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Food Allergy and Food Hypersensitivity in Children 0–3 years

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Abstract

Food allergy (FA) is a rising problem worldwide. No previous studies of FA and food hypersensitivity have been conducted in Latvia.

The objectives of the respective research include studies of the symptoms that are supposed to be caused by FA and identification of food allergens and tests used in diagnostics of FA in different age groups in children up to three years of age.

Data for the retrospective descriptive study were obtained from randomly sampled medical documents from allergy outpatient care in Children's Clinical University Hospital in Rīga. Children, 0–3 years old, participated in this study, divided into the following age groups: 0–6 months, 6–12 months, 12–18 months, 18–24 months and 2–3 years. For statistical analysis, MS Excel and IBM Statistics 20.0 programs were used.

Data from 100 children medical documents were collected; 61% boys, 39% girls. 65% of all patients were infants. In 92% of patients, symptoms had started in the first year of life. Patients most commonly had positive tests to egg and milk (71% and 40%, respectively). Specific IgE tests were used in 61% patients, skin prick tests in 51%, atopy patch tests (in combination with prick tests or specific IgE) in 20%. Oral food challenges were not performed. The most common complaints were skin symptoms (98%) and gastrointestinal problems (10%). 94% (n = 94) had atopic dermatitis, 3% (n = 3) anaphylactic reactions, 2% (n = 2) respiratory symptoms. 12% of patients had symptoms from more than one organ system.

This study has revealed that egg and milk are the most common food allergens found in children in the first three years of life. Skin problems could be the most worrying complaint for parents, which might explain why almost all patients who participated in this study had skin problems. Gastrointestinal symptoms are poorly recognised and evaluated as a sign of food allergy. Oral food challenges are necessary tests for diagnosis of food allergy and must be conducted in Children's Clinical University Hospital in Rīga.

Keywords: Food allergy, food hypersensitivity, specific IgE, skin prick test, atopy patch test.

Introduction

Food allergy (FA) is a rising health concern that affects both children and adults. Food hypersensitivity may be the first stage in the development of allergic diseases such as atopic eczema [Bath-Hextall, 2009]. The highest FA incidence occurs during the first year of life [Fiocchi, 2011], around the age of 6–9 months [Bath-Hextall, 2009].

The Guidelines for the Diagnosis and Management of Food Allergy in the United States: Report of the NIAID-Sponsored Expert Panel define FA as an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food [Bergmann, 2013]. It also indicates that sensitisation (as evidenced by the presence of allergen-specific immunoglobulin E (sIgE)) to food allergens alone is not sufficient to define FA [Boyce, 2010].

Existing studies of the incidence, prevalence and natural history of FA are difficult to compare owing to inconsistencies and deficiencies in study design and variations in the definition of FA [Alle, 2012]. Many studies define food allergies based on history, which is inaccurate. Other studies use IgE positivity or skin test positivity to specific foods as a marker of FA. Between 50% and 75% of patients with sIgE to food tolerate the food [Lack, 2008]. The prevalence of food allergies has been estimated to be 5–6% in infants and children younger than 3 years [Sicherer, 2013].

More than 170 foods have been reported to cause IgE-mediated reactions [Boyce, 2010]. Milk, egg, peanut, tree nuts, fish, shellfish, wheat and soy are considered to cause most of the food adverse reactions [Ito, 2012]. Cow's milk protein together with hen's egg protein are the key triggers of food allergy in infants and young children [Koletzko, 2012].

Clinical picture of food allergy is largely dependent on the pathogenesis (IgE or non-IgE response), patient's age and organ systems involved [Lozovskis, 2009]. The immune reaction may be IgE-mediated, non-IgE mediated, or mixed [Koletzko, 2012]. IgE-mediated reactions are characterised by an acute onset of symptoms generally within 2 hours after the ingestion of or exposure to the trigger food; they typically involve the skin, gastrointestinal tract, and respiratory tract [Burks, 2012]. Mixed IgE and non-IgE mediated reactions are atopic dermatitis, eosinophilic esophagitis and eosinophilic gastroenteritis. Food protein-induced enterocolitis syndrome, food protein-induced allergic proctocolitis, allergic contact dermatitis, Heiner syndrome are caused by non-IgE mechanisms [Burks, 2012]. Food generally appears to be the most common trigger of anaphylaxis in the community [Sicherer, 2011]. Reactions can occur following ingestion, inhalation or contact with foods [Fiocchi, 2011].

Double blind placebo controlled food challenge (DBPCFC) is the gold standard of FA diagnosis. However, a single-blind or an open-food challenge may be considered diagnostic under certain circumstances [Boyce, 2010]. The European Academy of Allergology and Clinical Immunology considers that the involvement of subjective factors is negligible among infants and children younger than three years of age and has approved the use of open food challenges in the diagnosis of FA [Yang, 2012]. Other frequently used tests are skin prick tests (SPT), detection of sIgE, atopy patch tests (APT).

The potential severity of the disease and the specific public health measures required for FA make it important to identify the specific risk factors for this condition [Cochrane, 2009]. The primary therapy for FA is strict avoidance of the causal food or foods [Burks, 2012]. However, such diets might induce nutritional deficiencies if applied indiscriminately and without a clear indication [Bergmann, 2013]. A diet that is not indicated or continued when the child may have already developed tolerance may impair growth and quality of life of both child and family, while incurring significant unnecessary health care costs [Koletzko, 2012].

Most children with FA eventually will tolerate milk, egg, soy, and wheat; far fewer will eventually tolerate tree nuts and peanut. The time course of FA resolution in children varies by food and may occur as late as teenage years [Boyce, 2010]. 90% of infants who are allergic to cow's milk may tolerate it by the end of their third year, whilst half their peers who are allergic to egg do not react to it at the same age [Fiocchi, 2011].

Aim

The aim of the research is to study the symptoms that are supposed to be caused by FA, identify food allergens and tests used in diagnostics of FA in different age groups in children up to three years of age.

Material and methods

It was a retrospective descriptive study. The study was conducted in Children's Clinical University Hospital in Rīga. Children 0–3 years old with suspected FA participated in this study. All patients were divided into the following age groups: 0–6 months, 6–12 months, 12–18 months, 18–24 months

and 2–3 years. The group selection was based on the differences in children's feeding habits and the fact that FA usually starts in infancy and in most cases tolerance to food is achieved up to the age of three years.

Data from randomly sampled medical documents from allergy outpatient care in Children's Clinical University Hospital in Rīga in year 2012 were collected for patients with FA diagnosis or positive allergy tests (sIgE, SPT, APT to food allergens or positive oral food challenge (OFC) tests). Evaluation was done concerning patients sex, age at the time of consultation and time of the onset of symptoms, and the type of symptoms in different age groups. Food allergens to which patients had positive allergy tests were assessed in total and in each age group. Cow's milk, egg, wheat, soy, nuts/peanuts, fish/crustaceans were counted separately, but other food allergens were put into one group since they were not among the most common food allergens. These included potato, carrot, banana, kiwi, broccoli, sweet pepper, tomato, pumpkin, apple, rye, oat, rice, chicken, pork and cacao. The evaluation was also done for the diagnostic methods used in study patients – SPT, sIgE, APT, OFC tests, diagnosis based on history and physical examination and other methods in each age group and in total.

Exclusion criteria: patients with celiac disease or other known gastrointestinal diseases, growth retardation due to a disease other than food allergy, airway diseases (other than bronchial asthma, allergic rhinitis, not associated with allergy), previously diagnosed skin diseases (except atopic dermatitis) or other known diseases with symptoms that resemble food allergy.

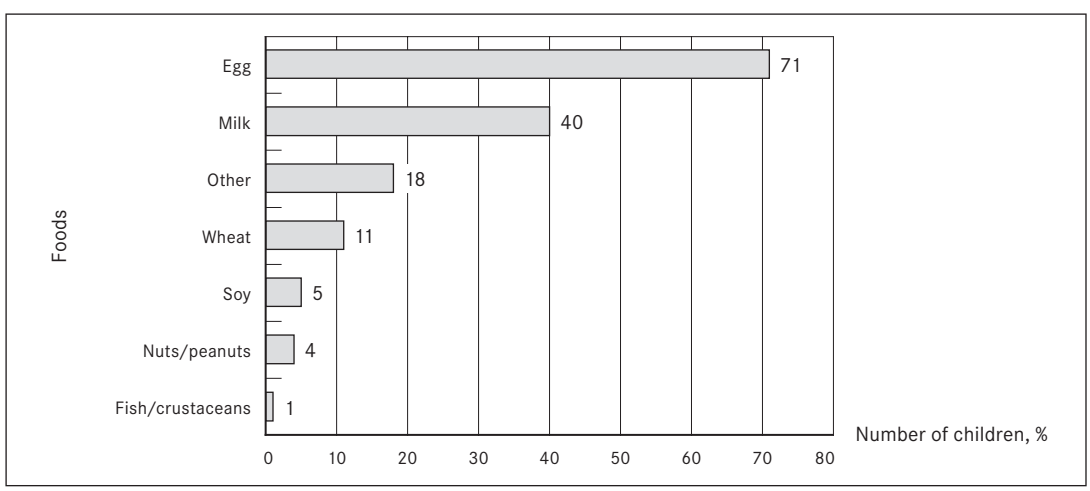
For statistical analysis, MS Excel and IBM Statistics 20.0 programs with comparative and descriptive methods were used with confidence interval (CI) 95%.

Results

Data from 100 children medical documents were collected. 61% (n = 61) were boys, 39% (n = 39) were girls. 65% (n = 65) of all patients were infants (0–6 months 31%, n = 31; 6–12 months 34%, n = 34). The number of patients in other age groups was as follows: 12–18 months 20 patients (20%), 18–24 months 6 patients (6%), 2–3 years 9 patients (9%). In 92% (n = 92) of cases symptoms had started in the first year of life. There was no information about the time of the onset of symptoms in five medical cards.

Food allergens. Egg was the most common food allergen among the study patients and the hypersensitivity to egg differed significantly from the second most common food allergen – milk (z score 4.53). Hypersensitivity to egg was found in 71% (n = 71) and to milk in 40% (n = 40) of patients. Hypersensitivity to other food allergens was as follows: wheat 11% (n = 11), soy 5% (n = 5), nuts/peanuts 4% (n = 4), fish/crustaceans 1% (n = 1) and other foods 18% (n = 18) (Figure 1).

Figure 1. The most common food allergens in children 0–3 years of age (% from the total number of patients)



There was no statistically significant association between the age of the patient and possible food allergen. The overall occurrence of each food allergen in age groups is shown in Table 1.

In 40% (n = 40) of cases hypersensitivity to several food allergens was detected; furthermore, it was more often seen in the first year of life – 25% (n = 10) of infants 0–6 months and 43% (n = 18) of infants 6–12 months of age. 15% (n = 6) of children in age group 12–18 months and 8% (n = 3) of children in both age group 18–24 months and age group 2–3 years were sensitised to more than one food allergen.

Diagnostic methods. In 5% (n = 5) of patients food allergy was diagnosed based on medical history and physical examination. Two of these patients had had anaphylactic reactions in the past (to nuts and broccoli).

SPT and evaluation of sIgE were statistically significant the most commonly used diagnostic methods in the study patients (z score 9.23). SPT (alone or in combination with other tests) were used in 51% (n = 51) of patients, sIgE (alone or in combination with other tests) in 61% (n = 61) of patients. APT (in combination with sIgE or SPT) were used in 20% (n = 20). OFC were not done (Figure 2).

The most common diagnostic methods used in each age group were as follows: in age group 0–6 month detection of sIgE (32%, n = 10), in age group 6–12 months SPT (41%, n = 14), in age group 12–18 months detection of sIgE (40%, n = 8), in age group 18–24 months and 2–3 years SPT (50%, n = 3 and 44%, n = 4).

Symptoms. The most common complaints were skin symptoms (98%, n = 98) and gastrointestinal (GI) problems (10%, n = 10). Anaphylactic reactions were observed in 3% (n = 3), respiratory symptoms in 2% (n = 2), general symptoms (growth failure, irritability) in 3% (n = 3). 12% (n = 12) of patients had symptoms from more than one organ system. Only two patients had isolated GI symptoms. None of the patients had respiratory symptoms as the only complaint (Figure 3).

The skin symptoms were as follows: 96% (n = 96) of patients had atopic dermatitis, 6% (n = 6) had urticaria, 1% (n = 1) had other skin eruptions. Three patients had several skin symptoms.

50% (n = 5) of patients with GI symptoms had regurgitation or vomiting, three patients had loose stool/diarrhoea, two patients had constipation. Blood and/or mucus in the stool was a complaint for one patient.

Skin symptoms were statistically the most significant common complaint in the study patients and their occurrence differed statistically significantly from GI disorders which were the second most common complaint (z score was 12.49 at the level of significance 0.05 (or 95% probability).

Atopic dermatitis was the most common symptom in all age groups.

Table 1. The comparison of food allergens in different age groups (% of patients in age groups)

Allergens	Age groups				
	0–6 months (n = 31)	**6–12 months (n = 34)**	**12–18 months (n = 20)**	**18–24 months (n = 6)**	**2–3 years (n = 9)**
	Percentage (%) and number (n) of patients in the age group				
Egg	74% (n = 23)	68% (n = 23)	70% (n = 14)	67% (n = 4)	78% (n = 7)
Milk	39% (n = 12)	56% (n = 19)	35% (n = 7)	17% (n = 1)	11% (n = 1)
Wheat	10% (n = 3)	12% (n = 4)	10% (n = 2)	17% (n = 1)	11% (n = 1)
Soy	3% (n = 1)	9% (n = 3)	5% (n = 1)	0	0
Nuts/peanuts	3% (n = 1)	0	0	33% (n = 2)	11% (n = 1)
Fish/crustaceans	0	3% (n = 1)	0	0	0
Other	10% (n = 3)	21% (n = 7)	20% (n = 4)	17% (n = 1)	33% (n = 3)

Figure 2. Diagnostic methods of FA and food hypersensitivity used in studied patients (% of total, n = 100)

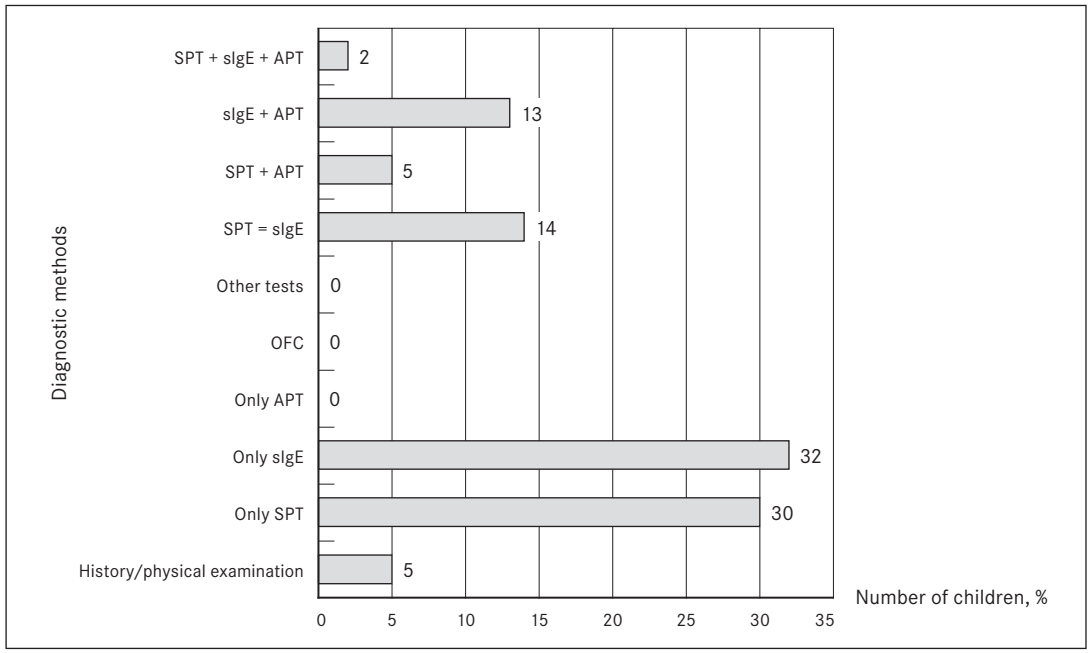
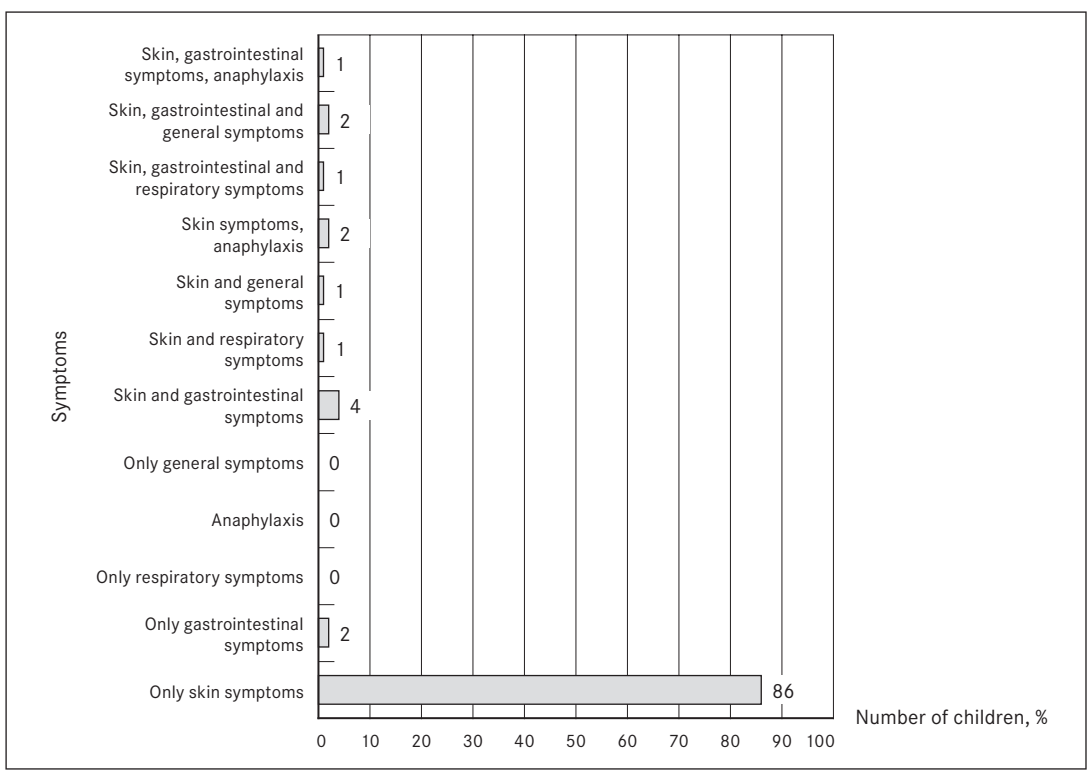


Figure 3. Symptoms in which FA were suspected (% of patients from total, n = 100)



Discussion

FA is a rising health concern worldwide that mostly affects young children but also can persist in adulthood. The highest FA incidence occurs during the first year of life [Fiocchi, 2011]. The results of this study revealed that most of the patients with suspected FA and diagnosed hypersensitivity to food allergens were infants. It is often discussed that milk is the most common food allergen in children. Several studies indicate that allergy and hypersensitivity to egg in infancy is more common than to other food allergens [Fiocchi, 2010; Salehi, 2009]. The results of *The Early Prevention of Asthma in Atopic Children (EPAAC ™)* study show that egg is the most important food allergen in children with atopic dermatitis in the first two years of life [Benedictis, 2009]. Egg was the most common food allergen among patients of this study and the hypersensitivity to egg differed significantly from the second most common food allergen – milk. Hypersensitivity to other food allergens in patients of this study was observed less frequently.

The US FA guidelines of 2010 [Boyce, 2010] advise that the nature of the reaction often suggests the underlying mechanism, either IgE-mediated (immediate) or non-IgE mediated (delayed), and will determine the diagnostic tests to be used. For detection of IgE-mediated reaction SPT and evaluation of sIgE antibodies are used. It is important to notice that neither SPT nor detection of sIgE when used alone are diagnostic of FA [Boyce, 2010]. In diagnosing FA or food hypersensitivity in patients of our study SPT or sIgE test were performed most often. SPT are easy to perform and the results are ready after 15 minutes. On the other hand, this test cannot be done on damaged skin or when antihistamines or systemic steroids are being used. Mehl, et al. study shows that the concordance between SPT and sIgE is surprisingly low for cow's milk and hen's egg on individual basis. In children who receive a negative test result the alternative test should also be used [Mehl, 2012]. A number of studies report that the APT may be useful in the evaluation of food allergy in patients with atopic dermatitis and eosinophilic eosophagitis [Boyce, 2010]. The US FA guidelines of 2010 indicate that insufficient evidence exists to support the use of the APT for the evaluation of food allergy. None of the patients in this study had APT performed as the only test for evaluation of possible FA. Negative SPT and/or sIgE test would explain why APT was done, since this test was generally used to assess delayed or non-IgE mediated reactions to a food allergen. Unfortunately, DBPCFC, which is the gold standard test for FA diagnosis was not performed for the study patients. This item should be seriously taken into consideration because without these tests FA diagnosis can be established only in several cases, for example, anaphylactic reactions after specific food and positive SPT for this food.

By evaluating the symptoms of the study patients, three of the patients reported anaphylactic reactions. Two of these patients had reaction to two of the most possible foods that cause anaphylactic reactions – nuts and fish. It was well seen that skin symptoms, particularly atopic dermatitis, was the most common problem of the study patients. Only two patients had isolated GI symptoms. Approximately 40% of infants and young children with moderate to severe atopic eczema have FA [Fiocchi, 2011]. It is indicated that 50–60% of children with cow milk allergy have GI symptoms [Vandenplas, 2007; Greef, 2012], and these symptoms are found in 41% of children with soy allergy [Savage, 2010]. Since there were no OFC performed for these study patients, it is difficult to link food allergens with symptoms, we can only discuss possible food hypersensitivity, not real FA. On the other hand, it was noticeable that FA was more often suspected in patients with skin problems. GI symptoms might be underestimated as potential symptoms for FA.

Conclusions

1. 92% of patients with suspected food allergy have developed symptoms till 12 months of age. 65% of patients at the time of consultation were infants.
2. Egg is statistically the most significant common food allergen among the study patients and hypersensitivity to egg differs statistically significantly from the hypersensitivity to the second most common food allergen – milk.

3. There is no statistically significant association between the age of the patient and possible food allergen.
4. Most frequently used diagnostic methods are skin prick tests or detection of sIgE antibodies.
5. Oral food challenges are not done, because they are time consuming and require specific preparation.
6. Skin problems are statistically the most significant common complaints for the study patients, the second most common complaint – gastrointestinal symptoms.
7. Gastrointestinal symptoms are not well recognised as a possible clinical manifestation of food allergy.
8. For diagnosis of food allergy and prescription of adequate elimination diet in Children's Clinical University Hospital in Rīga, it is necessary to perform oral food challenge tests.

Acknowledgement

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Thyroid Volume in Type 2 Diabetics: Relationship to Patients' Weight and Type of Treatment

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Abstract

Diabetes mellitus and thyroid diseases are closely connected, and 11% of patients with diabetes have thyroid diseases. The data is the same for type 1 and type 2 patients with diabetes mellitus; women face the disease two times more frequently than men. The latest researches have proved that metformin has lowered the level of TSH and thyroid volume.

The aim of the research is to prove the correlation between thyroid volume and the changes of weight in connection with type 2 diabetes, by analysing patients' medical situation, maintaining laboratory and clinical data, and therapy.

The research information (138 case histories) was divided into three groups: the first – 76 patients with type 2 diabetes and obesity who use metformin, the second control group – 39 patients with type 2 diabetes and obesity who are cured with insulin but do not use metformin, and the third – 23 patients with normal body mass index. The results of the research have been processed with the SPSS for Windows 22.0.

The correlation appraisal between body mass index and thyroid volume correlation was proved (r_s = 0.23; p = 0.006), and the results are statistically significant. It was proved that the patients with metabolic syndrome and insulin resistance have bigger thyroid volumes.

Thyroid volumes for patients with type 2 diabetes and obesity using metformin are nearly the same as for patients with type 2 diabetes and obesity who do not use metformin, (p = 0.857), and there is no significant statistical difference between these two groups.

TSH level for the first group is 1.67 ± 0.84 mU/L, for the second – 1.8 ± 0.81 mU/L, but for the third – 1.47 ± 0.86 mU/L (p = 0.443). The average TSH level for the first group compared to the second group is lower. The data proves that metformin may lower the TSH level.

Keywords: diabetes mellitus, thyroid volume, TSH, metformin.

Introduction

There are about 60 million people with diabetes in the European Region – about 10.3% of men and 9.6% of women aged 25 years and over. Prevalence of diabetes is increasing among all ages in the European Region, mostly due to increase in overweight and obesity, unhealthy diet and physical inactivity. Furthermore, high blood glucose kills about 3.4 million people worldwide annually. Almost 80% of these deaths occur in low- and middle-income countries, and almost half are people aged less than 70 years. WHO predicts that deaths due to diabetes will double between 2005 and 2030 [21].

Prevalence of diabetes is increasing in Latvia too. In 2007, the number of patients who were diagnosed with the second type of diabetes (T2DM) was 54 305; in the 2012, this number rose to 73 680 [1].

Diabetes mellitus and thyroid disease appear to be closely linked. A recent meta-analysis of 10 920 patients with diabetes mellitus revealed a mean frequency of thyroid disease of 11%. The data in type 1 diabetes mellitus did not differ from those in type 2 diabetes mellitus, but the prevalence in women was consistently more than twofold compared to men. There was a wide variability of the prevalence reported in different studies varying between 4.8% and 31.4%, partly explained by the different definitions used for the diagnosis of diabetes mellitus and thyroid disorders [5; 8].

Thyroid disease and type 1 and type 2 diabetes mellitus are strongly associated, and this has important clinical implications for insulin sensitivity and treatment requirements. Hyper- and hypothyroidism have been associated with insulin resistance, which has been reported to be the major cause of impaired glucose metabolism in T2DM. The state-of-art evidence suggests a pivotal role of insulin resistance in underlining the relation between T2DM and thyroid dysfunction [15; 19]. The pathophysiological basis of this association has only recently been better elucidated. The pathophysiological mechanisms underlying this linked regulation are increasingly being unravelled. They are exemplified in the regulation of 5´ adenosine monophosphate-activated protein kinase (AMPK), a central target not only for the modulation of insulin sensitivity but also for the feedback of thyroid hormones on appetite and energy expenditure. The present review will discuss these concepts and their consequences for the clinical care of patients with diabetes mellitus and thyroid disorders. Moreover, it refers to the added effect of metformin in suppressing thyroid stimulating hormone (TSH) [7; 8; 13; 14].

Aim

The aim of the research is to prove the correlation between thyroid volume and changes in weight in connection to the second type of diabetes, analysing patients' medical situation, maintaining laboratory and clinical data, therapy.

Material and methods

The research information about 138 case histories were divided into three groups: the first – 76 patients with type 2 diabetes and obesity who use metformin, the second control group – 39 patients with type 2 diabetes and obesity who are cured with insulin but do not use metformin, and the third – 23 patients with normal body mass index.

Patients were divided into three groups in order to compare patients with type 2 diabetes and obesity, metformin users, in patients with type 2 diabetes mellitus and obesity, and those not taking metformin, to determine how metformin is able to influence TSH levels and thyroid volume in patients who use metformin. The third control group was set up to explore overweight effects on the thyroid volume.

The first and the second group were examined by looking at the duration of diabetes, glicated hemoglobin, and C-peptide, all groups took into account the patient's age, sex, BMI, smoking, and smoking pack years, antibodies to TPO, TSH and free thyroxin, thyroid volume, and the number of nodes.

The results of the research have been processed with the SPSS for Windows 22.0 (Statistical Package for the Social Sciences).

Results

By assessing variability of age, the average age of the group is 41.41 years ± 11.796. Evaluation of groups was divided according to sexes – in the first group were 44 women (57.9%) and 32 men (42.1%), in the second group were 22 women (56.4%) and 17 men (43.6%), p = 0.879 between the first two groups, and in the third group were 17 women and 6 men.

