarian, Micha, Wallace, 2010; Moe, Wong, 2010; Nusier, El-Akawi, Abd-Alkareem, 2006].

Increase of molar relation cholesterol/phospholipids in erythrocyte membrane with patients suffering with hypertonic and ischemic cardiac diseases leads to changes in physico-chemical properties of this membrane, in particular, to increase of micro-viscosity of lipid bilayer, increase of its hardness and reduction of erythrocytes deformability [Mozaffarian, et al., 2010; Schalk, et al., 2006].

All these affect the kinetics and parameters of probe incorporation into the erythrocyte membrane.

It should be noted that in most cases investigated, the change in concentration of bound probe correlates with the dynamics of changes of parameters *N* (binding sites number) and fluorescence intensity in the maximum of the short wave band (*F*, 560 nm). These correlations probably mean that the changes in the concentration of the erythrocyte-bound probe and parameters of binding are determined basically by the physical and chemical properties of the membrane lipids. In adaptation reactions in pathologies and extreme effect on the organism the system of antioxidant protection of the lipids is enhanced, the share of cholesterol is increased and the lipid set in the erythrocyte membrane is changed [Moe, Wong, 2010; Mozaffarian, Micha, Wallace, 2010; Nusier, El-Akawi, Abd-Alkareem, 2004]. This leads to the change in its physico-chemical properties, in particular, to the increase in the microviscosity of the lipid bilayer, increase in the rigidity of the membrane and decrease in the deformability of the erythrocytes [Bluma, Kalnina, Ivanova, 1993]. All these affect the kinetics and parameters of probe incorporation into the erythrocyte membrane. It should be noted that the values of binding constant *K* and binding sites number *N* for each group of patients differ from the respective parameters for other groups and are similar to the obtained at increased adrenoreactivity of animals.

The changes in parameters of DSM binding to blood erythrocytes of patients, compared with erythrocytes of healthy individuals, are indicative of sensitivity of the probe to structural changes in erythrocyte membrane with the investigated diseases. The different degree of changes in parameters of the probe

binding is testifying to different pronouncement of structural changes in erythrocyte membranes in the investigated pathologies (the largest differences among healthy individuals were detected with patients suffering ischemic cardiac disease and neurocirculatory distonia). Reduction of the values of *R* and *K* with patients suffering cardiac infarction, compared with those suffering instable stenocardia, confirms the data found in the literature, stating that disorders in structural organization of membranes directly depend on the severity of pronouncement of ischemic cardiac disease.

The extent of changes in the parameters correlates with the severity of the disease and the extent of disturbances of the properties of the membrane revealed by other methods revealing the sensitivity of adrenoreceptors to beta adrenoblockers and change of serum albumin as a determinant of incident cardiovascular disease.

The changed adrenoreceptors sensitivity to adrenoreceptors was obtained by propranolol antihemolitic test parameters. Erythrocyte beta adrenoreceptors showed higher sensitivity to propranolol in patients with myocardial infarction. The changes are indicative to lower sensitivity of beta adrenoreceptors to the beta blocker. It may be used to define indications for beta adrenoblocker therapy [Somisky, Anshelevich, Okun, 1990].

Elevated level of beta adrenoreception is observed also in patients with disturbances of heart rhythm at the background of connective tissue dysplasia. At the background of intake preparation magnesium orotate significant decrease of degree of desentization of erythrocytes has been noted (significant decrease in rigidity of membrane) [Moskvina, Necaeva, 2011].

Serum albumin concentration and its structural/functional properties were also used to characterize cardiovascular diseases [Schalk, Visser,Bremmer, Penninx, et al., 2006; Grizunov, Dobrecov, 1998]. Biochemical changes of albumin, which occurred in the blood of myocardial infarction patients, were investigated, using routine laboratory methods [Nusier, El-Akawi, Abd-Alkareem, 2004] and fluorescent probe K-35 [Grizunov, Dobrecov, 1998]. Increased level of atherogenic lipids (cholesterol + triglycerides), decreased serum albumin total (TA) and “effective” (EA, equivalent of “healthy” albumin in patients’ plasma) concentrations were associated with cardio-vascular patients as compared with those of controls. The extent of changes in the parameters under study correlates with the severity of the disease, results of laboratory and pathological investigation. Parameters TA, EA and ratio EA/TA characterize the albumin binding sites number, properties, affinity for probe etc. [Zvagule, Kalnina, Kurjane, et. al., 2011].

Judging by the detected changes in DSM binding parameters depending on adrenoreactivity of animals, we can assume the probability of binding of a part of the probe to adrenoreceptors. Such assumption can be confirmed also by the data obtained in beta experiments performed earlier on rats. Administration of guanethidine (50 mg/kg) decreased the noradrenaline content in adrenergic neurons, which led to an augmentation of the effector organ’s adrenoreactivity in rats. The affinity of the erythrocytes’ beta adrenoreceptors to propranolol was enhanced, and parameters of their binding the fluorescent probe DSM were changed: number of binding sites increases, but constant of association decreases [Sominsky, Berdisheva, Bluma, Kalnina, 1989].

A decrease in the affinity of the probe-cation to the membrane can also be indicative of decay in the efficient negative charge of the membrane. According to the literature data, this is due to the decrease of such indices of cell metabolism as activity of ATPase and other membrane-bound enzymes, a decrease in the consumption of the ATPase energy pool. It should be noted that the shifts of the binding parameters of the probe in the pathologies studied and under pathologies are qualitatively similar with those observed in animals during the experimentally enhanced adrenoreactivity of the organism [Ivanova, Somisky, Arzamasov, et al., 1990].

The similarity in the character of membrane response to the change in the increased adrenoreactivity and in cardiovascular diseases suggests that along with the structural rearrangament of the erythrocytes membrane the structural and functional properties of the adrenoreceptors also change. The differences in absolute numbers of ABM binding constant and binding sites number in animals and humans are explained by differences in their physico-chemical properties.

The data suggest the possibility of using erythrocytes for in vivo estimation of functional state of adrenoreactive system in animals and humans.

Conclusion

The spectral characteristics and binding parameters of the probe DSM in norm and in various pathologies, at changing adrenoreactivity of the animal organism were found to differ. The similarity in the character of membrane response to the change in the adrenoreactivity of the organism in diseases of cardiovascular system suggests that along with structural rearrangement of the erythrocyte membrane the structural and functional properties of the adrenoreceptors also change. The data also suggest the possibility of using erythrocytes for in vivo estimation of functional state of adrenoreactive system in animals and humans.

References

1. Baulieu E. E., Raynod J. P. A proportional graph method for measuring binding systems // Eur J Biochem, 1970; 13(2): 293–304.
2. Bluma R. K., Kalnina I. E., Ivanova S. M. Use of fluorescent probes DSM and DSP-6 in studies of structural and functional changes of the erythrocytes membrane // Biol Mem, 1993; 6(5): 609–617.
3. Doyle A. E., Fraser J. R. Essential hypertension and inheritance of vascular reactivity // Lancet, 1961; 2: 509–515.
4. Currell G., Dowman A. Essential mathematics and statistics for science. – 2nd ed. – Wiley-Blackwell, John Willey & Sons, Ltd, Publication, 2010.
5. Evans B., Iweyama T., Burnstock G. Long lasting supersensitivity of the rat vas deferens to norephinephrine after chronic guanethidine administration // J Pharmacol and Exp Ther, 1973; 185(1): 60–69.
6. Lakowicz J. R. Principles of fluorescence spectroscopy. – 3rd ed. – New York: Springler Verlag, 2006.
7. Nusier M. K., El-Akawi, Abd-Alkareem. The association of blood biochemical parameters with myocardial infarction // Journal of Health Science, 2004; 50(6): 666–669.
8. Moe K. T., Wong P. Current trends in diagnostic biomarkers of acute coronary syndrome // Ann Acad Med, 2010; 39(3): 210–215.
9. Mozaffarian D., Micha R., Wallace S. Increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials // Published online 2010 March // doi: 10.1371/journal. pmed.1000252.
10. Schalk B. W. M., Visser M., Bremmer M. A., Penninx B. W. J. H., et al. Change in serum albumin and risk of cardiovascular disease and all-cause mortality // Am J Epidemiol, 2006; 164: 969–977.
11. Rasmussen B., Lake W., Allen J. E. The effect of catecholamines and prostaglandins upon human and rat erythrocytes // Biochem et Biophys Acta, 1975; 411(1): 63–73.
12. Zvagule T., Kalnina I., Kurjane N., Vanadzins J., et al. Alterations of albumin in Chernobyl clean-up workers blood plasma after myocardial infarction and group with epilepsy paroxysm // 14th International Congress of Radiation Research, August 28–September 01, 2011, Warsaw, Poland // Abstract Book: 250 (POS2626).
13. Альбумин сыворотки крови в клинической медицине // Под ред. Грызунова Ю. А., Добрецова Г. Е. / Книга 2. – Москва: ГЕОТАР, 1998. [Albumin blood serum in clinical medicine].
14. Dobrencov G. E., Dubur G. J., Morozova G. I., Dete A. K., et al. Флуоресцентный зонд 4-(п-диметиламиностирилпир идиний: оптические свойства // Биофизика, 1981; 26(2): 377–385.
15. Иванова С. М., Соминский В. Н., Арзамасов Г. С., Брантова С. С., et al. Влияние длительной антиортостти-ческой гипокинезии (АНОГ) на биохимические показатели эритроцитов и функциональное состояние их мембран // Тез. Докл. 9 Всесоюзной конференции “Космическая биология и авиакосмическая медицина”. Москва–Калуга, 1990. – С. 530–531.
16. Москвина Ю. В., Нечаева Г. И. Адренореактивность у пациентов с аритмическим синдромом, ассоциированным с дисплазией соединительной ткани, на фоне приема магния оротата // Кардиология, 2011; 3: 54–57.
17. Соминский В. Н., Бердышева Л. В., Блума Р. К., Калнина И. Э., et al. Использование эритроцитов крови для прижизненной оценки функционального состояния адренорецепторов // Физиол. журн. СССР, 1989; LXXV(2): 189–193.
18. Соминский В. Н., Аншелевич Ю. В., Окунь К. В. Адренореактивность у больных инфарктом миокарда по данным антигемолитической пробы // Кардиология, 1990; 30: 24–28.

Normative Data of the Population of Latvia for the SF-36 (The Short Form 36) Health Survey

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Abstract

The SF-36 (Short Form 36) health survey is a widely used measure of health-related quality of life. Population normative data are required to compare it with patients' health-related quality of life data, as well as to compare it with other countries' normative data. Published normative data for the SF-36 health survey exist for other countries, but have not been previously published for Latvia.

The aim of the survey was to develop age-, gender-, income- and native language-related normative data for the SF-36 health survey for the population of Latvia. Responses from the SF-36 were summed and then transformed to eight scores with a scale from 0 to 100. Higher scores on all scales indicate better function and subjective health in each individual aspect of health. To get more accurate results the survey data were weighted according to the general structure of the population of Latvia.

Cronbach's alpha coefficient for all SF-36 scales exceeds the minimum of 0.7. The correlations between the SF-36 scales range from 0.413 to 0.786. All SF-36 scales are affected by the age – younger persons have highest scores in all scales ($p = 0.000$). Gender is a significant factor, too; women have lower scale scores than men for all eight SF-36 scales ($p < 0.05$). Native language affects only two scales – persons with Latvian native language have lower scale scores than persons with other native language in bodily pain and general health scales ($p < 0.05$). Income per family member is a significant factor for the differences in all scales – more for physical-health scales, less for mental-health. Subjects with the lower income per family member have lower scores in all SF-36 scales ($p < 0.05$).

Keywords: SF-36, health-related quality of life, normative data, health survey, general population

Introduction

Health is a subjective and multidimensional concept, defined as "a state of complete physical, mental and social well-being" [Blake, et al., 2000]. Subjective health assessment by self-reported measures of health status is a standard part of epidemiological and community-based research surveys today. Such a wide use of subjective health assessment reflects the importance of individual's own perception of his / her health and the multidimensional nature of health. Thus, perceived health has become one of the most significant health indicators studied today [Maslić Seršić, et al., 2006].

Different diseases with the objective symptoms cause people psychological and social problems as well, affecting the patients' everyday life. It could be psychological difficulties, professional limitations, problems in family life, socialization difficulties, additional costs, etc. That is why it is very important to

evaluate not only the objective condition of patients (severity of the disease, symptoms, complications, etc.), but also the patient's subjective perceptions and health problems' impact on human life in general – patient's quality of life, or more precisely – health-related quality of life – defined as "a patient's subjective perception how the disease, its consequences and treatment can impact patient's physical, emotional and social well-being" [Baltins, 2003].

The number of tools available for measuring quality of life continues to grow, with the development of disease-specific and generic measurement instruments. Disease-specific questionnaires may be more sensitive to changes in their target population, but have limited application to other populations. Generic questionnaires have a wider applicability, allowing comparison between different patient groups and facilitating comparison with the general healthy population [Blake, et al., 2000].

Generic measures are not specific to any age, disease or treatment group. Generic instruments have major advantages. They reflect what is most important to patients, and they are applicable for patients with more than one condition. They can be used to compare patients with different conditions and, finally, they make it possible to draw comparisons between patients and general population. The last two advantages are of central importance, since the interpretation of health-related quality of life measures represents a considerable challenge. This challenge essentially implies answering such questions as – what do the numbers mean, what is a high or a law score, what is the clinical relevance of a score. Norm-based comparisons are a strategy for solving these challenges and can be performed on either an individual or a group level. Norms for expected or typical scores for a group generally reflect the average scores in the general population, and norm-based interpretations imply calculation of departure from the norm [Loge, et al., 1998].

The Short Form 36 (SF-36) is a generic measure of health related quality of life; it was constructed to produce a brief general measure of health related quality of life [Maslić Seršić, et al., 2006]. It is one of the most widely used health status questionnaires worldwide. The SF-36 was originally developed in the United States in the late 1980s for a longitudinal investigation into a self-reported health status of general population and patients with a wide range of chronic conditions. Interest in the SF-36 has grown steadily. It has been translated in more than 50 languages [Hoopman, et al., 2009].

The reliability and validity of the SF-36 have been well documented by the developers of the instrument [Hopman, et al., 2000]. United States studies have shown that the SF-36 scales have clinical validity for purposes of distinguishing patients with and without a chronic condition, discriminating between groups known to differ in medical and psychiatric conditions, discriminating levels of severity within medical diagnosis, and reflecting changes in health-related quality of life associated with changes in disease severity [Sullivan, et al., 1995].

The result of the survey could be used to provide a population-based measure of broader health status, for use in service planning and monitoring and in making comparisons with the health of populations elsewhere, and in measuring the health outcomes of clinical intervention [Bowling, et al., 1999].

Normative data are the key to determining whether a group or an individual scores below or above the average for their country, age or sex [Hopman, et al., 2000]. Published normative data exist for the United States [Hopman, et al., 2000], for almost all European countries [Gandek, et al., 1998], Australia, Canada [Hopman, et al., 2000] and for many other countries, but comparable normative data have not been previously calculated and published for Latvia.

The aim

The purposes of the survey were:

a) to gain population norms for SF-36 in a general Latvian population sample;
b) to calculate population scale measures depending on respondents' gender, age, income per family member and native language.

Material and methods

Company's "QualityMetric Incorporated" and organization's "Medical Outcomes Trust" standardized instrument SF-36 Health Survey was used to determinate health-related quality of life of the population of Latvia.

The SF-36 Health Survey represents a theoretically based and empirically verified operationalization of two general health concepts – physical and psychological; and their two general manifestations – functioning and well-being. Accordingly, the questionnaire contains four types of scales, or four conceptually different measures of health [Maslić Seršić, et al., 2006].

This relatively brief and simple questionnaire contains 36 items covering 8 health concepts chosen on the bases of reliability, validity and frequency of measurement in health surveys [Hopman, et al., 2000]. A set of common socio-demographic questions was added at the end of the questionnaire. The SF-36 questionnaire is short and quick to administer, the questionnaire's 36 questions take on average 10 minutes to be completed [Nante, et al., 1999]. Also the SF-36 survey is well adapted for studies in general populations [Peyre, et al., 2003].

The total result of the SF-36 is most often shown in the form of the profile defined with eight points that represent the measure of individual aspects of health. Responses are summed and then transformed to give eight scores with a scale from 0 to 100, which represent the measure of individual aspects of health. On all scales higher scores indicate better function and subjective health in that dimension [Blake, et al., 2000; Maslić Seršić, et al., 2006; Nante, et al., 1999].

The SF-36 includes eight health scales:

1) *Physical Functioning* (10 items) – the scale evaluates a human's physical condition and shows, if the person's health limits his physical activities, such as walking or climbing stairs. The higher is the score, the better is the physical condition of the person;
2) *Role Physical* (4 items) – the scale shows to what extent persons' health problems can affect their daily activities. The higher is the score, the less health limits person's daily activities;
3) *Bodily Pain* (2 items) – the scale assesses any limitations due to pain – the severity of pain and the extent to which pain interferes with normal work, including work outside home and housework. The higher is the score, the less pain the person feels;
4) *General Health* (5 items) – the scale evaluates personal health, the expectations of changes in health and treatment perspective. The higher the score, the better general health a person has;
5) *Vitality* (4 items) – the scale measures energy or tiredness. Low scores mean subjective tiredness of a person;
6) *Social Functioning* (2 items) – the scale measures the degree to which individual's emotional or physical problems of health disrupt his/her normal social activities. Low score means less social contacts due to health problems;
7) *Role Emotional* (3 items) – the scale measures the degree to which individual's emotional problems interfere with his/her work or other daily activities. The higher the score, the less emotional condition limits everyday activities;
8) *Mental Health* (5 items) – the scale assesses happiness, nervousness and depression in humans. Low scores of the scale point to depression. [Hopman et al., 2000; Blake et al., 2000; Loge et al., 1998, Amidzarova et al., 2008].

An additional item – *Health Transition* – reports health transition over the past year. Item content is reported elsewhere [Loge, et al., 1998].

This diversity allows providing a more nuanced assessment of patient's condition [Baltins, 2003].

In the next step it is possible to calculate two summary measures which reassume the two major domains of the SF-36 – the physical components' summary (PCS-36) and the mental components' summary (MCS-36) measure. These indexes have the advantage of being easily used, keeping the good properties of the SF-36 but reducing the number of statistical tests necessary for the eight SF-36 scales [Nante, et al., 1999].

The SF-36 survey versions for Latvia in Latvian and Russian were incorporated in the population-based Rīga Stradiņš University Health-related Quality of Life Assessment survey (2009) to develop demographic-adjusted norms for the population of Latvia.