In the first and second group, the medium duration of diabetes was 10.08 ± 8.46 years, but glicated hemoglobin (HbA1c) medium value was 9.2945 ± 1.7624%, which is significantly higher than in the normal range from 4.8 to 5.9% and the target glycemia. However, when comparing the first and second groups, the difference was not statistically significant: for the first group the median HbA1c level was 9.3 ± 1.77% (min 5.9%, max 14.9%), while the second – 9.27 ± 1.77% (min 5.9%, max 12.5%). On the other hand, the mean thyroid volume in all groups was 14.1934 ± 6.7282 mL. The first and the second group did not differ in the C-peptide level – 2.52 ± 1.27 ng/mL and 2.52 ± 2.62 ng/mL, respectively (min 0.54 ng/mL, max 6.18 ng/mL and min 0.1 ng/mL, max 12.1 ng/mL, respectively).

The average BMI was 33.7 ± 8.604 kg/m^2. Comparing the first and second groups of women, the first group of women had a higher BMI, 34.32 and 31.86 kg/m^2, respectively. Thyroid volume was 14.1934 ± 6.7282 mL (95% CI 13.06 to 15.32). After evaluating the correlation between BMI and thyroid volume in general population, it was statistically significant (r_s = 0.23; p = 0.006).

Comparing the thyroid volume in the first and second group, there was no statistically significant difference (p = 0.857). Comparing the thyroid volume between the first and second groups of women and men, there was not statistically significant difference (p = 0.989 and p = 0.9, respectively). This means that patients with type 2 diabetes and obesity receiving metformin have thyroid volume almost as high as patients have with type 2 diabetes and obesity not using metformin. Comparing the first to the third group of thyroid volume, there was a statistically significant difference (p < 0.001). Knowing that the first group has patients with type 2 diabetes mellitus and obesity, and the third one – patients with a normal weight, it can be concluded that the volume of the thyroid gland was bigger in first group (type 2 diabetes and metformin users) than third group (people with normal BMI).

When analysing the data, a reliable correlation between thyroid volume and TSH levels (r_s = −0.194, p = 0.024). The average TSH levels in all groups were 1.67 ± 0.84 mU/L (95% CI 1.53 to 1.81). The first group's TSH level was 1.67 ± 0.84 mU/L (min 0.48, max 4.1), for the second it was 1.8 ± 0.81 mU/L (min 0.5 max 3.5) and in the third it was 1.47 ± 0.86 mU/L (min 0.25, max 3.2) (p = 0.443). The average level of TSH in the first group was statistically significantly lower than in the second one, suggesting that perhaps metformin is able to reduce TSH levels.

Discussion

The study did not prove metformin's ability to reduce thyroid volume, which may be associated with a relatively small population. To further explore this topic, there is a need for a larger percentage of patients as well as patient questionnaires to find out how long and if metformin is used regularly. Hence, a large part of the study was based on the fact that metformin was administered for at least six months.

Following this study, it is interesting to compare patients with type 2 diabetes mellitus and obesity who use metformin, with patients with type 2 diabetes mellitus and normal weight who also are metformin users, to evaluate in which case metformin is able to reduce the volume of the thyroid gland.

A recent study included 2570 individuals for cross-sectional and 1088 individuals for longitudinal analyses. In cross-sectional data, females with T2DM were treated with anti-diabetic medication other than metformin and had a larger thyroid volume (β = 4.69; 95% CI 1.87 to 7.50) and a higher odds ratio (OR) for goitre (OR = 1.71; 95% CI 1.05 to 2.79) than females without T2DM. For males, no such association was detected. Females or males treated with metformin, T2DM were not associated with thyroid volume or goitre. In longitudinal analyses, incident T2DM not treated with metformin was significantly associated with a higher risk for incident goitre in the total population (incidence rate ratio (IRR) = 1.70; 95% CI 1.10 to 2.91). Individuals with T2DM having changed from metformin to other anti-diabetic agents during follow-up also had a higher risk for incident goitre than individuals without T2DM had (IRR = 2.71; 95% CI 1.74 to 4.20) [10].

Another result of the study was mean TSH level in the diabetes group (1.9 ± 0.9 mIU/L) was higher than in the control group (1.4 ± 0.8 mIU/L) and the pre-diabetes group (1.5 ± 0.8 mIU/L) (p < 0.0001 for both). Mean thyroid volume was higher in the pre-diabetes (18.2 ± 9.2 mL) and diabetes (20.0 ± 8.2 mL)

groups than in controls (11.4 ± 3.8 mL) ($p < 0.0001$ for both). Percentage of patients with thyroid nodules was also higher in the pre-diabetes (51.3%) and diabetes groups (61.8%) than in controls (23.7%) ($p < 0.0001$ for both) [2].

In conclusion, we would like to explore the effect of metformin on thyroid nodes that were not taken into account in this study because most of the thyroid USG descriptions did not accurately describe thyroid nodules number and size.

The recent study results suggest that patients with metabolic syndrome have significantly increased thyroid volume and nodule prevalence. Multivariate regression analysis model demonstrated that the presence of insulin resistance contributed substantially to this increased risk. Data provided the first evidence that insulin resistance is an independent risk factor for nodule formation in an iodine-deficient environment [3].

Conclusions

1. When analysing the results of the Latvian patients it was not proved that the metformin is capable of statistically significant reduction in thyroid volume compared to patients with type 2 diabetes mellitus and obesity using metformin and type 2 diabetes mellitus patients with obesity injecting insulin and who do not use metformin ($p = 0.857$).
2. It was possible to prove that patient's weight affects the thyroid volume, evaluating the weak correlation between average BMI and average capacity of thyroid volume ($r_s = 0.23$; $p < 0.006$).
3. Comparing the average TSH value between the groups (medium TSH volume in the first group is 1.67 ± 0.84 mU/L, but in second – 1.8 ± 0.81 mU/L, which may indicate a possible effect of metformin on TSH levels ($p = 0.443$)). It is possible that metformin is able to reduce TSH level, as well as statistically significant correlation between TSH level and thyroid volume ($r_s = -0.194$; $p = 0.024$).

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Immune Cell Subtyping in the Cerebrospinal Fluid of Patients with Neurological Diseases at Rīga Eastern Clinical University Hospital "Gaiļezers"

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Abstract

Cerebrospinal fluid (CSF) analysis is very important in differential diagnosis of CNS diseases. Normally the number of cells is increased in patients with inflammatory CNS diseases. In addition to the standard analysis of CSF, in this study we analysed the distribution of immunocompetent cells subtypes in various neurological diseases. $CD4^+$, $CD8^+$ lymphocytes (ly) prevails in healthy individual CSF, whereas the number of NK cells and B lymphocytes (Bly) is negligible.

In total, 15 patients were recruited. We analysed immune cells subtypes in the distribution of blood and cerebrospinal fluid. The study patient group was divided into four subgroups according to CNS diseases. The first group included 5 patients with a primary diagnosed multiple sclerosis, second group – 3 patients with viral type meningitis, third group – 3 patients who were diagnosed with Parkinson's disease and the control group consisted of 4 patients with non-inflammatory orthopaedic diseases.

In routine CSF test, we observed that elevated WBC count in patients with neuroinfections and MS, but WBC count in Parkinson's disease patients was normal or slightly elevated. The percentage of $CD4^+$ T cells and NK cells was higher in MS patients ($p < 0.05$). Higher percentage of T helper cells was observed in MS and neuroinfection groups, significantly higher than in healthy control group ($p < 0.05$).

The percentage difference of $CD8^+$ Tly subtype in all groups was not observed, but the percentage of activated T lymphocytes ($CD38^+$ cells) was significantly higher than in healthy controls ($p < 0.05$).

Patients with MS had significantly higher percentage of NK cells; that is significantly higher than in healthy controls ($p < 0.05$). Fraction of Bly was higher in all patient groups, comparing with healthy control group ($p < 0.05$).

There was no significant difference in mean percentage of apoptosis receptor $CD95^+$ bearing cells and CD4/CD8 ratio in all groups.

The analysis of immune cell subsets in the CSF adds valuable information to clinicians and is a promising tool for the differential diagnosis of neurological diseases. Additional studies are necessary in order to differentiate CSF cell populations in more detail.

Keywords: immunocompetent cell subtypes, CD3, CD4, CD8, CD38, CD16, CD19, CD95, index CD4/CD8, multiple sclerosis, neuroinfection, Parkinson's disease.

Introduction

Recently more and more attention is paid to neurodegenerative and inflammatory processes in the central nervous system. It is known that the immune system is highly organised and plays a key role in both inflammatory and in nerve cell degeneration [15, *216*]. It was believed that the CNS is immune privileged system, mainly because of a relative lack of antigen-presenting cells and "safe" blood-brain barrier that protects brain from periphery circulating cells. Recently, the views have changed due to the fact of a close and two-way communication between the central nervous system and the immune system [3, *89*]. Antigen presentation – the main process of immune response – takes place at the periphery with the help of the specific antigen-presenting cells (APC), such as dendritic cells (DC), macrophages and B cells. APC initiate the immune response by collecting antigen peptides, then processing and presenting them on their surface. As a result, T cells receive information about the antigen and become able to provide defence against foreign substances [6, *57*; 7, *72*; 19, *45*]. There is evidence that activated immune cells can migrate to the CNS and induce various processes with the help of neurotransmitters and immune mediators [21, *103*]. Microglia and astrocytes are immune cells in CNS that mainly interact with T cells [12, *1*]. As a result of such interaction, various diseases can develop, such as multiple sclerosis (MS), Parkinson's disease (PD), autoimmune encephalitis, and others [8, *1*; 9, *55*]. Activated T cells induce blood-brain barrier damage and facilitate other peripheral immune cell entry into the CNS [19, *47*].

Cerebrospinal fluid (CSF) analysis is very important in differential diagnosis of CNS diseases. Routine analysis includes cell count, total protein levels, and glucose levels. Normally the number of cells is increased in patients with inflammatory CNS diseases. In addition to standard analysis of CSF, in this study the distribution of immunocompetent cells subtypes in various neurological diseases was analysed. $CD4^+$, $CD8^+$ lymphocytes (ly) prevail in healthy individual CSF, whereas the number of NK cells and B lymphocytes (Bly) is negligible [5, *45*; 22, *516*]. According to literature, $CD4^+$ and Bly level can be increased in patients with MS [4, *1668*; 20, *95*].

Immune cells are not uniform in the body, depending on their function and receptors on their surface lymphocytes could be divided into different subtypes. Similar to peripheral blood, immune cell distribution in CNS may imply a pathological process and help identify the nature of pathology, especially in marginal cases.

The analysis of studies about immune pathogenesis of neurological diseases performed in Latvia demonstrated that the CD4/CD8 index, IL-12 and TNF-α is increased in patients with exacerbation of multiple sclerosis and correlate with CNS focus activity. All parameters were studied only in peripheral blood [1, *74*]. Immunocompetent cells distribution in different types of neurological abnormalities in CSF has been analysed in Latvia for the first time.

The aim

The aim of the respective study was to evaluate immunocompetent cell subtypes in CSF in patients with CNS diseases.

Material and methods

The study was conducted from 1st January 2014 to 1st June 2014 at Rīga Eastern Clinical University Hospital "Gaiļezers". In total, 15 patients were recruited. We studied immune cells subtypes in the distribution of blood and cerebrospinal fluid. The research patient group was divided into four subgroups according to CNS diseases (Table 1). The first group included 5 patients with primary diagnosed multiple sclerosis; three women and two men, mean age – 32.8 years. Inclusion criteria – patients with MS that was diagnosed and confirmed by McDonald criteria, and patients did not receive any immunomodulatory or immunosuppressive therapy before spinal tap was performed The second group, 3 patients with viral type meningitis, one man and two women, mean age – 36.3 years. The third group, 3 patients who were diagnosed with Parkinson's disease, two men and one woman, mean age – 52 years. All these patients were undergoing standard diagnostic procedure for their neurological disease.

Exclusion criteria for the study were: patients with MS who received any immunomodulatory or immunosuppressive therapy, patients with other aetiology for parkinsonism (as vascular or toxic) and patients with bacterial or unknown aetiology of meningitis.

Control group consisted of 4 patients with non-inflammatory orthopaedic diseases, one male and three female with a mean age of 38.5 years; healthy control group workup did not show any changes in CSF (WBC count, protein level, glucose level).

Lumbar puncture was performed using non-traumatic needle and 10 ml of cerebrospinal fluid was taken for analysis. T-helper ($CD3/CD4^+$), T cytotoxic cells ($CD3/CD8^+$), activated lymphocytes (CD38), natural killer cells ($CD16^+56^+$), Bly ($CD19^+$) and lymphocytes with receptor of ($CD95^+$). CD4/CD8 ratio was calculated. Immunocompetent cells were determined with laser flow cytometer (Becton Dickinson, USA) at Rīga Eastern Clinical University Hospital Centre of Laboratory Medicine.

Further data was analysed using IBM SPSS 20 statistical program and χ-test.

Table 1. Patient groups and characteristics

Group	Number of patients, n	Sex, m/f, n	Age, years	Phase of the disease	Treatment before analysis
Multiple sclerosis	5	2 / 3	18–45, mean – 32.8	Acute	No
Neuroinfection	3	1 / 2	18–52, mean – 36.3	Acute	No
Parkinson's disease	3	2 / 1	48–53, mean – 51.1	Firstly diagnosed	No
Controls	4	1 / 3	18–52, mean – 38.5	Acute	No

Results

In routine CSF test, it was observed that elevated WBC count persisted in patients with neuroinfections and MS, but WBC count in Parkinson's disease patients was normal or slightly elevated (Table 2) The results were demonstrated in percentage because of different lymphocyte count in all groups.

Percentage of $CD4^+$ T cells and NK cells was higher in MS patients ($p < 0.05$). Higher percentage of T helper cells was observed in MS and neuroinfection groups, significantly higher than in healthy control group ($p < 0.05$).

The percentage difference of $CD8^+$ T lymphocyte subtype in all groups was not observed, but the percentage of activated T lymphocytes ($CD38^+$ cells) was significantly higher than in healthy controls ($p < 0.05$).

Patients with MS had significantly higher percentage of NK cells; that is significantly higher than in healthy controls ($p < 0.05$). Fraction of Bly was higher in all patient groups, comparing with the healthy control group ($p < 0.05$)

There was no significant difference in mean percentage of apoptosis receptor $CD95^+$ bearing cells and CD4/CD8 ratio in all groups.

Table 2. Distribution of immune cell subsets in the CSF of typical neurological diseases

Diagnosis	White blood cells, µl	$CD3^+$, %	$CD4^+$, %	$CD8^+$, %	$CD38^+$, %	$CD16^+$, %	$CD19^+$, %	$CD95^+$, %	CD4/CD8 ratio
Multiple sclerosis	7	87	35.50	18.00	3.60	13.80	29.00	3.4	3.36
Neuroinfection	98	98	28.30	30.00	4.00	1.33	24.33	3.7	3.00
Parkinson's disease	4	95	22.28	31.66	6.33	2.00	30.33	4.3	3.10
Healthy control group	2	81	13.25	20.00	28.00	5.25	2.00	6.8	4.70

Discussion

The aim of the respective study was to analyse the immune cell subtypes in patients with various neurological diseases in CSF, thus patients with diseases such as multiple sclerosis, Parkinson's disease and neuroinfection were included in it. Each group of diseases had its own different pathogenesis and the immune cells had a different role in each of them; however, that role is still not completely understood.

Multiple sclerosis is an inflammatory demyelinating CNS disease that mainly affects younger women. Syndromes depend on the CNS damage regions. Nowadays MS aetiopathogenesis is not completely clear, recent views consider it to be a multi factorial disease. Pathogenesis shows an autoimmune reaction against myelin and neurons [25, *172*]. The main pathogenic role in the development of MS assigned to cytotoxic $CD8^+$ T cell response against myelin and neuronal antigens [2, *402*]. Comparing multiple sclerosis and neuroinfection CSF analysis with the control group showed a significantly higher $CD4^+$ subtype cell percentage in MS and neurounfection group. $CD4^+$ T cells are well known for participation in antibody class switching process, cytotoxic T cell development process and in forming memory cells [13, *5*]. However, $CD4^+$ role in disease pathogenesis has not been fully understood, yet many authors believe that this cell subtype possesses an antiviral and a direct cytotoxic effect [24, *16*]. Taking into consideration the fact that in this study $CD4^+$ cell percentage was increased in neuroinfection, MS groups also support the popular theory that these diseases have a common immune response mechanism and in both cases an important role in pathogenesis belongs to viruses [16, *28*].

It is clear that in the case of neuroinfection main pathogenic process involves leukocytes, macrophages and microglia that release free radicals, cytokines and excretory amino acids, resulting in lack of energy and cell death. Vascular inflammatory reaction, oedema and focal ischemia promote blood-brain barrier damage [17, *3*; 23, *530*]. In case demyelinating diseases were activated, T lymphocytes migrate to the CNS, the main mechanism of pathogenesis is related to blood-brain barrier damage. In either case, the process-stimulating factor could be promoted by a virus as human herpesvirus 6, 7 (HHV6, HHV7) or Epstein-Barr virus (EBV) [16, *29*].

In turn, Parkinson's disease (PD) pathogenesis is described by the inflammatory process of $CD4^+$ and $CD8^+$ T cell infiltration to substantia nigra, although well aware that disease results in brain's extensive neurodegeneration [10, *83*]. In our study, Parkinson's disease group showed a higher proportion of $CD8^+$ cells than in other groups, indicating prevailing cytotoxic processes. $CD8^+$ cells massive cluster of brain tissue in patients with Parkinson's disease was already performed in study in 1988 [14, *575*].

Presently, a lot of debate is about multiple sclerosis and neurodegenerative diseases common mechanisms, immune cells subtypes that could play a major role in these processes and could influence the differential diagnosis for neurological diseases thus allowing to understand some steps in pathophysiology of these conditions and in its turn help to find new drugs for these diseases.

HHV6, HHV7 and EBV could be trigger factors for neuroinflammation and neurodegeneration, but still no specific claims can be proven. We are planning to test CSF for antibodies in these viruses in the future to understand if these viruses could be the stimulating factors.

Despite the small number of patients in our study, the first CSF immunocompetent cell analysis showed trends that are specific to patients with various neurological diseases. Further research is necessary with larger patient groups and, in addition, aetiopathogenic factor analysis (e.g., latent viruses or viral infections demonstration in CSF) to draw conclusions about the role of immune cells in neurological disease development.

Conclusions

1. The analysis of immune cell subsets in the cerebrospinal fluid adds valuable information to clinicians and is a promising tool for the differential diagnosis of neurological diseases.
2. Additional studies are necessary in order to differentiate cerebrospinal fluid cell populations in more detail.

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Breast Cancer Patients Survival Rates at Pauls Stradiņš Clinical University Hospital

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Abstract

Breast cancer is the most common malignant tumour in women in Latvia. The incidence of breast cancer increases gradually. Since 2006, each year in Latvia more than 1000 women are diagnosed with primary breast cancer. In 2010, breast cancer mortality rate per 100 000 women in Latvia was 23.7. Mortality rate per 100 000 women demonstrates the situation of breast cancer in the country, it describes disease long-term prognosis, risk factor influence on cancer development, and treatment possibilities.

The purpose of this study was to calculate breast cancer patients' survival rates at Pauls Stradiņš Clinical University Hospital (PSCUH), depending on the disease onset, cancer morphological type and molecular subtype, and to compare the results with the survival rates in developed countries.

The retrospective study was performed for 377 breast cancer patients, who were treated at PSCUH during the period from 2005 to June 2010 (80% of all surgically treated breast cancer patients in the period). Patients' data were collected from National Cancer Registry and Institute of Oncology databases. To calculate the relative 3- or 5-year survival rates, survived days from the day of diagnosis for each patient were performed separately. For statistical analysis Kaplan–Meier estimator and the ratio between number of survived patients and number of patients at the beginning of the study were used.

Breast cancer patients' relative 5-year survival was 75.5%. Relative 5-year survival rates, depending on the stage were as follows: stage 0 – 100%; stage I – 98%; stage II – 83%, stage III – 47%; stage IV – 0%. 3-year survival rate for ductal and lobular cancer was 86.7% and 90%, respectively. Depending on molecular subtype, the highest 3-year survival rate has Luminal A cases (93.1%), and the lowest rate has triple negative cases (77.1%).

PSCUH breast cancer patients' relative 5-year survival rate at stages I and II were comparable with the survival rates from Western countries, but stage III survival rate demonstrated a tendency to be slightly lower.

Keywords: breast cancer, ductal cancer, lobular cancer, molecular subtype.

Introduction

Breast cancer is the most common cancer in women worldwide and the second most common cancer overall [2]. In 2012, nearly 1.7 million new breast cancer cases were diagnosed [2, 3]. In Europe, in 2012 there were around 470 000 new breast cancer cases [2]. The incidence of breast cancer varies greatly around the world. The highest age-standardised incidence rate for breast cancer in Europe in 2012 was in Belgium – 111.9 cases per 100 000 inhabitants, and the lowest incidence rate was in Bosnia Herzegovina with only 37.4 cases per 100 000 people [3].

In Latvia, breast cancer is the most common malignant tumour in women. In Latvia, since 2006, each year more than 1000 new cases are diagnosed. In 2013, 1133 women in Latvia were diagnosed with primary breast cancer, the age-standardised incidence rate for breast cancer was estimated to be 56.3 cases per 100 000 people. 2/1133 (0.2%) cases were diagnosed in stage 0, 301/1133 (27%) in stage I, 405/1133 (36%) in stage II, 263/1133 (23%) in stage III and 68/1133 (6%) in stage IV. In 5% of cases, the stage was not known and in 3% of cases cancer diagnosis was registered after death [3].

In 2010, breast cancer mortality rate per 100 000 women in Latvia was 23.7. Mortality rate of breast cancer in Latvia is higher compared to other European countries such as Spain, Portugal, Norway, Sweden, but lower if compared to Denmark [3, 4].

Aim

The main goal of this study was to analyse PSCUH breast cancer patients' relative specific 5-year survival rate, depending on the stage of the disease, and relative specific 3-year survival rate, depending on the breast cancer morphological type, grade and molecular subtype, and to compare the results with the survival rates in Latvia on the whole as well as in developed countries.

Material and methods

The retrospective study was performed for 377 breast cancer patients, who were treated in PSCUH during the period from 2005 to June 2010 (80% of all breast cancer patients, who were treated in the Department of Surgery, PSCUH in the period). The patients' data were collected from two databases – the National Cancer Register database and the Institute of Oncology database. Inclusion criteria were as follows: histologically confirmed breast cancer and survival data available. Distribution of patients according to the disease onset at the time of diagnosis has been represented in Table 1. In all cases, 7th edition of TNM classification was used. Distribution of patients according to histological type, grade and molecular subtype, if available, has been demonstrated in Figures 1, 2, 3.

The group of 377 patients was used to calculate the relative overall 3-year survival rate for all patients, who were treated in PSCUH in the period from 2005 to June 2010. For cases where respective data were available, breast cancer specific survival, depending on morphological type, grade, and molecular subtype were calculated. In our study we used following definitions for molecular subtypes of breast cancer: Luminal A type (positive oestrogen and/or progesterone receptors, HER2 receptor is negative, Ki67 < 14%) Luminal B type (positive oestrogen and/or progesterone receptors and positive HER2 receptor, or Ki67 > 13%), HER2 type (only HER2 receptor is positive), triple negative type (none of the receptors is positive) [1].

Table 1. Distribution of patients by stage (n = 377)

Stage	Percentage	Number of patients, n
In situ	3	11
Stage I	23	88
Stage II	42	161
Stage IIA	–	101
Stage IIB	–	60
Stage III	29	109
Stage IIIA	–	47
Stage IIIB	–	34
Stage IIIC	–	28
Stage IV	2	8

Group of 216 patients, who were treated in PSCUH in the period from 2005 to 2008, was used to calculate relative 5-year survival and breast cancer specific survival, depending on the onset of the disease.

To statistically calculate the relative 3- and 5-year survival, for each patient survived days from the day of diagnosis were calculated separately. Last update on status alive or dead was performed on 01.01.2014. For statistical analysis Kaplan–Meier estimator and the ratio between number of survived patients and the number of patients at the beginning of the study were used.

Figure 1. Distribution of patients by breast cancer morphological type (n = 377)

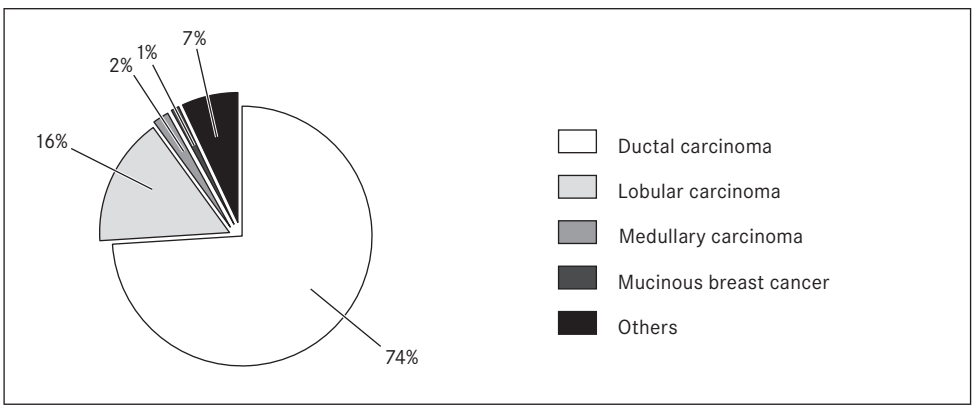


Figure 2. Distribution of ductal breast cancer patients by grade (n = 279)

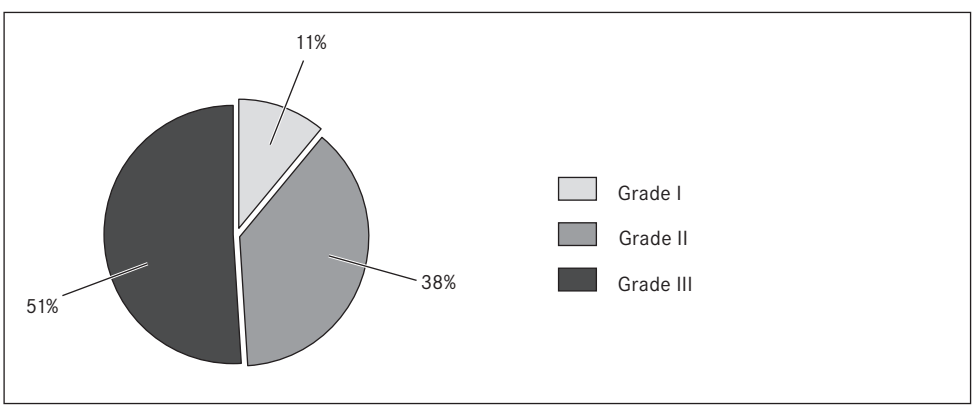
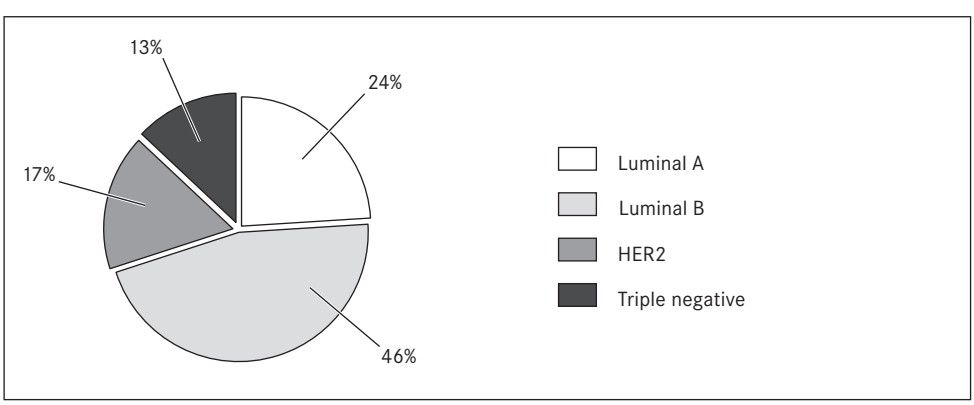


Figure 3. Distribution of patients by breast cancer molecular subtype (n = 362)



Results

From 377 patients, 77 died from breast cancer, during the study period. 48 out of 77 cases, died from the disease within 3 years from the time of diagnosis. Breast cancer specific 3-year survival for all stages is 87.3% (Figure 4).

Analysing the group of 216 patients, who were treated in PSCUH in the period from 2005 to 2008, 53 cases of death from breast cancer were detected within 5 years from the time of diagnosis. Breast cancer specific 5-year survival is 75.5% (Figure 5).

Data on breast cancer specific 5-year survival rate according to the stage of disease and respective USA and UK data can be seen in Table 2.

Figure 4. Distribution of 377 patients by disease status on 01.01.2014

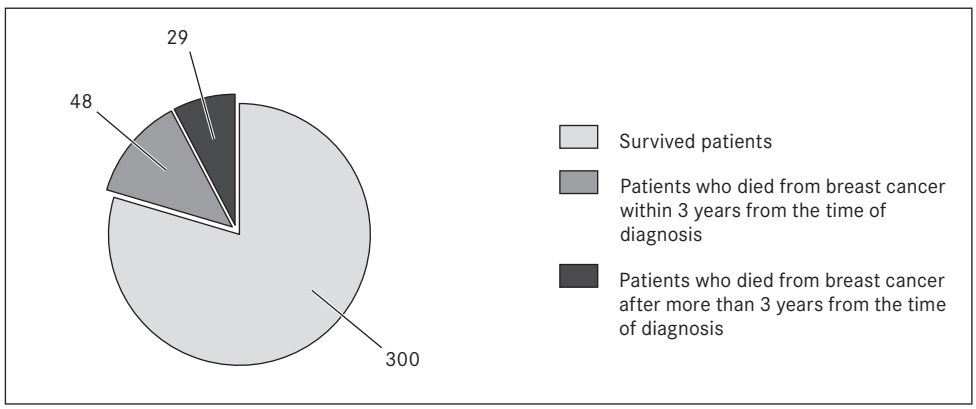


Figure 5. The distribution of 216 patients by disease status on 01.01.2014

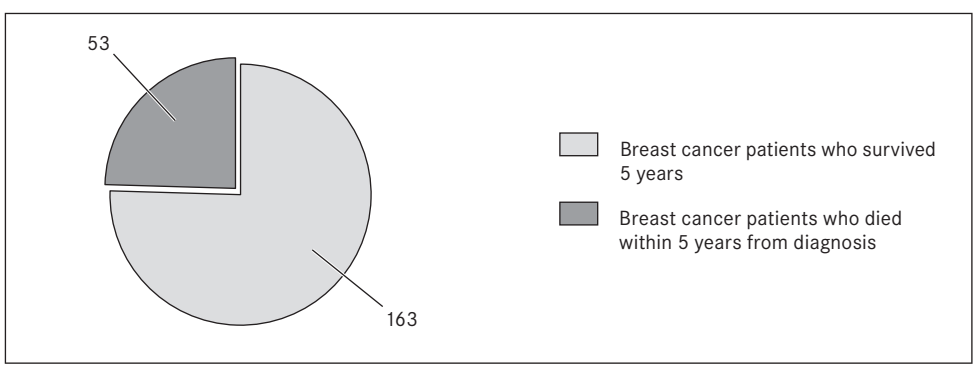


Table 2. 5-year survival rate of breast cancer patients, depending on the stage of the disease

Stage	PSCUH* 5-year survival rate	5-year survival rate in Latvia for breast cancer diagnosed in 2005	5-year survival rate in the USA (by NCI, 2012 data) [9]	5-year survival rate in the UK (by cancer research, UK 2013) [8]
Stage 0	100%	–	~ 100%	~ 100%
Stage I	98%	88%	~ 100%	> 90%
Stage II	83%	74%	85%	> 70%
Stage III	47%	43%	66%	> 50%
Stage IV	0%	4%	21%	> 13%

* Pauls Stradiņš Clinical University Hospital.

The relative 3-year survival of ductal carcinoma (86.7%) was slightly lower than relative 3-year survival of lobular carcinoma (90%).

The breast cancer specific 3-year survival of ductal carcinoma according to grade was as follows: Grade I – 96.6%; Grade II – 91%; Grade III – 81%.

The breast cancer specific 3-year survival, depending on cancer molecular subtype is as follows: Luminal A type – 93%; Luminal B type – 90%; HER2 type – 79%, triple negative type – 71% (Figure 7).

Figure 6. Survival of breast cancer patients according to the stage of the disease

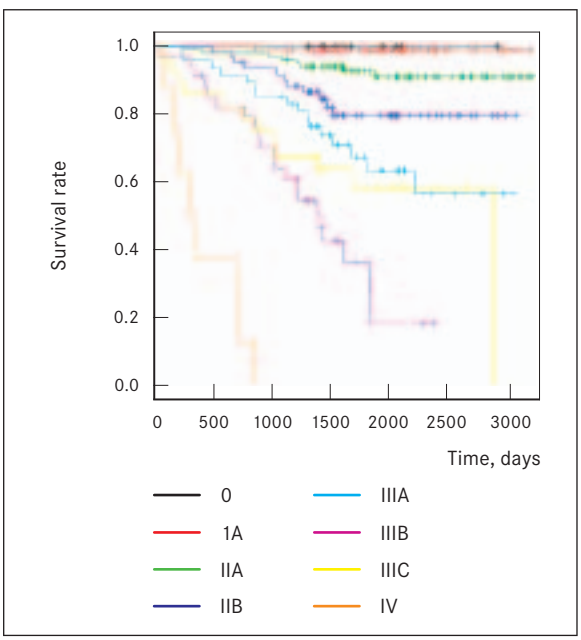
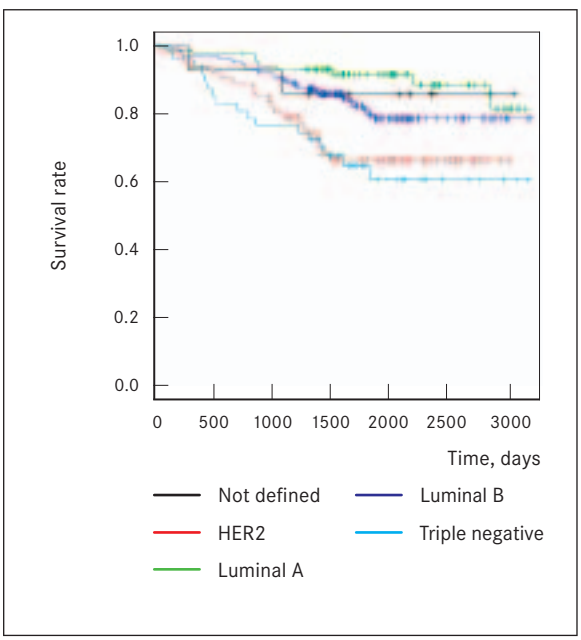


Figure 7. Survival of breast cancer patients, depending on molecular type



Discussion

According to National Cancer Registry (NCR) data, breast cancer 5-year survival for cases diagnosed in 2005 for all stages together is 61.7%. This is considerably less than our PSCUH data – 75.5%. However, we have to admit that in our study are included only the operated breast cancer cases and for stage IV the disease surgery is performed very rarely, mainly for hygienic reasons. Therefore, the proportion of stage IV cases in our study was only 2%, but nationwide – 6%. Metastatic breast cancer has poor prognosis and undoubtedly worsen overall 5-year survival, which is reported by NCR. Also higher rate of stage 0 (3% *vs.* 0.2%) and early stage (stage I and II) (65% *vs.* 63%) disease in our material comparing to NCR data could have some influence on better 5-year survival data.

We compared PSCUH data and nationwide 5-year survival data for breast cancer cases diagnosed in 2005 (see Table 2) according to the stage of disease. For stage I 5-year survival is 98% and 88%, for stage II – 83% and 74%, for stage III – 47% and 43% and for stage IV – 0% and 4%, respectively. The data show the tendency that 5-year survival for PSCUH cases in stages I–III is better than nationwide. One probable cause of such bias could be that nationwide data for breast cancer cases diagnosed in 2005 were used, but the study group also included cases diagnosed between 2005 and 2008. In 2006–2008, there was a comparably larger health care budget, which could have improved also the treatment results of breast cancer.

PSCUH data with 5-year survival data was also compared according to the stage of the disease from the USA and the UK. PSCUH stage 0–II data are very similar to the data from the USA reported by NCI as well as the UK data. We admit that number of stage 0 cases in our study is too small to draw any conclusions; however, it allowed detecting a tendency of 100% breast cancer specific 5-year survival, which is in accordance with natural course of non-invasive cancer. 5-year survival data for stage III

disease in PSCUH and in Latvia altogether is rather similar. However, we detected that 5-year survival in stage III–IV cases is worse in PSCUH cases compared to the USA and the UK. Again, the number of stage IV disease in PSCUH material is too small for any conclusions, but the data by NCR show very clear difference between Latvia, the USA and the UK – 4%, 21% and > 13%, respectively. There could be several possible explanations for poorer survival in stage III and IV breast cancer cases in PSCUH and Latvia treated in 2005–2008 including limited accessibility to cancer specialists, patients' compliance and limited resources for breast cancer drugs.

The histopathological type and grade of the tumour have prognostic significance. Certain morphological types of breast cancer, such as medullary, mucinous and tubular have a more favourable long-term prognosis Our study did not show statistically significant difference of survival between the patients with diagnosed lobular breast carcinoma and patients with diagnosed ductal carcinoma, which is in concordance with the data of other authors [5, 7]. The 3-year survival rate correlated also with the tumour grade – the best for grade I cancers and the worst for grade III cancers, which is consistent with the data of other publications.

Breast cancer survival rate as well as long-term prognosis also depends on the cancer molecular subtype [5, 6]. Research showed that the highest survival rate was in patients with Luminal A molecular subtype. Patients with diagnosed Luminal B subtype, which was most common in our study, had lower survival compared to Luminal A, but overall had good long-term prognosis. On the other hand, patients with HER2 and triple negative molecular subtypes had much poorer long-term prognosis and significantly lower survival. Our results on survival in different breast cancer molecular subtypes confirm the earlier published data.

Conclusion

Breast cancer patients relative 5-year survival rate at stages I and II was comparable with the survival rates from developed countries, but at stages III and IV relative 5-year survival of breast cancer cases in Pauls Stradiņš Clinical University Hospital and Latvia showed a tendency to be worse. Those results may suggest, that treatment possibilities and patients' compliance for advanced breast cancer cases (stages III and IV) in 2005 probably were worse, compared to the United Kingdom and the USA.

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Diagnostics of Subclinical Optic Nerve Damage by Optical Coherence Tomography in Multiple Sclerosis Patients

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Abstract

Evaluation of the afferent visual system may give insight into ongoing neuro-degenerative processes in multiple sclerosis (MS) patients. The aim of our study is to assess the utility of optical coherence tomography (OCT) for sub-clinically damaged optic nerve diagnostics and to characterise retinal nerve fibre layer (RNFL) changes in MS patients without previous optic neuritis.

The cross-sectional study included 43 relapsing/remitting MS patients (86 eyes) without visual acuity changes and historical evidence of optic nerve inflammation. Measures included clinical characteristics and the RNFL measures in six standard sectors (temporal (RNFL T), temporal upper (RNFL TS), temporal lower (RNFL TI), nasal (RNFLT N), nasal upper (RNFLT NS), nasal lower (RNFLT NI).

In the eyes of MS patients, there was found a significantly decreased average RNFL thickness in RNFL T, RNFL TS, RNFL TI, RNFLT N and RNFLT NS sectors compared to controls ($p < 0.05$). However, this difference was not significant in RNFL NI sector ($p = 0.97$). Basing on the ROC curve analysis, it was detected that the major difference was observed in the RNFL T and RNFL TI sectors (as follows: AUC = 0.72, AUC = 0.75, $p < 0.05$). RNFL reduction in RNFL T sector was identified in 33.72% of MS patients (n = 29).

Significant thinning of RNFL obtained by OCT was found in one third of MS patients without previous demyelinating event in optic nerves. The most pronounced changes were observed in temporal sector without significant difference in nasal sector. Presumably, attention for further neurodegenerative process analysis should be focused on the temporal sector monitoring in the asymptomatic MS patients group.

Keywords: multiple sclerosis, sub-clinical, optic neuritis, optical coherence tomography.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory and autoimmune central nervous system disorder that is mainly diagnosed clinically, basing on the evidence about lesion dissemination in space and time [Polman, 2011]. An episode of acute optic neuritis (ON) is observed in about 50% of MS patients during the course of disease [Frohman, 2005; Balcer, 2006]. Damage of the vision pathways has increased an interest among MS specialists, as it may be an ideal model for neurodegenerative process monitoring

[Sakai, 2011]. Optical coherence tomography (OCT) creates images from retina, the only place, where direct axonal tissue layer examination is possible regardless of the myelin sheath thickness [Jindahra, 2011]. It is repeatedly shown that the retinal nerve fibre layer (RNFL) damage, diagnosed with OCT method may be observed after acute ON episode and even in the eyes without a history of ON. Quite often in these studies, RNFL assessment of the fellow, ON intact eye in patients with a history of ON was carried out [Parisi, 1999; Trip, 2005; Fisher, 2006; Garcia-Martin, 2011].

Aim

The aim of our study is to assess the utility of OCT for sub-clinically damaged optic nerve diagnostics and to characterise RNFL changes in MS patients without ON history.

Material and methods

The cross-sectional study included 43 relapsing/remitting multiple sclerosis patients with *Snellen* acuities of 20/20 in both eyes and without ON symptoms or clinical signs in history.

Patients were recruited from the Multiple Sclerosis Centre at Pauls Stradiņš Clinical University Hospital. Patients suffering from other neurological diseases but MS, ophthalmological or systemic ones, which could affect the afferent visual system, were not included in the study. Clinically definite MS diagnosis was established by an experienced neurologist based on McDonald's criteria. An experienced ophthalmologist performed a complete ophthalmologic examination: determination of visual acuity, measurement of ocular tension, bio-microscopic examination of the anterior pole and visual field detection.

The OCT (Heidelberg Engineering SPECTRALIS) measured RNFL thickness in six standard sectors (temporal (RNFL T), temporal upper (RNFL TS), temporal lower (RNFL TI), nasal (RNFLT N), nasal upper (RNFLT NS), nasal lower (RNFLT NI)) for all subjects, using Tru Track Active Eye Tracking technology. All scans were performed by the same ophthalmologist. Poor quality scans were rejected. Presence of the defect was determined based on the machine normative database; a red coloured quadrant was classified as abnormal.

Descriptive statistics were performed according to data type. The statistical difference of quantitative variables for both groups was analysed using the Student's t test. The level of significance for all statistical tests was set at $p < 0.05$. The receiver operating characteristic (ROC) curve and the area beneath it were analysed. We used MedCalc 12.0 software to estimate the statistical significance of the differences between the ROC curves areas.

Results

In the study, 86 eyes of MS patients were analysed, mean age (m = 39.58, SD = 10.81), the minimum age of patients was 17 years, the maximum age – 65 years, modal, or the most common age was 36 years. The control group consisted of 16 individuals (32 eyes). Basing on the independent t test, it was concluded that patients of the MS group (m = 39.58, SD = 10.81) were statistically significantly ($p < 0.001$) by 6.58 years older than the control group (m = 33.00; SD = 9.47).

In the eyes of MS patients without a history of ON, significant decrease of average RNFL thickness in RNFL T, RNFL TS, RNFL TI, RNFLT N and RNFLT NS sectors was found, compared to controls ($p < 0.05$). However, in RNFL NI sector RNFL thickness did not differ statistically significantly ($p = 0.97$) between the two groups. Analysis of the mean RNFL in different eye sectors in MS patients and control group has been represented in the Figure 1.

Basing on the ROC curve analysis, making the assessment of differences in the characteristics of MS patients and control group, it was found that the major difference is observed in the RNFL T and RNFL TI sectors (as follows: AUC = 0.72, AUC = 0.75, $p < 0.05$) (Fig. 2). Overall, RNFL reduction in RNFL T sector was seen in 33.72% of MS patients (n = 29).

Figure 1. The mean RNFL thickness in different sectors in MS patients without ON and control group

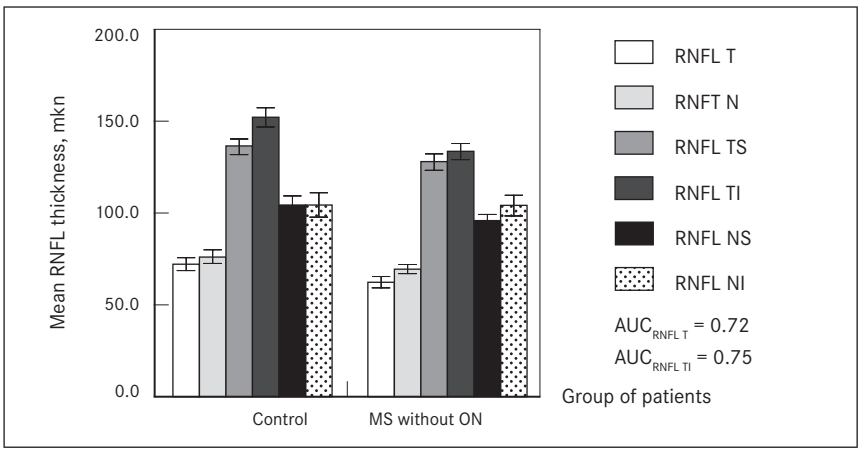
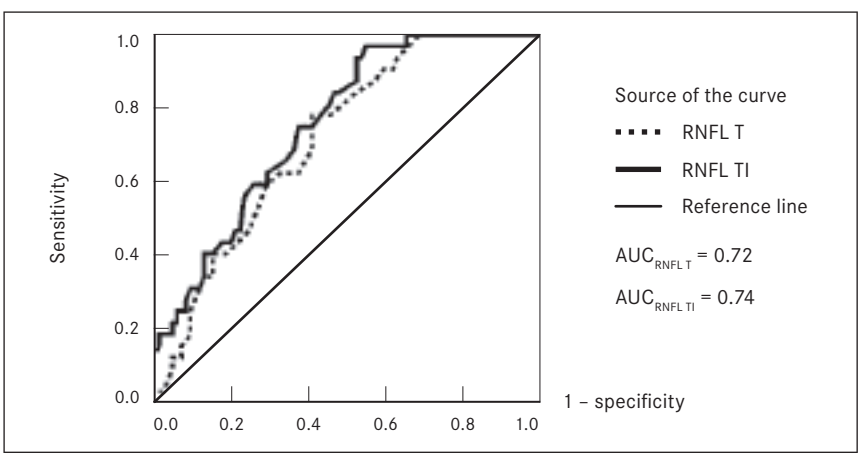


Figure 2. Assessment of RNF T and RNFL TI measurement suitability using ROC curves



Discussion

The course of MS disease is very unpredictable and varies from patient to patient. There is an increasing necessity for biological marker that could assess ongoing neuro-degeneration. OCT is highly reproducible, objective, non-invasive and easy test for axonal tissue damage evaluation. Similar to previously published studies [Parisi, 1999; Trip, 2005; Fisher, 2006; Garcia-Martin, 2011], our results show that eyes of MS patients without a previous ON episode have considerable abnormalities in OCT studies. Our findings suggest that the most marked changes affect the temporal RNFL sectors, both in the upper and lower quadrant, but practically no RNFL changes are observed at the lower nasal sector.

In the cases after an ON episode, axonal damage appears to be secondary to demyelination [Sakai, 2011]. However, the above-mentioned findings suggest about other than ON axonal damage mechanism that continues progressively and sub-clinically in the MS patient's afferent visual system independently from the direct demyelinating process. These results support the need to apply OCT test to MS patients even without a history of ON in order to obtain information about axonal tissue damage. Relying on the data we have obtained, about 30% of asymptomatic MS patients have already had significant axonal tissue involvement before ON episode. It is likely that the damage mostly related to retrograde axonal degeneration, probably due to MS lesions in the *radiatio optica* area [Jindahra, 2009], but this mechanism is not fully understood yet and needs further evaluation.

Conclusions

Significant thinning of RNFL obtained by means of OCT was found in one third of MS patients without previous demyelinating event in optic nerves. The most pronounced changes were observed in the temporal sector without significant difference in the nasal sector. Targeted analysis by OCT in temporal sector in asymptomatic MS patients group in the future could provide additional information about neurodegenerative processes in different clinical and therapeutic situations.

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Occurrence of *Legionella Pneumophila* in Water Distribution Systems in Dental Practices in Latvia

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Abstract

Legionella pneumophila is the major agent of Legionnaire's disease and Pontiac fever. Legionellosis are normally acquired by inhalation or aspiration of legionellae from a contaminated environmental source. The pathogens present in dental unit waterline could be spread by aerosols created by dental equipment, presenting a risk for both a patient and members of a dental team.

The aim of the study was to investigate the occurrence of *Legionella* contamination of water distribution systems in dental practices. A total of 185 samples were collected from 74 dental practices. Samples were taken from water taps in dental practices (n = 79) and from dental unit waterline (n = 106). Overall, 20 out of 74 (27%) of dental practices were found *Legionella pneumophila* positive. Occurrence of *Legionella pneumophila* was significantly higher in samples from water taps than in samples from dental unit waterlines – 25 of 79 (25%) and 5 of 106 (5%), accordingly. From all *Legionella pneumophila* positive samples, 23 (92%) represented *L. pneumophila* serogroup 2–15. Two samples from dental practices in Rīga were contaminated with *L. pneumophila* serogroup 1. The level of contamination of samples from water taps ranged from 2×10^2 CFU/L to 1.1×10^4 CFU/L, and the level of contamination of samples from dental unit waterlines ranged from 3×10^2 CFU/L to 2.4×10^3 CFU/L. Both samples from water taps and dental unit waterlines were positive in three dental practices (4%). In two cases, samples from water taps were negative, though *Legionella pneumophila* was found in samples from dental unit waterlines.

The study showed no correlation between the year of installation of dental unit and occurrence of *Legionella pneumophila*, since it was isolated from samples taken from dental units installed in the year 1998 and up to the year 2013. *Legionella pneumophila* was found in one dental practice dental unit waterline with independent distilled water supplying system.

Keywords: Legionella pneumophila, dental units, Latvia.

Introduction

Legionella pneumophila is a facultative intracellular bacterium that multiplies within phagocytic cells [Diederen, 2008]. *Legionella pneumophila* is the major agent of Legionnaire's disease and Pontiac fever. Legionellosis are normally acquired by inhalation or aspiration of legionellae from a contaminated

environmental source. Moreover, *Legionella* strains can survive in moist environments for long periods and can be ubiquitously found in natural moist environments and man-made systems. In natural environments, *Legionella* is present in low density but its concentration can significantly increase in artificial habitats depending on the type of materials, on the presence of biofilms and available nutrients [Veronesi, 2007]. Bacterial biofilm in dental unit waterlines (DUWL) is a widespread problem [Tuttlebee, 2002]. Each dental chair unit (DCU) is equipped with an elaborate loom of interconnected narrow-bore flexible plastic tubing called dental unit waterlines (DUWLs), which supply water to all of the DCU-supplied instruments [O'Donnel, 2011]. The water used in DUWL acts as a coolant for high speed drills and as irrigant during dental procedures, most often it is supplied directly from municipal water supplies [Walker, 2004]. The general problem of microbial contamination of DUWL is well known [Atlas, 1995; Pankhurst, 1998]. Due to the texture and composition of the plastic tubing, microbial biofilms form readily, resulting as high bacterial contaminations in outputs water. The pathogens present in DUWL could be spread by aerosols created by dental hand-pieces, presenting a risk for both a patient and members of a dental team [Laheij, 2012].

Aim

The aim of the study was to investigate the occurrence of *Legionella* contamination of water distribution systems in dental practices, and whether dental treatment might pose a risk for patients and for dental team. In addition, analysis of hot tap water samples for presence of *Legionella* were carried out in order to assess the prevalence of *Legionella* in water supply system in the entire building.

Material and methods

A total of 185 samples were collected from 74 dental practices from February 2014 until June 2014. Samples were taken in Rīga (n = 71) and four regions of Latvia, randomly representing Latgale (n = 40), Kurzeme (n = 34), Vidzeme (n = 25) and Zemgale (n = 15). The samples were taken from water taps in dental practices (n = 79) and from dental unit waterline (n = 106). Water samples were collected in sterile bottles before routine working hours. At least two samples were collected in each dental practice, one sample from DUWL (cup filler) and one hot tap water sample from the sink in the same room. In dental practices, which have more than one or two dental chair units, up to 10 DUWL samples were taken per practice. During the sampling, the dental personnel was asked for additional information about the year of installation of DCU and methods for treatment of DUWL incoming water.

Isolation and identification of *Legionella pneumophila* was carried out by using standard ISO 11731. One litre of water sample was filtrated and concentrated using membrane filtration with 0.45 µm pore-size polyamide filter (Millipore, USA). The filter membranes were cut into pieces and resuspended in 5 ml sterile distilled water, then shaken for two minutes (Vortex Genie) and kept in room temperature for 10 minutes. A total of three 0.1 ml untreated, heat-treated and acid-treated aliquots of the sample were spread on Buffered Charcoal Yeast extract medium (GVPC, Oxoid, UK). The plates were incubated at 36 °C in a humidified environment for 10 days, and examined every day beginning on day 3. At least three characteristic colonies from each GVPC plate were selected for subculture onto plates Buffered Charcoal Extract agar medium with L-cysteine (BCYE, OXOID, UK) and Buffered Charcoal Extract agar medium without L-cysteine (BCYE-Cys, OXOID, UK) and incubated for at least 48 hours at 36 °C. Colonies grown on BCYE were subsequently identified by latex agglutination test (*Microscreen Legionella CE*, Microgen Biologics, UK). *Legionella* Rapid Latex Test Kit allows for separate identification of *L. pneumophila* serogroup 1 and serogroups 2–15 and identification of 10 non-*Legionella pneumophila* species. Colonies from all plates were counted, confirmed and the estimated number of *Legionella* was expressed as CFU/litre *Legionella* species and serogroup.

Microbiological analysis was carried out in Laboratory of Medical Microbiology (Institute of Food Safety, Animal Health and Environment "BIOR").

Results

Overall, 20 out of 74 (27%) dental practices were found *Legionella* positive (Fig. 1, Fig. 2). However, *Legionella* was not found in samples from dental practices in Zemgale. In other districts, the occurrence of *Legionella* ranged from 13% in Latgale up to 48% in Rīga (Table 1).

Samples were taken in different administrative districts of Rīga (Figure 2), where *Legionella* was found in 9 of 15 (60%) administrative districts.

Overall, *Legionella* was isolated in 25 out of 185 samples (14%). The occurrence of *Legionella* was significantly higher ($p = 0.04$) in samples from water taps than in samples from dental unit waterlines – 25 of 79 (25%) and 5 of 106 (5%), accordingly (Table 1).

From all *Legionella* positive samples, 23 (92%) represented *L. pneumophila* serogroup 2–15. Two samples from dental practices in Rīga were contaminated with *L. pneumophila* serogroup 1. The level of contamination of samples from water taps ranged from 2×10^2 CFU/L to 1.1×10^4 CFU/L, and the level of contamination of samples from dental unit waterlines ranged from 3×10^2 CFU/L to 2.4×10^3 CFU/L (Table 2).

Both samples from water taps and dental unit waterlines were positive in three dental practices (4%). In two cases, samples from water taps were negative, though *Legionella* was found in samples from dental unit waterlines.

Table 1. Occurrence of *Legionella* in samples from water taps and dental equipment in regions of Latvia

District	Number of dental practices, n	Number of practices with at least one positive sample, n (%)	Samples from water taps. Number of samples / positive samples, n (%)	Samples from dental unit waterline. Number of samples / positive samples, n (%)
Rīga	23	11 (48)	27 / 11 (41%)	44 / 3 (7%)
Latgale	16	2 (13)	16 / 2 (13%)	24 / 0 (0%)
Kurzeme	15	3 (20)	15 / 3 (20%)	19 / 2 (11%)
Vidzeme	13	4 (31)	14 / 4 (31%)	11* / 0 (0%)
Zemgale	7	0 (0)	7 / 0 (0%)	8 / 0 (0%)
TOTAL	74	20 (27)	79 / 20 (25%)	106 / 5 (5%)

* In two dental practices in Vidzeme, only tap water samples were taken.