The survey was carried out in January, 2009. 1005 residents of Latvia were interviewed during the survey, 10 filled-in questionnaires were declared invalid, and, as a result, 995 cases were statistically processed for the survey. Face-to-face interviews took place in respondents' residences to enhance subjects' understanding of the questions and minimize missing values [Pappa, et al., 2005]. Respondents of various age, gender and ethnicity groups aged from 18 to 74 years were selected in a sample through random route procedure. Interviews took place in 20 regions of Latvia, including the district centres, other cities, villages and farmsteads. The performer of the field work – "Data Serviss" Ltd.

To get more accurate results, the survey data was weighted according to the general structure of the population of Latvia. A particular weight corresponded to the number of persons represented by the respondent for the entire population.

The data were analyzed using the statistical package SPSS version 15.0 statistical software. Eight health status measurement scales were set up by using a programme "Quality Metric Health Outcomes Scoring version 2.0".

Descriptive statistics were generated to evaluate data completeness to characterize the response distribution. Basic descriptive statistics, such as mean, median, standard deviation, standard error, range of scores and 95% confidence interval were calculated for each SF-36 scale.

Reliability was tested via Cronbach's α coefficient of internal consistency.

Analysis of variance (ANOVA) was used to test the statistical significance of the observed differences in proportional data of the study. Values of $p < 0.05$ were considered statistically significant.

Correlation analysis (Pearson correlation coefficient) was used to evaluate any relationship between SF-36 scales.

Results and conclusions

Sample characteristics

The socio-demographic profile of the respondents is presented in Table 1. The mean age of respondents was 43.66 ± 15.47 years. The largest age group was 36–45 years (20.8%), the smallest – 66 and over (10.9%).

57.4% of respondents were females, 42.6% – males.

Proportion of respondents' native language was 61.8% / 38.2% for Latvian and other languages, where all other languages like Russian, Belorussian, Ukrainian, etc. were included.

The significantly larger group was with the income of 186–250 Ls per family member – 23.4%, the smallest – 326 Ls or more – 19.1%. For more details see Table 1.

Data quality

Cronbach's α (alpha) coefficient of internal consistency was used to estimate the reliability of the eight SF-36 scales. Cronbach's alpha is an inter-item internal consistency coefficient which measures the overall correlation between items in the scale. Internal consistency is considered acceptable when a coefficient is higher than 0.7 [Blake, et al., 2000]. In all cases, coefficients' values exceed the minimum standard of 0.7. Internal consistency estimates ranges from 0.719 (Vitality) to 0.954 (Role Physical). For more details see Table 2.

The correlations between the SF-36 scales ranged from 0.413 (Physical Functioning and Mental Health) to 0.786 (Mental Health and Vitality). The correlations between the scales constituting the physical heath summary measure range from 0.581 (Role Physical and General Health) to 0.731 (Physical Functioning and Role Physical). Between the scales constituting the mental health the correlations ranged from 0.477 (Role Emotional and Mental Health) to 0.786 (Mental Health and Vitality). All correlation coefficients were statistically significant ($p = 0.000$). For more details see Table 4.

Table 1. Socio-demographic profile of the SF-36 survey respondents (N = 995)

Socio-demographic profile	Percentage
Age groups, years*:	
18–25	16.3
26–35	17.7
36–45	20.8
46–55	20.6
56–65	13.6
66 and over	10.9
Gender:	
Male	42.6
Female	57.4
Native language:	
Latvian	61.8
Other	38.2
Income per family member:;	
145 Ls or less	19.2
146–185 Ls	19.9
186–250	23.4
251–325 Ls	18.4
326 Ls or more	19.1

* Mean ± standard deviation – 43.66 ± 15.47

Table 2. Reliability estimates (Cronbach's alpha) for the SF-36 scales

SF-36 scale descriptors	Number of items	N	Cronbach's alpha
Physical Functioning	10	987	0.917
Role Physical	4	993	0.954
Bodily Pain	2	985	0.853
General Health	5	995	0.744
Vitality	4	992	0.719
Social Functioning	2	984	0.726
Role Emotional	3	993	0.923
Mental Health	5	992	0.758

Normative data

Table 3 represents the descriptive statistics for normative data for the population of Latvia. General Health scale has the lowest score (61.24 ± 20.45). The highest score is for Physical Functioning scale – 88.27 ± 18.37. The scales which measure both positive and negative aspects of well-being (General Health, Vitality and Mental Health) have lower mean scores, in comparison to the scales measuring health-related limitations (Physical Functioning, Role Physical, Bodily Pain, Social Functioning and Role Emotional) that showed higher scores.

The full range of 0–100 was observed for all scales, with the exception of the scale Mental Health, where the observed range was 5–100. The median exceeded the mean for all scales, with the exception of the scale Bodily Pain, where median is less than the mean for 0.98 points. For more details see Table 3.

Table 3. Descriptive statistics and features of score distributions for SF-36 (n = 995)

Basic descriptive statistics	Physical Functioning	Role Physical	Bodily Pain	General Health
Mean	88.27	82.12	74.98	61.24
Standard error	0.58	0.70	0.77	0.65
Median	95.0	93.75	74.0	62.0
Standard deviation	18.37	22.04	24.18	20.45
Range	0–100	0–100	0–100	0–100
95% CI	87.1–89.4	80.7–83.4	73.4–76.5	59.9–62.5
N	994	993	989	995
Basic descriptive statistics	**Vitality**	**Social Functioning**	**Role Emotional**	**Mental Health**
Mean	64.08	80.21	82.41	66.31
Standard error	0.54	0.66	0.65	0.53
Median	67.63	87.5	91.67	70
Standard deviation	16.92	20.92	20.57	16.80
Range	0–100	0–100	0–100	5–100
95% CI	63.0–65.1	78.9–81.5	81.1–83.7	65.2–67.3
N	993	993	993	993

Normative data by demographic indicators

Age. Age was an important health status factor and affected physical health relatively more than mental health. Respondents of 66 years or more had the lowest scores in all eight scales and younger persons had higher scores in all SF-36 scales. The tendency is equal to all scales – the older the person, the lower score he has. The difference by age groups for all scales reached statistical significance ($p = 0.000$), thus we can say that oldest persons have the lowest scale scores in Latvia. The biggest significant difference was found in the Physical Functioning (range – 34.38, $p = 0.000$). The smallest significant difference was found in the Mental Health scale (range – 9.73, $p = 0.000$) (Table 5).

Gender. Women have lower scale scores than men for all eight SF-36 scales. All gender differences are statistically significant ($p < 0.05$), that is why we can say that women in general have lower results of health-related quality of life than men. The results confirmed the assumption that women report worse health than men, a fact described and confirmed in many SF-36 surveys [Sullivan, et al., 1995; Loge, et al., 1998; Pappa, et al., 2005; Peyre, et al., 2003]. The range of scores difference is from 2.52 (Mental Health, $p = 0.018$) till 8.55 (Bodily Pain, $p = 0.000$). (Table 6)

Native language. With the exception of two scale scores, differences in other scales did not reach statistical significance. Persons with the Latvian language as the native tongue have lower scale scores than persons with other native languages in Bodily Pain (range – 4.44, $p = 0.004$) and General Health (range – 5.14, $p = 0.000$) (Table 7).

Income per family member. There is a significant effect of income per family member on all the scale scores ($p < 0.05$). Subjects with lower income per family member had lower scores in all SF-36 scales. The greatest differences in relation to income per family member were found in Physical Functioning (range – 12.57, $p = 0.000$), and the smallest were found in the Mental Health scale (range – 3.76, $p = 0.041$). The tendency for SF-36 physical-health summary measure is, that the subject group with income 146–185 Ls per family member has the lowest scores, but the subject group with income 326 Ls per family member or more has the highest scores (Table 8).

Table 4. Correlations between SF-36 scales

SF-36 scale descriptors		Physical Functioning	Role Physical	Bodily Pain	General Health	Vitality	Social Functioning	Role Emotional	Mental Health
Physical Functioning	Pearson Correlation Sig. (2-tailed) N	1 994							
Role Physical	Pearson Correlation Sig. (2-tailed) N	0.731 0.000 993	1 993						
Bodily Pain	Pearson Correlation Sig. (2-tailed) N	0.619 0.000 988	0.650 0.000 989	1 989					
General Health	Pearson Correlation Sig. (2-tailed) N	0.592 0.000 994	0.581 0.000 993	0.604 0.000 989	1 995				
Vitality	Pearson Correlation Sig. (2-tailed) N	0.533 0.000 993	0.539 0.000 993	0.552 0.000 989	0.601 0.000 993	1 993			
Social Functioning	Pearson Correlation Sig. (2-tailed) N	0.488 0.000 993	0.551 0.000 993	0.577 0.000 989	0.469 0.000 993	0.580 0.000 993	1 993		
Role Emotional	Pearson Correlation Sig. (2-tailed) N	0.533 0.000 992	0.718 0.000 993	0.521 0.000 989	0.434 0.000 993	0.500 0.000 993	0.537 0.000 993	1 993	
Mental Health	Pearson Correlation Sig. (2-tailed) N	0.413 0.000 993	0.432 0.000 993	0.473 0.000 989	0.528 0.000 993	0.786 0.000 993	0.603 0.000 993	0.477 0.000 993	1 993

Table 5. Mean SF-36 scale scores by age groups

Age group		Physical Functioning	Role Physical	Bodily Pain	General Health	Vitality	Social Functioning	Role Emotional	Mental Health
18–25 years	N	165	165	163	165	165	165	164	165
	Mean	97.76	91.10	85.34	71.57	70.86	87.59	88.49	70.87
	Std. deviation	6.45	15.37	20.58	18.12	14.39	17.68	17.55	16.96
26–35 years	N	192	192	190	192	192	192	192	192
	Mean	96.44	90.52	82.39	69.06	67.98	83.02	86.85	69.09
	Std. deviation	8.20	14.20	20.09	17.16	16.28	18.71	15.74	16.64
36–45 years	N	188	188	188	188	188	188	188	188
	Mean	93.49	85.13	78.96	64.59	64.40	80.54	83.05	67.15
	Std. deviation	11.58	18.31	21.10	17.93	14.33	20.08	17.79	14.90
46–55 years	N	191	192	191	192	192	192	192	192
	Mean	88.76	80.44	74.26	58.86	62.20	79.12	81.80	64.61
	Std. deviation	14.03	21.36	22.48	19.26	17.10	21.35	20.26	16.47
56–65 years	N	139	137	137	139	137	137	137	137
	Mean	79.30	76.28	64.88	51.94	60.18	76.94	79.40	62.70
	Std. deviation	20.59	24.96	25.77	18.72	17.81	20.79	23.74	16.79
66 and more years	N	119	119	119	119	119	119	119	119
	Mean	63.38	60.89	55.46	43.73	55.40	70.51	70.37	61.14
	Std. deviation	25.45	26.40	24.72	19.54	18.37	24.58	25.87	17.82
Total	N	994	993	989	995	993	993	993	993
	Mean	88.27	82.12	74.98	61.24	64.08	80.21	82.41	66.32
	Std. deviation	18.37	22.04	24.18	20.45	16.92	20.92	20.57	16.80
p value		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 6. Mean SF-36 scale scores by gender

Gender		Physical Functioning	Role Physical	Bodily Pain	General Health	Vitality	Social Functioning	Role Emotional	Mental Health
Male	N	464	463	460	464	463	463	463	463
	Mean	91.53	85.41	79.55	63.88	66.19	82.67	85.01	67.66
	Std. deviation	15.34	20.07	21.78	19.81	16.20	19.82	18.87	16.49
Female	N	530	531	529	531	531	531	530	531
	Mean	85.40	79.25	71.00	58.92	62.23	78.07	80.14	65.14
	Std. deviation	20.25	23.26	25.44	20.73	17.33	21.63	21.71	16.99
Total	N	994	993	989	995	993	993	993	993
	Mean	88.27	82.12	74.98	61.24	64.08	80.21	82.41	66.32
	Std. deviation	18.37	22.04	24.18	20.45	16.92	20.92	20.57	16.80
p value		0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.018

Table 7. Mean SF-36 scale scores by native language

Native language		Physical Functioning	Role Physical	Bodily Pain	General Health	Vitality	Social Functioning	Role Emotional	Mental Health
Latvian	N	583	583	581	583	583	583	583	583
	Mean	88.43	82.34	73.15	59.11	63.93	80.17	82.45	66.08
	Std. deviation	17.74	21.24	24.42	20.32	16.45	20.81	19.79	16.33
Other	N	411	410	408	412	410	410	409	410
	Mean	88.03	81.80	77.59	64.25	64.29	80.27	82.36	66.65
	Std. deviation	19.25	23.14	23.62	20.27	17.58	21.10	21.65	17.44
Total	N	994	993	989	995	993	993	993	993
	Mean	88.27	82.12	74.98	61.24	64.08	80.21	82.41	66.31
	Std. deviation	18.37	22.04	24.18	20.45	16.92	20.92	20.57	16.80
p value		0.735	0.704	0.004	0.000	0.740	0.939	0.945	0.594

Table 8. Mean SF-36 scale scores by income per family member

Income per family member		Physical Functioning	Role Physical	Bodily Pain	General Health	Vitality	Social Functioning	Role Emotional	Mental Health
145 Ls or less	N	188	186	185	188	186	186	185	186
	Mean	86.88	79.60	72.43	57.72	63.06	78.02	80.58	64.33
	Std. deviation	18.25	23.23	25.48	18.86	17.49	22.30	22.29	17.75
146–185 Ls	N	188	188	187	188	188	188	188	188
	Mean	81.09	75.51	68.53	55.49	61.13	77.46	79.27	64.12
	Std. deviation	23.92	25.98	26.04	22.45	17.34	21.82	22.35	17.19
186–250 Ls	N	223	223	222	223	223	223	223	223
	Mean	88.17	82.77	75.47	62.38	64.88	81.63	82.41	67.88
	Std. Deviation	18.52	21.84	24.03	20.43	16.90	19.69	22.04	16.46
251–325 Ls	N	176	176	176	176	176	176	176	176
	Mean	92.46	86.13	78.02	64.15	66.04	82.35	86.19	67.78
	Std. deviation	13.00	18.89	23.13	18.59	17.20	21.00	16.48	16.66
326 Ls or more	N	191	192	189	192	192	192	192	192
	Mean	93.66	87.01	80.68	66.89	65.63	81.66	85.06	67.47
	Std. deviation	12.65	17.08	20.51	19.77	15.71	19.35	17.23	15.80
Total	N	965	964	960	966	964	964	964	964
	Mean	88.41	82.20	75.02	61.35	64.16	80.26	82.66	66.36
	Std. deviation	18.33	22.01	24.24	20.47	16.99	20.87	20.46	16.83
	p value	0.000	0.000	0.000	0.000	0.029	0.058	0.005	0.041

Discussion

These survey results show values for the eight dimensions of the SF-36 health survey for a random sample of the population of Latvia. The survey was designed so that the sample drawn was unbiased and be a representative of the general population.

The aim of health-related quality of life was to establish norms for future comparison. These norms could serve as anchors for interpretation of scores, for instance, as age- or gender-specific cohorts in cross-sectional studies. They also could help in interpreting the clinical significance of the observed changes in prospective studies [Loge, et al., 1998].

The number of completed questionnaires in Latvian research was less than in other similar SF-36 surveys in other countries [Sullivan, et al., 1995; Loge, et al., 1998; Pappa, et al., 2005; Peyre, et al., 2010], but the representativeness of the research results was ensured by calculating the sampling error and weighing the obtained results. As the number of general population of Latvia aged 18–74 years is N = 1 694 900 and assuming that the maximum sampling error is 3%, the sample size n = 1000 is considered sufficient and it is enough[1] to calculate valid normative data for the population of Latvia.

All the physical- and mental-health scales were affected by age (poorer health with increasing age) and by gender (women showing poorer health compared with men). The results of the research carried out in Latvia in general are similar to the results of other countries' SF-36 surveys [Sullivan, et al., 1995; Loge, et al., 1998; Maslić Seršić, et al., 2006; Pappa, et al., 2005; Peyre, et al., 2010]. Native language affected only two scales – Bodily Pain and General Health. Income per family member is a significant factor for differences in all scales – more for physical-health scales, less for mental-health.

In practice the instrument has several advantages, including brevity and flexibility of administration methods. Standard criteria for scoring and transforming the raw responses ensure accurate group comparisons suitable for use in a wide variety of populations [Blake, et al., 2000].

Conclusions

In summary, the normative data that we present are valid and are based on a representative sample of residents of Latvia aged 18 years and over. The SF-36 survey can be considered a suitable instrument for assessing health-related quality of life for the population of Latvia.

References

1. Baltiņš M. Lietišķā epidemioloģija. – Rīga: Zinātne, 2003. – 354. lpp. (In Latvian). [Baltins M. Applied Epidemiology, Riga, Zinatne, 2003. – 354 pp.].
2. Blake C., Codd M. B., O'Meara Y. M. The Short Form 36 (SF-36) Health Survey: normative data for the Irish population // Ir J Med Sci, 2000; 169(3): 195–200.
3. Bowling A., Bond M., Jenkinson C., et al. Short Form 36 (SF-36) Health Survey questionnaire: which normative data should be used? Comparisons between the norms provided by the Omnibus Survey in Britain, the Health Survey for England and the Oxford Healthy Life Survey // J Public Health Medicine, 1999; 21(3): 255–270.
4. Gandek B., Ware J. E. Jr., Aaronson N. K., et al. Tests of data quality, scaling assumptions, and reliability of the SF-36 in eleven countries: results from the IQOLA Project // J Clin Epidemiol, 1998; 51(11): 1149–1158.
5. Hoopman R., Terwee B. C., Deville W., et al. Evaluation of the psychometric properties of the SF-36 health survey of the among Turkish and Moroccan ethnic minority populations in the Netherlands // Qual Life Res, 2009; 18(6): 753–764.
6. Hopman W. M., Towheed T., Anastassiades T., et al. Canadian normative data for the SF-36 health survey // CMAJ, 2000; 163(3): 265–271.

[1] The following formula is used for calculation of sample size: $n = \frac{1.96^2 W(1-W)N}{\Delta^2 N + 1.96^2 W(1-W)}$, where n = required number of respondents in the sample, Δ = sampling error (3% or 0.03), N = general population (1 694 00), W = 0.5.

7. Loge J. H., Kaasa S. Short Form 36 (SF-36) health survey: normative data from the general Norwegian population // Scand J Soc Med, 1998; 26(4): 250–258.
8. Maslić Seršić D., Vuletić G. Psychometric evaluation and establishing norms of Croatian SF-36 health survey: framework for subjective health research // Croat Med J, 2006; 47(1): 95–102.
9. Nante N., Groth N., Guerrini M., et al. Using the SF-36 in a rural population of elderly in Italy: a pilot study // J Prev Med Hyg, 1999; 40: 1–7.
10. Pappa E., Kontodimopoulos N., Niakas D. Validating and norming of the Greek SF-36 health survey // Qual Life Res, 2005; 14(5): 1433–1438.
11. Peyre H., Coste J., Leplčge A. Identifying type and determinants of missing items in quality of life questionnaires: application to the SF-36 French version of the 2003 decennial health survey // Health Qual Life Outcomes, 2010; 8: 16.
12. Sullivan M., Karlsson J., Ware J. E. Jr. The Swedish SF-36 health survey – I. Evaluation of data quality, scaling assumptions, reliability and construct validity across general populations in Sweden // Soc Sci Med, 1995; 41(10): 1349–1358.
13. Амиджарова В. Н., Горячев Д. В., Коршунов Н. И., и др. Популяционные показатели качества жизни по опроснику SF-36 // Научно-практическая ревматология, 2008; 4(1): 36–48. (In Russian).
14. Amidzarova V. N., Gorjacev D. V., Korsunov N. I., et al. Population indicators of life quality based on SF-36 // Scientifically-practical Chrestomathy, 2008; 4(1): 36–48.

Information Transfer from Hospital to Primary Medical Care after Elderly Patients' Discharge from Hospital in Bulgaria

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Abstract

The medical surveillance of the elderly patients in the post-hospital period depends on the communication and coordination between the hospital and primary medical care. The information transfer from hospital to primary medical care is investigated within a research of the health needs of people aged 65 years and over after hospital treatment. The study includes 362 patients discharged from two multiprofile hospitals in northeastern Bulgaria and their 181 general practitioners (GP). Information from the patients is received through a semi-structured interview on the 7th and 30th day after discharge at their homes in 12 towns and 64 villages. Information from the GPs is received through a phone interview on the 7th day after patients' discharge. Results show that the regulated way for discharge summary to reach the primary medical care through the patient does not guarantee its reception by the GP and thus impedes the continuity of care. GPs prefer direct information transfer from hospital, with electronic receiving the discharge summary considered as the most appropriate.

Keywords: elderly, hospital/GP communication, discharge summary, continuity of care.

Introduction

The policy of shortening the length of hospital stay requires a continuation of treatment and medical supervision after patients' discharge from hospital. The complete medical supervision in the post-hospital period depends on the information exchange and coordination between the hospital and primary medical care.

Communication between hospital and primary medical care is often cited as a weakness in relation to discharge planning [1]. Deficiencies in information transfer at hospital discharge are common and may adversely affect continuity of care [2, 3]. Timeliness of communication is a key safety/quality requirement because the delay in information transfer can lead to post-discharge care delay [4]. The continuity of the care after discharge from hospital depends on the quality of the communication document and the speed at which it reaches the general practitioner (GP).

According to the current health regulations in Bulgaria, the hospital is responsible for providing coordination with the primary medical care after patient's discharge from hospital in order to complete the treatment process. The document establishing the link between the hospital and the primary medical care is the discharge summary. It includes the reason for hospitalization, diagnostic findings, therapy and procedures, patient's condition at discharge, and recommendations for post-discharge follow-up. The discharge summary is made in two copies handed in to the patient on the day of discharge from

hospital. One of the copies is for the patient himself, and the other one must be passed on to the GP by the patient (or his representative). The responsibility of the GP for delivering continuous medical care for the patients in his list is one of the specific quality indicators of the primary medical care [5, 6].

The aim

The aim of the paper is to reveal the real way of transferring information about hospital stay of elderly patients between the hospital and primary medical care after elderly patients' discharge in Bulgaria; how the speed of the discharge summary transfer influences timeliness and continuity of medical supervision needed for the elderly patients after their discharge from hospital; and what GPs' views are about the most suitable way to receive information from hospital about discharged elderly patients in order to ensure adequate medical care for them in the post-hospital period.

Material and methods

The presented results are a part of a research of health needs of people aged 65 years and over after hospital treatment. Included in the research are all the patients at the age of 65 and over, discharged within a 45-day period from therapeutic (internal medicine and neurology) and surgical (surgery and orthopaedics) wards of two multi-profile hospitals in northeastern Bulgaria, excluding those who have denied participation and the dead up till the 7th day after discharge. Information from the patients is received through a semi-structured interview during a visit in their homes on the 7th (n = 362) and 30th day (n = 339), 23 persons have been excluded due to death) after discharge from hospital, in 12 towns and 64 villages. The needs for a GP check-up are assessed on the day of discharge by each patient's hospital physician. The communication between the hospital and the primary medical care is researched through a phone interview with the GPs of all the patients on the 7th day after patients' discharge – interviewed are 181 GPs, serving 357 patients (three patients do not have GP, phone calls to two GPs failed).

Results and discussion

The hospital wards do not have information about GPs, except in cases of hospitalization with a referral by a GP, but there is such a way of directing to the hospital for less than half of the respondents – 153 (42.3%). This fact shows the insufficient conditions for communication between the hospital and the primary medical care.

The discharge summary as an instrument for communication and compatibility between the hospital and primary medical care has reached in the early post-hospital period (up till the 7th day after discharge from hospital) to the GPs of 169 (47.3%) patients through various ways: brought in person by the patient, brought by patient's relatives, received on a home visit. The discharge summary of more than half of the patients has not been received by their GPs up till the 7th day after discharge from hospital (Figure 1).

Receiving an official document about the hospital stay provides an opportunity for the patients to read (personally or their relatives) the instructions for treatment continuation, for the necessary examinations, for the right of performing a control examination in the hospital. The research of the time for receiving the discharge summary shows that 139 (38.4%) patients received the discharge summary on the day of discharge, 35 (9.7%) received it up to 3 days after discharge, 54 (14.9%) – from 3 to 7 days after discharge, 134 (37.0%) did not receive it up till the 7th day after discharge from hospital. Personally acquainted with the discharge summary contents up till the 7th day after discharge are 119 (32.9%) patients, for 60 (16.6%) it has been read by a relative, for two it has been read by their GPs, half of the patients are not acquainted with its contents. Out of 339 patients who were survived on the 30th day after discharge, 35 (10.3%) had not received a discharge summary yet – all of them live in populated areas, different than those where the hospitals are. Those patients had not performed a control examination in the hospital because they have not been informed about their right and the dates for a control examination, which are noted in the discharge summary.

The absence of a regulated direct communication between the hospital and primary medical care leads to violation of the timeliness of the medical care for the patients after discharge. There are assessed needs for a GP check-up up till the 7th day after discharge (assessed by treating hospital physicians) for 206 (56.9%) patients. Comparison between the needs of a GP check-up and the realized check-ups shows the weakest realization during the first 1–2 days after discharge – some of the patients are not examined at all, other patients' examinations are delayed. Without an assessed need of check-up, 87 patients with non-impaired mobility have visited GPs. For 83 patients with an assessed need of check-up, such has not been performed (Table 1).

The carried out check-ups are ambulatory or realized during a home visit after calling the GPs by the patients or their relatives. Only 12 patients are visited by the initiative of the GPs themselves (without calling), with those visits being realized after receiving the discharge summary through patients' relatives, who have visited the GPs on another occasion. The last mentioned fact shows the potential possibility for active surveillance of the elderly patients in the post-hospital period that can be more widely realized with timely informing the GPs about patients' discharge.

The responsible behaviour of the GPs towards their elderly patients is illustrated by their opinions for the benefits of the direct communication with the hospital care. The official information about the discharge of a particular patient, included in the research, would assist the GPs in several directions: 113 (62.4%) – to visit the patient; 23 (12.7%) – to phone the patient to get informed about his condition after discharge; the rest of the GPs would like to know the condition of each discharged patient who has been directed by them for hospitalization, or the reason for hospitalization of each patient for whose hospital admission they are not informed.

Figure 1. Discharge summary received by GPs within 7 days after patients' discharge from hospital (n = 357)

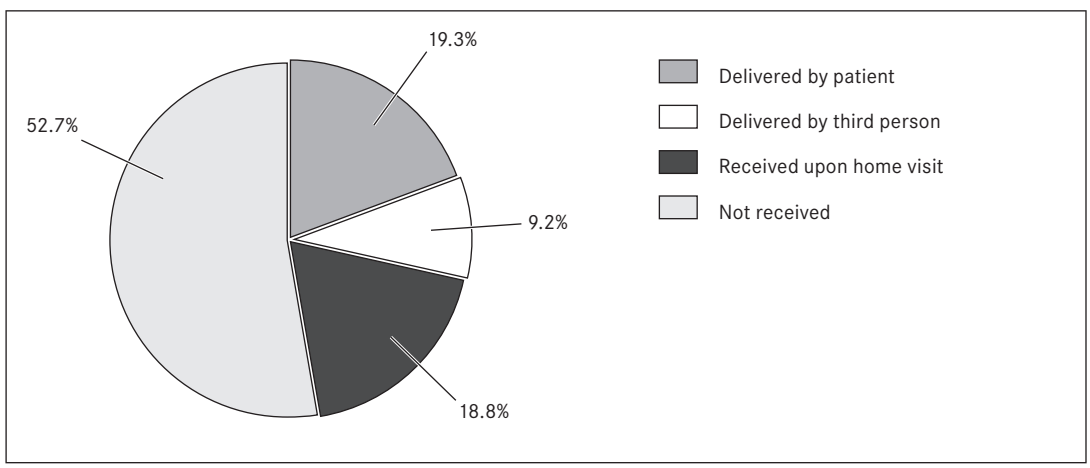
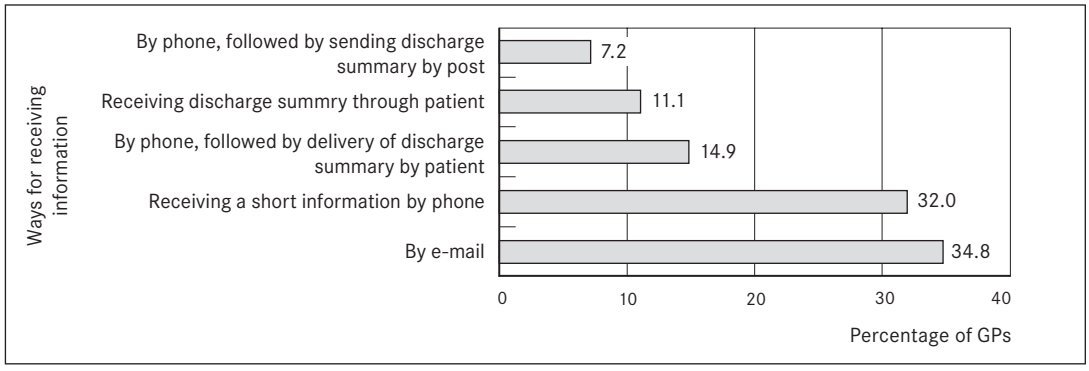


Table 1. Cross-comparison between GP check-up needs and check-ups carried out within 7 days after patients' discharge from hospital (n = 362)

Check-ups carried out	GP check-up needs (assessed by hospital physicians)				
	Up to 24 h	Up to 48 h	3–7 days	None	Total
On the same day	0 (0.0%)	1 (0.3%)	1 (0.3%)	4 (1.1%)	6 (1.7%)
On the next day	0 (0.0%)	3 (0.8%)	28 (7.7%)	22 (6.1%)	53 (14.6%)
On the third day	1 (0.3%)	1 (0.3%)	20 (5.5%)	14 (3.9%)	36 (9.9%)
After the third day	3 (0.8%)	1 (0.3%)	64 (17.7%)	47 (13.0%)	115 (31.8%)
None	3 (0.8%)	3 (0.8%)	77 (21.3%)	69 (19.1%)	152 (42.0%)
Total	7 (1.9%)	9 (2.5%)	190 (52.5%)	156 (43.1%)	362 (100.0%)

According to the GPs, the most suitable ways for receiving information from the hospital about discharged elderly patients in order to guarantee continuous medical surveillance are: receiving the discharge summary by e-mail, and officially informing by phone about the discharge (Figure 2). Only 20 (11.1%) GPs consider the receiving the discharge summary by the patient suitable, with the note that the patient should receive it from the hospital on the day of discharge.

Figure 2. The most suitable way to receive information from hospital about discharged elderly patients, according to GPs (n = 181)



The diversity of additional comments of the GPs for their communication with the hospital care can be summarized in the following groups:

1. The direct reception of information about the hospital stay and the patient's condition at discharge would be of great help for the GPs, because for the elderly people in rural areas travelling to the town is a "real event", and if the discharge summary is not given on the day of discharge, they will never come back to take it.
2. The direct informing by the hospital about the discharge of elderly patients would allow the GPs to visit the patient without calling during the regulated visit in the corresponding village.
3. The informing by phone about discharged elderly patients who need bandages or injections would assist the GPs to timely plan their home visits and necessary consumatives.
4. It would be convenient for each patient to have an electronic medical record in which all examinations and hospitalizations are documented and where the discharge summary is attached.

The research of the usual communication between the hospital and the primary medical care shows that the active party in this communication is the GPs: 69 (38.1%) GPs phone the hospital "seldom" to receive information about the hospitalized patients, 8 (4.4%) do that "often", the rest do not do that. Within the present study none of the GPs has established a direct contact with the hospital regarding a hospitalized patient.

The results of the presented study show that the regulated way for the hospital stay information to reach the GP through the patient does not guarantee its reception by the GP. The discharge summary itself often is not received by the patients on the day of discharge. Provided that the GP does not know about the discharge of the patient (or even about his hospital admission if he has not issued personally a hospitalization referral), it is not clear how he will achieve a continuity of the medical surveillance. The role of care manager in critical period such as the post-hospital one is left to the elderly patients themselves due to insufficient communication and coordination between the hospital and the primary medical care.

Conclusions

A direct communication between the hospital and the primary medical care is missing.

The documented information about the hospital stay (discharge summary) often is not provided to the patients on the day of discharge and for that reason in numerous cases it does not reach the elderly patients who live in other settlements (different than that of the hospital), respectively their GPs. To provide for reception by the GPs of information about the hospital stay of the patients according to the current national regulations, there is the need to organize the work in hospital in a way that guarantees an actual provision of the discharge summary to the patients on the day of discharge.

The regulated way for receiving information from the hospital through the patient impedes the GPs in achieving a continuity of the medical surveillance of the elderly patients after discharge from hospital.

The GPs consider the information reception by e-mail as the most suitable way to receive information about discharged elderly patients.

Adoption of the electronic communication system among the different units of the medical care would facilitate the timely provision of post-hospital medical care for the elderly patients.

References

1. McInnes E., Mira M., Atkin N., et al. Can GP input into discharge planning result in better outcomes for the frail aged: results from a randomized controlled trial // Family Practice, 1999; 16(3): 289–294.
2. Kripalani S., LeFevre F., Phillips C. O., et al. Deficits in communication and information transfer between hospital-based and primary care physicians // JAMA, 2007; 297(8): 831–841.
3. van Walraven C., Seth R., Laupacis A. Dissemination of discharge summaries: not reaching follow-up physicians // Can Fam Physician, 2002; 48: 737–742.
4. Principles of Quality GP/Hospital Communication. General Practice Advisory Council – Queensland // www.health.qld.gov.au/hssb/hou/integration/GPAC_GP.pdf
5. National Framework Contract between National Health Insurance Fund, Bulgarian Medical Association and Bulgarian Dental Association for 2006 // State Gazette No 106/2005, amendment and addition 68/2006. [in Bulgarian].
6. Regulation No 29 about the major requirements to which must comply the structure, activity and the internal order of the hospitals, dispensaries and medical-social care units. // State Gazette No 108/1999, amendment and addition 80/2000, 61/2001, 99/2003, 7/2007, 55/2008. [in Bulgarian].

The Link between Hyperhomocysteinemia and Methylenetetrahydrofolate Reductase 677C→T Polymorphism in Children and Adolescents with Psychotic Disorders

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Abstract

The aim of the study was to get information about serum level of homocysteine (hereinafter – Hcy) and examine the associations between the level of Hcy and methylenetetrahydrofolate reductase (hereinafter – MTHFR) 677C→T polymorphism for patients with schizophrenia spectrum disorders.

For the studies 82 patients from Children Psychiatric Hospital were used. Patients were selected according to their diagnosis (in line with ICD-10) and current clinical status.

The level of Hcy was stated by isocratic HPLC system with fluorometric detection (Shimadzu LC-20, model RF-10AxL).

DNA isolation from venous blood was made at Rīga Stradiņš University, Human Genetics Research Laboratories by the use of standard phenol-chloroform method. In the determination of MTHFR 677C→T polymorphism polymerase chain reaction was used.

Mean level of Hcy was 11.99 μmol/L.

It has been found that the highest level of Hcy was observed in patients with episodic-recurrent course of disease, especially if there was acute onset and pronounced affect.

No associations between schizophrenia, the level of Hcy and MTHFR gene polymorfism were discovered.

The obtained data are indicative of potential link among the level of Hcy and schizophrenia, and its severity and course. But there was not found an association between the level of Hcy, schizophrenia illness and the MTHFR 677C→T polymorphism.

Keywords: schizophrenia, homocysteine, MTHFR, children and adolescent psychiatry.

Introduction

Hcy was first described by Butz and du Vigneud in 1932. They obtained the product by treating methionine with concentrated acid.

Methionine is the immediate precursor of S-adenosylmethionine (SAM), the methyl donor of numerous methylation reactions in the brain, many of which are directly involved in the synthesis and metabolism of monoamines such as dopamine, norepinephrine and serotonin [1]. This suggests that the association between elevated homocysteine and schizophrenia is biologically plausible.

Another way of investigating the association between Hcy and mental disorders is via the MTHFR gene. MTHFR converts 5.10-methylene tetrahydrofolate to 5-methyltetrahydrofolate which is needed for the remethylation of homocysteine to methionine [2].

MTHFR is a critical component of the 1-carbon cycle, and the MTHFR polymorphisms C677T affect nucleotide synthesis and DNA methylation. This gives a plausible biologic explanation for potential associations between genetic variation in folate metabolism and both depression and schizophrenia [3].

The obtained information points to the fact that the risk to come down with psychiatric illness correlates with elevated level of Hcy [4]. Men who have MTHFR C677T polymorphism for genes have greater risk to become ill with schizophrenia than women, for their part it is greater risk to get bipolar affective disorders [5].

Elevated Hcy is associated with an increased risk of occlusive vascular disease, birth defects (neural tube defects), and complications during pregnancy and functional psychiatric disorders (major unipolar depression, anxiety disorders, bipolar affective disorders, and in gender – specified manner – schizophrenia) [6].

High level for prenatal Hcy is a valid risk factor of schizophrenia and affective disorder, because Hcy has partial antagonism with NMDA receptors. This antagonism can lead to the dysfunction of NMDA receptors in these patients. It is associated with the changes in glycine concentration resulting in disturbances of the functioning of placenta and pregnancy complications [7].