Figure 1. Sampling points and *Legionella* positive results in Latvia

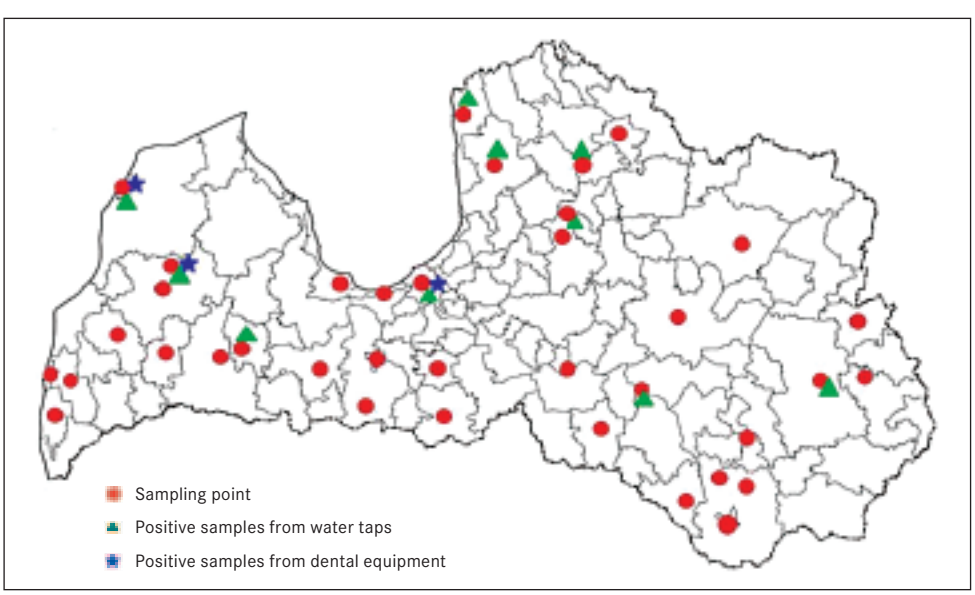

Figure 2. Sampling points and *Legionella* positive results in districts of Rīga

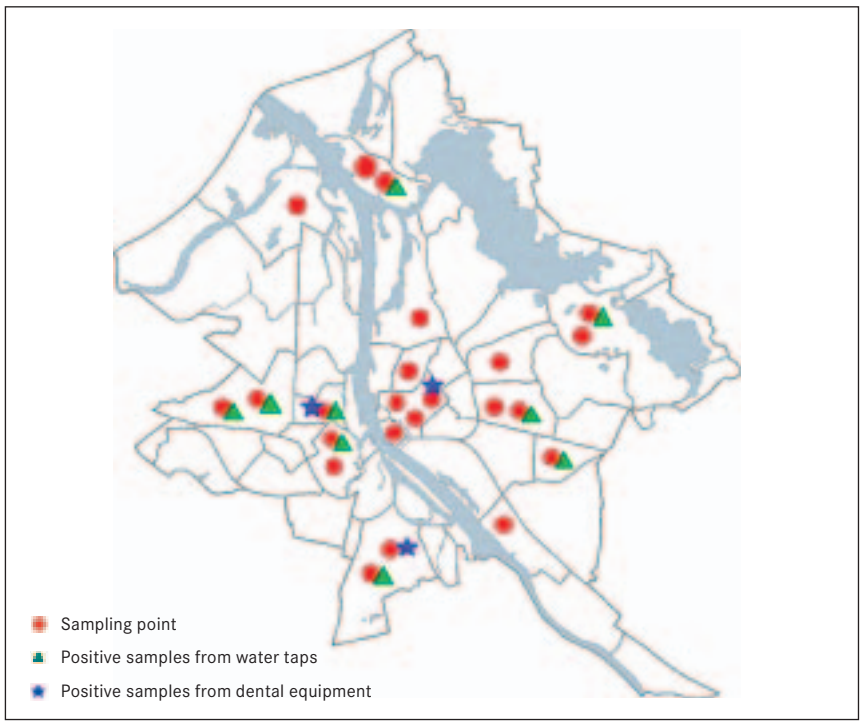

Table 2. *Legionella* positive samples and level of colonisation

ID number	District	Samples from hot water taps		Samples from dental unit waterline	
		CFU/L	Serogroup	CFU/L	Serogroup
19	Kurzeme	4×10^3	2–15	1×10^3	2–15
20	Vidzeme	6×10^3	2–15	ND	ND
21	Latgale	9×10^2	2–15	ND	ND
22	Vidzeme	3.5×10^3	2–15	ND	ND
36	Vidzeme	1×10^3	2–15	ND	ND
37	Vidzeme	6×10^2	2–15	ND	ND
38	Latgale	3×10^3	2–15	ND	ND
39	Kurzeme	8×10^2	2–15	3×10^2	2–15
46	Rīga	7×10^3	2–15	ND	ND
47	Rīga	1.1×10^4	2–15	ND	ND
49	Rīga	8×10^3	1	ND	ND
50	Rīga	ND	ND	2.4×10^3	2–15
51	Rīga	2.5×10^3	1	ND	ND
54	Rīga	4×10^2	2–15	ND	ND
58	Rīga	8×10^2	2–15	ND	ND
61	Rīga	2×10^2	2–15	ND	ND
		4×10^2	2–15	ND	ND
		9×10^3	2–15	1.1×10^3	2–15
66	Rīga	7×10^2	2–15	ND	ND
68	Rīga	6×10^3	2–15	ND	ND
70	Rīga	ND	ND	1.2×10^3	2–15
74	Kurzeme	2×10^3	2–15	ND	ND

ND – not detected.

Table 3. Installation period of dental chair units included in sampling plan

District	Period of installation, years	Average age of DCU, years
Rīga	2000–2014	4.7
Latgale	2000–2009	8.3
Kurzeme	1998–2014	6.4
Vidzeme	1995–2014	7.5
Zemgale	1997–2013	6.9

The study showed no correlation between the year of installation of dental unit and the occurrence of *Legionella*, since it was isolated from samples taken from dental units installed in years between 1998 and 2013. Some DCUs use independent water reservoir bottles to provide distilled water to the DUWLs. Thus, *Legionella* was found in one dental practice DUWL with such water supplying system. The only method used for municipal water additional treatment, were filters. No influence of additional filters on occurrence of *Legionella* was observed.

Discussion

This study showed that 27% of dental practices had at least one *Legionella* positive sample. Overall, *Legionella* was found in 5 of 106 DUWL samples, which is significantly lower than in other countries with a higher average annual temperature, where the occurrence of *Legionella* in DUWL systems varied from 16.1% in Greece [Mavridou, 2006], 33% in South Africa [Singh, 2005], 33.3% in Italy [Montagna, 2006] and 86.7% in Jordan [Ma'ayeh, 2008].

Water temperature could be the main reason for significant differences. It is difficult to maintain cool water temperature below 20 °C in countries with high average air temperature. Optimum temperature range for proliferation of legionellae is 32–35 °C [Levesque, 2004; Wadovsky, 1985]; however, in Latvia, cold water temperature rarely exceeds 20 °C. In some countries with similar climate, the results may vary. No *Legionella* positive dental unit reservoir samples were found in Poland [Szymanska, 2004]; in London and Northern Ireland the prevalence of *Legionella* was very low (0.37%) [Pankhurst, 2003]; however, a significantly higher occurrence was observed in Sweden (15%) [Dahlen, 2009], Switzerland (20%) [Barben, 2009] and Germany (27.8%) [Arvand, 2013]. Differences in the occurrence of Legionella can be explained by different sampling strategies. In the retrospective study, DUWL samples were mainly taken from cup-fillers, while in other researches samples were taken from high-speed hand-piece tube, syringe or micromotors. It has been confirmed that cup-filler samples can be twice less contaminated with *Legionella* than samples from instrument channels [Arvand, 2013].

Some DCUs use independent water reservoir bottles to provide water to the DUWLs. These bottles were manually filled with distilled or sterile water.

One of *Legionella* positive samples was taken in dental practice, which does not use municipal water, but DCU is supplied by distilled water from a single reservoir. However, it does not protect against contamination. Even DUWL supplied by sterile or distilled water, at the moment of filling will become colonised to the same extent as those supplied by tap water. Once the bacteria gained access to the system, there will be enough nutrients from the plastic tubing and the turnover of the bacteria themselves to support biofilm growth. This does create difficulties for some practitioners, despite the use of sterile water source [Walker, 2004].

Our results showed no correlation between the year of installation of dental unit and the occurrence of *Legionella*; it was isolated from samples taken from dental units installed between years 1998 and 2013. Most DCUs often are not used for more than 12 hours per day, 5 days per week, and thus water stagnation is a significant contributory factor to DUWLs output water contamination [O'Donnell, 2011]. Historically, the majority of DUWL have been supplied by municipal tap water, which is still the case today in Latvia. With such systems, even within 5 days of installation, the microbial counts can reach 2.0×10^5 CFU/ml in the water at the distal outlets [Walker, 2004; Barbeau, 1996]. Complex design of dental chair equipment, resulting in the stagnation of water within the equipment lines where bacteria, including *Legionella*

pneumophila could proliferate within biofilm is a major factor affecting microbial contamination of water lines [Smith, 2002]. DCU manufacturers can significantly contribute to controlling the problem of DUWL biofilm [Coleman, 2007].

The occurrence of *L. pneumophila* was considerably higher in hot tap water (25%) compared to other European countries, where the occurrence of *Legionella* in water distribution systems varied from 22.6% in Italy [Borella, 2004], 26% in Germany [Zietz, 2001] to 30% in Finland [Zacheus, 1994].

A total of 15 dental practices, where *Legionella* was found in hot tap water samples, were not contaminated in DUWL. This may suggest that incoming municipal water could be a source of infection for DUWL biofilms, which is in accordance with previous studies [Valcina, 2013] and using other sampling strategies and methods of analysis, *Legionella* prevalence in DUWL could be higher. However, it has to be emphasised that the classical cultivation method used in this study did not allow determining the presence of non-cultivable legionellae [Delgado-Viscogliosi, 2005].

Statistically significant differences ($p = 0.02$) were observed in the distribution of *L. pneumophila* in different districts of Latvia. Zemgale was the only region where *Legionella* was not detected in any sample.

From all *L.pneumophila* positive samples, 8% represented *L. pneumophila* serogroup 1 and 92% *L. pneumophila* serogroup 2–15. Both cases of serogroup 1 were observed in Rīga, in territories, which received treated surface water. The data are consistent with results of other studies. In Poland, *L. pneumophila* 2–15 serogroup was isolated from 73% and serogroup 1 from 19.8% of *Legionella* spp. positive samples [Stojek, 2011], in Italy 75.6% and 22.6%, respectively [Borella, 2004].

Currently, only one case has been reported about an 82-year-old woman who died of Legionnaires disease in Italy in 2011 [Ricci, 2011]. Nevertheless, dental personnel and the increasing number of immunocompromised dental patients that present routinely at dental surgeries are being exposed to potentially opportunistic pathogenic bacteria through ingestion and inhalation of dental unit water [Walker, 2004]. The potential occupational hazard to a dental team is considered greater than that of the patient population due to sustained and daily contact with contaminated DUWL aerosols [Pankhurst, 2007].

Conclusions

1. Our study showed that several dental unit water lines contained *Legionella pneumophila* (5%), which poses a risk for both patients and dental team. However, the actual risk of legionellosis based on our results has to be studied further.
2. High contamination of hot tap water with *Legionella pneumophila* (25%) can indicate that incoming water may cause a threat to dental unit water line systems.
3. Regular monitoring of microbial contamination of dental unit waterlines is essential to control and reduce the microbial burden within dental unit water lines as well as to highlight the risk of occupational exposure in general dental practices.

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Analysis of Seroreactivity to Recombinant *B. burgdorferi* Antigens BBA65 and BBA73

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Abstract

Because of remarkable heterogeneity of *Borrelia burgdorferi*, the identification and characterisation of possible antigens is essential for the improvement of current laboratory diagnostics for Lyme borreliosis and vaccine development. Several new *B. burgdorferi* outer surface proteins have been identified over the past decade in an effort to characterise immunodominant antigens and evaluate them as possible vaccine candidates. In the present study, two recently identified recombinant immunogens of the PFam54 family (BBA65 and BBA73) were tested for the serodiagnostic potential.

The results of this study indicate that these proteins did not show sufficient antigenic properties to be used as single antigens; however, these proteins could be used as additional markers in multi-antigen cocktail-based diagnostic tests for Lyme borreliosis. The detected antigenic properties of studied proteins indicate their possible involvement in the pathogenesis of LB, therefore further investigation of the functions is needed.

Keywords: serodiagnostic marker, recombinant protein, Lyme borreliosis.

Introduction

Lyme borreliosis (Lyme disease, LB) is the most common vector-borne disease in the temperate zone of Northern hemisphere and an important emerging zoonosis in Europe, North America and Far East countries. LB is caused by the spirochetes of *Borrelia burgdorferi* sensu lato (*B. burgdorferi* s. l.) complex that is maintained in nature in enzootic cycles involving ticks of the *Ixodidae* family, as well as a range of mammalian and avian hosts [Piesman, 2004]. The disease is transmitted to humans after a bite of an infected *Ixodes* tick. LB usually begins with a slowly expanding skin lesion, called erythema migrans (EM) at the tick-bite site, and followed within days or weeks by disseminated infection if left untreated [Steere, 2004]. The disseminated borrelial infection may manifest with a wide range of clinical signs such as multiple EM skin lesions, borrelial lymphocytoma, nervous system involvement (neuroborreliosis), and arthritis. Cardiac manifestations were reported as well [Strle, 2009]. Because of the diversity of clinical symptoms, LB is often considered as a differential diagnosis [Wilske, 2007]. LB diagnosis is based primarily on clinical presentation and an assessment of tick-exposure risk. Serological two-tier testing is supporting diagnostic measure to confirm the diagnosis, in which the first tier is usually a sensitive enzyme linked immunosorbent assay (ELISA) and the second is a confirmatory Western blot [Stanek, 2012]. However, the limitations of antibody tests must be appreciated. Weak or absent antibody

response in early LB, false positive results caused by the over-reading of the IgM immunoblots, and high background rates of seropositivity may confound the interpretation of seroreactivity. Beside this, the heterogeneity of the causative agent must be considered [Wilske, 2007]. Recently, in the study of Ang et al. it was shown that the assays used to detect anti-Borrelia antibodies have widely divergent sensitivity and specificity, and the choice of ELISA-immunoblot combination severely influences the number of positive results [Ang, 2011]. Thus, identifying and testing of novel target borrelia proteins for antigenicity is still in progress and offers a possibility to improve laboratory testing of LB.

One of the strategies to seek for a new potential serodiagnostic markers is to look for the Borrelia surface proteins that are markedly expressed during mammal infection. Similarly, surface protein expression increase in mammals comparing to tick environment could be interesting to characterise. Many studies have shown the differential gene expression regulated by changes in environmental conditions, the most studied of these cues is temperature shift. A large number of *B. burgdorferi* ORFs with significantly higher expression at 35 °C relative to 23 °C have been identified by global gene expression profiling study [Ojaimi, 2003]. In further studies, several outer surface exposed lipoproteins encoded by these genes have been identified that are actively expressed and/or immunogenic during Borrelia infections making them possible antigenic markers and vaccine candidates for Lyme disease [Adusumilli, 2010; Brooks, 2006]. Genes belonging to the paralogous gene family 54 were shown to be the most highly regulated group; moreover, members of this group, namely proteins BBA65, BBA66, BBA71 and BBA73 were detectable only in infectious *B. burgdorferi* B31 isolates [Hughes, 2008].

Aim

The aim of this study was to evaluate the possible use of two outer surface exposed *B. burgdorferi* proteins that are members of the paralogous gene family 54 (BBA65 and BBA73) as a serodiagnostic agents for LB diagnosis.

Material and methods

Human serum samples. A collection of human sera samples from patients who were diagnosed with LB and healthy control sera samples from healthy individuals without evidence of a current LB infection were used in this study. The study was approved by the LU EKMI (Institute of Experimental and Clinical Medicine, University of Latvia (Latvian: *Latvijas Universitātes Eksperimentālās un klīniskās medicīnas institūts*) ethical committee (26.05.2014). All samples were tested for LB by enzyme immunoassay for the *in vitro* diagnostic (Borrelia IgG + VlsE ELISA and Borrelia 14 kDa + OspC IgM ELISA, IBL International, Germany, data not shown). Based on these tests, LB sera samples were divided in three groups: IgM positive and IgG negative samples (early stage of disease, 14 samples), IgM positive and IgG positive samples (acute stage, 8 samples) and IgM negative, IgG positive samples (late stage, 13 samples). 10 LB IgM and IgG negative sera samples were included in the control sera group.

Cloning and expression of recombinant proteins. Genes coding for target proteins (BBA65 and BBA73) were amplified from *B. burgdorferi* s. l. strain B31 excluding the coding sequence for the hydrophobic region of N-terminal signal sequence (residues 1–24 for BBA65 and residues 1–27 for BBA73). Amplified genes were cloned in pETm_11 expression vector with integrated 6xHis tag and TEV protease cleavage site (EMBL, Heidelberg, Germany) and plasmids were transformed in competent *E. coli* RR1 cells by standard technique. The recombinant plasmids were isolated from positive clones by Plasmid Miniprep kit (Fermentas, Lithuania), and correct constructs were verified by sequencing. The N-terminal 6xHis tagged recombinant proteins were expressed in *E. coli* BL21 (DE3) by standard techniques. Briefly, the plasmid of the correct construct was transformed into *E. coli* cells and obtained transformants were grown overnight on LB agar plates containing kanamycin (10 mg/ml) at 37 °C. Seed material was grown from individual clone overnight without additional aeration in 2 × TY media supplemented with kanamycin (10 mg/ml). For expression experiments, seed material was inoculated in modified 2 × TYP media (TY supplemented with kanamycin (10 mg/ml), glucose (4 g/l) and 133 mM phosphate buffer pH 7.4)

and cultivated with vigorous aeration until OD600 0.8–1.0 was reached. The recombinant protein expression was induced by adding IPTG (Isopropyl β-D-1-thiogalactopyranoside, 0.2 mM, Sigma-Aldrich, USA). The expression of target protein was confirmed by SDS-PAGE and Western blot analysis of total protein samples with Penta His antibody (Qiagen, Germany) followed by incubation with horseradish peroxidase-labelled anti-mouse antibody (ECL Anti-Mouse IgG, Horseradish Peroxidase-linked, GE Healthcare, UK). The colour was developed using 3.3-diaminobenzidine (SERVA, Germany).

Purification of recombinant proteins. The cells were harvested by centrifugation and lysed by sonication. The cell debris was removed by centrifugation and the recombinant proteins with a six-histidine tag were purified from the lysate using affinity chromatography with Ni-NTA agarose (Qiagen, Germany) column following the manufacturer's instructions. The recombinant proteins were eluted with a high imidazole concentration. Buffer exchange was performed for eluted proteins into 20mM Tris-HCl pH 8.0 using Amicon centrifugal filter units (Millipore, UK). As a next purification step ion-exchange chromatography on a Mono Q 10/100 GL column (GE Healthcare, UK) was performed followed by 6-histidine tag cleavage by TEV protease was carried out to obtain a pure protein. Protein samples were analysed by SDS-PAGE and the gels were stained with Coomassie brilliant blue R-250.

Western blot analysis. A Western blot assay was used to evaluate the possible antigenic property of the recombinant proteins obtained in this study. Two LB negative and two LB positive (acute stage) sera samples were used. The analysis was performed by standard techniques. Briefly, purified proteins were separated using polyacrylamide gel electrophoresis (PAGE) and transferred to Hybond™C Extra nitrocellulose membrane (GE Healthcare, UK). Non-specific binding sites were blocked by 5% non-fat dried milk in phosphate-buffered saline (PBS) for one hour at room temperature. The membrane was briefly rinsed in diluent and wash buffer (PBST, 0.1% Tween 20 in PBS) and incubated with human sera samples diluted 1 : 500 in PBST for an hour at room temperature. After rinsing in two changes of wash buffer, the membrane was incubated in anti-human IgM or IgG peroxidase-labelled antibodies diluted 1 : 10000 in PBST (Goat Anti-Human IgG (HRP); Goat Anti-Human IgM (HRP), Abcam, UK) for an hour at room temperature. After rinsing in two changes of wash buffer, the colour was developed using 3.3-diaminobenzidine (SERVA, Germany). Both recombinant proteins were probed by one sera sample simultaneously.

Solid-phase binding assays (ELISA). Microtitre wells (Maxisorb 96-well plates, Nunc, Thermo Fisher Scientific, Germany) were coated overnight at 4 °C with 100 µl of purified recombinant protein diluted in coating buffer (100 pg total protein). Wells were blocked overnight at 4 °C with 5% non-fat dried milk in PBS (blocking buffer). After washing 3 times with PBST human sera samples diluted 1 : 100 in blocking buffer were added to wells and incubated for 2 hours at room temperature. After washing 3 times with PBST, wells were incubated with horseradish peroxidase-labelled anti-human IgM or anti-human IgG antibody diluted 1 : 20 000 in blocking buffer (Goat Anti-Human IgM (HRP); Goat Anti-Human IgG (HRP), Abcam, UK) for 1 hour at room temperature. Wells were again washed four times with PBST, and the colour was developed using tetramethylbenzidine (Sigma-Aldrich, USA). Absorbance was read at 450 nm using Multilabel Counter 1420 (Perkin Elmer, USA). The mean optical density (OD) value for the control sera plus three Standard deviations (SD) was considered the cut-off value to determine positivity in each sample. Two independent experiments were performed.

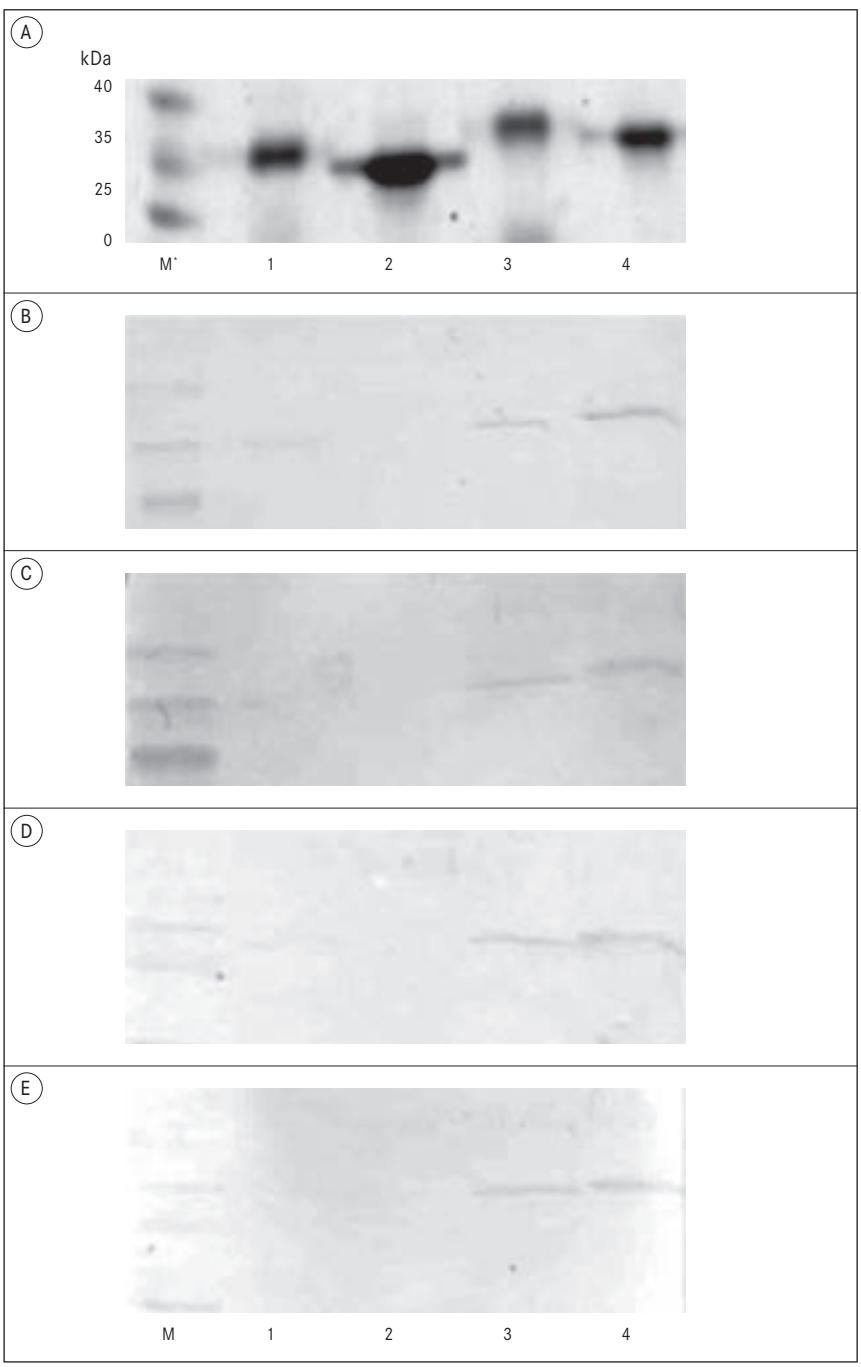
Statistical analyses. Statistical analysis was performed using MedCalc (MedCalc Software, Belgium). In ELISA, a serum sample was considered positive if it reacted positively in two parallel tests for LB samples.

Results

Expression and purification of *B. burgdorferi* recombinant proteins. The selected outer surface lipoproteins of *B. burgdorferi* contained signal peptide at the N-terminal part of a protein with the average length of 20 amino acids as predicted by SignalP 3.0. [Bendtsen, 2004]. Therefore, the lipoproteins were expressed in *E. coli* as truncated constructs lacking the hydrophobic signal peptide. A sufficient expression of His-tagged soluble recombinant proteins in *E. coli* was followed by the downstream purification

procedures. The combination of Ni-NTA affinity chromatography and ion-exchange chromatography was used and the purity of the isolated proteins as estimated from the Coomassie blue staining of the SDS-PAGE gels was at least 90% (Figure 1).

Figure 1. Testing of the seroreactivity of LB sera samples by Western blot



A – PAAG analysis of recombinant proteins;
B – Western blot of LB negative sample, IgG antibodies;
C – Western blot of LB negative sample, IgM antibodies;
D – Western blot of LB positive sample, IgG antibodies;
E – Western blot of LB positive sample, IgM antibodies.
* M – Protein weight marker; 1 – BBA65 with 6-histidine tag; 2 – BBA65 without 6-histidine tag; 3 – BBA73 with 6-histidine tag; 4 – BBA73 without 6-histidine tag.

Western blot analysis of BBA65 and BBA73 antigens. The antigenic properties of two recombinant proteins of the paralogous gene family 54 (BBA65 and BBA73) were tested by immunoblot analysis as described above by using LB positive and negative sera samples. Both antigens were tested simultaneously by individual sera sample in order to avoid the possible variability of Western blot conditions protein to protein. The results showed that BBA65 and BBA73 antigens were not able to distinguish LB positive and negative samples under the conditions used (Figure 1). There was not a detectable antibody response against recombinant BBA65 protein by immunoblot assay in patients with LB. By contrast, BBA73 showed positive response with LB positive as well as LB negative samples indicating lack of specificity for this antigen in this study under the conditions used.

Diagnostic effectiveness of BBA65 and BBA73 antigens. To evaluate the overall diagnostic effectiveness of BBA65 and BBA73 antigens for different stages of LB in human patients the quantitative ELISA was used. The immobilised antigens were probed with LB-positive sera samples, and both IgM and IgG antibodies were detected. A set of 10 sera samples from healthy individuals was used to define the cut-off value in each experiment. Samples with an absorbance higher than the cut-off value in two tests were considered positive. BBA65 antigen had an IgM antibody response only in case of early LB when 14.3% of samples were positive (Table 1). By contrast, several samples representing all three stages of LB had IgG antibodies against the BBA65 protein: 14.3%, 12.5% and 15.4% samples of early, acute and late LB stage were positive, respectively. Further, IgM and IgG antibodies against the BBA73 protein were detected. Similarly to BBA65 antigen, several samples representing all three stages of LB had IgG antibodies against the BBA73 protein: 7.1%, 12.5% and 15.4% samples of early, acute and late LB stage were positive, respectively (Table 1). In addition, BBA73 antigen had IgM antibody response in case of early and late LB when 21.4% and 7.7% of samples were positive, respectively. None sera samples of acute LB phase had IgM antibodies against BBA73 protein.

In total, the results show that of 35 Latvian patients with LB, 5 (14.3%) and 4 (11.4%) had positive IgG antibody response to BBA65 and BBA73 antigen, respectively (Table 1). For IgM antibodies, 5.7% (2 of 35) and 11.4% (4 of 35) of LB samples were positive in case of BBA65 and BBA73 antigen, respectively.

Table 1. Testing of the seroreactivity of LB sera samples by ELISA

Stage of disease	Number of samples, n	Positive sera samples, n (%)			
		BBA65		BBA73	
		IgM	IgG	IgM	IgG
Early	14	2 (14.3)	2 (14.3)	3 (21.4)	1 (7.1)
Acute	8	0	1 (12.5)	0	1 (12.5)
Late	13	0	2 (15.4)	1 (7.70)	2 (15.4)
Total	35	2 (5.70)	5 (14.3)	4 (11.40)	4 (11.4)

Discussion

The identification of novel antigens has a range of potential applications for the improvement of the current laboratory diagnosis of LB and in the area of vaccine development. In the present study, we evaluate the possible antigenicity of two recombinant Borrelia proteins of paralogous gene family 54 – BBA65 and BBA73 in human patients with LB. It is known that many members of paralogous family 54 are affected by changing culture conditions, and several proteins of this family were shown to have immunogenic properties during murine and human infection [Angel, 2010; Barbour, 2008; Gilmore, 2007; Gilmore, 2008; Hughes, 2008; Ojaimi, 2003; Ouyang, 2008; Revel, 2002]. BBA65 and BBA73 are outer-membrane localised proteins of *B. burgdorferi*. It was shown that antibodies specific for these proteins are detectable over the course of persistent infection in mice [Gilmore, 2007; Hughes, 2008]. However, there is little information about the presence of anti-family 54 proteins antibodies in sera of LB patients. Serologic

analysis of Borrelia proteins in humans revealed a limited set of immunogens of this protein family. Little reactivity with another family 54 members, BBA64 and BBA66 was observed with either early-disseminated or late Lyme sera pools [Nowalk, 2006]. Similar results for these two antigens were obtained in the study of Barbour et al [Barbour, 2008]. Our results indicated that two members of paralogous gene family 54 – BBA65 and BBA73 – did not show sufficient antigenic properties in Latvian patients with LB to be used as single antigens in serodiagnostic tests. In Western blots analysis, there was not a detectable antibody response against recombinant BBA65 protein in patients with LB under the conditions used. By contrast, BBA73 showed the lack of specificity for this antigen in this study. The estimation of possible diagnostic effectiveness of BBA65 and BBA73 antigens by ELISA shows that 14.3% and 11.4% LB sera samples had positive IgG antibody response to BBA65 and BBA73 antigen, respectively. Importantly, the antibodies against these antigens were detected in sera samples representing all three stages of LB. By contrast, only 14.3% of early LB samples were positive for BBA65 antigen. 21.4% and 7.7% of samples of early and late LB were positive for BBA73 protein, respectively. It is obviously, that paralogous could possess similar biochemical properties but distinct functional implications; however, it seems that temporal expression profile of many paralogous gene family 54 proteins inconveniences their usage as possible serodiagnostic markers for LB. In addition, the results indicated that due to the remarkable variation of antigenic profile, an optimal multi-antigen cocktail could be more effective to cover the heterogeneity of antibody responses and thus achieve the highest possible serodiagnosis test sensitivity and specificity.

Conclusions

Two studied proteins of paralogous gene family 54 – BBA65 and BBA73 – could be used as additional markers in multi-antigen cocktail-based diagnostic tests for Lyme borreliosis due to the remarkable variation of antigenic profile. The detected antigenic properties of studied proteins indicate their possible involvement in the pathogenesis of Lyme borreliosis, therefore further investigation of the functions is needed.

Acknowledgements

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Innate and Acquired Immunity Features of Psoriatic Nails: Expression of hβD-2, hβD-3, hβD-4 and IL-1, IL-6, IL-10

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Abstract

Psoriasis is a chronic inflammatory skin disease where nail involvement is an important aspect although often underestimated. Our knowledge of nail immunology is still very scarce. To develop more targeted therapies, it is relevant to understand the immunologic mechanisms of psoriatic nails.

The aim of the study was to determine the presence and distribution of human beta defensins and cytokines in psoriatic nail apparatus biopsies comparing with healthy nails.

We obtained 8 biopsies of nail psoriasis and control group with material of 5 healthy nail apparatus from fresh cadavers with a punch method and fixed in Stefanini's solution, dehydrated and embedded in paraffin. Subsequently we performed staining with haematoxylin and eosin and immunohistochemistry for human β-defensins (hβD-2, hβD-3 and hβD-4) as well as interleukins (IL-1α, IL-6 and IL-10) and evaluated the results semi-quantitatively grading the intensity of positively stained structures in the visual field.

Both in psoriatic nails and the control group, hβD-2 and hβD-3, not hβD-4, was positively expressed with predominance in psoriatic nails. In psoriatic nails, IL-1α was absent, while it was moderately positive in control group. Structures positive for IL-6 and IL-10 in psoriatic nails were found in greater extent compared to the control group.

The increased expression of hβD-2 and hβD-3 in the psoriatic nail highlights the potential role of antimicrobial peptides for the innate immunity involvement in the pathogenesis of nail psoriasis.

In psoriatic nails, the pro-inflammatory (IL-1α) cytokine expression is decreased, but expression of IL-6 as well as the anti-inflammatory cytokine IL-10 is increased indicating stimulation of nail growth and increase of anti-inflammatory immunity.

Keywords: nails, psoriasis, antimicrobial peptides, cytokines.

Introduction

Around 3% of the population has psoriasis, and the nails are involved in up to half of the patients with psoriasis, besides often the skin surrounding the affected nails remains "normal". Although nails are involved in tactile sensitivity, ensure small object retrieving, plays aesthetic role, its impact on patients' quality of life has often been overlooked [21].

Despite its high prevalence and significant impact on the patients' quality of life, nail psoriasis treatment remains a challenge for physicians and no consistent approach has been advocated [29].

As conventional therapies are often ineffective or inconvenient for patients, the effects of biologic agents on nail psoriasis have been investigated. Thus, the effectiveness of biological treatment in nail psoriasis appears to offer great promise for the future management of this distressing condition.

Even the understanding of healthy human nail immunology is scarce; regarding nail psoriasis there is only fragmentary knowledge that has been concluded from the results of the efficacy of the biologic treatment. The mechanisms of nail psoriasis should be investigated to apply targeted treatment options. It should not be ignored that although the nail is an appendage of the skin, there are important distinctive immunological features between the nail and skin, therefore the characteristics of the skin may not be automatically referred to the nail as well. Clinical manifestations of nail psoriasis vary according to the part of the nail affected by the inflammation.

Histologically, the changes are similar to those observed in psoriasis plaques on the skin. However, in contrast to other areas of the skin, the nail commonly shows evidence of spongiosis and serous exudates, and hypergranulosis may be observed [25].

The normal nail immune system of human infants shows some similarities to human hair immune system, both exhibiting defined compartments that represent as the sites of relative immune privilege [9, 22, 6]. It has been proclaimed that in the human nail apparatus there are decreased levels of key protagonists (natural killers and mast cells) of innate immunity [9]. Therefore an important role that still has to be clarified in the human nail innate immunity may be played by the antimicrobial peptides (AMPs) of which cathelicidins and β-defensins are the most well-characterised AMPs found in the human skin [2]. However, in the human nail apparatus from antimicrobial peptides only the presence of cathelicidin group peptide LL-37 has been described [16].

Aim

The aim of the study was to determine the presence and distribution of human beta defensins (hβDs) – hβD-2, hβD-3 and hβD-4 and interleukins (IL) – IL-1α, IL-6, IL-10 – in psoriatic nail apparatus biopsies compared with healthy nails.

Material and methods

The pilot study included eight (n = 8) patients' aged 18 to 70 years nail unit tissue samples obtained with a punch (diameter of five millimetres) biopsy technique from the nail unit according to our selection criteria with pathohistologically confirmed diagnosis of nail psoriasis. Exclusion criteria were other skin or its derivate diseases than psoriasis (including coexisting onychomycosis samples with negative culture for fungi and negative Periodic Acid Schiff (PAS) reaction were selected) in anamnesis, local or systemic anti-bacterial, anti-fungal, anti-inflammatory, immunosuppressive or exposure to an artificial UV radiation source within the last month.

As a control material five (n = 5) samples of fresh (samples were obtained within twelve hours after death) cadaverous' nail units were obtained. The included sample material was clinically unaffected nails' units with pathohistological appearance of normal nail apparatus and negative Periodic Acid Schiff reaction; without skin diseases clinically or in anamnesis, with no anti-bacterial or immunosuppressive treatment within the last two weeks.

The study was approved by the Ethical Committee at Rīga Stradiņš University (permit issued on 26.01.2012.)

Human nail biopsies were fixed in Stefanini's solution, dehydrated, and embedded in paraffin. Four-micrometre-thick sections were prepared from each tissue specimen and stained routinely with haematoxylin and eosin; Periodic Acid Schiff reaction was performed.

Immunohistochemistry (IMH) method was performed for human beta defensin-2 (hβD-2) (VJU01.1 : 100; R & D Systems, USA), human beta defensin-3 (hβD-3), human beta defensin-4 (hβD-4), human 1 alpha interleukin (Il-1α) (SC-9983, 1 : 50; Santa Cruz Biotechnology, USA), interleukin 6 (IL-6) (NYRhIL6, 1 : 50; Santa Cruz Biotechnology, USA) and interleukin 10 (IL-10) (AB34843, 1 : 400, Abcam, UK).

The results were evaluated semiquantitatively grading the appearance of positively stained structures in the visual field [27]. Few positive structures in the visual field were labelled with +, moderate number of positive structures in the visual field was labelled with ++, numerous positive structures in the visual field were labelled with +++ and abundance of positive structures in the visual field was marked with ++++.

For statistical analysis, non-parametric statistics with Mann–Whitney U-test were used.

For visual illustration of our findings, we used Leica DC 300F digital camera and image processing, and analysis software Image Pro Plus (Media Cybernetics, Inc., Rockville, MD, USA).

Results

Hyperkeratosis, parakeratosis, spongiosis, focal hypergranulosis and dilated vessels in the papillary dermis, as well as infiltration of neutrophils were observed in the psoriatic nail bed and connective tissue.

In psoriatic nails hβD-2 and hβD-3 positive structures observed in epithelia varied from numerous (+++) to abundance (++++) in view field (Figure 1, Table 1); in connective tissue hβD-2 was moderate (++), but for hβD-3 there were few positive cells (+). In the nail bed of healthy nails, there was moderate (++) amount of hβD-2 and hβD-3 containing structures (Figure 2). Structures containing hβD-2 and hβD-3 were absent in the connective tissue of the healthy nail. In control group, hβD-2 and hβD-3 positive epithelial cells of the nail bed displayed a patchy distribution prevailing close to the nail plate whereas in psoriatic nails it was evenly expressed both in the nail bed and in connective tissue. Noticeably more positive structures stained for hβD-2 and hβD-3 could be observed per visual field in psoriatic nails. Structures positive for hβD-4 were absent in psoriatic nails as well as in control group nails.

Statistically significant difference ($p < 0.05$) comparing appearance of hβD-2, hβD-3 and hβD-4 in psoriatic nail group with the control group nails could be observed for all the defensins both in nail bed and in connective tissue (Table 1).

In psoriatic nails, IL-1α positive structures were absent (Figure 3), while in the control group there was moderate (++) number of IL-1α positive cells in the nail bed and few (+) IL-1α positive cells in the connective tissue (Figure 4).

Table 1. Statistic analysis of cytokines and anti-microbial peptides appearance data

Method	Psoriasis affected nails						Control group nails	
	Nail bed	Mann–Whitney U test	p	Connective tissue	Mann–Whitney U test	p	Nail bed	Connective tissue
IL-1	0	15.0	0.435	0	0	0.010	++	+
IL-6	+++	6.0	0.021	++	2.0	0.014	++	0
IL-10	+++	5.0	0.018	+	8.0	0.143	++	+
hβD-2	+++	2.5	0.006	++	0.0	0.002	++	0
hβD-3	+++	5.0	0.024	+	5.0	0.033	++	0
hβD-4	0	0.0	0.000	0	0.0	0.000	0	0

Designation of semiquantative method: 0 – negative reaction; 0 / + – few positive elements in some preparations; + – few positive structures in the visual field; ++ – moderate number of positive structures in visual field; +++ – numerous positive structures in the visual field; ++++ – abundance of positive structures in the visual field.

Mann–Whitney U-test: displays the ranking differences between the control group and studied group, that is, the lower the Mann–Whitney U test, the higher the ranking difference.

P-significance: if significance is < 5%, then the probability to accept zero hypothesis is applied – the values in the groups are the same size, is less than 5%, i.e., saying, that the hypothesis of the uniformity of value size in groups may be indicated at 5% confidence level.

There were more IL-6 positive structures in psoriatic nails than in the control group nails (Table 1). Numerous (+++) IL-6 positive epithelial cells were observed in the psoriatic nails' bed and moderate (++) in connective tissue cells (Figure 5). Meanwhile in the control group there were only few (+) IL-6 positive cells (Figure 6).

There were more IL-10 positive structures in psoriatic nails than in the control group nails (Table 1). In psoriatic nails, IL-10 appeared positive in moderate (++) to numerous (+++) numbers in the nail bed with predominance in the basal layer, but only few (+) IL-10 positive cells were found in the connective tissue (Figure 7), whereas in the control group it showed moderate (++) number of IL-10 positive epithelial cells in the nail bed and varied from few (+) to absence (0) in the connective tissue (Figure 8). Statistically significant difference ($p < 0.05$) comparing interleukin appearance in psoriatic nail group with the control could be observed for IL-6 both in nail bed ($p = 0.021$) and connective tissue ($p = 0.014$), IL-1 in connective tissue ($p = 0.010$) and IL-10 in nail bed ($p = 0.018$) (Table 1).

Figure 1. Abundance of hβD-2 containing cells in the psoriatic nail (hβD-2 IMH, × 250)

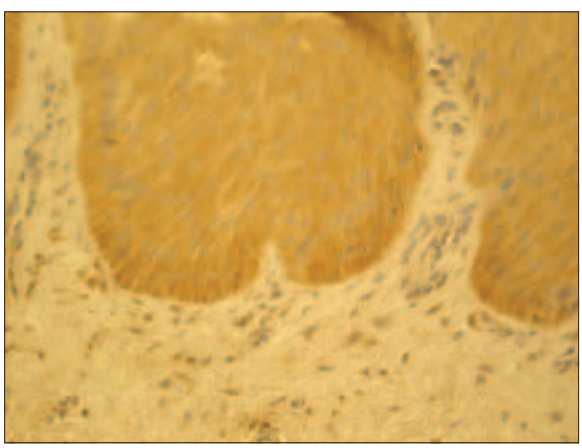


Figure 2. Few hβD-2 containing cells in the control group nail apparatus (hβD-2 IMH, × 250)

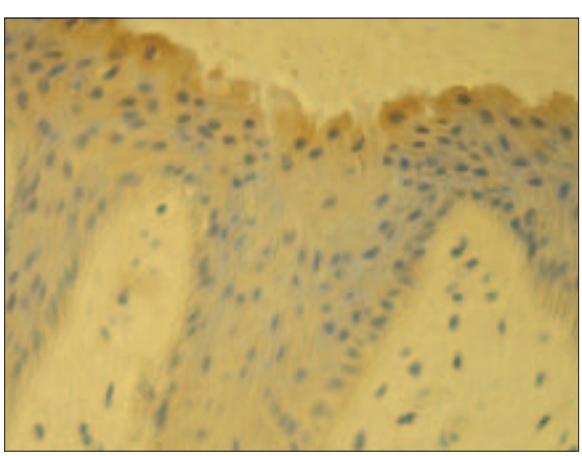


Figure 3. Absence of IL-1 expression in the psoriatic nail (IL-1 IMH, × 250)

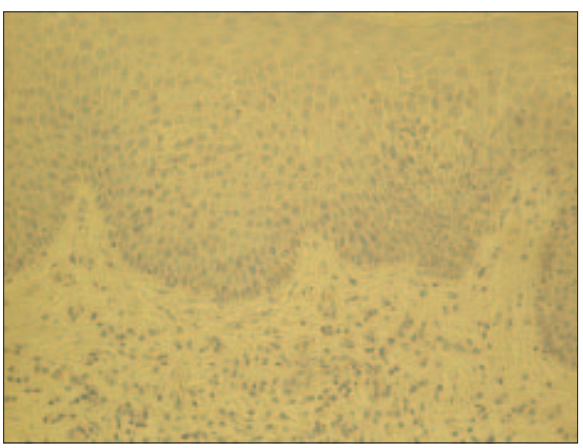


Figure 4. Moderate numbers of IL-1 containing cells in the control group nail (IL-1 IMH, × 250)

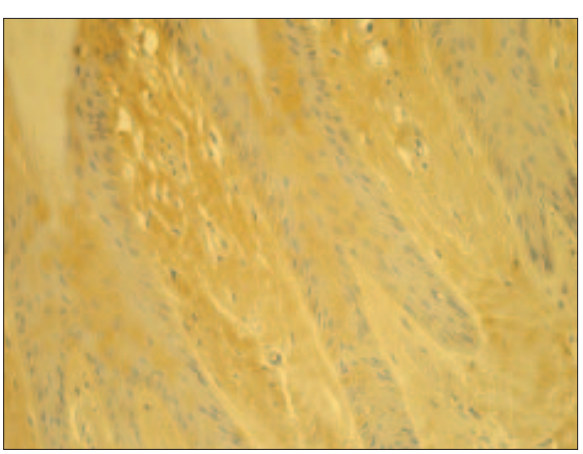


Figure 5. Abundance of IL-6positive structures in epithelia and moderate amount in connective tissue in psoriatic nail (IL-6 IMH, × 250)

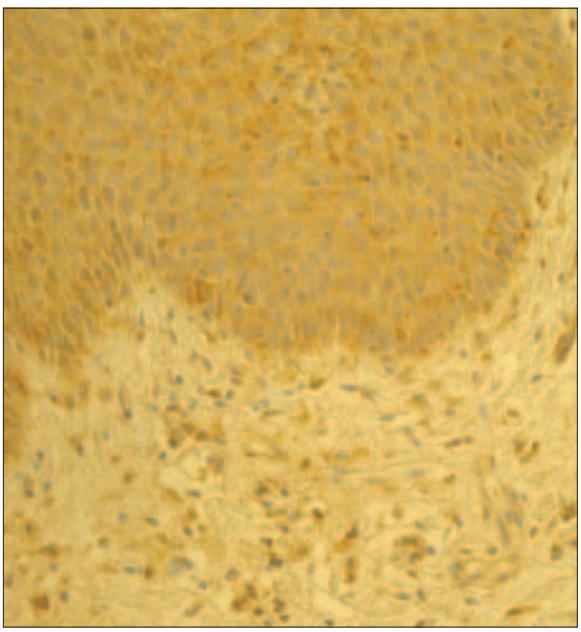


Figure 6. Moderate IL-6 positive structures in epithelia and negative expression in connective tissue in control group nail (IL-6 IMH, × 250)

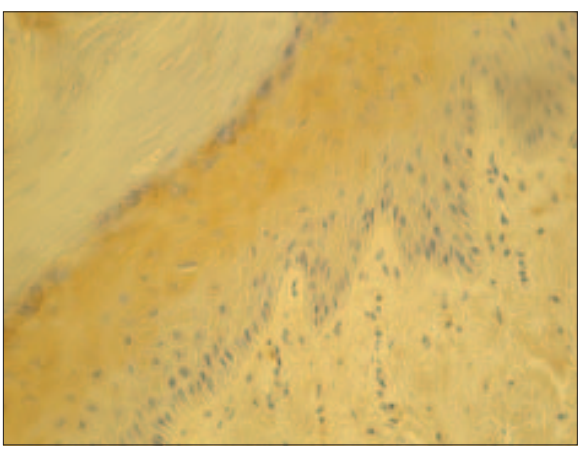


Figure 7. Abundance of IL-10 positive cells in the nail bed and few positive structures in connective tissue in the psoriatic nail (IL-10 IMH, × 250)

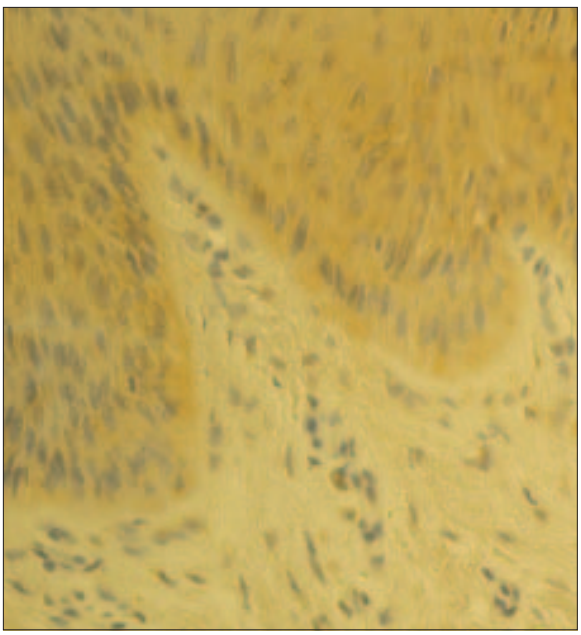
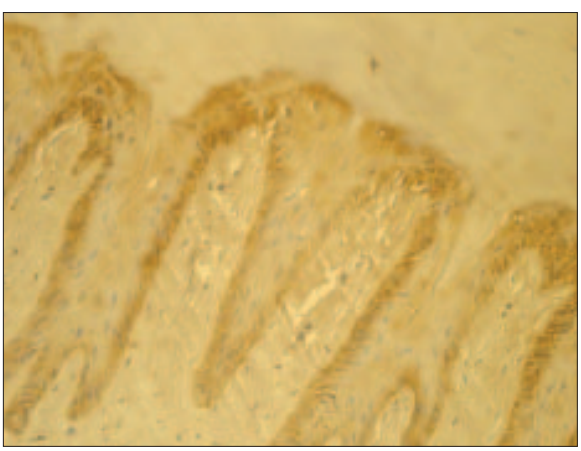


Figure 8. Moderate amount of IL-10 positive cells in epithelia in the control group nail (IL-10 IMH, × 250)



Discussion

It has been described that human nail matrix represents a site of relative immune privilege, partly due to the low number of the potent immunosuppressants (ACTH, a-MSH, IGF-1, TGF-b1). The relative immune privilege of the proximal nail matrix may serve to suppress inflammatory/autoimmune damage of the most critical component of the actual nail growth to prevent the loss of nails [9]. However, there is no functional evidence that defines compartments of the nail apparatus immune privilege. In addition, the specific arrangement of the nail immune system (NIS) is poorly understood.

It should be considered, whether a well-developed immune system might be promoted by components of innate immune system, in part with the expression of anti-microbial peptides. In nail apparatus, from anti-microbial peptides only cathelicidin LL-37 has been described [16]. Expression of

hβD-2 and hβD-3, but not hβD-4, was detected both in healthy and psoriatic nails of our study group patients. Furthermore, in control group hβD-2 and hβD-3 positive structures in the epithelia of the nail bed had a patchy distribution prevailing close to the nail plate. These findings suggest that a significant part of the human nail immune system could be ensured by anti-microbial peptides of the defensin group. Those peptides in healthy nails were distributed mainly closer to the nail plate where the microbial agent is exposed. Thus, we hypothesised that in addition to anti-microbial peptide cathelicidin LL-37 also other antimicrobial peptides that are capable to ensure an effective innate immunity defence system in the presence of intense exposure to various microbiological agents, are also present in nails, despite the relative immune privilege in the nail matrix [9, 16].

In several studies exploring psoriatic skin, it has been shown that AMPs such as hβD-2, psoriasin, and LL-37 are strongly over-expressed in psoriatic plaques [8, 31, 16, 19, 20]. Similarly, in all our patients' samples of a chronic inflammatory nail disease by psoriasis affected nails noticeably more positive structures for hβD-2 and hβD-3 were expressed evenly both in the nail bed and connective tissue. However, it is increasingly evident that these peptides not only act as endogenous antibiotics but also display additional roles, such as regulation of inflammatory and immune responses, chemo-attracting immune or inflammatory cells to wound or infection/inflammation sites, acceleration of angiogenesis, promotion of wound healing, and re-epithelisation, and many others that still have to be explored [13].

In addition, it has been suggested that in the psoriatic skin an endogenous antimicrobial peptide may play an important role in breaking innate tolerance that could induce auto-immunity in psoriasis [4]. On the other hand, the pathogenesis of nail psoriasis has been proposed to have an auto-inflammatory basis rather than auto-immune where subclinical micro-damage of the enthesis results in a diffuse soft tissue inflammation including nail lesions. The consequences of inflammatory disease in this anatomical region should be considered in understanding the development of nail psoriasis [11]. Therefore, to explore the cytokine expression in psoriatic nails compared with healthy nails, we chose cytokines the role of which has well been described in psoriatic skin plaque – IL-1, IL-6 and IL-10.

Although IL-1 expression in the psoriatic epidermis appears altered, data on this finding are often conflicting. Some studies showed that IL-1α levels in psoriatic lesions were decreased or below detection limits in comparison to non-lesional and healthy skin [15, 3, 32] whereas increased levels of IL-1α were noted in supernatants of monocyte cultures obtained from patients with psoriasis [15]. We observed that IL-1α was expressed to a higher amount in the epithelial cells of the healthy nail bed than in the connective tissue. However, in the psoriatic nail bed and connective tissue IL-1α was absent. Therefore, we suggest that this cytokine is not locally expressed in psoriatic nails.

Staining with IL-6 showed positive expression in epithelia and connective tissue in psoriatic nails with predominance in the nail bed of our study group patients, while in the control group it was weakly expressed in the epithelia only. Higher IL-6 levels were observed in psoriatic lesions compared to non-lesional and normal healthy skin [18, 23, 7]. Therefore, we speculate that IL-6 similarly as in skin is an important cytokine in psoriatic nail disease due to the stimulation of other cytokine expression [18, 23, 7].

We found positive IL-10 expression both in epithelia and in connective tissue in the control group, prevailing in nail bed. However, we found even more pronounced IL-10 expression in epithelia of the nail bed in psoriatic nails. It was weakly expressed in the connective tissue of the psoriatic nails in some samples. We speculate that it indicates some significant differences in psoriatic nails in comparison to psoriatic skin plaques reflecting the unique pathways in psoriatic nail disease. Low levels of IL-10 in both psoriatic skin and in blister fluid were demonstrated by other authors [24, 14]. A total lack of IL-10R expression on keratinocytes in psoriatic lesions has been described [1, 5, 12, 17, 28, 30, 26].

Our findings provide perception of the site-specific inflammation of psoriatic nails where both elevations of antimicrobial peptides of innate immunity, common pro-inflammatory cytokine IL-6, as well as anti-inflammatory IL-10 can be observed.

An improvement in understanding the human nail immune system could ensure better comprehension of psoriatic nail disease and promote development of more targeted treatment options.

Conclusions

From anti-microbial peptides of defensin group, hβD-2 and hβD-3, but not hβD-4, are characteristic for human nail apparatus. The increased expression of these defensins in the psoriatic nail highlights the potential role of hβD-2 and hβD-3 for the innate immunity involvement in the pathogenesis of nail psoriasis.

In psoriatic nails, the pro-inflammatory (IL-1α) cytokine expression is decreased, but expression of IL-6 as well as the anti-inflammatory cytokine IL-10 is increased indicating stimulation of nail growth and increase of anti-inflammatory immunity.