Hcy and Hcy-acid have abilities to increase intracellular calcium level of ions and active oxygen compounds within cerebellum of rats as like as NMDA (N-Methyl-D-Aspartate acid). It rouses premature apoptosis for cells. These mechanisms of actions are background for Hcy and its derivates for neurotoxic impact [8].

Grounded on the above mentioned, it is urgent to establish, which mental affections are linked with changes of Hcy level in blood plasma; whether changes of Hcy concentration depend on clinical state of affection (affection is in progress, remission or continuous progredient).

The aim

The aim of the study was to gain information about serum level of Hcy and MTHFR gene polymorphism and increased frequency of the TT genotype for patients with schizophrenia spectrum disorders and their possible links.

Material and methods

The investigation was carried out in the department of Pharmacology of Rīga Stradiņš University and in Children Psychiatric Hospital. DNA isolation from venous blood was made at Rīga Stradiņš University, Human Genetics Research Laboratories by the use of standard phenol-chloroform method.

For the studies, 82 patients from Children Psychiatric Hospital were used. These patients were with schizophrenia spectrum disorders (1st group – 18; 2nd group – 37; 3rd group – 27). Patients were selected according to their diagnosis (in line with ICD-10) and current clinical status.

Each diagnosis and clinical status was coded depending on its severity and course of disease – 1st group: paranoid schizophrenia – continuous; 2nd group: paranoid schizophrenia – episodic with residual symptoms and schizoaffective disorders; 3rd group: simple schizophrenia and schizotypal disorders.

There are no patients with severe somatic pathology (renal insufficiency, usage of glucocorticoids), patients with evidence of genetic diseases and patients without compliance, as well as the patients whose parents or guardians did not agree with the children inclusion in the studies.

The patients' treatment regimen designated by their attending physician was not changed. Medications were appointed in accordance with established indications and dosing principles, evaluated both contraindications, and possible side effects.

Data were registered in special form including information about family anamnesis, child up-growth period, beginning of disease, its course and individuality, all previous therapies and their results, as well as currently received treatment and its effectiveness.

The level of Vitamin B_{12} and folic acid in the blood were analyzed in the Laboratory of NMS. The level of Hcy in the blood was analyzed in the Research Laboratory of Pharmacology department of Rīga Stradiņš University by isocratic HPLC system with fluorometric detection (Shimadzu LC-20, model RF-10AxL). The present method allows the rapid, simple and specific determination of total Hcy (tHcy) using isocratic HPLC system with fluorometric detection. The HPLC system was a Shimadzu LC-20 Prominence with a fluorescence detector (model *RF*-10AxL, Shimadzu).

Sample preparation and chromatographic separation were performed according to the recommendations of use provided with the commercially available Chromsystems GmbH (Germany) kit for HPLC analyses of tHcy in plasma.

The reduction step for releasing homocysteine from its protein-bound state, protein precipitation and precolumn derivatisation followed by HPLC separation and fluorescent detection is the most widely applied technique [2].

In the determination of MTHFR 677C→T polymorphism polymerase chain reaction was used.

During the investigation, none of the patients took any vitamins – neither dietary supplements, nor vitamin preparations. All analyses were taken early in the morning on an empty stomach.

Statistical analysis. Several methods and statistical indicators for statistics processing were used: average value, average standard deviation and average standard errors.

Validity in difference in average measurements of two groups was estimated according to Stjudent t-test. Significance level $p < 0.05$.

The data were obtained using STATA Data Analyses Tool: Regression Statistics; Two Sample t-test with equal variances, Bartlett's test for equal variances, Bonferroni test.

Results

The level of Vitamin B_{12} and folic acid has been found according to norm for all patients.

Mean level of Hcy was 11.9 µmol/L for patients with schizophrenia spectrum disorders.

It was found that the highest level of Hcy was observed in patients with paranoid schizophrenia-continuous (13.9 µmol/L) and episodic-recurrent course of disease and schizoaffective disorders (13.29 µmol/L), especially if there was an acute onset and pronounced affect ($r = -0.36$; $p < 0.001$). The lowest level of Hcy was observed in patients with simple schizophrenia and schizotypal disorders (9.25 µmol/L).

In genetic investigations, patients were divided into three groups depending on the existing genotype for T allele: CC – healthy, CT – heterozygous and TT – homozygous. There was not observed any genotype prevalence in the studied patient groups ($p < 0.01$). Yet more, there was no correlation between elevated Hcy levels and MTHFR gene 677C→T polymorphism ($p < 0.01$).

Discussion

Studies, carried out at the moment, have not yet convincingly demonstrated the role of Hcy in the origin of mental disorders. The results of these studies reflect a partial, episodic, and sometimes even weak clinical correlation between hyperhomocysteinemia and psychiatric diseases. Not always studies conducted simultaneously or repeatedly show confirmation in this correlation.

If schizophrenia begins before the age 12, it is called juvenile schizophrenia. It occurs in 1 out of 10 000 children, but the incidence of schizophrenia and schizophrenia-related disorders occur to 1–2 out of 1000 in adolescence [9].

The fact, that schizophrenia is already presented before puberty, suggests that the cause of the disease can be found as neurodevelopmental abnormality.

Seeing the clinical status of schizophrenia, affective disorders (aggravation of affect) are found to be substantial, which can be very pronounced in case of schizoaffective disorders as well as for episodic schizophrenia, and for continued paranoid schizophrenia.

It is established that enlarged Hcy level is commonly linked with MTHFR gene TT genotype. The MTHFR gene is located at the end of the short arm of chromosome 1 (1p36.3). The enzyme plays a central role in the folate metabolism by irreversibly converting 5.10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, the predominant circulating form of folate. 5-methylenetetrahydrofolate donates a methyl group to Hcy in the generation of S-adenosylmethionine, a major source of methyl groups in the brain [10].

Two common single MTHFR nucleotide polymorphisms have been reported, a C→T transition at nucleotide 766 in exon 4 and an A→C transversion in exon 7 at position 1298. Both of these polymorphisms are functional and result in diminished enzyme activity. For the C677T polymorphism, homozygote variants have 30% enzyme activity in comparison with homozygotes for the wild-type C allele, while heterozygotes retain 65% of wild-type MTHFR enzyme activity [11]. The consequences of the C677T polymorphism have been demonstrated in population studies, where the lower levels of the red blood cell folate, plasma folate, and vitamin B_{12} have been reported among healthy persons with genotype 677 TT by the side of persons with other genotypes [12].

There was an increased risk of schizophrenia among homozygote variants (TT) (reliability of statistical data is low) in one study. Subjects with schizophrenia showed a significantly increased frequency of the T allele. A cumulative meta-analysis showed that a moderate and significant association between schizophrenia and MTHFR C677T has remained over time. But no association between MTHFR C677T and anxiety disorders was found [13].

To day, practically there are no studies about hyperhomocysteinemia linked with psychic disorders in children and adolescent psychiatry. There are some separate researches about connection of Hcy level and affection in case of schizophrenia in adolescents by using numerically small groups of patients (age 14 to 21). It is established that Hcy level is higher for patients with schizophrenia than for healthy patients from the control group. However, this concurrence is observed only in boys [14].

In our study it was found that significantly higher level of Hcy is observed during exacerbation of the disease. This allows discussing that there is no relationship only between Hcy and schizophrenia spectrum disorders, but also between Hcy and the current affective state, in respect of basic diagnosis.

In our study no relationship between MTHFR gene homozygous (TT) or heterozygous (CT) genotype variant and schizophrenia illness was found, as well as a certain form of the disease. Similarly, there was not observed the role of TT genotype in the elevated level of Hcy.

Conclusion

The data obtained are indicative of potential link among the level of Hcy and schizophrenia, and its severity and course, therefore approving the hypothesis that Hcy can be one of the risk factors of schizophrenia getting forward. These data also indicate that the level of Hcy is inwrought with affective disorders and anxiety in the course of disease.

The data, obtained up to now, do not point at more frequent occurrence of any MTHFR gene 677C→T genotype (CC, CT or TT), in case of some forms of schizophrenia. Nevertheless, it could be believable, that for obtaining more accurate genetic data, it would be desirable to investigate a larger number of patients.

References

1. Bottiglieri T., Laundy M., Crellin R., Toone B. K., et al. Homocysteine, folate, methylation, and monoamine metabolism in depression // Journal of Neurology, Neurosurgery and Psychiatry, 2000; 69: 228–232.
2. Klerk M., Verhoef P., Clarke R., Blom H. J., et al. MTHFR Studies Collaboration Group: MTHFR 677C→T polymorphism and risk of coronary heart disease: a meta-analysis // JAMA, 2002; 288: 2023–2031.
3. Coppen A., Bolander-Gouaille C. Treatment of depression: time to consider folic acid and vitamin B_{12} // Journal of Psychopharmacology, 2005; 19: 59–65.
4. Muntjewerff J. W., Kahn R. S., Blom H. J., et al. Homocysteine, methylenetetrahydrofolate reductase and risk of schizophrenia: a meta-analysis // Molecular Psychiatry, 2006; 11: 143–149.
5. Kempisty B., Bober A., Luczak M., et al. Distribution of 1298A > C polymorphism of methylenetetrahydrofolate reductase gene in patients with bipolar disorder and schizophrenia // European Psychiatry, 2007; 22: 39–43.
6. Murray C. J., Lopez A. D. The global burden of disease: a comprehensive assessment of mortality and disability from disease, injuries and risk factors in 1990. – Boston, MA: Harvard School of Publics Health, 1996.
7. Bolander-Gouaille C., Bottiglieri T. Homocysteine. Related vitamins and neuropsychiatric disorders. – 2nd ed. – France: Springer Verlag, 2007. – Pp. 15–29.
8. Makhro A. V., Bulygina E. R., Boldyrev A. A. Effects of homocysteine and homocysteine acid on cerebellar granule cells // Neurochemical Journal, 2007; 1(2): 127–132.
9. Sadock B. J., Sadock V. A. Synopsis of psychiatry. – 10th ed. – Lippincott Williams & Wilkins, 2007. – Pp. 467–526.
10. Cantoni G. L. S-Anenysylmethionine: a new intermediate formed enzymatically from 1-methionine and adenosine triphosphate // Journal of Biological Chemistry, 1953; 204: 403–416.
11. Rozen R. Molecular genetics of methylenetetrahydrofolate reductase deficiency // Journal of Inheritable Metabolic Disorders, 1996; 19: 589–594.
12. Molloy K. J., Daly S., Mills J. L., et al. Thermolabile variant of 5.10- methylenetetrahydrofolate reductase association with low red-cell folates: implications for folate intake recommendations // Lancet, 1997; 349: 1591–1593.
13. Reif A., Pfuhlmann B., Lesch K. P. Homocysteinemia as well as methylenetetrahydrofolate reductase polymorphism are associated with affective psychoses // Progress of Neuropsychopgarmacology and Biological Psychiatry, 2005; 29: 1162–1168.
14. Nevo G. A., Meged S., Sela B. A. Homocysteine levels in adolescent schizophrenia patients // European Neuro-psychopharmacology, 2006; 16(8): 588–591.

Combined Method of Imaging with 20 MHz Ultrasound, Spectrophotometric Intracutaneous Analysis and Pathohistology in Determination of Malignancy Characteristics of Basal Cell Skin Cancer – Review of Literature

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Abstract

Basal cell carcinoma (BCC) is the most common skin cancer that occurs mainly in fair skinned patients. Excessive cumulative dose of sunlight and family history of this cancer can play a significant role. BCC grows locally with invasion and destruction of local tissues, and rarely metastasizes. At diagnostics the clinical features exceptionally may not confirm the appropriate diagnosis. 20 MHz skin ultrasound, spectrophotometric intracutaneous analysis (SIAscopy) and pathohistological investigation support the appropriate diagnostic means. According to the personal practical experience and the review of literature, we analysed whether SIAscopy (as modification of dermatoscopy) plus high-frequency ultrasonography (HFUS) 20 MHz is the recommendable combination of choice for non-invasive BCC diagnostics. Pre-surgical ultrasound with compact linear 20 MHz probe is known to report the morphology and thickness of tumors. Pre-operative imaging may aid surgical planning by identifying the extent and location of a neoplasm, which can be interesting at zones with higher risk of recurrences such as the face. According to our experience the optical definition of the margins is almost precise; that is proved by cancer cell free pathological findings in most cases after excision of BCC. Combination of several non-invasive methods of diagnostics offers the possibility to evaluate the level of malignancy and the method of therapy properly.

Keywords: basal cell carcinoma, dermatoscopy, skin ultrasound, spectrophotometric intracutaneous analysis, biopsy.

Introducion

Non-melanoma skin cancers subdivide in basal cell skin cancer (BCC) and squamous cell skin cancer (SCC). Basal cell carcinoma (basalioma, basal cell carcinoma of the skin, basocellular epithelioma) is a low-grade cancer, locally invasive and destructive, that rarely metastasizes. It localises mostly on sun-exposed skin areas in a growing number of patients all over the world. Biopsy is considered as

the routine and most reliable diagnostic modality for BCC, whereas excision is the treatment of choice for most types of BCC. Routine BCC surface scrape cytology is informative. At diagnostics the clinical features exceptionally may not confirm the appropriate diagnosis. Dermatoscopy, skin ultrasound, spectrophotometric intracutaneous analysis (SIAscopy) and pathohistology support the appropriate diagnostic means. The listed methods vary by means of sensitivity and specificity, mechanism of action and ability to penetrate the dermis. However, alone none of them give a proper picture concerning the malignant characteristics of the cancer. All the investigative methods have certain advantages and disadvantages. Most optical methods have low penetration options into the skin – up to 2 mm, that does not allow determining the depth of invasion. Combining several diagnostic methods, e.g., optical coherence tomography, reflectance confocal microscopy, skin ultrasound, the cost for the investigation grows, as well as the possibility to define borders of the cancer and choice of appropriate method of therapy. Even with a precise preoperative diagnosis, complete treatment of non-melanoma skin cancer is not always achieved [Acarturk and Edington, 2005; Telfer, et al., 2008]. Thus relapses of BCC occur due to left residual, not removed or eliminated cancer cells [Griffiths, et al., 2005].

The aim of the study

The aim of our current study is to analyse the scientific literature available on combination of scope of pre-surgical high resolution ultrasound and spectrophotometric intracutaneous analysis (SIAscopy) in basal cell carcinoma confirmed by cytological investigation, and pathohistology post-surgical treatment.

Discussion

BCC was first described by Necam (in 1901) as basalioma. Krompecher in 1903 named it basal cell skin cancer, and developed the first classification, specifying the malignant traits of different forms of it. Monographers present different classification of BCC: Broders (in 1919), Montgomery (in 1929 and 1935), Albertini (in 1941), Gottron (in 1964), Ehlers (in 1965), Howard (in 1971), Lever (in 1975), Fanta (in 1976), Bauer (in 1979), etc.

BCC consists of basaloid cells that mimic basal cells in human epidermis. BCC is a locally destructive neoplasm that causes cosmetic and functional disfigurations. Rarely metastasizes (0.01–0.1%), concerning *morphea* and basosquamous forms of BCC. The high mitotic activity of BCC does not correlate with the clinically slow growth. Mean time of duplication of a BCC cell is 9 days, but the lesion enlarges only by 1–6 mm in a year. The extensive cell death is due to apoptosis, especially in less malignant BCC forms. On the opposite, aggressive BCC forms do not present with such obvious degenerative changes [Elder, 2005].

BCC occurs mostly on sun exposed skin, although genetic, physical, chemical factors also share the responsibility. UV radiation caused mutations in PTCH1 are proved in most BCCs but 10% of sporadic BCCs have mutations in SMOOTHENED which encode the protein whose function is inhibited by the PATCHED1 protein. Obviously, the relevant dysfunction driving BCCs is abnormal hedgehog signalling, irrespective of which gene controlling that signalling is mutated. Genetic analysis of sporadic basal cell carcinoma has been propelled by the identification of mutations in PTCH1 (chromosome 9q22.3) as the cause of the basal cell nevus syndrome (BCNS), a rare autosomal dominant disorder. Genetic factor, so called, genetic instability, is mostly responsible for BCC development. Decrease of T-cell and overall cytotoxic functional activity has been found in BCC patients. While in nodular BCC angioneogenesis plays the crucial role, supplying cancer tissues with high metabolic activity [Elder, 2005]. As a rule, nodular forms of BCC develop in elderly patients, localising in head and neck region. Superficial forms of BCC affect patients of more early age and localise on the skin of the body surface (Figures 1–8 (photos from the authors' personal archive)).

Figure 1. Gigantic destructive BCC of nasal region of immunocompromised patient

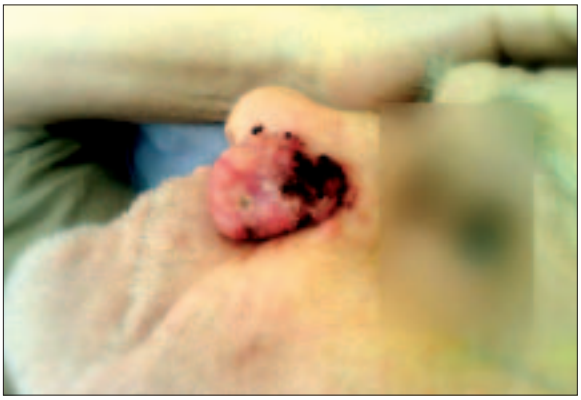


Figure 2. Micronodular BCC

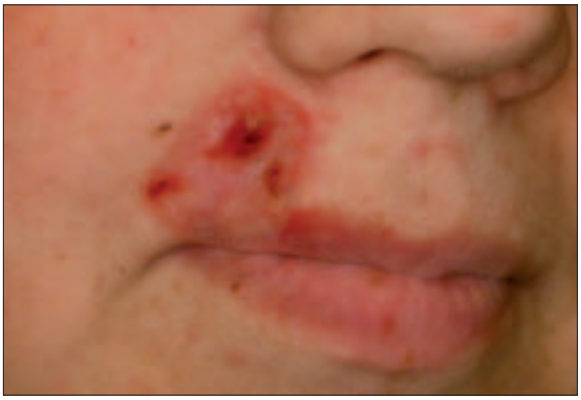


Figure 3. Nodular (solid) BCC

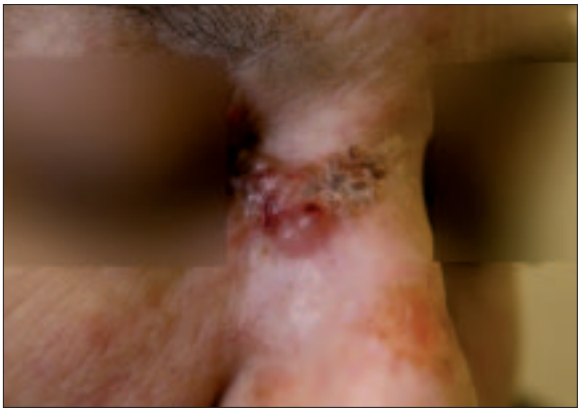


Figure 4. Melanoma-like nodular (solid) pigmented BCC

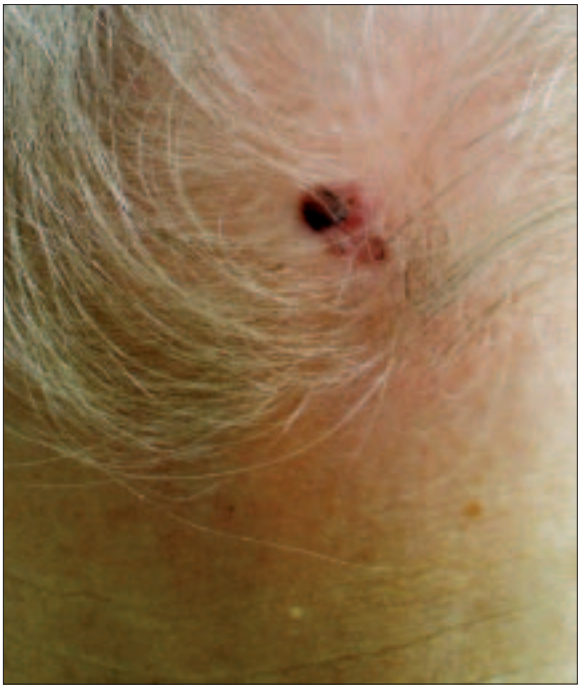


Figure 5. Nodular (solid) BCC in retroauricular region

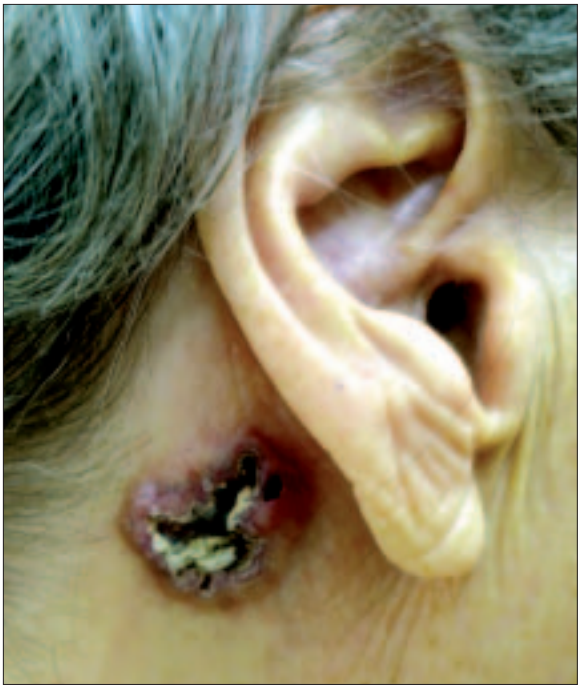


Figure 6. Nodular BCC

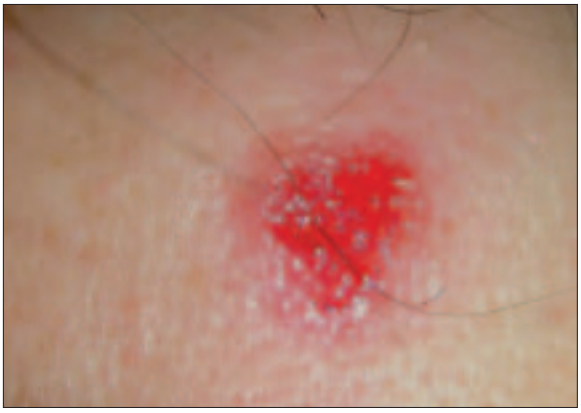


Figure 7. Relapse of BCC in 4 years after cryotheraphy

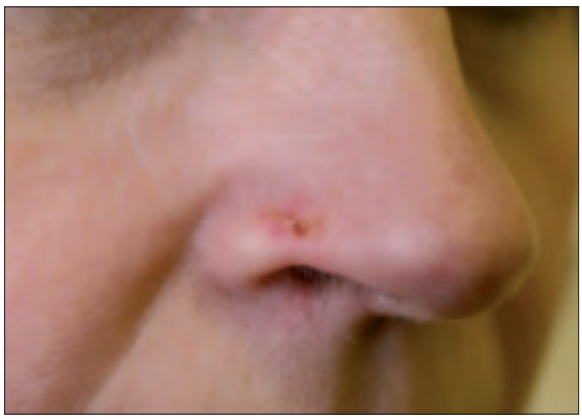
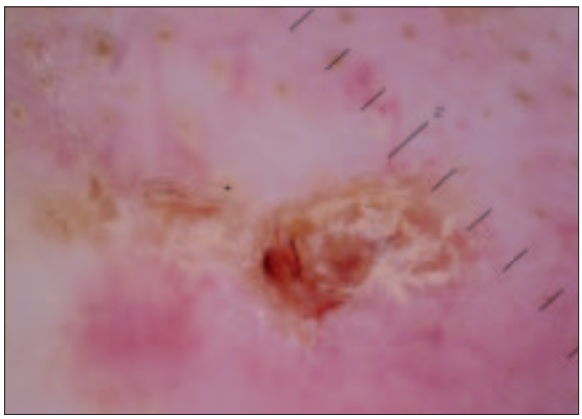


Figure 8. Dermatoscopic analysis of relapse of BCC 4 years after cryotheraphy



Classification of BCC. According the WHO classification [LeBoit, et al., 2006], BCCs are grouped as keratinocytic tumours, subdividing as follows: superficial basal cell carcinoma, nodular (solid) basal cell carcinoma, micronodular basal cell carcinoma, infiltrating basal cell carcinoma, fibroepithelial basal cell carcinoma, basal cell carcinoma with adnexal differentiation, basosquamous carcinoma, keratotic basal cell carcinoma.

The main clinical forms are: nodular, superficial, sclerotic (sclerodermaform, *morphea*), and fibroepithelioma of Pinkus. The nodular form subdivides: micronodular, ulcerating, pigmented, cystic, conglobatic, infiltrating. Superficial form of BCC subdivides – pigmented, Pedget, and ulcerative. The nodular, superficial spreading and infiltrating variants are the 3 most commonly encountered types of BCC in descending order of prevalence.

Clinical examination. The first step in evaluating a lesion suspicious for NMSC is the clinical examination. However, the diagnostic accuracy is difficult to assess and depends on the educational level and training of the observer. Reported sensitivity of clinical diagnosis varies from 56% to 90% and specificity from 75% to 90% [Ulrich, et al., 2008].

Cytology. The advantage of this method is rapid screening diagnostics of cancer cells in out-patient clinic. It is easy to perform, painless, causes no complications, provide high sensitivity (88%–90%) in case of correct application. Scraping material of tumour surface performed with a sterile surgical scalpel is smeared on a glass slide in a thin film. Necrotic mass should be thoroughly cleaned from the surface of the suspicious lesion. In case of no ulceration of epidermis, aspiration of cancer tissue and application of collected material on a glass slide is performed. Negative result of a single time performed cytological investigation, if clinically skin cancer is suspected, should not be considered as a refusal from further investigations. In some cases, healing of surface of nodulo-ulcerative type of BCC can occur with normal epidermal cells; however, in the depth of the lesion atypical cells are present.

Biopsy. Biopsy of the cancer lesion is mandatory as "the golden standard" to distinguish the histological type of BCC and avoid diagnostic mistakes [Derveniece, et al., 2002; Saldanha, et al., 2003]. Basal cell carcinomas tend to share the common features of a predominant basal cell type:

- peripheral palisading of cell nuclei,
- a specific stroma, and typical clefting artifact between the epithelium and the stroma.

Routinely processed basal cells display a characteristic artfact consisting of a cleft caused by detachment of the tumour nest from the surrounding stroma. This clear space is characteristic of BCCs, and occasionally its presence helps in diagnosis. In addition, there are variable degrees of cytologic atypia and mitotic activity; some degree of these latter changes is virtually always present. Tumor cells are homogeneous without displaying atypical features or abundant mitosis. Classic basal cell carcinoma is characterised by nests of basaloid cells displaying hyperchromatic nuclei and a scanty cytoplasm. These nests

are connected to the epidermis or infiltrate the dermis and occasionally the subcutaneous and underlying tissues. The tumour nests show peripheral palisading of cells. Between the tumour cells there is often mucinous material that separates the cells and occasionally produces pseudoglandular spaces. These spaces can resemble an adenoma especially in glandular BCC. Surrounding the tumor nests, there is a prominent reaction of the stroma characterised by the deposition of new collagen by young fibroblasts and abundant mucous substances. Vascular or lymphatic invasion by BCCs is seldom seen [Day, 2000; Wortsman and Wortsman, 2010].

Fibroepithelioma of Pinkus is a basal cell carcinoma in-situ, characterised by thin strands of basaloid epithelial cells projecting from the epidermis with frequent anastomosis. The stroma is often fibrous, and this feature plus the presence of adjacent classical BCC helps distinguish this entity from a reticulate seborrheic keratosis. Morpheaform BCC is an infiltrating BCC with a marked fibrous reaction of the stroma. The tumour cells are arranged in strands, one or two cells thick, and they often display atypical nuclear features. This type of tumour appears to be more aggressive than classic BCCs and should be excised with adequate margins.

Pigmented BCCs show solid nests of basaloid cells, many of which contain melanin. The pigment is also seen between tumour cells and in melanophages. Micronodular BCC is characterised by small nests, often with little stromal reaction. This is an aggressive tumour which is often seen infiltrating deeply in the soft tissue. Syringomatous or eccrine-like BCCs show areas of eccrine differentiation. The tumour cells in basal cell nevus syndrome display the morphology of regular basal cell carcinomas. Some of these tumours are occasionally calcified.

Baso-squamous carcinoma shows features of both BCC and SCC, and they may be difficult to classify under only one heading. These tumours, in general, behave more aggressively than classic BCC, and, in fact, account for most of the few cases recorded in literature of basal cells with lymphatic or hematogenous metastasis.

Nevertheless, pathologic information on skin lesions is always available from conventional skin biopsy, an *ex vivo* test with inherent cosmetic problems. Furthermore, biopsy specimens may occasionally give inconclusive diagnosis because of insufficient sampling and cannot inform on the relations *in vivo* between lesional tissue and local structures [Wortsman and Wortsman, 2010].

The main disadvantage of biopsy method – delay in acquisition of the results and certain subjectivity in interpretation of the data. Skin biopsy causes certain inconvenience. The procedure is invasive and scar producing on the biopsy spot. Histological treatment of the tissue (fixation, staining) can alter the result and induce artifacts. Additionally, the result can be false negative due to incorrect choice of the place *in vivo* between the lesion and surrounding tissue. Biopsy does not allow controlling the lesion *in vivo* within longer time period. It provides no assistance to define the excision line at the extirpation of the cancer [Day, 2000]. Results of a regular biopsy can be obtained in several days and request services of pathologist and supportive staff. Moh's surgery is highly expensive, requesting proper environment and trained specialists.

Dermatoscopy and correlation of dermoscopic phenomena with the pathomorphological findings in BCC. Dermatoscopy applies polarised light in investigation of skin with magnification 10–100 times. Sensitivity of this method in diagnostics of BCC (pigmented forms): 93% and specificity – 89%. Argenziano et al., 1998, published a thorough investigation on arborising blood vessels as the main indication in 82% of BCC with positive finding in 94%. Dermatoscopy is a non-invasive technique that improves the accuracy of the clinical diagnosis of pigmented skin lesions and helps physicians to correlate clinical dermatology and dermatopathology findings by means of visualising the morphological features that are not visible to the naked eye. Dermatoscopic evaluation has an important role in the diagnosis of pigmented BCC, which is a clinical subtype that accounts for 2–5% of all BCC [Wang, et al., 2000].

Dermatoscopic diagnostic criteria particularly for pigmented BCC are defined: maple leaf-like areas; spoke-wheel areas; large blue-grey ovoid nests multiple blue-grey globules; arborising vessels; and ulceration. Only some of these dermatoscopic diagnostic criteria have been defined precisely in terms of histopathological counterparts. Non-specific dermatoscopic features that can be observed in pigmented

BCC include depigmentation, blue-whitish veil, multiple blue-grey dots, multiple brown dots, brown/black globules, peripheral black dots or globules, red-blue lacunae, pseudopods, radial streaming and milia-like cysts [Menzies, et al., 2000].

Certain dermatoscopic features are in agreement with histopathological findings [Tabanlıoglu Onan, et al., 2000]. Maple leaf-like areas visible in dermatoscopy of pigmented BCC histologically resemble pigmented basaloid cell nests in the papillary dermis. The multifocal tumour nests, connected to each other by lobular extensions and to the epidermis multifocally, are mostly localised to the papillary dermis and rarely to the reticular dermis. Tumour nests contain pigment aggregates inside tumour stroma. Spoke-wheel areas are multifocal tumour nests that have multiple connections to the epidermis with finger-like extensions. They localise superficially in the epidermis and papillary dermis, occasionally tumour nests with radial extensions that localise to the papillary dermis are detected. They rarely have connections to follicles with basaloid cell extensions and some form bridging connections between the epidermis and follicular epithelium. Pigment aggregates are concentrated at the centre of the tumour body and localised to the tumour stroma. Large blue-grey ovoid nests are multifocal, well-bordered tumour nests with a few small buddings at the periphery. These tumour nests, some of which originate from the epidermis, contain pigment aggregates inside the tumour stroma and are usually localised to the papillary and reticular dermis. Multiple blue-grey globules are small, round tumour nests with pigment inside and in the tumour stroma, localised to the papillary dermis and reticular dermis.

Arborising vessels visible in dermatoscopy as dilated vessels scattered in the papillary and reticular dermis, histopathologically correlate with dilated vessels in the papillary dermis. Ulceration both in dermatoscopic and histological investigation is detected as superficial loss of epidermis. Brown dots signify brown pigment deposition at the dermoepidermal junction and tumour nests that are connected by thin bands and localised to the papillary dermis. Blue-whitish veil purport brown pigment deposition of melanin-loaded melanocytes and melanophages in the dermis. Orthokeratosis, hypergranulosis and rarely parakeratosis can be detected just above the brown pigment deposition.

In pigmented BCC lesions three previously histopathologically undefined specific dermatoscopic criteria can be identified: spoke-wheel areas, large blue-grey ovoid nests and multiple blue-grey globules. The histopathological findings of spoke-wheel areas, particularly connections of tumour nests to follicular epithelium with basaloid cell extensions, are typical streaks.

Fair skin patients with skin type I–II usually present with non-pigmented BCC, while pigmented BCCs are more characteristic to individuals with skin type III–V.

Few data are available in literature concerning dermatoscopic diagnostics of non-pigmented BCC. The dermatoscopic diagnostic criteria for non-pigmented BCC are not specific enough to set the diagnosis and choose the optimal therapy. Bright homogenous zones in white or pink colour, short fine telangiectasias, ulceration, arborising blood vessels are the typical findings [Altamura, et al., 2010].

Seldom are multiple blue-grey globules, maple leaf-like areas, blood vessels that mimic dots and pins visible. Uneven linear and polymorphic blood vessels consisting of rough lines, red dots and pins are characteristic features of hypopigmented and non-pigmented melanoma. This does not support these features to state as characteristics for BCC. Altamura, et al., 2010 have investigated blood vessels in 531 skin cancers of different type and concluded that in the diagnostics of malignant skin cancers (melanoma, BCC, squamous cell carcinoma) high prognostic value in positive outcome of treatment confirm the presence of rough linear (81%) and polymorphic (68%) blood vessels. Thus the value of dermatoscopy in the diagnostics of non-pigmented BCCs is low and is based on the detection of specific vascular pattern of short, fine, teleangiectatic and arborising blood vessels (Figures 9, 10 (photos from the authors' personal archive)).

The optics of human skin explain how a ray of light in human skin can be absorbed, reflected, remitted, re-emitted, scattered or transmitted and any combination of these [Anderson and Parrish, 1981]. Spectrophotometric Intracutaneous Analysis (SIAscopy) is a scanning technique applying remitted light in the visible and infrared spectra to investigate the skin. SIAgraphs are high-resolution images of the collagen and haemoglobin content of the papillary dermis and the melanin content of the epidermis and papillary dermis. Epidermis is formed from keratinocytes, the stratum corneum, melanocytes and

the epidermal appendages. Optical properties of the epidermis concern the difference in refractive index between air and the stratum corneum, n = 1.0 and n ~ 1.5, respectively. Typically 5% of the light is reflected back across the entire spectrum uniformly from infrared (1200 nm) to ultraviolet (200 nm) and this also applies to light returning from deeper regions of the skin. The surface of the skin is not smooth or planar and so light striking it will not be specular (it does not maintain an image). In addition, the light passing into the stratum corneum will be refracted and made diffuse by this rough surface [Anderson and Parrish, 1981]. Skin surface microscopy alters these events by using a lipophilic and planar surface, oil and a cover slip for instance, to match the refractive index at the stratum corneum and prevent reflection of the incident light. The light in the skin may be scattered or absorbed. Anderson and Parrish (1981) explain that scattering within the epidermis is essentially weak and less than 5% is remitted by to the surface in the Caucasian skin. Thus the major interaction with light seen in the epidermis is absorption. From optical properties of the dermis it is apparent that light is absorbed and remitted in the dermis according to a complex function of absorption and scattering and that chromophores absorb not only according to the quantity of the molecule and its absorption properties but also the depth at which the chromophore is situated in the dermis determines the range of wavelengths (colours) that the chromophore is exposed to. This is a fundamental point in the understanding of spectrophotometric analysis [Moncrieff, et al., 2002]. At SIAscopy (Figure 11 (copy from figure 2.20, http://www.astronclinica.com)) light is passed through band-pass filters, through a handset that is in contact with the skin surface and into the region of skin that is of interest. The light that is remitted is captured using a dedicated charged couple device (CCD) and the signal is then digitised. This information is passed on to a computer that runs software that performs the task of SIA. In essence the algorithm takes the two infrared and one RGB measurement for each pixel and compares these values to the skin model to produce four SIAgraphs that are pixel maps depicting the following features: papillary collagen thickness, papillary dermal blood content, total melanin content and papillary dermal melanin content. Dedicated software displays the SIAgraphs on a monitor screen and the whole process takes approximately 15 seconds to perform [Moncrieff, et al., 2002].

SIAscopy in the differentiation for BCC is informative, but not as the only method to relay on (Figures 12, 13 (photos from the authors' personal archive)).