Acknowlegements

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Human Parvovirus B19 Infection Status in Patients with Myalgic Encephalomyelitis/Chronic Fatigue Syndrome and Fibromyalgia

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Abstract

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and fibromyalgia (FM) are chronic diseases with unclear aetiology. Human parvovirus B19 (B19) is immunomodulatory single-stranded DNA virus, which belongs to *Parvoviridae* family, *Parvovirinae* subfamily and *Erythrovirus* genus. B19 is considered as a possible pathogen or trigger factor in development of ME/CFS and FM.

The aim of this study was to compare frequency of B19 specific antibodies and to estimate B19 infection status in patients with ME/CFS and FM.

Thirty six patients with ME/CFS and 22 patients with FM were analysed for the presence of B19 specific IgM and IgG class antibodies in blood plasma. B19 genomic sequence was detected by nested polymerase chain reaction (nPCR). In addition, 60 apparently healthy individuals were analysed by nPCR.

B19 genomic sequence was found in 13.9% (5/36) of patients with ME/CFS, 27.3% (6/22) of patients with FM and only in 6.7% (4/60) of apparently healthy individuals.

B19 specific IgG class antibodies had 63.9% (23/36) of patients with ME/CFS and 81.8% (18/22) of patients with FM; however, B19 specific IgG and IgM class antibodies had one patient with ME/CFS.

Assessing B19 specific IgG and IgM class antibody reaction patterns for ME/CFS patients with B19 viremia, infection status after infection was revealed in one, whereas past infection in three cases, from which two had developed NS1 antibodies, that indicates persistent B19 infection. One ME/CFS patient with B19 viremia had had infection long ago. Both presented NS1 antibodies.

One patient with FM and B19 viremia was revealed status after B19 infection, four had past infection and one – infection long ago.

In patients with ME/CFS and FM more frequently B19 infection statuses after infection (months) and past infection (months to years) were found allowing to suggest the possible involvement of the viral infection in the development of mentioned diseases.

Keywords: parvovirus B19, myalgic encephalomyelitis, chronic fatigue syndrome, fibromyalgia.

Introduction

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a complex disease incorporating central nervous system and immune system disorders, cell energy metabolism and ion transport dysfunction, as well as cardiovascular abnormalities [Carruthers et al., 2011]. ME/CFS is characterised by severe chronic fatigue, accompanied by such clinical symptoms as sore throat, tender cervical or axillary lymph nodes, muscle pain, joint pain without swelling or redness, impaired memory or concentration, headache of new type, un-refreshing sleep, post-exertional malaise lasting more than 24 hours [Fukuda et al., 1994].

ME/CFS definition was first mentioned in 1988. After six years, this definition was revised and later other definitions were constituted. Eventually International Consensus Panel developed criteria suggesting using also term "myalgic encephalomyelitis", due to widespread inflammation and multi-systemic neuropathology of the disease [Carruthers et al., 2011].

Data on prevalence of ME/CFS varies depending on the used diagnostic criteria. Using Fukuda criteria and Reeves empirical criteria ME/CFS is determined from 0.24% up to 2.54% of population [Fukuda et al., 1994; Reeves et al., 2005].

Fibromyalgia (FM) is a syndrome that has a varied and inconsistent clinical spectrum. FM symptoms are described as chronic widespread pain at multiple tender points, joint stiffness and systemic symptoms such as mood swings, fatigue, cognitive dysfunction and insomnia without other explanatory diagnosis [Bellato et al., 2012].

Although knowledge and understanding about the syndrome has increased, the aetiology and pathogenesis is still unclear and diagnosing is complicated. Due to these conditions, it is believed that FM is not diagnosed for three out of four people who are suffering from this syndrome [Clauw, Arnold, McCarber, 2011]. Published reports have shown that FM affects 2–5% of population in developed countries, furthermore majority of them are young to middle-aged women [Guymer, 2013]. Ratio between women and men who are diagnosed with this disorder is 9 : 1 [Staud, 2011]. The diagnosis is based on symptoms and existence of this medical condition has been questioned protractedly, since the causal factor of FM is still unclear and there are no standardised tests and biological markers for confirming it [Bellato et al., 2012].

Only in 1990, American College of Rheumatology (ACR) developed widely used diagnostic criteria for diagnosing FM that includes: 1) a history of widespread musculoskeletal pain present for at least three months, 2) tenderness in at least 11 of 18 defined tender points. Both criteria must be confirmed [Wolfe et al., 1990].

It is believed that important role in this disorders association with infectious agents might play cytokines and glia cells that express receptors for bacteria and viruses [Bellato et al., 2012]. Infectious agent that has been associated with ME/CFS and FM are hepatitis C virus, human immunodeficiency virus, Coxsackie B, Epstein-Barr virus, human herpesvirus 6, human parvovirus B19 (B19) and bacteria such as Borrelia, Mycoplasma and Chlamydia. However, association of single specific infectious agent and mentioned diseases has not been established [Nicolson, 2002; Ablin et al., 2006; Bansal et al., 2012; Bellato et al., 2012; Chapenko et al., 2006; Chapenko et al., 2012].

B19 is immunomodulatory single-stranded DNA virus, which belongs to *Parvoviridae* family, *Parvovirinae* subfamily and *Erythrovirus* genus. B19 was first discovered in 1975 in healthy donors' blood serum [Cossart et al., 1975]. B19 genome consists of linear single-stranded DNA, 5596 bases in length. Right side of virus genome is coding viral capsid proteins VP1 and VP2, furthermore VP2 protein constitutes 95% of the virion [Ozawa et al., 1987; Deiss et al., 1990]. Left side of B19 genome encodes non-structural proteins – NS1 that takes part in the production of infectious virus by regulating transcription and participating in replication and formation of virions DNA capsid [Momoeda et al., 1994]. After entering the cell, B19 migrates to the nucleus, where mRNA transcription and DNA replication accomplishes [Richman et al., 2002]. DNA molecules of positive and negative polarity are encapsidated and virions are released during cell lytic cycle [Green et al., 2000]. Virus

replicates mainly in primary target cells – erythroid progenitor cells in the bone marrow that are permissive to B19 infection [Morey et al., 1993]. Antigenic determinant within P blood group – globoside is expressed not only on erythroblasts, but also on megakaryocytes, heart tissue, liver, lungs, kidneys, endothelium, aorta and gastro-intestinal smooth muscle tissues and synovium [Brown et al., 1993; Soderlung-Venermo et al., 2002].

B19 was first related with human disease in 1981 [Pattison et al., 1981]. It was only parvovirus associated with human diseases until 2005, when Allander with colleagues in Sweden discovered new human parvovirus called human bocavirus [Allander et al., 2005]. B19 is frequently detected in children and young people, therefore 60 to 80% of adults has antibodies against B19 [Brown et al., 1993; Cooling et al., 1995]. B19 can cause such symptoms as rashes (exanthema subitum), infectious erythema, arthralgia, aplastic crisis with reduced red blood cell lifespan and aplasia in immunocompromised patients, various skin lesions, neutropenia, hepatobiliary diseases and neurologic diseases [Kerr, 2000].

Production of virus-specific antibodies represents protection against B19. Human normal immunoglobulin can eliminate virus from peripheral blood, therefore improving clinical signs in immunosuppressed individual [Kurtzman et al., 1989; Schwarz et al., 1990].

After primary infection, B19 can remain in organism and it has been associated with different clinical manifestations, including arthritis, fatigue and autoimmune processes [Campadelli-Fiume et al., 1999; Clark, 2000; Kerr, Tyrrell, 2003].

B19 has been considered as one of possible trigger factors for ME/CFS [Shmuel et al., 2007].

Aim

The aim of this study was to compare frequency of B19 specific antibodies and to estimate B19 infection status, as well as detect frequency of B19 genomic sequence by nPCR in patients with myalgic encephalomyelitis/chronic fatigue syndrome and fibromyalgia.

Material and methods

Study was done according to safety standards and with Rīga Stradiņš University Ethics Committee's permit issued on 27.09.2012. All enrolled patients gave their informed consent prior to the study.

Fifty eight patients: 36 patients (24/36 (67%) female and 12/36 (33%) male) with clinically diagnosed ME/CFS corresponding to 1994 Fukuda Centers for Disease Control and Prevention criteria and 22 patients (21/22 (95%) female and 1/22 (5%) male) with FM diagnosed according to 1990 American College of Rheumatology criteria were enrolled in this study.

Presence of B19 specific IgM and IgG class antibodies were detected in blood plasma by recomLine Parvovirus B19 IgM and IgG commercially available kits (MIKROGEN DIAGNOSTIK). Specific antibodies against six antigens of B19 (Vp-2p- main capsid antigen (conformation epitope); VP-N-N-terminal half of the structure proteins VP-1 and VP-2; VP-1S-specific segment (differentiation to VP-2); VP-2r- main capsid antigen (linear epitope); VP-C- C-terminal half of the structure proteins VP-1 and VP-2; NS-1-non-structure protein) were identified thereby various reaction patterns allow determining status of B19 infection.

DNA was extracted from whole blood and from cell-free blood plasma by phenol-chloroform method. Quantity of extracted DNA was measured spectrophotometrically using Nanodrop spectrophotometer. To assure the quality of DNA from whole blood and to exclude possible contamination of plasma by cellular DNA, PCR was carried out. B19 genomic sequence was detected by nested PCR (nPCR) [Hokynar et al., 2000].

In addition, 60 age and gender matched apparently healthy individuals were analysed by nPCR.

Statistical analysis was done by Fisher's exact test.

Results

B19 genomic sequence was detected in 13.9% (5/36) of patients with ME/CFS: three in DNA isolated from whole blood, one in DNA from cell-free blood plasma and one in both DNA form whole blood and from cell-free blood plasma; 27.3% (6/22) of patients with FM (four in DNA isolated from whole blood and two in DNA from cell-free blood plasma. Only 6.7% (4/60) of apparently healthy individuals had B19 genomic sequence: two in DNA isolated from whole blood and two in DNA from cell-free blood plasma (Figure 1).

B19 specific IgG class antibodies were detected in 63.9% (23/36) of patients with ME/CFS and 81.8% (18/22) of patients with FM blood plasma samples. However, B19 specific IgG and IgM class antibodies had only one patient with ME/CFS.

B19 specific IgG class antibodies against NS1 protein were detected in 12/36 (33.3%) patients with ME/CFS and 2/22 (9.1%) patients with FM (Figure 2).

Assessing B19 specific IgG and IgM class antibody reaction patterns for patients with ME/CFS and FM with B19 genomic sequence found in DNA from whole blood and/or blood plasma, such B19 infection status as acute infection was found in none of patients with ME/CFS and FM. For one patient with ME/CFS and B19 genomic sequence detected in DNA from whole blood, infection status "after infection" (weeks to months) was revealed. B19 infection status "after infection" (months) had 11/36 (30.6%) patients with ME/CFS and 5/22 (22.7%) patients with FM, from them in one patient with FM B19 genomic sequence was detected in DNA isolated from whole blood.

Figure 1. Presence of B19 genomic sequence in DNA extracted from whole blood (WB) and cell-free blood plasma from patients with FM and ME/CFS and apparently healthy individuals

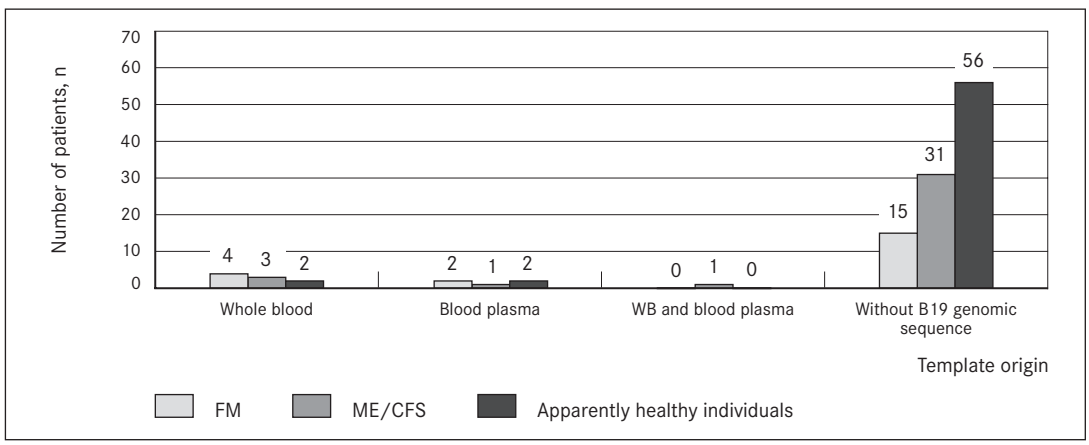
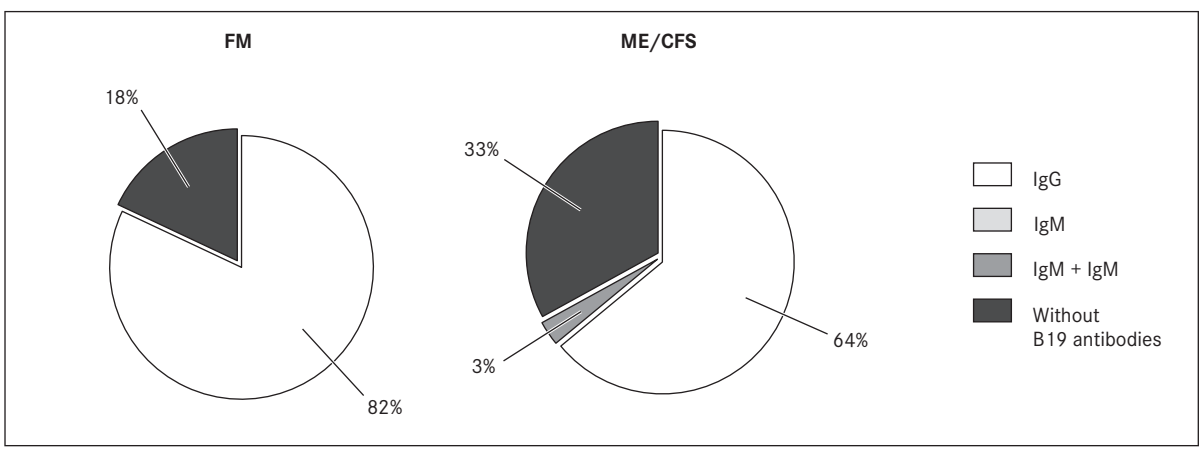


Figure 2. Frequency of B19 specific IgM and IgG class antibodies in blood plasma from patients with FM and ME/CFS



Past infection (months to years) was estimated in 8/36 (22.2%) patients with ME/CFS and 9/22 (40.9%) cases of FM. B19 genomic sequence was present in DNA from whole blood in 2/8 ME/CFS cases, from which one was with developed NS1 antibodies, that indicates persistent B19 infection. Another 1/8 ME/CFS patients with past infection (months to years), B19 genomic sequence was detected in DNA from cell-free blood plasma and B19 NS1 antibodies were presented. B19 genomic sequence was found also in 4/9 FM patients with past infection (months to years): 2 in DNA from whole blood and 2 in DNA from cell free blood plasma.

Four out of 36 (11.1%) patients with ME/CFS and 4/22 (18.2%) patients with FM had B19 infection status "infection long ago" (years). From them one patient with ME/CFS was with B19 genomic sequence in DNA from whole blood and blood plasma with developed NS1 antibodies. In addition, one patient with FM and B19 infection long ago (years) had B19 genomic sequence in DNA from whole blood (Figure 3 and Figure 4).

Figure 3. B19 infection status in viremic cases of patients with ME/CFS according to the presence of IgM and/or IgG class antibodies

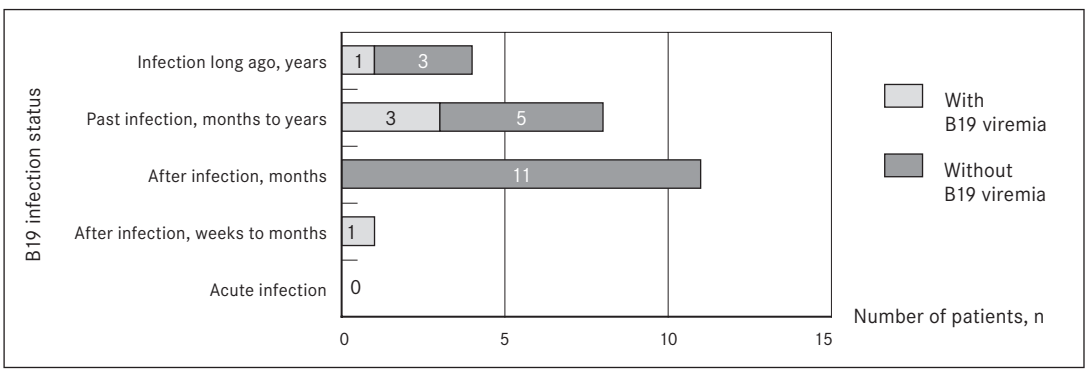
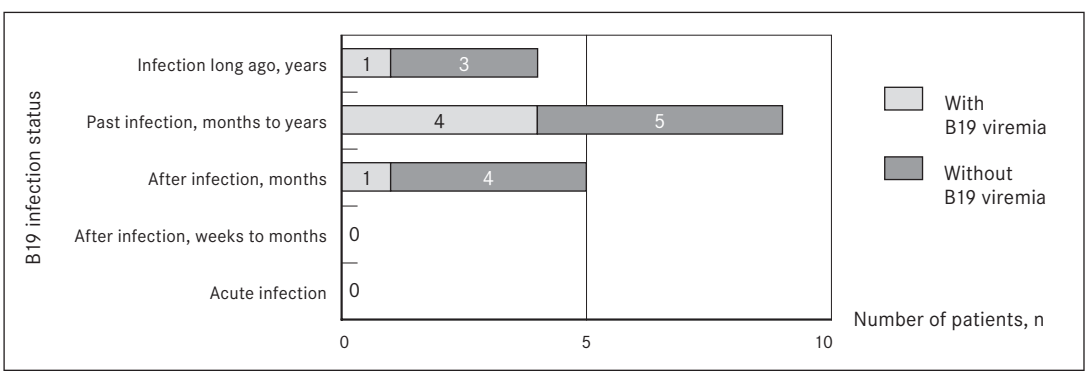


Figure 4. B19 infection status in viremic cases of patients with FM according to the presence of IgM and/or IgG class antibodies



Discussion

ME/CFS is a multi-factorial disease accompanied by severe chronic fatigue without pathophysiological explanation that reduces or subtracts ability to work. There is no consensus on presence, form and level of immune dysfunction in case of ME/CFS [Bansal et al., 2012]. ME/CFS is often followed by prolonged stress or virus infection that is considered as one of possible ME/CFS causal agents due to the fact, that most of patients report on sudden start of the illness with "flu-like" symptoms. Some virus infection can result in post-infectious fatigue and many patients with ME/CFS have immunological disturbances that could result from virus infection or be promoted by the infection. Still involvement of virus infection in aetiopathogenesis of ME/CFS remains ambiguous [Morinet, Corruble, 2012].

FM is a syndrome involving chronic, debilitating disorders that affect individuals' quality of life, reducing ability to work, which further results in an influence on national economic situation. Various factors such as dysfunction of central and autonomic nervous system, neurotransmitters, hormones, immune system, external stressors, psychiatric and other aspects are considered to be involved in the pathogenesis of FM syndrome [Bellato et al., 2012]. Scientists consider that possible cause of fibromyalgia can be currently unknown infectious agent such as virus [Ablin, Shoenfeld, Buskila, 2006]. Using internet survey, data revealed that for 26.7% out of 2596 of respondents with FM onset of the disease is associated with acute illness, which can possibly be caused by virus reactivation [Bennett et al., 2007]. Virus infection is considered as one of the possible causes of FM because most patients report on sudden onset of the disease and symptoms that are present in patients with chronic bacterial or viral infection. Despite the continuing studies of pathogenesis and aetiology of FM, no consensus about origin of this disorder is revealed.

According to previous study in our laboratory B19 specific IgG class antibodies was identified in 73/94 (77.7%) of apparently healthy individuals plasma samples, IgM class antibodies – in 15/94 (16%) from them 4 (4.3%) had only IgM and 11 (11.7%) had both IgM and IgG class antibodies in blood plasma [Kozireva et al., 2008]. In this study, the presence of B19 specific IgG class antibodies was statistically significantly higher in patients with ME/CFS than in apparently healthy individuals ($p = 0.0036$). However, significant difference was not revealed in the occurrence of B19 infection status between patients with ME/CFS and FM. It should be noted that determining B19 specific IgG and IgM class antibody reaction patterns for patients with ME/CFS and FM past infection (months to years) had more patients with FM (40.9%), than with ME/CFS (22.2%). Whereas status after infection (months) was defined for more patients with ME/CFS (30.6%) than with FM (22.7%). Furthermore, patients with B19 viremia, infection statuses "after infection" (months), "past infection" (months to years) as well as "infection long ago" were found. As for most of patients with ME/CFS and FM onset of disease occurred at least six months ago, B19 infection could serve as a trigger factor.

The study results are in accordance with other researchers' findings of B19 IgG class antibodies frequency – 66.7% in case of ME/CFS and 81.8% – in FM group. Other B19 seroprevalence studies find no significant difference between patients and control group. Some report that B19 seroprevalence varies from 60% to 80% of population [Cooling et al., 1995], but another report shows finding of B19 specific IgG class antibodies in 74% and IgM – in only one patient with ME/CFS. Seroprevalence of B19 is typical to general population [Zhang et al., 2010]. Kerr with colleagues analysing markers for B19 infection in 200 apparently healthy individuals and 200 patients with ME/CFS corresponding to 1994 Fukuda diagnostic criteria also found no difference in B19 seroprevalence (anti-B19 VP2 IgG class antibodies detected in 75% and 78%, respectively). Anti-B19 VP2 IgM class antibodies are present in four patients [Kerr et al., 2010].

Data from this study show the presence of B19 specific anti-NS1 protein antibodies in 12 (33.3%) patients with ME/CFS and 2 (9.1%) patients with FM, indicating possible persistence of B19 infection. In case of ME/CFS results are similar to study, where Kerr found IgG class antibodies against NS1 protein more frequently in patients with ME/CFS (41.5%) than in healthy controls (7%) related with high expression level of CFS-associated NHLH1 and GABPA genes. Anti-B19 NS1 IgM class antibodies are detected in three patients and one control group donor. Presence of B19 NS1 antibodies indicates of chronic or severe B19 infection, thereby part of patients' immune system cannot sufficiently control the virus [Kerr et al., 2010].

In this study, B19 genomic sequence was detected statistically significantly more in patients with FM (6/22) than in apparently healthy individuals (4/60) ($p = 0.020$). Whereas no difference was found in presence of B19 genomic sequence in patients with ME/CFS (5/36) comparing with apparently healthy individuals ($p = 0.288$), as well as between groups of patients with ME/CFS and FM ($p = 0.301$).

Results show that B19 is more detected in patients with FM and ME/CFS than in apparently healthy individuals. Nevertheless, patients' groups are comparatively small; therefore, to demonstrate statistically approved difference, investigated cohorts should be enlarged. Published studies report that using real-time PCR B19 genomic sequence is detected in 11 patients with ME/CFS and in none of control group blood donors [Kerr et al., 2010]. It is outlined that B19 can cause typical clinical symptoms of

ME/CFS; therefore some studies report on this virus as one of possible trigger factors for ME/CFS, which tends to coincide with our study results [Matano et al., 2003; Shmuel et al., 2007]. Others conclude that at least part of patients B19 could cause ME/CFS, because it is detected in 40% of patients and in less than 15% of apparently healthy individuals [Fremont et al., 2009]. Meanwhile others find no association of parvovirus B19 infection with this disease, because the presence of B19 is not revealed in all cases of ME/CFS [Sanders, Korf, 2008]. The contrary fact is that in Brazil B19 genomic sequence was not detected in 141 Brazilian children with exanthema subitum by age four [Magalhaes et al., 2011].

Although some researchers have studied and discussed the possibility of FM and ME/CFS being one single disease, it has been proven that these are two different diseases [Abbi, Natelson, 2013]. Though finding no statistical difference comparing groups of patients with FM and ME/CFS could be explained by the similarity between FM and ME/CFS.

ME/CFS and FM could be caused by various factors and some viruses or other infectious agents may contribute to a subset for these diseases, confirming hypothesis of B19 as a trigger factor for ME/CFS and FM [Kerr et al., 2010]. At least some studies show that for part of patients B19 could be one of the trigger factors for this disease [Fremont et al., 2009].

Conclusion

In patients with myalgic encephalomyelitis/chronic fatigue syndrome and fibromyalgia B19 infection statuses "after infection" (months) and "past infection" (months to years) more frequently were found allowing to suggest the possible involvement of the viral infection in the development of mentioned diseases.

This study did not detect any significant difference in the frequency of the presence of B19 specific antibodies and infection status between patients with myalgic encephalomyelitis/chronic fatigue syndrome and fibromyalgia; however, it is necessary to analyse a larger cohort to draw general conclusion about the possible association of different B19 infection statuses with disease aetiopathogenesis.

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Human Herpesvirus 6 and Parvovirus B19 Infection in Patients with Chronic Autoimmune Thyroiditis and in Practically Healthy Blood Donors

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Abstract

The human pathogenic beta-herpesvirus 6 (HHV-6) and parvovirus B19 (B19) are immunomodulating viruses that are unique due to broad cell tropism including thyroid tissue. HHV-6 and B19 are triggers of many autoimmune diseases and have been associated with autoimmune thyroid gland diseases. Autoimmune thyroid gland diseases are multifactorial diseases and different factors have been proposed to contribute to their aetiology; however, the primary cause is still unknown.

This work aimed to estimate the frequency of HHV-6 and B19 infection in patients with chronic autoimmune thyroiditis and practically healthy blood donors. 60 patients with struma nodosa after thyroidectomy and 60 practically healthy blood donors were included in this study. Thyroid-specific autoantibodies against thyroid peroxidase (TPOAb), TSH receptor (TRAb) and thyroglobulin (TGAb) were detected using ELISA. HHV-6 and B19 genomic sequence in DNA extracted from whole blood and cell-free plasma was detected using nested polymerase chain reaction (nPCR).

High titre of thyroid-specific autoantibodies was detected significantly more often in patients with thyroid gland pathologies than in practically healthy donors (27/60 *vs.* 0/59; p = 0.0001). HHV-6 genome sequence was found in 15 out of 27 (55.5%) patients with chronic autoimmune thyroiditis which is significantly more often comparing to the practically healthy donors – 8 out of 59 (13.5%) (p = 0.0001). HHV-6 genome sequence was found also in 1 out of 5 (20.0%) patient with chronic thyroiditis without autoimmune component and in 18 out of 28 (64.2%) patients without chronic thyroiditis. B19 genome sequence was found significantly more often in patients with chronic autoimmune thyroiditis – 25.9% (7 out of 27) in comparison with practically healthy donors 6.7% (4 out of 59; p = 0.031). However, B19 genome sequence was detected also in one patient with chronic thyroiditis without autoimmune component and 10 out of 28 patients without chronic thyroiditis. No statistical significance was observed in virus frequency between patients according to high or normal range of thyroid-specific autoantibodies.

The high detection rate of HHV-6 and B19 infection suggest a potential pathogenic role of these viruses in development of thyroid gland diseases.

Keywords: HHV-6, B19, thyroiditis, thyroid-specific autoantibodies.

Introduction

Autoimmune thyroiditis is a common disease characterised by lymphocytic infiltration and the presence of thyroid-specific autoantibodies. Despite the intensive investigation of chronic inflammatory and autoimmune processes, there are many uncertainties so far concerning their aetiological factors. Viral infections are frequently cited as a major environmental factor involved in thyroid gland diseases [Prumel, 2004]. Several reports have provided an important information linking HHV-6 to autoimmune diseases including multiple sclerosis, autoimmune connective tissue diseases and Hashimoto's thyroiditis [Nora-Krukle, 2011; Broccolo, 2013; Caselli, 2012]. In 1986, Ablashi, Gallo and Salahuddin discovered human herpesvirus 6 (HHV-6), a member of the Roseolovirus genus, Herpesviridae family, β-herpesvirus subfamily [Ablashi, 1987]. HHV-6 is lymphotropic, immunomodulating virus characterised by widespread tissue tropism and has been found also in the thyroid gland tissue. Infection with HHV-6 is a global concern, and it is the aetiological agent of many diseases. However, little literature on HHV-6 involvement in thyroiditis is available. An excellent review has been published where researchers from Italy have shown that HHV-6 infection may play a role in the pathogenesis of Hashimoto's thyroiditis [Caselli, 2012]. Human parvovirus B19 (B19) is the member of the family Parvoviridae and is a cause of a childhood exanthema, but it has also been well documented that B19 is a common human pathogen, which has been linked to autoimmune diseases such as autoimmune neutropenia, thrombocytopenia, haemolytic anaemia and rheumatoid arthritis [Desailloud, 2009]. During the last few years, studies have suggested the association of B19 infection with autoimmune thyroiditis [Wang, 2010; Page, 2014]. According to Mori and his colleagues review, intra-thyroidal persistence of B19 DNA in a patient with Hashimoto's thyroiditis has been detected. The cell types responsible for the B19 DNA persistence are not determined and immune cells infiltrating the thyroid gland may be the source of B19 DNA. However, the possibility that thyroid epithelial cells harbour B19 DNA cannot be excluded [Mori, 2007].