SIAscopy is a three stage process. First, light is incident on and enters the skin. Within the skin light is scattered by collagen. It is also absorbed by the chromophores in various amounts depending on its wavelength. Consecutively, some light escapes the skin and is collected by the SIAscope for analysis. Second, the light incident on the skin is emitted by calibrated LEDs (light emitting diode) of various known wavelengths. Red, green, blue and infrared LEDs are used. Calibration takes place at capture of every SIAscan, so that the exact quantity of each wavelength of light entering the skin is known. The light that escapes the skin is collected by a camera chip. This is again highly calibrated and its response to all wavelengths is known.

By measuring the amount of the light incident on the skin and its amount returned, it becomes possible to determine skin properties up to 2 mm in depth (www.astronclinica.com).

Reflectance confocal microscopy. Reflectance Confocal Microscopy (RCM) – an innovative method used during the screening and diagnostics of variety of dermatoses. It offers bias in the diagnostics of skin lesion, and BCC as well. BCC has five major criteria, including elongated monomorphic basaloid nuclei, polarisation of these nuclei along the same axis, prominent inflammatory infiltrate, increased dermal vasculature with tortuosity of the tumour vessels and pleomorphism of the overlying epidermis with loss of normal honeycomb pattern. RCM has high sensitivity and specificity with regard to some of BCC subtypes that do not elevate more than 1 mm above the skin level [Ulrich, et al., 2008].

RCM method applies low potency diod laser with wavelength 830 nm and zoom lens 30 × magnifications with aperture 0.9. The maximal wattage of the laser system is 40 mW. With axle resolution 3–5 μm and lateral resolution about 1 μm, obtained with the RCM regular histological pictures. The depth of investigation reaches 300 μm, which corresponds with the level of the upper reticular dermis. On the contrary to vertical histological sections, the sections-images obtained by RCM are horizontal. The images of RCM look like a scale of brightness. The contrast is based on the different parameters of light deflection of skin chromophores (melanin, hemoglobin, and cellular microstructures) that provide the necessary contrast for morphological visualisation of the different skin layers.

Figure 9. Superficial BCC on the trunk

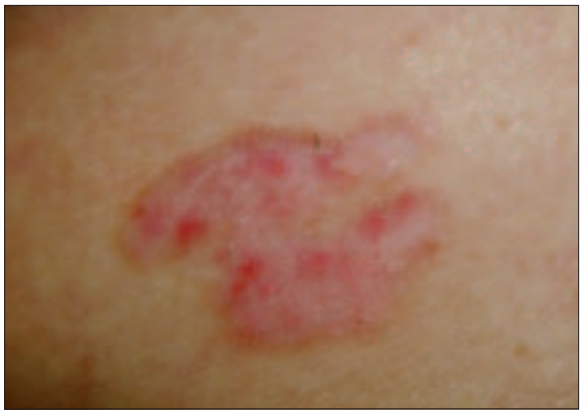


Figure 10. Dermatoscopy with characteristic signs of BCC

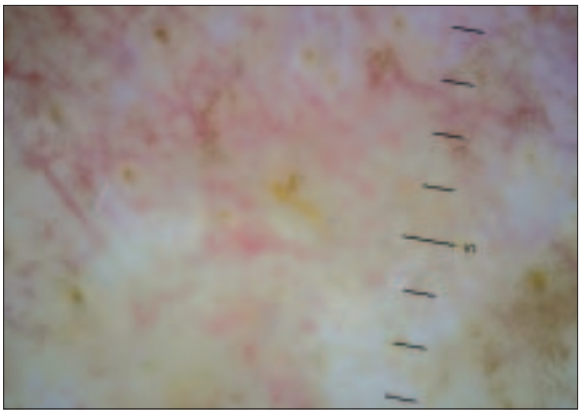


Figure 11. SIAscopy – optical model of the skin
Source: www.astronclinica.com

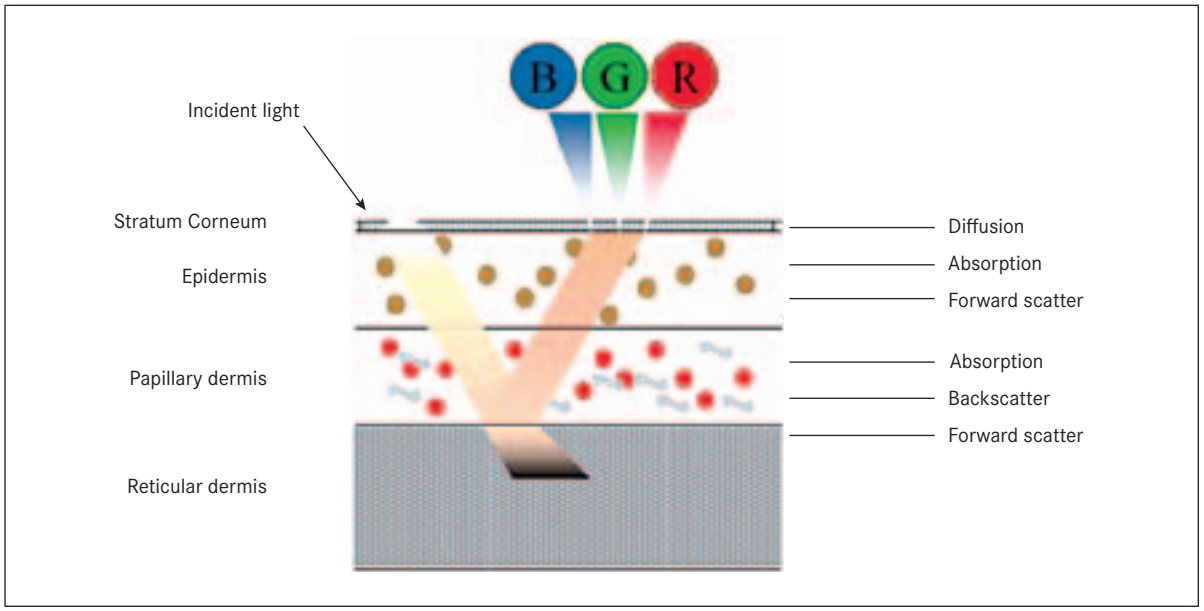


Figure 12. SIAscopy of superficial BCC – investigation of blood vessels

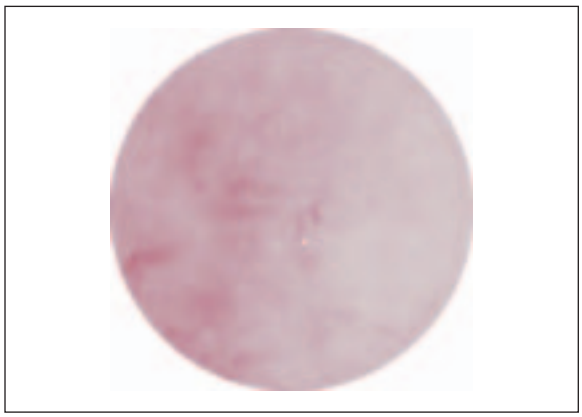
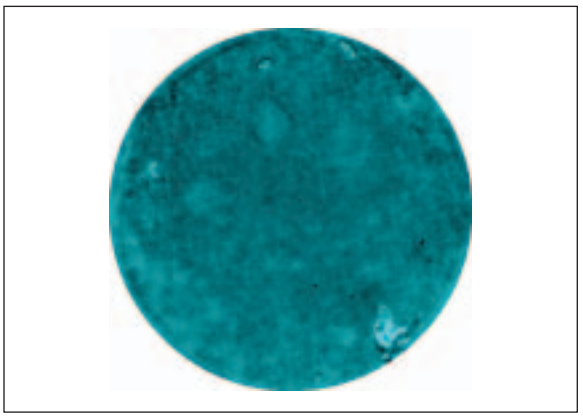


Figure 13. SIAscopy of superficial BCC – dermal collagen tissue investigation



There are common features of BCC in RCM. Parakeratosis in most cases and superficial disruption in case of ulcerated BCC is present in epidermis, particularly in stratum corneum. There is certain atypia with disruption of honeycomb pattern and pleomorphism in stratum granulosum and spinosum. In stratum basale at dermo-epidermal junction monomorphic cells with elongated nuclei are found. Typically for BCC polarisation of these elongated nuclei along the same axis visualises. It is called "streaming": all tumor cells are oriented along the same axis. The second typical feature is palisading when peripheral cells in the tumour nodule are oriented in a parallel manner forming an outer line perpendicular to the stroma. In the dermis of BCC there are: increased vascularity, vessels with large calibre and high blood flow, increased tortuosity, inflammatory infiltrate composed of small highly refractile vessels around cells, monomorphic polarised cells and formation of tumour nodules. In particular, nodular BCC presents with nests of aggregated tumour cells in the upper dermis, which are often adjacent to large and dilated blood vessels. Applying the horizontal mapping function on the level of the upper dermis lighten judgment process. Characteristic morphology with multiple tumour nodules or digitiform structures is separated from the dermal collagen by fibrosis. The tumor aggregates present as formation of crowded cells with variable refractivity. Cells in the periphery of tumour nodule usually show peripheral palisading and typical elongated nuclei. Visualised by confocal microscopy, peritumoral clefting appears as dark spaces surrounding tumour nodules. Similar clefts are detected also on routine histology. Inflammatory process can alter the typical findings; it is usually defined by the presence of small, highly refractive cells in the dermis. In superficial BCC, aggregation of polarised cells with elongated nuclei with orientation along the same axis are visualised in the basal cell layer and the superficial dermis, as well as increased dilatation and tortuosity of the vasculature and variable degree of inflammatory cells. In pigmented variants of BCC, the presence of highly refractive cells may suggest that the lesion is melanocytic, but the presence of specific BCC features: monomorphic cells with elongated nuclei or polarisation confirm the diagnosis of BCC. On RCM imaging, dendritic highly refractive cells in the upper dermis can be visualised, corresponding to melanocytes on histopathologic exam. Furthermore, bright oval-to-stellate structures with indistinct borders can be imaged by RCM, correlating to melanophages on routine histology [Ulrich, et al., 2008].

RCM in infiltrative BCC shows aggregation of monomorphic cells with elongated nuclei in the upper dermis, surrounded by a dense and cell-rich stroma. Peripheral palisading is usually absent and the borders between tumour cell aggregates and stroma are poorly defined [Bobadilla et al., 2008]. The diagnosis of infiltrative BCC remains more challenging when compared with nodular and superficial BCC as the features are often less pronounced [Desai, et al., 2007]. We found almost no studies on residual or recurrent BCC after previous excision. To date, no studies have been performed regarding this question. According to our experience, it might be very challenging to detect residual or recurrent BCC in surrounding scar tissue. The formation of the collagen bundles within the scar may resemble polarisation along the same axis or peritumoral fibrosis.

Shortcomings of the RCM method. Limited depth of penetration into the tissue (300 μm – 0.3 mm) that does not give sufficient visualisation of the skin structure and the pathologic alterations of the deeper dermal tissues. Furthermore, horizontally located cuttings do not allow the evaluation of the vertical invasion and the depth of the cancer. In hyperkeratotic forms certain amount of light is absorbed and / or disseminated (BCC with squamous differentiation, squamous cell cancer or keratoacantoma) that decreases the possibility of visualisation. It is necessary to inject the fluorescent contrast agent in to the cancer tissue for proper visualisation [Ulrich, et al., 2008].

Optical coherence tomography. OCT is an optical method analogue to B regimen of ultrasound, applying low intensity infrared light of 1–1.5 mWt, which gives no harm to the tissue investigated. The two dimensional visualisation of the vertical skin cutting provided by OCT offers the possibility to evaluate the following: the height of the layers, structure and grade of homogeneousness of the investigated areas, contrast of the layers and zones, parameters of the margins separating optical layers and zones. Malignant skin cancers: melanoma, squamous cell skin cancer, BCC, present resembling OCT signs. Accordingly, OCT should not be considered the method for setting differential diagnosis.

The OCT system used was developed at the Technical University of Denmark. The OCT probe is applied directly to the skin, using ultrasound gel as an optical coupling medium to improve image quality. Infrared radiation used by OCT lies in the "optical window" where the radiation is least scattered and absorbed compared with other wavelengths. The radiation source is a super luminescent diode with a centre wavelength of 1318 nm. Axial resolution is 8 mkm, and lateral resolution is 24 mkm. The maximal penetration depth in skin is 2.0–2.5 mm, and therefore only NMSCs with thickness < 2 mm were included. OCT measures reflection of infrared radiation from the skin, instead of acoustic waves, and the signal strength is mapped as a function of position. Accurate assessment of tumour size is important when planning treatment of nonmelanoma skin cancer (NMSC). Imaging with optical coherence tomography (OCT) has the potential to diagnose and measure depth of NMSC. Morgensen, et al. (2009) found that OCT is more precise than 20 MHz HFUS for mean maximal thickness measurement in BCC lesions thinner than 2 mm. This is in accordance with another study using OCT to measure tumour thickness in BCC lesions < 1–2 mm in depth, where OCT measurements correlated well with histology.

High-frequency ultrasonography (HFUS) is a frequently used method of non-invasive diagnostics exhales targeted investigation of echostructures of the skin and underlying fat tissues with determination of the depth and peculiarities of each layer. HFUS image in normal-appearing skin shows clear separation of the layers with the epidermis being generally seen as a thin hyperechoic line, except in the palmar and plantar areas where it is thicker and bilaminar. Normally, the dermis is seen as a hyperechoic band of variable thickness, being predominantly thin in the forearm and thick, as a result of high collagen content, in the lumbar region. The subcutaneous tissue is hypo-echoic because of the presence of fat lobules, which are surrounded by hyperechoic fibrous septa [Uhara, et al., 2007]. Subcutaneous blood vessels appear as thin ducts (venous or arterial) with low resistance and distinct peak systolic flow velocity [Wortsman and Wortsman, 2010].

The diagnostic efficacy of HFUS in certain skin pathologies reaches up to 100% [Wortsman and Wortsman, 2010]. At the diagnostics and monitoring of BCC this method is highly informative, showing the size, form, and depth of invasion into the surrounding tissue. Accordingly, the obtained information is sufficient to choose the treatment modality, the scope of planned intervention. Furthermore, HFUS is used to control the efficacy of the therapy performed, as well as for the determination of relapses.

Recently Lithuanian colleagues have published a detailed comparison of several HFUS equipments currently used for the diagnostic purposes of the skin (Table 1).

Table 1. Characteristics of the commercially available 20–50 MHz frequencies ultrasonic equipment for skin investigation (2D imaging. B-scan) [copy from paper of Jasaitiene, et al., 2011]

Equipment, characteristics	**Episcan 1–200, Longport, Inc.**	**DermaScan C, Cortex Technology**	**DUB-USB, Taberna *pro medicum***
Analogue-to-digital converter	200 MHz, 8 bits (256 levels of amplitude	–	100 MHz, 8 bits (256 levels of amplitude)
Scan (penetration) depths, mm	3.8–22.4	10–20 (depends on focal distance of the transducer)	8–10
Axial resolution, mkm	Up to 40	60	72
Latteral resolution, mkm	–	130	–
Central frequency of the transducer, MHz	20, 50	20	22
Scan lateral length, mm	15	–	12.8
Scan rate, frame per second	1	8	2.5
Type of visualisation	A-scan, B-scan (envelopes)	A-scan, B-scan (envelopes)	A-scan, B-scan (envelopes)
Combination with PC	Combined with embedded PC in minitower design	Combined with embedded PC in minitower design	Separate portable device, connection with PC via USB 2.0

The sonographic appearance of BCC is often stereotyped: well delimited from the surrounding dermis anechoic tumour, including large focally dense echoes, hyperechogenic spots, which in histopathology visualise as calcinates, horny cysts, apoptotic cell clusters in the centres of BCC nests [Uhara, et al., 2007].

The high value of HFUS in the diagnostics of malignant neoplasms has been proved in many investigations based on the correlation between ultrasound and histological investigations, especially in detection of thickness and horizontal borders [Fornage, et al., 1993; Gupta, et al., 1996]. Most of them were focused on the investigation of melanoma, and only one, published by Bobadilla, et al., 2008, on BCC. The data obtained in this study proved the high correlation (r = 9.0) between pre-operative HFUS detected excision lined and histologically proved cancer margins. In operative cases, where HFUS has been performed on pre-operative basis, the excision borders are histologically free from cancer cells, and 2-year follow up (71% cases, n = 29) showed absence of relapses.

HFUS pre-operatively precisely shows the morphology of the cancer, its relationship with the major blood vessels, and other crucial tissue structures. HFUS can clearly identify and define the pattern of blood vessels inside the lesions and extra-lesionally. Thereby, the risks of bleeding, relapse, metastasizing can be minimalised, as well as assist to observe patients of high risk groups. Other modalities include investigations for relapses, control of the result of cancer treatment with non-invasive methods: chemo-, cryotherapy, etc (Figures 14–16 (photos from the authors' personal archive)). HFUS helps to differentiate dermatological from non-dermatological skin lesions. Although almost all investigations point to the overestimation of borders of skin cancer in all dimensions for 1–2 mm, particularly in depth, due to infiltrates of lymphoid cells, this should still be considered as a "positive mistake" leading to radical excision of the cancer [Jasaitiene et al., 2011]. Bobadilla et al., 2008, has also stressed this finding in analysing correlation between the histological and ultrasound estimation of BCC tumour thickness.

In vivo RCM has quite little ability to penetrate the derma (0.5 mm (500 mkm), restricting its application purely for investigations of epidermal lesions and upper papillary skin layer. Other methods, as magnetic resonance (MR) or positron-emission tomography have limited resolution to isolate objects exceeding 5 mm in diameter. Intravenous contrast infusion is required for visualisation purpose, besides these methods are more expensive. In comparison, HFUS can investigate a lesion in diapason within 1 mm, in vertical – about 0.1 mm. The depth of investigation can vary from 6 to 20 mm. As most skin lesions involve subepidermal structures, they are not visualised for low (less than 3 mm) penetration providing equipment.

Therefore, HFUS comparing with all other equipment available gives the best balance for horizontal resolution and profundity.

Concerning the size of the equipment and the possibility for the adaptation for investigation of tissue structures in different depths, modern HFUS equipment shows definite advantages.

The routine algorithms for investigators need to be precise taking into consideration the native skin properties and polymorphism of skin pathologies. Since HFUS investigations are performed in one visit and in different depths, the investigator should have good knowledge of dermatology.

The ultrasound results are evaluated in correlation with spectrophotometric intracutaneous analysis (SIAscopy) and pathohistology. SIAscope operates by emission of visible light radiation (from 400 to 950 nm) into the skin and tumour, by the measurement of the reflected light quantity for each wavelength. It is possible because individual components of the skin vary in their optical properties. From the spectral measurements SIAscope extracts information regarding location, quantity and distribution of melanin, collagen and haemoglobin (i.e. vascularity) within the layers of the skin. The data are then displayed which are graphical representations of digital information. Pathohistology is the routine method to confirm the diagnosis of BCC [Raasch, et al., 2006]. The intraclass correlation coefficient (ICC) has been created by our scientific group to compare tumour thickness measurements, SIAscopy data and pathohistology, to perform excision of facial BCC with tumour-free borders at the first surgery. Dermatologists apply regular dermatoscopy as a routine method in the evaluation of skin lesions. In BCC reliable information can be achieved mainly on superficial or pigmented forms [Scalvenzi, et al., 2008].