Aim

This study aimed to estimate the frequency of HHV-6 and B19 infection in patients with chronic autoimmune thyroiditis and practically healthy blood donors.

Materials and methods

All laboratory tests were approved by the Ethical Committee of Rīga Stradiņš University (Rīga, Latvia). Patients with struma nodosa thyreotoxicum or euthyreosis were referred to thyroidectomy and patients' material was received after thyroidectomy performed at Rīga Eastern Clinical University Hospital, Clinic "Gaiļezers". In total 60 patients: 32 patients with chronic thyroiditis (27 of them with chronic autoimmune thyroiditis) and 28 patients without markers of chronic thyroiditis were included in this study. The exact diagnosis of the thyroid gland pathology was established by histological analysis of resected tissue and analysis of thyroid gland specific autoantibodies. The age of the patients ranged from 40 to 78 (mean age – 50) and only 5 of them were men. 60 age-matched practically healthy blood donors were included as the control group. Blood plasma was separated from the EDTA whole blood, aliquoted and stored at –70 °C. DNA was isolated from 0.5 ml of whole blood and 0.2 ml cell-free plasma by proteinase K digestion followed by phenol-chloroform extraction and ethanol precipitation. The plasma samples were treated with DNase1 before DNA purification. B-globin PCR was performed in order to assess quality of the extracted DNA [Vandamme, 1995]. Negative b-globin PCR result for DNA isolated from plasma shows that there is no presence of cell DNA in the sample. Nested polymerase chain reaction (nPCR) with the corresponding primer pairs was used for the detection of B19 and HHV-6 genomic sequences [Feraj, 2001; Secchiero, 1995]. DNA samples negative for B19 and HHV-6 specific sequences and water controls were included in each experiment to exclude the possibility of contamination during PCR.

The amplified DNA was analysed in 1.7% agarose gel with ethidium bromide staining and analysed using BioSpectrum Imaging System. Autoantibodies against thyroid peroxidase (TPOAb), thyroglobulin (TGAb) and thyroid stimulating hormone (TRAb) were detected using ELISA kit (Euroimmun).

The data were analysed using Fisher's test and Student's t test.

Results

Statistical analysis showed no significant difference in age and gender between the patients and control group individuals. Plasma samples from 60 patients with struma nodosa after thyroidectomy and 60 practically healthy blood donors were investigated for thyroid-specific autoantibodies against TPOAb, TRAb and TGAb using the corresponding Euroimmun Immunoassays. 27 out of 60 (45%) patients had high titre of TRAb (2.0 – 40.0 ± 13.07 IU/ml); TGAb (107.0 – 1070.0 ± 312.68 IU/ml) and TPOAb (90.0 – 500.0 ± 164.13 IU/ml). 33 (55%) patients were within the normal range of TRAb (< 1.8 IU/ml) and TGAb (< 100 IU/ml) and TPOAb (< 50 IU/ml) (Figure 1).

One individual was excluded from the control group because of high titre of autoantibody against TPO (395.0 IU/ml) (suspected patient of chronic autoimmune thyroiditis).

The presence of HHV-6 and B19 was analysed in whole blood and cell-free plasma DNA samples from all patients with and without chronic thyroiditis and control group individuals according to the titres of thyroid specific autoantibodies. HHV-6 specific genomic sequence was detected in 34 out of 60 (56.6%) whole blood DNA samples from patients: 15 out of 27 (55.5%) whole blood DNA samples from patients with chronic autoimmune thyroiditis who had high titres of thyroid specific autoantibodies; in one out of 5 (20.0%) whole blood DNA samples from patients with chronic thyroiditis who were within the normal range of TGAb, aTPOAb and/or TRAb autoantibodies. Besides, in one patient with high titre of thyroid-specific autoantibodies, HHV-6 genomic sequence was detected also in cell-free plasma DNA sample, which is a marker of active viral infection. HHV-6 genomic sequence was found also in 18 out of 28 (64.2%) whole blood DNA samples from patients without chronic thyroiditis. HHV-6 genomic sequence in whole blood DNA samples was found in 8 out of 59 (13.5%) healthy blood donors. HHV-6 was not found in any of the cell-free plasma DNA sample of patients without thyroiditis and control group individuals (Figure 2).

B19 genomic sequence was detected in 8 out of 32 (25.0%) patients with chronic thyroiditis: in 7 out of 27 (one whole blood DNA; 5 cell-free plasma DNA; one whole blood and plasma DNA sample) patients with chronic autoimmune thyroiditis and in 1 out of 5 (one plasma DNA sample) patients with chronic thyroiditis without autoimmune component. DNA B19 genomic sequence was found also in 10 out of 28 (35.7%) patients without chronic thyroiditis (3 whole blood DNA; 6 cell-free plasma DNA; one whole blood and plasma DNA sample) and 4 out of 59 (6.7%) practically healthy donors (2 in whole blood DNA samples and 2 in cell-free plasma DNA samples (Figure 3).

Figure 1. The presence of TGAb, TPOAb and TRAb antibodies in patients with and without chronic thyroiditis and practically healthy blood donors

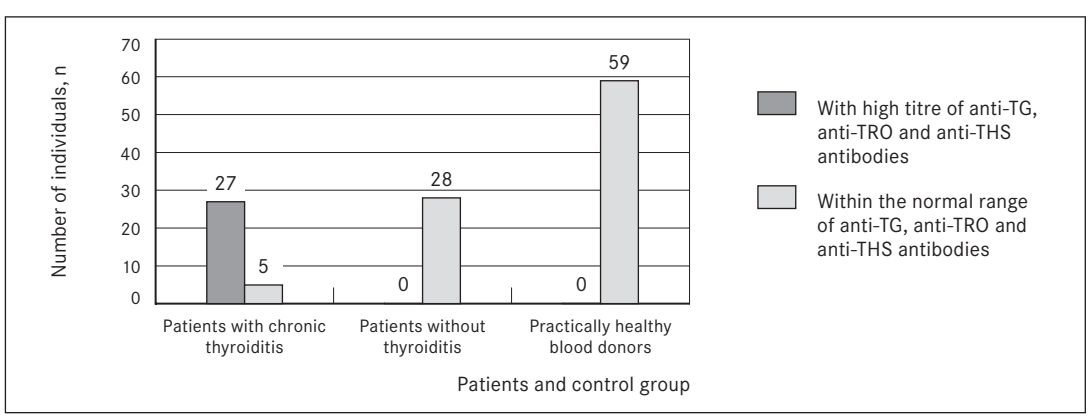


Figure 2. The presence of HHV-6 genome sequence in whole blood and cell-free plasma DNA samples from patients with and without chronic thyroiditis and practically healthy blood donors

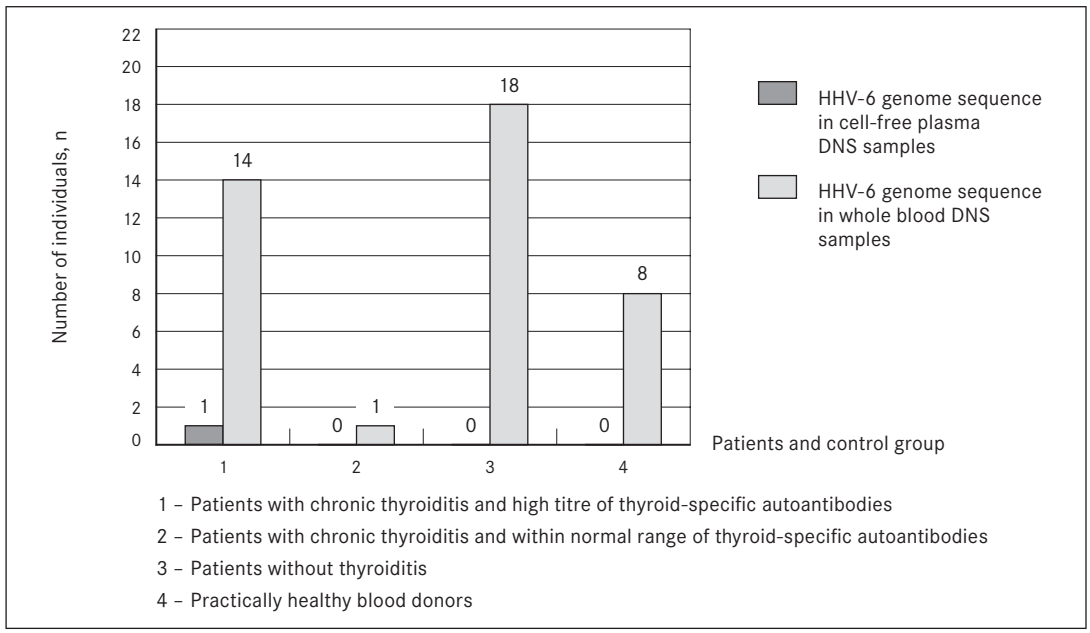
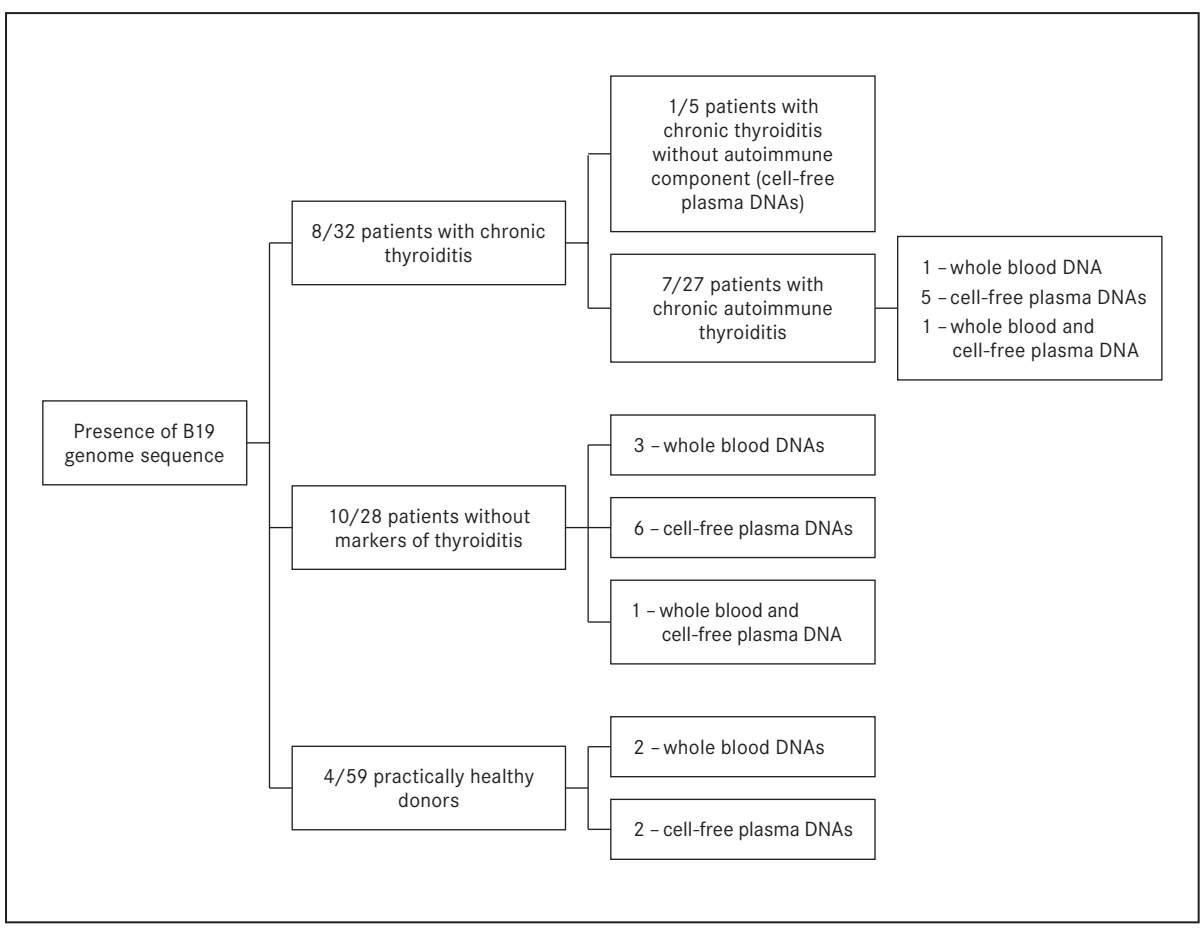


Figure 3. The presence of B19 genome sequence in DNA samples from patients with and without chronic thyroiditis and practically healthy blood donors



Discussion

Viral infections have been frequently cited as important environmental factor implicated in chronic autoimmune thyroiditis, but no specific virus infection has yet been associated to the disease. Despite long time since HHV-6 and B19 were discovered, these viruses offer continuous challenge to virologists.

We found that patients with chronic autoimmune thyroiditis are characterised by statistically significantly higher titres of thyroid-specific autoantibodies expression than practically healthy donors ($p = 0.0001$) confirming previously published data that from serological point of view autoantibodies against thyroid peroxidase, TSH receptor and thyroglobulin are the main markers of autoimmune thyroid gland diseases.

Nested polymerase chain reaction is an appropriate and sensitive method for the detection of viral markers in DNA samples, and it is the beginning of understanding the relationship between HHV-6 and/or B19 and thyroid gland diseases. HHV-6 was found in 55.5% patients with chronic autoimmune thyroiditis which is statistically significantly more often than in practically healthy donors – 13.5% ($p = 0.0001$). The presence of HHV-6 was also demonstrated in 20.0% of patients with chronic thyroiditis without autoimmune component and 64.2% of patients without chronic thyroiditis confirming Thomas and co-authors' previously published data where they showed no differences in the incidence of HHV-6 infection in patients with or without thyroid autoimmune diseases [Thomas et al., 2008]. Very recently, experimental evidence supporting a role for HHV-6 in the aetiology of Hashimoto's thyroiditis has been provided [Caselli, 2012]. The presence and transcriptional state of HHV-6 was detected in thyroid fine needle aspirates and peripheral blood mononuclear cells from patients with Hashimoto's thyroiditis [Caselli, 2012].

Following primary infection, HHV-6 persists lifelong in the human host and viral reactivation is associated with a range of complications. However, much more research is needed to answer one of the most important issues – whether the virus causes clinical activity of the disease, or these viruses become activated due to pathological mechanism of the disease. It is very complex to evaluate the impact of each virus because many viruses are able to simultaneously exist in a body. For example, Chapenko and co-authors demonstrated that in renal transplant patients, human herpesvirus 7 is activated first, and only then HHV-6 [Chapenko et al., 2009], which could be explained by the fact that in case of HHV-7 infection CD46 and CD59 expression level increases in target cells [Takemoto et al., 2007]. In addition, there are studies, which show that other viruses have also been found in patients with thyroid gland diseases – Epstein-Barr virus Simian vacuolating virus 40, mumps virus and retroviruses [Kannangai, 2010; Desailloud, 2009].

Infection with B19 has been linked to a variety of diseases including erythroid, thyroid, neurological and autoimmune diseases. Lately, Ignatovich and Hobbs showed that the infection of primary $CD36^+$ cells with B19 coincides with down-regulation of thyroid, retinoid, and oestrogen hormone receptors [Ignatovich, 2013]. Our results demonstrate that B19 genome sequence was found significantly more often in patients with chronic autoimmune thyroiditis – 25.9% comparing with practically healthy donors – 6.7% ($p = 0.031$). B19 was also found in one patient with chronic thyroiditis without autoimmune component and in 35.7% patients without markers of chronic thyroiditis. Active B19 infection was observed in 6 patients with chronic thyroiditis (5 with chronic autoimmune thyroiditis and one patient with chronic thyroiditis without autoimmune component) and only in two practically healthy donors; however, the difference between patients and control group is not statistically significant ($p = 0.159$). It must not be ruled out that B19 is using cellular mechanisms, which are responsible for the immune response and therefore promoting the development of thyroid-specific autoantibodies.

Conclusion

HHV-6 and B19 infection was demonstrated in patients with chronic autoimmune thyroiditis significantly more often in comparison to practically healthy blood donors. At the same time, HHV-6 and B19 infection was also detected in patients with other thyroid gland pathologies, without statistical significance in virus infection frequency between patients with chronic autoimmune thyroiditis and other thyroid gland pathologies. To draw a conclusion on the involvement of HHV-6 and B19 infection in etiopathogenesis of chronic autoimmune thyroiditis resected thyroid tissues should be analysed for the presence of virus-specific DNAs and RNAs as well as for viral antigens expression.

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Right Atrial Tissue Morphology in Different Acquired Heart Diseases: A Pilot Study

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Abstract

Despite scientific progress, diseases of circulatory system are still the most common cause of mortality in Latvia and all Europe. Approximately 1–2% of adult population in developed countries has heart failure, with the prevalence rising to ≥ 10% among persons 70 years of age or older. One of the main principles of heart failure treatment is to target the underlying cause, for example, revascularisation and valve surgery. Understanding the pathophysiology and its clinical implications are important for the cardiovascular surgeon, because the therapeutic options may improve a patient's outcome.

The aim of this study was to identify appearance and distribution of apoptosis, homeostasis regulating factors, innervations and ischemia markers in right atrial tissue in different acquired heart diseases.

In Pauls Stradiņš Clinical University Hospital during elective heart surgery were taken right atrial tissue fragments from 6 patients. The mean age was (mean ± SD) 70.3 ± 9.1 years (range 58–83 years) and there were 4 female patients. Tissues were processed for apoptosis, protein-gene peptide 9.5 (PGP 9.5), human atrial natriuretic peptide (hANUP), vascular endothelial growth factor (VEGF), chromogranin A and endothelin by means of biotin-streptavidin immunohistochemistry.

In all patients, a moderate to severe myocyte degeneration and vacuolisation was observed. In three patients, significant vascular sclerosis was found. The apoptotic index ranged from 24% to 80%. Mean number of TUNEL positive cardiomyocytes was significantly different among all specimens. All examined patients showed different expression of PGP 9.5. Numerous hANUP secreting cells were detected in all specimens, except patient No. 2, where this factor was detected in moderate cells. Few vascular endothelial growth factors were observed in endothelial cells in all specimens. Only in 2 patients, blood vessels contained endothelin positive endotheliocytes. Mostly this factor was seen in epicardium

Different expression of PGP 9.5, hANUP, VEGF, chromogranin A and endothelin in right atrial tissue might characterise pathogenesis of different acquired heart diseases.

Large variation of apoptotic index seems to correlate to larger volume and longer untreated history of the affected disease in our pilot study patients in Latvia.

Keywords: heart, apoptosis, protein-gene peptide 9.5, atrial natriuretic peptide, vascular endothelial growth factor, chromogranin A, endothelin.

Introduction

Despite scientific progress, diseases of circulatory system are still the most common cause of mortality in Latvia and Europe [Nichols, 2012]. Approximately 1–2% of adult population in developed countries has heart failure, with the prevalence rising to ≥ 10% among persons 70 years of age or older [John, 2012]. One of the main principles of heart failure treatment is to address the underlying cause, for example, revascularisation and valve surgery. Understanding the pathomorphology and its clinical implications are important for the cardiovascular surgeon, because the therapeutic options may improve a patient's outcome [Khoynezhad, 2004].

Programmed cell death (apoptosis) is a regulated mode of cell death in multicellular organisms [Hengartner, 1994]. Apoptosis of cardiac muscle cells has been identified as an essential process in the progression to heart failure [Empel, 2005]. Therefore, apoptosis could show heart failure before clinical symptoms have appeared.

Protein gene product 9.5 (PGP 9.5) is a specific protein used to visualise neuropeptide-containing innervations [Pilmane, 2011].

The heart secretes two natriuretic peptides, atrial (ANUP) and B-type (BNP). ANUP is secreted from atrial granules into the circulation in response to acute or chronic atrial stretch to physiologically act as antihypertensive and antihypervolaemic factor [Kuhn, 2012]. B-type natriuretic peptide is a cardiac neurohormone specifically secreted from the ventricles in response to volume expansion and pressure overload [Maisel, 2002]. The physiologic actions of BNP are similar to those of ANP and include reducing both cardiac preload and afterload by their natriuretic, diuretic, and vasodilatory actions [Nakagawa, 1995].

Vascular endothelial growth factors are major molecules controlling vascular growth and function, vascular homeostasis, permeability, and vasodilatation. They have been shown to be important for neovascularisation of the chronically ischemic adult heart [Karu, 2013].

Chromogranin A (ChgA) is a protein that is stored and released together with neurotransmitters and hormones in the nervous, endocrine and diffuse neuroendocrine systems [Glattard, 2006]. Cardiac ChgA is stored in atrial granules with cardiac natriuretic peptides. ChgA measurement has gained interest in cardiovascular disease, because increased plasma concentrations are associated with risk of clinical deterioration and death with acute coronary syndromes or chronic heart failure [Goetze, 2013]. ChgA levels have been found to reflect sympathetic activity, indicating that circulating ChgA levels may represent overall neuroendocrine activity. Increased activity in the sympathetic nervous system is a recognised risk factor for poor outcome in heart failure [Røsjø, 2010].

Endothelin is primarily released by endothelial cells, but it can also be synthesised and released by a variety of cell types, such as cardiac myocytes. Endothelins play an important role in cardiac and vascular pathology associated with heart failure [Zolk, 2000]. Endothelin is a potent vasoconstrictor and has inotropic, chemotactic and mitogenic properties. The overall action of endothelin is to increase blood pressure and vascular tone [Agapitov, 2002].

The aim of this study was to identify appearance and distribution of apoptosis, homeostasis regulating factors, innervations and ischemia markers in right atrial tissue in different acquired heart diseases.

Material and methods

In Pauls Stradiņš Clinical University Hospital's Heart Surgery Centre during elective open heart surgery right atrial tissue fragments (~ 2 mm^2) from 6 patients were taken. The material was obtained from the venous cannulae insertion site before cardioplegic solution was given. Tissue fixation was carried out immediately in the operating room and for this purpose already pre-prepared eppendorf

tubes with saturated picric acid solution (2% formaldehyde and 0.2% picric acid in 0.1 M phosphate buffer (pH 7.2)) were used. Tissue fragments were transported to morphology laboratory, Institute of Anatomy and Anthropology at Rīga Stradiņš University. For 12 hours, tissue fragments were washed in 10% sucrose phosphate buffer, embedded in paraffin and cut into 8 μm thick slices.

All tissue specimens were obtained in accordance with the ethical requirements of Rīga Stradiņš University.

Review pictures. Tissue for routine light-microscopical examination was stained with haematoxylin and eosin. To assess the cellular structure, all specimens were observed using Leica VM 6000B microscope.

Apoptosis. The cellular apoptosis was observed by terminal deoxynucleotidyl transferase-mediated nick-end labelling method (TUNEL). All TUNEL positive cardiomyocytes in 3 randomly chosen non-overlapping fields of each specimen were counted. Also apoptotic index (the number of apoptotic cardiomyocytes as a percentage of all cardiomyocytes in one visual field) was determined.

Protein-gene peptide 9.5 (PGP 9.5). The slides were prepared to detect neuron specific protein-protein-gene peptide 9.5 (439273A, working dilution 1 : 200, Invitrogen, USA).

Human atrial natriuretic peptide (hANUP). In order to assess the hormonal response, we determined hANUP (8515/6, working dilution 1 : 10, Dako, Denmark).

Vascular endothelial growth factor (VEGF). To identify chronic ischemia, we determined the presence of VEGF in blood vessels (SC7296, working dilution 1 : 50, Santa Crus Biotechnology, Inc., USA).

Chromogranin A. We also determined the neuroendocrine cell marker – chromogranin A (910216A, working dilution 1 : 100, Invitrogen, USA).

Endothelin. The slides were prepared also to detect endothelin releasing cells (ab2786, working dilution 1 : 250, Abcam, England).

Quantification of structures. For the quantification of structures, the semiquantitative counting method was used. The designations were as follows: 0 – negative reaction; 0 / + – occasionally positive structures in the view field; + – a few positive structures in the view field; + / ++ – few to moderate positive structures in the view field; ++ – moderate count of positive structures in the view field; ++ / +++ – moderate to numerous positive structures in the view field; +++ – numerous positive structures in the view field; ++++ – abundance of positive structures in the view field [Pilmane, 1998].

Patients. The mean age was (mean ± SD) 70.3 ± 9.1 years (range 58–83 years) and there were 4 female patients (Table 1). All patients were examined to the common procedure before heart surgery.

Table 1. Patient demographics

No.	Age, years	Sex
1	58	Female
2	62	Male
3	66	Male
4	73	Female
5	80	Female
6	83	Female

All patients had open-heart surgery (Table 2). 2 of them (No. 4, No. 6) had isolated aortic valve replacement because of aortic valve stenosis, 2 patients (No. 2 and No. 3) had coronary artery bypass grafting (CABG). One patient (No. 5) had concomitant CABG and mitral valve repair and one patient (No. 1) had reoperation – tricuspid valve replacement after mitral commissurotomy for rheumatic mitral valve stenosis in 1973 and mitral valve replacement, and tricuspid valve repair in 2000.

Table 2. Type of operation. Coronary artery bypass grafting (CABG)

No.	Type of operation
1	"REDO" tricuspid valve replacement
2	CABG
3	CABG
4	Aortic valve replacement
5	CABG + mitral valve repair
6	Aortic valve replacement

Echo and coronarography data are shown in Table 3 and Table 4. Almost all patients, except No. 5, had good left ventricular ejection fraction (LVEF) 55.2 ± 7.6%. Patient No. 5 had a slightly (40%) decreased ejection fraction. Two patients (No. 3 and No. 4) had moderate and severe pulmonary hypertension. All patients' right atrial size was normal.

All patients, except No. 6, had anamnesis of coronary heart disease. Two of them (No. 1 and No. 4) had previous percutaneous transluminal coronary angioplasty (PTCA) and had no symptoms of coronary heart disease at the time of operation.

Table 3. Preoperative echo data

No.	Echo						
	RAA, cm^2	LAVI, ml/m^2	RVSP, mmHg	LVEF, %	AR	MR	TR
1	–	–	55	55	–	–	4
2	17	35	22	58	–	1	1
3	15	32	30	58	–	–	–
4	13	33	30	65	2	1	1
5	17	46 × 67 mm	66	40	–	4	2
6	17	37 × 52 mm	–	55	1	1	1

RAA – right atrial area; LAVI – left atrial volume index, RVSP – right ventricular systolic pressure, LVEF – left ventricular ejection fraction, AR – aortic regurgitation, MR – mitral regurgitation, TR – tricuspid regurgitation.

Table 4. Preoperative coronarography data

No.	Coronarography									
	LM, %	LAD, %			LCX, %			RCA, %		
		prox	mid	dist	prox	mid	dist	prox	mid	dist
1	–	–	–	–	–	stent	–	–	–	–
2	–	–	90	90	–	90	95	–	75	90
3	–	95	95	–	–	–	–	–	100	–
4	–	stent	–	–	–	–	–	–	–	–
5	–	75	100	–	100	–	–	100	100	–
6	–	–	–	–	–	–	–	–	–	–

LM – left main coronary artery, LAD – left anterior descending artery, LCX – left circumflex artery, RCA – right coronary artery.

Statistical analysis. All statistical analysis was performed with IBM SPSS Statistics 22. Statistical significance was considered at the level of $p < 0.05$. Data are given as mean ± standard deviation (SD).

Results

Review pictures. In all specimens, myocardial degeneration with diffuse vacuolation of cardiomyocytes was detected especially in perinuclear region (Figure 1).