Figure 14. HFUS of superficial BCC before therapy

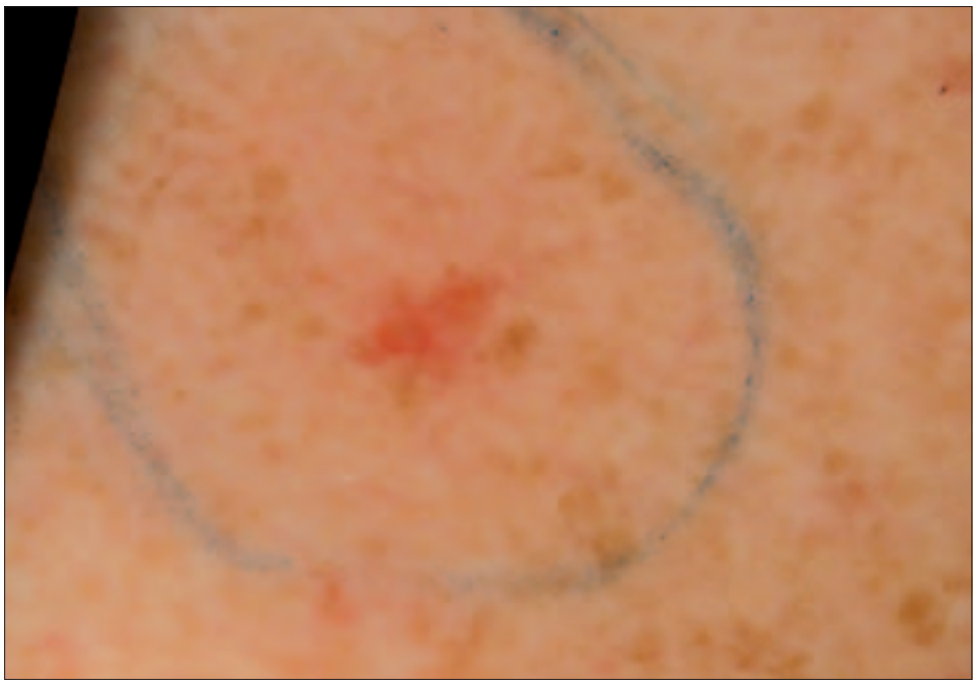


Figure 15. Superficial BCC 4 weeks after local cryotherapy + ALDARA cream (5% imiquimod)

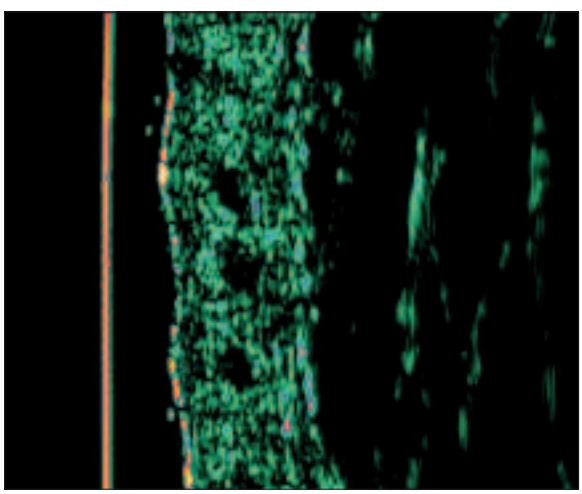
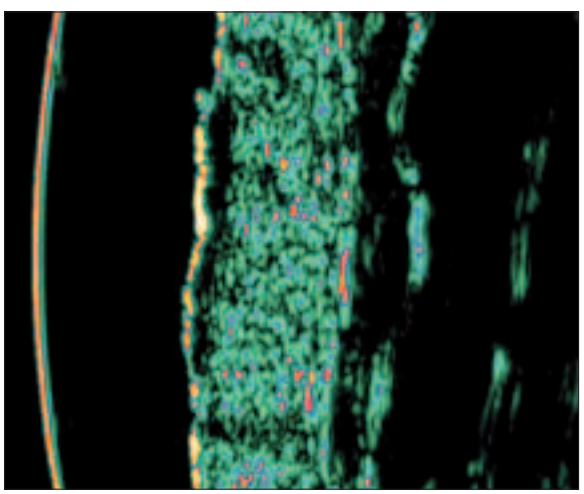


Figure 16. HFUS of superficial BCC 4 weeks after local cryotherapy + ALDARA cream (5% imiquimod)



Both OCT and especially HFUS tend to overestimate tumour depth if compared with histology. Simple correlations may be high even though the absolute differences between methods may be large [Mogensen, et al., 2009].

To conclude, none of the methods for skin investigation alone provide full information for proper planning of BCC treatment. Both HFUS 20-MHz and SIAscopy can be useful to plan BCC surgery – to recognise lesions, layers of involvement and vascularity patterns in a non-invasive way. Currently, only HFUS enables to define the lateral borders and depth of invasion of BCC precisely enough. Imaging with 20-MHz ultrasound has a good thickness correlation with pathohistological findings and may be used as a technique to monitor disease changes following non-invasive medical BCC treatments. Only combination of several non-invasive methods of diagnostics offers the possibility to evaluate the level of malignancy and the method of therapy properly.

References

1. Acarturk T. O., Edington H. Nonmelanoma skin cancer // Clin Plastic Surg, 2005; 32: 237–248.
2. Altamura D., Menzies S. W., Argenziano G., et al. Dermatoscopy of basal cell carcinoma: morphologic variability of global and local features and accuracy of diagnosis // J Am Acad Dermatol, 2010; 62: 67–75.
3. Anderson R. R, Parrish J. A. The optics of human skin // J Invest Dermatol, 1981; 77: 13–19.
4. Argenziano G., Fabbrocini G., Carli P., De Giorgi V., et al. Epiluminescence microscopy for the diagnosis of doubtful melanocytic skin lesions. Comparison of the ABCD rule of dermatoscopy and a new 7-point checklist based on pattern analysis // Arch Dermatol, 1998; 134(12): 1563–1570.
5. Bobadilla F., Wortsman X., Munoz C., Segovia L., et al. Pre-surgical high resolution ultrasound of facial basal cell carcinoma: correlation with histology // Cancer Imaging, 2008; 8: 163–172.
6. Day G. R. How blurry is that border? An investigation into algorithmic reproduction of skin lesion border cut-off // Comput Med Imaging Graph, 2000; 24(2): 69–72.
7. Derveniece A., Hartmane I., Cema I., Mikazans I., et al. Ultraviolet-radiation-caused skin cancer development and immune system response // Proceedings of the Latvian Academy of Sciences / Section B, 2002; Vol. 56; No. 3(620): 93–102.
8. Desai T. D, Desai A. D., Horowitz D. C., Kartono F., et al. The use of high-frequency ultrasound in the evaluation of superficial and nodular basal cell carcinomas // Dermatol Surg, 2007; 33: 1220–1227.
9. Elder D. E., Elenitsas R., Johnson B. L. Jr., Murphy G. F. Lever's histopathology of the skin. – 9th ed. – Philadelphia, PA: Lippincott Williams & Wilkins, 2005. – Pp. 836–848.
10. Fornage B. D., McGavran M. H., Duvic M., Waldron C. A. Imaging of the skin with 20 MHz US // Radiology, 1993; 189: 69–76.
11. Griffiths R. W., Suvarna S. K., Stone J. Do basal cell carcinomas recur after complete conventional surgical excision? // The British Journal of Plastic Surgery, 2005; 58: 795–805.
12. Gupta A. K., Turnbull D. H., Foster F. S., Harasiewicz K. A., et al. High frequency 40-MHz ultrasound: a possible noninvasive method for the assessment of the boundary of basal cell carcinomas // Dermatol Surg, 1996; 22: 131–136.
13. http://www.astronclinica.com – last reviewed on 30.08.2011.
14. Jasaitiene D., Valiukeviciene S., Linkeviciute G., Raisutis R., et al. Principles of high-frequency ultrasonography for investigation of skin pathology // Journal of the European Academy of Dermatology and Venereology, 2011; 25(4): 375–382.
15. LeBoit P. E., Burg G., Weedon D., Sarasain A. (Eds.). WHO histological classification of keratinocytic skin tumours: World Health Organization Classification of Tumours. Pathology and Genetics of Skin Tumours. – Lyon: IARC Press, 2006. – Pp. 13–19.
16. Menzies S. W., Westerhoff K., Rabinovitz H., Kopf A. W., et al. Surface microscopy of pigmented basal cell carcinoma // Arch Dermatol, 2000; 136(8): 1012–1016.
17. Mogensen M., Nürnberg B. M., Forman J. L., Thomsen J. B., et al. In vivo thickness measurement of basal cell carcinoma and actinic keratosis with optical coherence tomography and 20-MHz ultrasound // The British Journal of Dermatology, 2009; 160(5): 1026–1033.
18. Moncrieff M., Cotton S. D., Claridge E., Hall P. N. Spectrophotometric intracutaneous analysis – a new technique for imaging pigmented skin lesions // The British Journal of Dermatology, 2002; 146(3): 448–457.
19. Raasch B. A., Buettner P. G., Garbe C. Basal cell carcinoma: histological classification and body-site distribution // The British Journal of Dermatology, 2006; 155: 401–407.
20. Saldanha G., Fletcher A., Slater D. N. Basal cell carcinoma: a dermatopathological and molecular biological update // The British Journal of Dermatology, 2003; 148: 195–202.
21. Scalvenzi M., Lembo S., Francia M. G., Balato A. Dermoscopic patterns of superficial basal cell carcinoma // Intermational Journal of Dermatology, 2008; 47: 1015–1018.
22. Tabanlioglu O. D., Sahin, S., Gokoz O., Erkin G., et al. Correlation between the dermatoscopic and histopathological features of pigmented basal cell carcinoma // Journal of the European Academy of Dermatology and Venereology, 2000, 24: 1317–1325.
23. Telfer N. R., Colver G. B., Morton C. A. Guidelines for the management of basal cell carcinoma // The British Journal of Dermatology, 2008; 158(7): 35–48.
24. Uhara H., Hayashi K., Koga H., Saida T. Multiple hypersonographic spots in basal cell carcinoma // Dermatologic Surgery, 2007; 33(10): 1215–1219.
25. Ulrich M., Astner S., Stockfleth E., Röwert-Huber J. Noninvasive diagnosis of non-melanoma skin cancer: focus on reflectance confocal microscopy // Expert Rev Dermatol, 2008; 3(5): 557–567.
26. Wang S. Q., Katz B., Rabinovitz H., Kopf A. W., et al. Lessons on dermoscopy #4. Poorly defined pigmented lesion. Diagnosis: pigmented BCC // Dermatol Surg, 2000; 26(6): 605–606.
27. Wortsman X., Wortsman J. Clinical usefulness of variable-frequency ultrasound in localized lesions of the skin // Am Acad Dermatol, 2010; 62(2): 247–256.

Effect of Concomitant Used Drugs on the Antiplatelet Therapy

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Abstract

Antiplatelet drugs represent a key class of drugs that are proven value in arterial thromboembolic disorders. In cases when antiplatelet therapy fails, possible interaction with other concomitant used drugs should be considered.

The aim of this study was to identify most frequent existent possible acetylsalicylic acid and clopidogrel interaction with co-administrated drugs and to work out a recommendation for the optimization of pharmacotherapy

This study included 70 patients from practices of family physicians in Rīga and Ogre, two cities of Latvia, who used antiplatelet drugs concomitant with other drugs during the time from July, 2010 to January, 2011.

The average age of the patients was 62 ± 5 years. The most common indications for antiplatelet therapy were primary prophylaxis of myocardial infarction in patients with cardiovascular risk factors (n = 33) and stent placement following percutaneous coronary intervention (n = 20). All patients received acetylsalicylic acid antiplatelet therapy. 37 patients received acetylsalicylic acid in combination with clopidogrel. From the obtained data about concomitant drugs that possibly interact with acetylsalicylic acid nonsteroidal anti-inflammatory drugs (39%) with diclofenac (20%) and ibuprofen (11%) as most common and gastric-acid reducing agents (50%) should be noticed.

Among the patients who received dual antiplatelet therapy, 60% also used proton pump inhibitors (omeprazole (38%)) and 60% received large daily doses of lipophilic statin – atorvastatin.

Clinicians should probably judge patients taking such combination therapies as acetylsalicylic acid with diclofenac, which is at high risk for bleeding, and also combinations of acetylsalicylic acid and ibuprofen, clopidogrel and omeprazole and/or atorvastatin that have a reduced efficacy of antiplatelet therapy.

Keywords: acetylsalicylic acid, clopidogrel, antiplatelet drug resistance, drug-drug interaction.

Introduction

Oral antiplatelet drugs such as acetylsalicylic acid and clopidogrel are a cornerstone of pharmacotherapy in cardiovascular atherotrombotic diseases. Low-dose acetylsalicylic acid is usually the first choice antiplatelet drug. According to the European Society of Cardiology (ESC) guidelines, acetylsalicylic acid is indicated in all with cardiovascular diseases (including diabetes) and lifelong treatment with low dose (75–150 mg) is recommended [Graham, 2007]. Furthermore, acetylsalicylic acid in combination with clopidogrel has become the golden standard for patients receiving coronary stent or suffering from acute coronary syndrome [Feher, 2010].

The antiplatelet effect of acetylsalicylic acid (ASA) is mediated through its irreversible inactivation of cyclooxygenase (COX) 1, which is responsible for the formation of thromboxane, a potent vasoconstrictor and platelet aggregator, from arachidonic acid [Michos, 2006].

Clopidogrel – the thienopyridine derivative – irreversibly binds the platelet surface P2Y12 ADP receptor, thereby inhibiting ADP-induced platelet activation. Clopidogrel is a prodrug requiring oxidation by the hepatic cytochrome P450 (CYP450) to generate an active metabolite. Only a small proportion of clopidogrel undergoes metabolism by CYP450; it is mostly (85%) hydrolyzed by esterase to an inactive carboxylic acid derivative. The other pathway converts clopidogrel to its active metabolite by at least two CYP-dependent steps. Of the several CYP enzymes identified, CYP1A2, CYP3A4/5, and CYP2C19 are considered to be the main contributors to active metabolite formation [Feher, 2010].

All antiplatelet drug interactions with concomitant used drugs can be divided into pharmacokinetic and pharmacodynamic effects. Pharmacokinetic mechanism of drug interaction with clopidogrel is mainly the inhibition of certain CYP450 enzymes. Pharmacodynamic mechanisms for interactions with antiplatelet agents are synergism and competitive antagonism with acetylsalicylic acid (Table 1). Pharmacokinetic interaction with acetylsalicylic acid is a changed absorption of acetylsalicylic acid.

Table 1. Mechanisms of possible antiplatelet drug-drug interaction

Antiplatelet drugs	Mechanism of interaction	Drugs
Acetylsalicylic acid	Changed absorption of acetylsalicylic acid [Charlot, 2011; Kasprzak, 2009]	Proton pump inhibitors (PPIs) Ranitidine
	Competitive inhibition of COX-1 [Schuijt, 2009]	Ibuprofen
	Synergic antiplatelet action [Abajo, 2008]	Non-steroidal anti-inflammatory drugs (NSAIDs)
		Selective serotonin reuptake inhibitors (SSRIs)
Clopidogrel	Inhibition of the CYP450-mediated metabolic bioactivation [Bates, 2011]	Atorvastatin (CYP450 3A4)
		Omeprazole, Esomeprazole (CYP450 2C19)

The aim

The aim of this study was to identify most frequent existent possible acetylsalicylic acid and clopidogrel interaction with co-administrated drugs and to work out a recommendation for the optimization of pharmacotherapy.

Material and methods

This study included 70 patients from practices of family physicians in two cities of Latvia, Rīga (47 patients) and Ogre (23 patients), who used antiplatelet drugs, concrete, acetylsalicylic acid and clopidogrel concomitant with other drugs during the time from July, 2010 to January, 2011. A questionnaire was devised with the aim to gather such data from outpatient cards as sex, age, weight, height, blood pressure, clinical diagnoses, past history of cardiovascular disease, duration of antiplatelet drug use, dosage of antiplatelet and concomitant drugs and information about drug using-habits. Also the information about cardiovascular risk factors, lipid-lowering therapy, gastric acid-reducing therapy, selective serotonin reuptake inhibitor therapy, non-steroidal anti-inflammatory drug therapy and about concomitant used non-prescription drugs was obtained.

The collected information about used drugs included following medicines:

- acetylsalicylic acid-containing medicines – *Aspirin Cardio* 100 mg gastro-resistant tablets (Bayer), *Hjertemagnyl* 150 mg/21 mg and 75 mg/10.5 mg film-coated tablets (Nycomed GmbH), *Thrombo ASS* 100 mg, 75 mg and 50 mg gastro-resistant tablets (G. L. Pharma);
- clopidogrel-containing medicines – *Clopidogrel GSK* 75 mg film-coated tablets (GlaxoSmithKline), *Plavix* (brand name drug) (Sanofi Pharma Bristol-Myers Squibb SNC), *Trombex* 75 mg film-coated tablets (Zentiva);
- diclofenac sodium-containing medicines – *Diclac* 100 mg modified release tablets (Sandoz), *Diclac ID* 150 mg modified release tablets (Sandoz), *Dicloberl* 50 mg gastro-resistant tablets (Berlin-Chemie AG / Menarini Group), *Diclomelan* 50 mg gastro-resistant tablets (G. L. Pharma), *Diclomelan* retard 100 mg sustained-release tablets (G. L. Pharma), *Diclovit* hard capsules (G. L. Pharma);
- ibuprofen-containing medicines – *Ibugesic* 400 mg film-coated tablets (Cipla Ltd.), *Ibumetin* 400 mg film-coated tablets (Nycomed GmbH), *Ibuprofen-Teva* 400 mg coated tablets (Teva Pharma);
- meloxicam-containing medicine – *Movalis* 15 mg tablets (Boehringer Ingelheim International GmbH);
- etoricoxib-containing medicines – *Arcoxia* 60 mg and 90 mg film-coated tablets (brand name drug) (Merck Sharp & Dohme B. V.);
- omeprazole-containing medicines – *Gasec Gastrocaps* 20 mg gastro-resistant capsules (Mepha), *Lomac* 20 mg capsules (Cipla Ltd.), *Omeprazol Stirol* 20 mg capsules (Stirolbiofarm), *Omeprazole* 20 mg capsules (JSC Olainfarm);
- pantoprazole-containing medicines – *Controloc* 20 mg gastro-resistant tablets (Nycomed GmbH), *Nolpaza* 20 mg gastro-resistant tablets (KRKA);
- esomeprazole-containing medicine – *Nexium* 20 mg gastro-resistant tablets (brand name drug) (AstraZeneca);
- ranitidine-containing medicines – *Raniberl* 150 mg film-coated tablets (Berlin-Chemie AG / Menarini Group), *Ultak* 150 mg film-coated tablets (Cipla Ltd.), *Ranitidin Olainfarm* 150 mg film-coated tablets (JSC Olainfarm);
- atorvastatin-containing medicines – *Atilen* 40 mg film-coated tablets (Teva Pharma), *Atoris* 20 mg and 40 mg film-coated tablets (KRKA), *Caduet* 5 mg/10 mg film-coated tablets (brand name drug) (Pfizer), *Lopamol* 40 mg film-coated tablets (Actavis), *Sortis* 40 mg and 80 mg film-coated tablets;
- citalopram-containing medicine – *Citalopram-Teva* 10 mg film-coated tablets (Teva Pharma);
- escitalopram-containing medicine – *Elicea* 10 mg film-coated tablets (KRKA).

We used SPSS version 14th for the data processing. The level of statistical significance was defined as a p value < 0.05.

Results

70 patients (47 female, 23 male) were included in this study (Table 2). The mean age was 62.29 (SD ± 1.77).

All patients received acetylsalicylic acid antiplatelet therapy. 37 (52.9%) patients received dual antiplatelet therapy, i.e. acetylsalicylic acid and clopidogrel combination. Daily dose of clopidogrel is 75 mg. Frequently used daily dose of acetylsalicylic acid was 100 mg as utilized by 48 (68.6%) patients.

The most common indication for antiplatelet therapy was primary prophylaxis of myocardial infarction in 33 (47.1%) patients with cardiovascular risk factors. Among them 14 (20.0%) patients were diagnosed with hypercholesterolemia, 11 (15.7%) patients with hypertension, 5 (7.1%) patients with diabetes and 3 (4.3%) patients were smokers ($p = 0.01$). From all the population 3 (4.3%) patients were obese and 45 (64.3%) patients were overweight ($p = 0.008$).

Other antiplatelet therapy indications were stent placement following percutaneous coronary intervention (n = 20), unstable angina (n = 15), recent myocardial infarction (n = 7) and recent stroke or transient ischemic attacks (n = 6).

27 (38.6%) patients from all the population received concomitant non-steroidal anti-inflammatory drug (NSAIDs) therapy that possibly interacted with acetylsalicylic acid. In this group 14 (20.0%) patients used diclofenac, 9 (12.9%) patients – ibuprofen, 2 (2.9%) patients – meloxicam and 2 (2.9%) patients – etoricoxib (p = 0.005).

35 (50.0%) used gastric acid-reducing agents: 25 (35.5%) used proton pump inhibitors (PPI) and 10 (14.5%) – H2 receptor antagonist ranitidine. In addition, 4 (5.7%) patients received selective serotonin reuptake inhibitor (SSRIs) therapy, (3 (4.3%) patients– citalopram and, 1 (1.4%) patient – escitalopram) (p=0.06) (Figure 1).

All 37 patients with dual antiplatelet therapy received concomitant statin therapy. 31 (83.8%) patients used atorvastatin and 22 (59.5%) of them received high daily doses: 54.1% – 40 mg per day and 5.4% – 80 mg per day respectively (p = 0.006) (Figure 2).

22 (59.5%) patients from those with dual antiplatelet therapy also received proton pump inhibitor therapy, the most common of which was omeprazole for 14 (37.8%) patients (p = 0.013) (Figure 3).

Table 2. General characteristics of patients

General characteristics of patients	n (%)
Sex:	
Female	47 (67.1%)
Male	23 (32.9%)
Duration of antiplatelet drug use:	
≤ 6 months	40 (57.1%)
> 6 months	30 (42.9%)
Antiplatelet therapy:	
Monotherapy (acetylsalicylic acid)	33 (47.1%)
Dual therapy (acetylsalicylic acid + clopidogrel)	37 (52.9%)
BMI (kg/m^2):	
≤ 24.9 (normal)	22 (31.4%)
25–29.9 (overweight)	45 (64.3%)
≥ 30 (obese)	3 (4.3%)

Figure 1. Drugs with possible interaction with acetylsalicylic acid (aspirin)

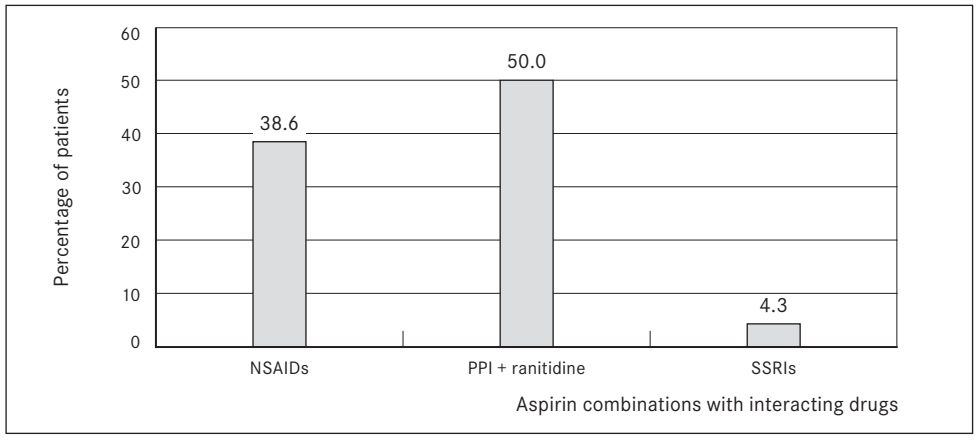


Figure 2. Daily doses of atorvastatin

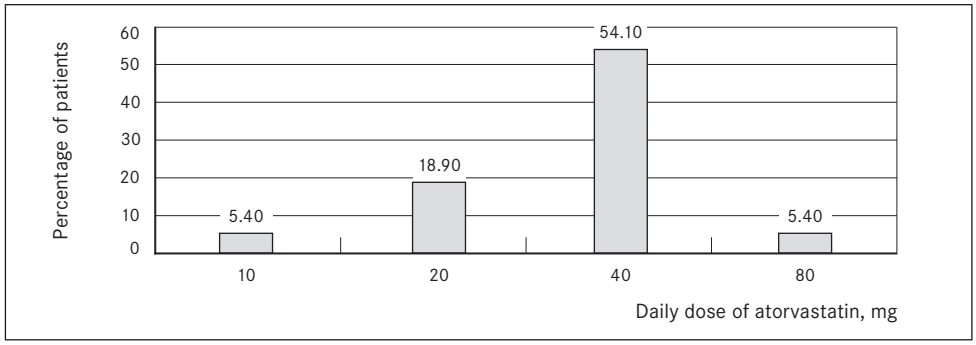
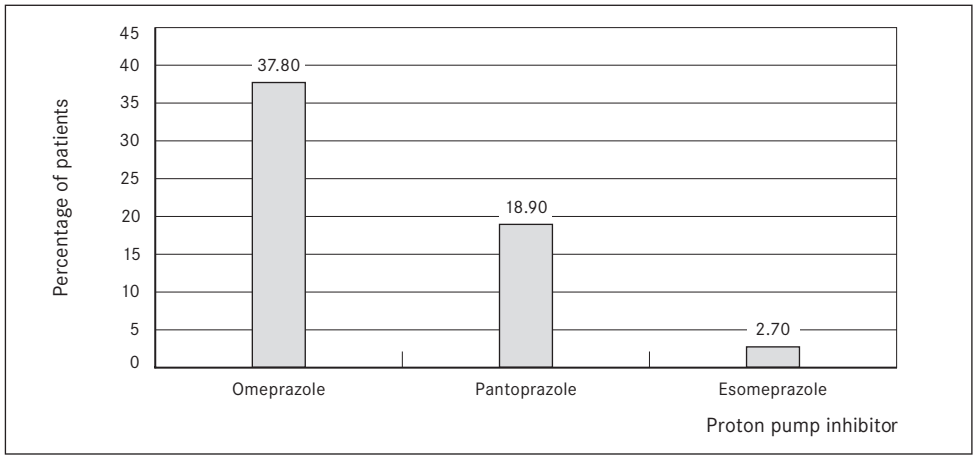


Figure 3. Frequently used proton pump inhibitors



Discussion

The present study demonstrates that antiplatelet drug interaction with concomitant drugs is a common phenomenon in practices of family physicians in Rīga and Ogre.

Antiplatelet treatment carries substantial side effects: mainly gastrointestinal ulceration and bleeding. For that reason, patients receiving a dual antiplatelet therapy after coronary stenting are commonly treated with gastric acid reducing agents like proton pump inhibitors for gastrointestinal protection, as also indicated by our study. Buffered or enteric coated tablets containing acetylsalicylic acid are proposed as another approach to gastroprotection of patients requiring antiplatelet treatment [Kasprzak, 2009].

Gastric acid-reducing agents change absorption of acetylsalicylic acid depending on the acetylsalicylic acid tablet coating. Studies show that increased gastric pH reduces the extent of acetylsalicylic acid absorption from non-enteric coated acetylsalicylic acid tablets [Charlot, 2011]. Acid suppression with PPI diminishes the gastric acetylsalicylic acid absorption because acetylsalicylic acid is not absorbed in stomach when its pH is greater than 6.5. Rising gastric pH can also increase the potential for mucosal esterase to hydrolyze acetylsalicylic acid to its inactive salicylic acid form.

Other studies show that acetylsalicylic acid concomitant use with gastric-acid reducing agents promotes premature dissolution of acetylsalicylic acid from enteric coated acetylsalicylic acid tablets. As pH > 5.5 does not normally exist in stomach, addition of PPI causes earlier destabilization of the methacrylic acid sheath (mostly used for enteric-coated acetylsalicylic acid forms) and after sheath depolymerization acetylsalicylic acid reached the duodenum already in its soluble form, thus assuring rapid absorption [Kasprzak, 2009].

Our study utilised acetylsalicylic acid tablets with two different coatings – *Thrombo ASS* and *Aspirin Cardio* as enteric coated tablets and *Hjertemagnyl* as non-enteric coated tablets.

Acetylsalicylic acid as a non-steroidal anti-inflammatory drug (NSAID) can interact with other members of that class. Co-administration of acetylsalicylic acid with another NSAID could produce a pharmacodynamic interaction. In contrast to acetylsalicylic acid, most other NSAIDs bind to COX-1 catalytic site reversibly. As long as an NSAID is present in the COX-1 channel, it obstructs the access of acetylsalicylic acid to the serine residue and thus the irreversible inactivation of platelet COX-1. Accordingly, NSAIDs may impair the thromboprophylactic action of acetylsalicylic acid. Whether this occurs depends on the affinity of the NSAID for the COX-1 enzyme [Schuijt, 2009]. On the other hand, such a pharmacodynamic interaction could result in increased incidence of gastrointestinal and non-gastrointestinal hemorrhage [Vardeny, 2008].

Ibuprofen has greater affinity for the COX-1 enzyme than diclofenac. Acetylsalicylic acid and ibuprofen concomitant use could lead to decreased antiplatelet effect because of competition of both for the same binding site on COX-1 enzyme [Schuij, 2009]. Clinicians should avoid this combination if possible. In case ibuprofen is needed, health care professionals should consider counseling patients about the appropriate timing of ibuprofen dosing if they are also taking acetylsalicylic acid for cardioprotective effects. Experts recommend patients who use immediate release acetylsalicylic acid (non-enteric coated acetylsalicylic acid tablets) and take a single dose of ibuprofen 400 mg to dose the ibuprofen at least 30 minutes or longer after the ingestion of non-enteric coated acetylsalicylic acid tablet, or more than 8 hours before the ingestion of non-enteric coated acetylsalicylic acid tablet to avoid attenuation of acetylsalicylic acid's effect [FDA, 2006]. However, this can not be attributed to enteric coated low dose acetylsalicylic acid tablets because in that case the absorption of acetylsalicylic acid is delayed. The likely drug interactions explanations suggest that use of ibuprofen containing drugs as non-prescription drugs should be more controlled.

Co-administration of selective serotonin reuptake inhibitors with NSAIDs may increase the risk of upper gastrointestinal tract and other bleeding. The mechanism of interaction is the depletion of serotonin from platelets caused by SSRIs showing antiplatelet action [Abajo, 2008].

Drug-drug interactions that involve the cytochrome P-450 system have been postulated to reduce the efficiency of clopidogrel. The most studied possible interactions involving the CYP450 system are with the inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) – atorvastatin and with the proton pump inhibitor – omeprazole.

Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase the rate-limiting enzyme of cholesterol synthesis in the liver. CYP3A4 is important for the elimination of lipophilic statins (lovastatin, simvastatin, and atorvastatin), but hydrophilic statins (fluvastatin, pravastatin, and rosuvastatin) are not significantly metabolized by this isoenzyme. Atorvastatin is the most commonly prescribed statin, and its hepatic metabolism has the potential to result in significant interactions with clopidogrel especially in high doses [Bates, 2011]. The obtained results indicate that atorvastatin is the most commonly prescribed statin; therefore adjustment of drug administration could be a solution.

Proton pump inhibitors (PPIs) are prodrugs that are activated in gastric parietal cells. PPIs irreversibly inhibit the gastric H^+/K^+ ATPase (the proton pump) that accomplishes the final step in acid secretion. Omeprazole, the most widely used PPI, is metabolized primarily by CYP2C19 and CYP3A4. Omeprazole has a greater affinity for CYP2C19 than CYP3A4, compared with the other PPIs that are also metabolized by these isoenzymes, and therefore has a greater potential for drug-drug interactions mediated by CYP2C19 [Bates, 2011; Feher, 2010].

There are conflicting and inconsistent data regarding the adverse clopidogrel-omeprazole interaction [Gilard, 2008; Kwok, 2010]. Clinicians should be cautious combining those drugs. There is a possibility to replace omeprazole with other PPI that is not metabolized by the same CYP enzymes (e.g., pantoprazole) or adapt drug administration regime, i.e. morning and evening administration.

Our study shows that family physicians prescribe both brand name and generic drugs. The possible difference in clinical effects of brand name or generic drugs with similar active substance is under-investigated.

Conclusions

1. The most common interactions with acetylsalicylic acid are with non-steroidal anti-inflammatory drugs: prescription drug diclofenac and non-prescription drug ibuprofen; with gastric acid-reducing agents: prescription drug omeprazole and non-prescription drug ranitidine. The most frequent existent possible interactions with clopidogrel are with proton pump inhibitor omeprazole and with atorvastatin in large doses.
2. Clinicians should probably judge patients taking such combination therapies as acetylsalicylic acid with diclofenac, which is at high risk for bleeding, and also combinations of acetylsalicylic acid and ibuprofen, clopidogrel and omeprazole and/or atorvastatin that have reduced efficacy of antiplatelet therapy.
3. Enteric coated tablets containing acetylsalicylic acid is a better choice than non-enteric coated form. It is an approach to gastroprotection of patients requiring acetylsalicylic acid treatment and does not have risk of the reduced absorption of acetylsalicylic acid for those patients who receive concomitant gastric acid-reducing agent therapy.
4. Clinicians should focus on potential harm from ulcers and hemorrhage before deciding to omit omeprazole in patients taking clopidogrel. Replacing omeprazole with pantoprazole and adapted drug administration regime could be a solution to clopidogrel-omeprazole interaction.
5. The drug-drug interaction may also be connected with more drugs than the patient is taking. That is an area that is relatively under-investigated.
6. The use of non-prescription drugs should be more controlled. Physicians and pharmacists must gather data about all drugs a patient is using, work out individualized pharmacotherapy. Collaboration of pharmacist and physician in the management of drug interactions can enhance the efficacy and safety of antiplatelet therapy.

References

1. Abajo F., García-Rodríguez L. Risk of upper gastrointestinal tract bleeding associated with selective serotonin reuptake inhibitors and venlafaxine therapy interaction with nonsteroidal anti-inflammatory drugs and effect of acid-suppressing agents // Archives of General Psychiatry, 2008; 65(7): 795–803.
2. Bates E., Lau W., Angiolillo D. Clopidogrel-drug interactions // Journal of the American College of Cardiology, 2011; 57(11): 1251–1263.
3. Charlot M., Grove E., Hansen P. R., et al. Proton pump inhibitor use and risk of adverse cardiovascular events in acetylsalicylic acid treated patients with first time myocardial infarction: nationwide propensity score matched study // http://www.bmj.com/content/342/bmj.d2690.full.pdf (accessed 4.06.2011).
4. Feher G., Feher A., Pusch G., et al. Clinical importance of acetylsalicylic acid and clopidogrel resistance // World Journal of Cardiology, 2010; 2(7): 171–186.
5. Graham I., Atar D., Borch-Johnsen K., et al. European guidelines on cardiovascular disease prevention in clinical practice: executive summary // European Heart Journal, 2007; 28(19): 2375–2414.
6. Gilard M., Arnaud B., Cornily J. C., et al. Influence of omeprazole on the antiplatelet action of clopidogrel associated with acetylsalicylic acid: the randomized, double-blind OCLA (Omeprazole CLopidogrel Acetylsalicylic acid) study // Journal of the American College of Cardiology, 2008; 51(3): 256–260.
7. Kasprzak M., Koziński M., Bielis L., et al. Pantoprazole may enhance antiplatelet effect of enteric-coated acetylsalicylic acid in patients with acute coronary syndrome // Cardiology Journal, 2009; 16(6): 1–9.
8. Kwok C. S., Loke Y. K. Meta-analysis: the effects of proton pump inhibitors on cardiovascular events and mortality in patients receiving clopidogrel // Alimentary Pharmacology & Therapeutics, 2010; 31(8): 810–823.
9. Michos E. D., Ardehali R., Blumenthal R. S., et al. Acetylsalicylic acid and clopidogrel resistance // Mayo Clinic Proceedings, 2006; 81(4): 518–526.
10. Schuijt M., Huntjens-Fleuren H., de Metz M., Vollaard E. The interaction of ibuprofen and diclofenac with acetylsalicylic acid in healthy volunteers // British Journal of Pharmacology, 2009; 157(6): 931–934.
11. U.S. Food and Drug Administration. Information for healthcare professionals: Concomitant use of ibuprofen and acetylsalicylic acid // http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsand Providers/ ucm125222.htm (accessed 04.06.2011).
12. Vardeny O., Solomon S. D. Cyclooxygenase-2 inhibitors, nonsteroidal anti-inflammatory drugs, and cardiovascular risk // Cardiology Clinics, 2008; 26(4): 589–601.

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