Cardiomyocytes and their nuclei in all specimens varied in size. In all patients, especially in Case No. 4, pyknotic nuclei were detected. In three patients (No. 2, No. 3, No. 6) significant vascular sclerosis was found (Figure 2), but in patient No. 5 endothelial cell proliferation was observed.

Figure 1. Vacuolar degeneration of cardiomyocytes (arrows, patient No. 4, H/Eo, × 400)

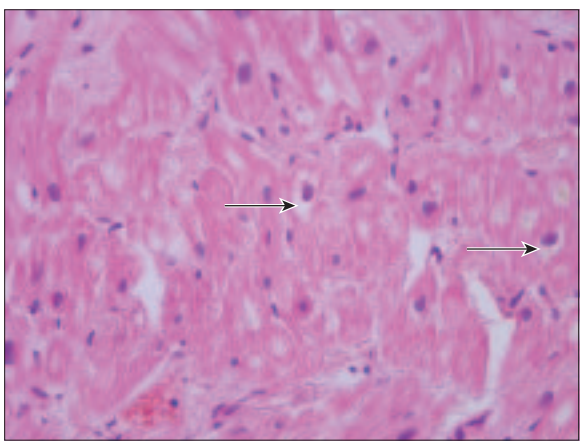
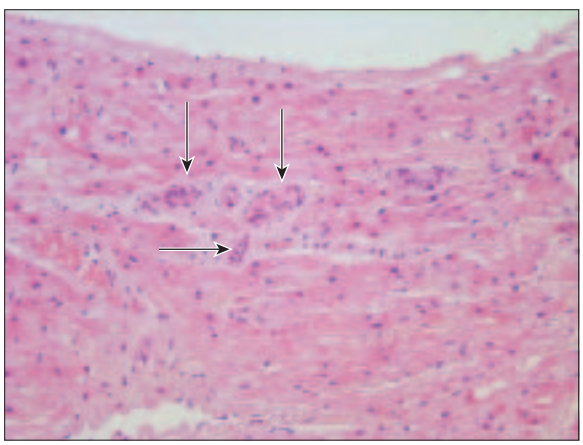


Figure 2. Almost completely sclerotised arterioles in myocardium (arrows, patient No. 3, H/Eo, × 200)



Apoptosis. The apoptotic index ranged from 24 to 80% (Figure 3). The smallest (24%) apoptotic index was determined in specimen No. 1.

Mean number of TUNEL positive cardiomyocytes (Table 5) was significantly different among all specimens (p = 0.000).

Figure 3. The apoptotic index of acquired heart disease affected right atrial tissue

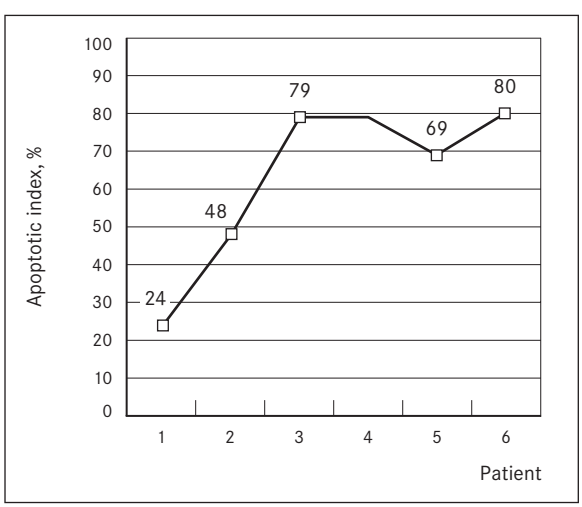


Table 5. The number of apoptotic cardiomyocytes

No.	The number of apoptotic cells, mean ± SD
1	48.67 ± 14.19
2	36.00 ± 15.00
3	121.67 ± 26.50
4	121.00 ± 15.87
5	141.33 ± 8.33
6	107.33 ± 14.74

Protein-gene peptide 9.5. All examined specimens showed mainly numerous PGP 9.5 containing innervations (Table 6). However, an increased number of PGP 9.5 immunoactive innervations was observed in patient No. 4 (Figure 4), but in patient No. 5 PGP 9.5 was in few to moderate nerve fibres.

Interestingly, PGP 9.5 was found also in endothelium and in endocardial cells with quite good expression (Figure 5). Additionally, in patient No. 1, in scar tissue (after previous heart surgery, mitral valve replacement and tricuspid valve repair through right atriotomy), we found PGP 9.5 positive nerves only around the blood vessels.

Human atrial natriuretic peptide. Numerous hANUP secreting cells were detected in all specimens, except patient No. 2, where this factor was detected in moderate cells (Table 6).

Figure 4. Protein-gene peptide 9.5 positive nerve fibres among connective tissue in myocardium (arrows, patient No. 4, IMH, × 200)

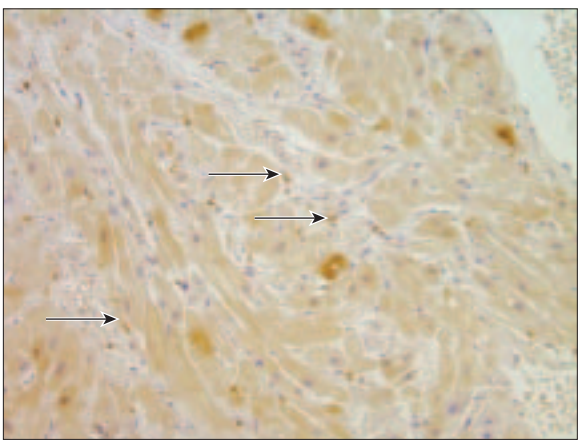


Figure 5. Rich PGP 9.5 expression in endocardial layer (arrow, patient No. 5, IMH, × 400)

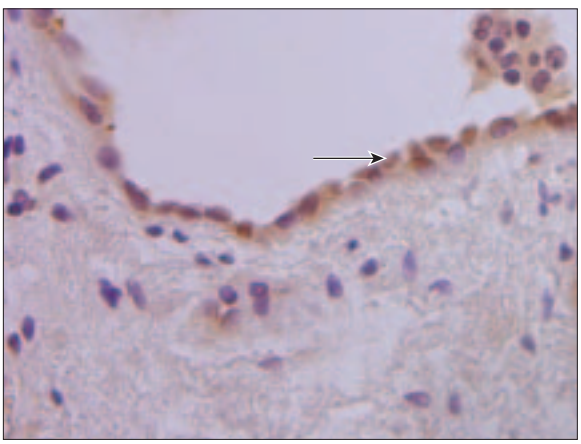


Table 6. Immunohistochemical analysis

No.	PGP 9.5	hANUP	VEGF		Chromogranin A	Endothelin
			Endothelium	Endocardium		
1	+ / ++	+++	+	0	++	+
2	+++ / ++++	+++	+	+++	+	+
3	+++	+++	+	+	0	0 / +
4	+++	++	+	++	++	++
5	+++	+++	+	+	++ / +++	++
6	+++	+++	+	0	0 / +	0 / +

PGP – protein-gene peptide 9.5. hANUP – human atrial natriuretic peptide. VEGF – vascular endothelial growth factor.

The designations were as follows: 0 – negative reaction; 0 / + – occasionally positive structures in the view field; + – a few positive structures in the view field; + / ++ – few to moderate positive structures in the view field; ++ – moderate count of positive structures in the view field; ++ / +++ – moderate to numerous positive structures in the view field; +++ – numerous positive structures in the view field; ++++ – abundance of positive structures in the view field.

Vascular endothelial growth factor. Few vascular endothelial growth factors were observed in endothelial cells in all specimens (Table 6). In patient No. 4 VEGF mostly was found in endocardium (Figure 6).

Chromogranin A. There were some blood vessels, which few endothelial cells contained chromogranin, in patient No. 5 (Table 6). In specimens No. 2 and No. 4 were no signs of chromogranin in blood vessels at all, but few positive cells were found in the endocardium. In patient No. 6 no chromogranin was observed. There were no chromogranin containing blood vessels in specimen No. 3., but factor positive cells particularly were pronounced in epicardium (Figure 7).

Endothelin. Only in 2 patients (No. 5 and No. 4) blood vessels contained endothelin positive endotheliocytes (Table 6). Mostly this factor was seen in epicardium (Figure 8).

Figure 6. Moderate nucleus of VEGF immunoreactive cells in endocardial layer (arrows, patient No. 4, IMH, × 200)

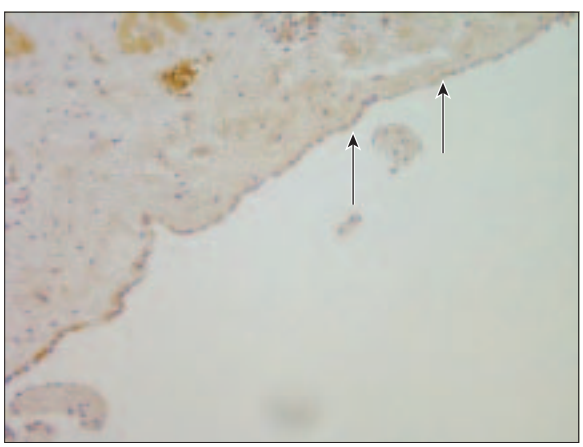


Figure 7. Chromogranin A containing epicardium (arrows, patient No. 3, IMH, × 400)

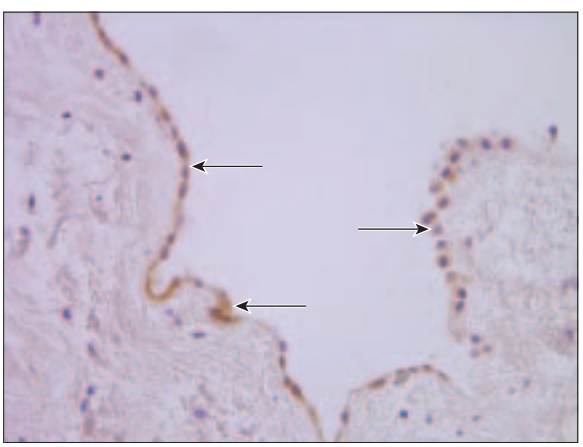
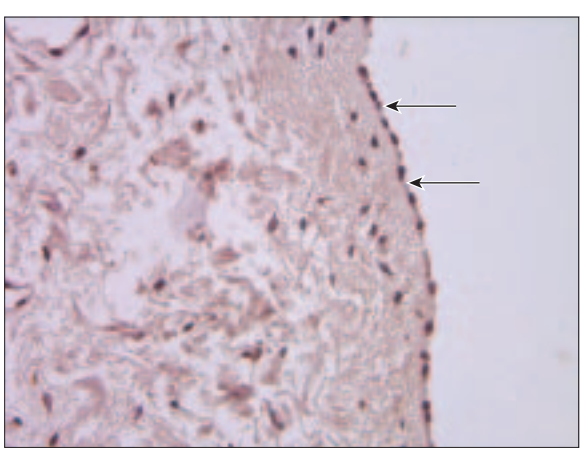


Figure 8. Endothelin in epicardium (arrows, patient No. 4, IMH, × 400)



Discussion

Review Pictures. In all patients, a moderate to severe myocyte degeneration and vacuolisation was observed, and it is a marker of heart failure [Ahmed, 2010]. Besides, preoperatively performed echo showed good or only slightly reduced left ventricular ejection fraction. It seems that these patients have a latent heart failure, without the true left ventricular ejection fraction decline yet.

Apoptosis. Cardiomyocyte apoptosis has been associated with the pathogenesis of heart failure [Empel, 2005]. There were statistically significant differences in apoptotic index and apoptotic cell count in right atrial tissue among all patients in our study, but no relationships between reduced left ventricular ejection fraction or clinical heart failure classes and apoptosis were observed. It is noteworthy that the lowest apoptotic index was found in youngest patients (age 58 and 62 years/apoptotic index 24% and 48%).

It is interesting that apoptotic index in our patients was very high (range from 24 to 80%) if we compare to other studies. So, Narula (1996) determined apoptotic index in 7 explanted hearts from patients undergoing heart transplantation at Massachusetts General Hospital and it ranged from 5 to 35.5% [Narula, 1996]. Thus, we speculate on the differences between these patient groups, predicting that probably our patients received surgical treatment later than American patients.

Protein-gene peptide 9.5. Protein-gene peptide 9.5 is a specific marker of nervous tissue [Chow, 2001]. Relatively good innervations in all specimens were observed with one exception – patient No. 5, proving probably "weak heart syndrome" in relation to all other patients. On the other hand, protein-gene peptide was particularly pronounced in patient No. 4. It was the patient, who underwent aortic valve replacement because of aortic valve stenosis due to calcific degeneration, suggesting that more pronounced remodeling of heart muscle compensatory connects to the increase of neuropeptide containing innervations.

Human atrial natriuretic peptide. Atrial myocardiocytes contain neuroendocrine granules and these granules secrete atrial natriuretic hormone when the atria are stretched excessively. Numerous hANUP secreting cells in all specimens were detected, except patient No. 2, where such cells were only slightly fewer. Atrial natriuretic peptide is involved in the maintenance of arterial blood pressure and intravascular volume homeostasis [Kuhn, 2012]; it concludes that there is active hormonal response to ensure homeostasis in all cases reviewed, except the patient No. 2.

Vascular endothelial growth factor. Vascular endothelial growth factor (VEGF) is an angiogenic protein that can stimulate collateral vessel development in the ischemic myocardium [Pearlman, 1995]. Karu et al. (2013) demonstrated that patients with stable coronary artery disease have significantly lower serum levels of vascular growth factors compared with data of healthy volunteers [Karu, 2013]. In this study, no relationship between coronary heart disease and expression of vascular endothelial growth factor in right atrial tissue has been observed. Additionally, the presence of only few VEGF containing cells in our patients indicates the lack of serious tissue ischemia. The increase of VEGF in some patient endocardial layer raises the question about the involvement of other heart structures except myocardial blood vessels in the tissue suffering from the heart disease and request further research.

Chromogranin A. Heart failure is a syndrome comprising cardiac dysfunction and neurohumoral activation [Goetze, 2014]. In 2007, Pieroni et al. for the first time demonstrated that human ventricular myocardium produces and releases chromogranin A [Pieroni, 2007]. This study allowed to determine chromogranin A in right atrial tissue and it was also found in endothelium (patient No. 5), endocardium (patient No. 2 and No. 4) and epicardium (patient No. 3). That means that probably these heart structures can change a phenotype and produce neuroendocrine prohormones important for the diseased tissue functions, from the one side, and, from the other side, stimulate remodeling of heart tissue [Pieroni, 2007].

Endothelin. Increased production and biological activity of the potent vasoconstrictor and pro-inflammatory peptide endothelin is a hallmark of endothelial dysfunction [Böhm, 2007], while the endothelial dysfunction is associated with accelerated progression of heart failure [Fischer, 2005]. In the retrospective study, most of all endothelin was found in patients No. 4 and No. 5 and one of them had the poorest left ventricular ejection fraction – 40% and the highest pulmonary hypertension (RVSP 66 mmHg). The role of endothelin end endothelial dysfunction in pulmonary hypertension development has been demonstrated in several studies [Galie, 2004].

Conclusions

Different expression of PGP 9.5, hANUP, VEGF, chromogranin A and endothelin in right atrial tissue might characterise pathogenesis of different acquired heart diseases.

Large variation of apoptotic index seems to correlate to a larger volume and longer untreated history of affected disease in our pilot study patients in Latvia.

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Work Ability and Stress Factors of Latvian Office Workers

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Abstract

Worldwide, the number of people who are employed in offices has increased. Abilities of an office work employee have changed over the years due to changes in the working environment, equipment, work culture and responsibilities. Good work ability increases wellbeing and supports employment. The aim of the study was to investigate the subjectively estimated work ability, stress-causing factors and to determine the training programme's (Metal Age®) impact on these stress factors and work ability of Latvian office workers.

The study was carried out using questions from General Nordic Questionnaire, Occupational Stress Questionnaire and Work Ability Index Questionnaire. Respondents were randomly divided into two groups: intervention group and control group. Both groups were interviewed twice – in March/April 2012 (first stage) and in May/June 2013 (second stage). The intervention group had a training course between the surveys using Metal Age® (ME®) method but the control group did not. Metal Age® is a structured method for developing wellbeing at work and through that improving profitability and productivity in organisations.

Latvian office workers displayed moderate and good work ability (WAI average score: 34.5–38.6). The best work ability was shown in the age group between 20 and 49 (WAI average score: 34.8–39.4); work ability decreased with age – WAI average score up to 33.6. WAI is affected by marital status and work experience, but it is not dependant on the type of work (mental or mixed mental and physical). Employees' stress-causing factors included bad relationship with their workmates; competitive and strenuous atmosphere at workplace; psychological violence or bullying at workplace; the workplace not always being supportive and open to new ideas; suspending carrying out ongoing task because of an urgent matter; insufficient amount of discussion at workplace concerning the aims and tasks related to one's work; possibility to accidentally ruin some valuable equipment or work result. Approximately half of the respondents considered their work being psychologically strenuous, and employees experienced stress at their workplace; the majority of employees could not relax after work. The closest relationship was observed between WAI and "get into situations that invoke negative feelings" ($r = 0.26$) and "carrying out ongoing tasks because of other interventions or more urgent matters" ($r = -0.24$).

After ME® training, some of the stress-causing factors improved: the possibility to influence the situation at a workplace; work became more autonomous; more attention was paid to the relationships with workmates at workplace; there was less hurry to finish one's task, or suspend carrying out an ongoing task; the number of employees who consider their job being psychologically strenuous decreased.

Keywords: office workers, work ability, stress at work.

Introduction

It is proved that work-related stress affects workers' satisfaction with their work and productivity, their mental and physical health, absenteeism, the impact on family function and the potential for employer liability. Scientists in their research are focused on the issue how to prevent work related stress. Tennant considers that "work and family are two domains from which most adults derive satisfaction in life; equally it is a common source of stressful experiences" [1].

The forms and conditions of work have changed during the past few decades. Physical work has shifted more towards mental work that increasingly involves working in offices. The changes in the functional capacity knowledge and attitudes of workers are reflected in the content of work ability. For work ability improving and developing is necessary knowledge on the dimensions of work ability in modern society [2].

Worldwide, millions of office workers work with computers. Reports of adverse health effects due to computer usage have received considerable media attention. The literature review summarises the evidence for the relationship between the duration of work time spent using a computer and the incidence of hand-arm and neck-shoulder symptoms and disorders as well as reduction in work ability [3]. Population's work ability has changed over the years due to changes in working life, public health, structure of the population, culture and societal norms. Comprehensive and up-to-date knowledge on different dimensions of work ability is essential for the promotion of longer careers, employment growth and wellbeing of the population of working age. Good work ability increases wellbeing and supports employment. One of the problems in current industrialised world is early retirement. Despite increased life expectancy and improved health in communities, in recent decades in many European countries the period of people's active work life has decreased [4]. Improvement of work ability is one of the most effective ways to enhance the ability and prevent disability and early retirement [5, 6]. Work ability is defined as the ability of a worker to perform their job, taking into account how demanding the work is, its physical and mental conditions [7].

In order to increase work participation and prolong the working life among workers, the concept of work ability was developed in the early 1980s in Finland, and was later adopted in different European and Asian countries [8–11]. The Work Ability Index (WAI) is by far the most used, and well-accepted instrument to measure work ability [12]. WAI has demonstrated the possibility of wide application by its availability in 24 languages and for different professions [12–24].

Work ability is built on the balance between a person's resources and work demands. The bases for work ability are health and functional capacity, at the same time, work ability is also determined by professional knowledge and competence (skills), values, attitudes, and motivation, and work itself [25]. Improvement of work ability is one of the most effective ways to enhance the ability and prevent disability and early retirement. Few studies have addressed determinants of work ability and stress factors at work. Sjögren-Rönkä showed that low stress at work and better self-confidence were directly related to higher work ability [26]. Job experience and satisfaction also associated with good work ability among office workers [27]. The improvement of work ability is closely linked with an improvement in the quality of job and life. The quality of life can be defined as a picture of a particular fragment of one's life (e.g., professional life) in comparison to an ideal model including less stressful work environment. The quality of life and work ability in various chronic diseases has been the subject of many scientific publications [28, 29]. However, knowledge of the determinants of work ability and stress factors are not sufficiently widely studied.

Aim

The aim of this study was to investigate the subjectively estimated work ability, the stress-causing factors and to determine the training programme's (Metal Age®) impact on these stress factors and work ability of Latvian office workers.

Material and methods

This study was carried out in the frame of Interreg 4A project "Workability and Social Inclusion". The Work Ability and Social Inclusion (WASI) project is mainly based on Metal Age® (ME®) method, which was developed to increase wellbeing at work, including measuring the effects of intervention on organisational leadership and stress management. Metal Age® is a structured method for developing wellbeing at work and through that improving profitability and productivity in organisations. The Metal Age® method brings to workplaces specific, tailored and practical actions for developing the wellbeing among the personnel. An important part of the Metal Age® method is ensuring the continuity of the development process through follow-up sessions [30].

Altogether 636 respondents from 13 companies from Latvia were invited to take part in the survey. Respondents were randomly divided into two groups: intervention group and control group. Both groups were interviewed twice – in March/April 2012 (first stage) and in May/June 2013 (second stage). The intervention group had a training course between the surveys using Metal Age® (ME®) method, but the control group had not.

According to Statistical classification of economic activities in the European Community (NACE Rev. 2.), two of the companies work in the field of financing and insurance, two in manufacturing of food products, three – in public administration and defence sector, two – in communications (radio broadcasting and telecommunications), two – in transport industry and one in education sector. All job tasks of the respondents involved only or mostly office work with ICT and customers. 424 respondents' answers were used for this study (response rate 66.7%). 33.3% had a lot of missing data, and they were excluded from the study. The study sample included the selection of participants who responded to all the survey questions: 212 answers of all participants – the intervention group and 212 – the control group. The participation in the study was voluntary.

The results obtained in this study were analysed within each group comparing the results in two survey periods.

Approval was obtained from the Ethics committee.

Questionnaire. The questionnaire has been an important tool in research on psychological and social factors at work. Furthermore, the questionnaire is a common tool in organisational development at worksite level.

For the study, respondents' answers from the 3 questionnaires were used:

1. General Nordic Questionnaire (QPSNordic) – 8 questions – "Leadership".
2. Occupational Stress Questionnaire (OSQ) – 33 questions and
3. Work Ability Index Questionnaire (WAI) – 23 questions [11, 31, 32].

The Questionnaire was modified and adapted for office workers. It contains general information – demographic data (age, gender, marital status, education, work experience, type of work) and 41 questions about stressors in the following groups: modifying factors/resources at work, leadership, supervision, social relations and esteem, workplace atmosphere, work demands, responsibility (hazards) and environment, stress and wellbeing, need for support and interventions in job. Respondents were offered the following answers: "always", "quite often", "often", "time to time", "rather seldom", "never". In Occupational Stress Questionnaire, frequencies analysis answers were grouped in two groups: "1 – not stressful" and "2 – stressful".

The WAI is a self-administered questionnaire used in clinical occupational health and research to assess work ability during health examinations and workplace surveys. The WAI can be used for individual employees and groups of workers.

The WAI is an assessment of the ability of a worker to perform his/her job, taking into account the specific psychosocial and physical work related factors, mental and physical capabilities, and health. The index consists of a questionnaire on physical and mental demands of an individual in relation to their work, diagnosed diseases and limitations in work due to disease, sick leave, work ability prognosis, and psychological resources [12].

The work ability index (WAI) consists of the following seven items and range from 7 to 49 points:

1. Current work ability compared with the lifetime best comprises the work ability score that is often used as a separate indicator of work ability and has been described above (0–10 points).
2. Work ability in relation to the demands of the job (2–10 points).
3. Number of current diseases diagnosed by a physician (1–7 points).
4. Estimated work impairment due to diseases (1–6 points).
5. Sick leave during the past year (1–5 points).
6. Own prognosis of work ability two years from now (1, 4 or 7 points).
7. Mental resources (1–4 points).

The work ability index is calculated by summing the points of the seven items (possible score ranging between 7 and 49 points). The index can be divided into four classes represented in the table below.

Table 1. Work ability index distribution

Points	Work ability	Objective of measures
7–27 points	Poor	Restore work ability
28–36 points	Moderate	Improve work ability
37–43 points	Good	Support work ability
44–49 points	Excellent	Maintain work ability

For easier data editing, subjects at or below 36 points were classified as having low work ability and they need to improve, subjects at or above 37 points classified as having satisfying work ability.

Results were analysed using IBM SPSS-20 statistical package. P values under 0.05 were considered significant; however, p value under 0.1 was taken into account as close to statistical credibility. Descriptive statistics were used to describe the characteristics of the study groups, included the analysis of age, gender, marital status, education, work experience and work type. Spearman correlation analysis was performed for WAI and stress questions. Wilcoxon or related t-test was used to compare related samples depending on data distribution. Pearson's Chi square test for the comparison of independent categorical variables or McNemar test for related samples was used.

Results

Characteristics of the study groups (control and intervention) are shown in Table 2.

Both study groups are similar in age, gender, education and type of work. In the intervention group 68.9% subjects who responded were women and in the control group – 69.3%. The smallest number of people was in the age group > 60 years, 11 and 13 individuals as well as groups who live in divorced or widowed circumstances.

Most of participants had higher education, the intervention group – 83.5% and the control group – 82.1%. Individuals who participated in the study had a relatively small length of service – up to 9 years (~ 67%), of 10–19 years (~ 26%). 88.7% of the intervention group respondents and 89.6% control group respondents noted that they performed mental work, ~ 10% in both groups performed a mixed-type work and only 1 person performed physical work.

Work ability index (WAI). Both groups (control and intervention) were interviewed in two periods. For the intervention group, the second period was after ME® training. Results of average WAI in the control and intervention group were compared in each group. The average work ability in first survey period for both groups was moderate (respectively, m = 35.3; SD = 3.2 and m = 34.5; SD = 4.8). After the second survey period, the average WAI was changed to statistically significant for the control group (m = 44.8; SD = 1.0) and for the intervention group (m = 37.0; SD = 5.6) ($p < 0.001$).

Distribution in work ability index for all workers is represented in Table 3. WAI results in the control group in the first period varied between 25 and 42 (excellent – 0% from all respondents, good – 33.0%, moderate – 64.6%, poor – 2.4%); in the intervention group it ranged between 19 and 48 (excellent – 1.9% from all respondents, good – 26.4%, moderate – 63.2%, poor – 8.5%).

Distribution of respondents in WAI groups (excellent, good, moderate, poor) changed in the second survey period. WAI results in the control group in the second period varied between 24 and 47 (excellent – 18.4% from all respondents, good – 47.2%, moderate – 31.2%, poor – 3.3%); in the intervention group it ranged between 19 and 49 (excellent – 9.4% of respondents, good – 49.1%, moderate – 33.5%, poor – 8.0%).

Table 4 shows work ability index depending on age, marital status, education and work experience and type of work in control and intervention groups (both periods). There were no statistically significant changes in the work ability to the mentioned indicators in the control group in the first and second stage. The exceptions are the respondents with secondary and special education, which increased WAI during the second stage.

Working ability of the respondents of the intervention group had a statistically significant increase, depending on education, marital status and work experience (< 9 years) after ME® training period.

Table 2. Characteristics of the studied groups

Parameters	Control group		Intervention group	
Total	n = 212	100%	n = 212	100%
Sex:				
women	147	69.3	146	68.9
men	65	30.7	66	31.1
Age group:				
< 29	72	34.0	79	37.3
30–39	68	33.1	62	29.3
40–49	25	11.8	27	12.7
50–59	34	16.0	33	15.6
> 60	13	6.1	11	5.2
Marital status:				
married or cohabiting	142	67.0	141	66.5
single	43	20.3	39	18.4
separated, divorced, widowed	27	12.7	32	15.1
Education:				
secondary and special education	38	17.9	35	16.5
higher education	174	82.1	177	83.5
Work experience, years:				
< 9	142	67.0	141	66.5
10–19	55	25.9	54	25.5
20–29	8	3.8	11	5.2
30–39	2	0.9	3	1.4
40 and > 50	5	2.4	3	1.4
Type of work:				
mental work	190	89.6	188	88.7
physical work	1	0.5	1	0.5
mixed work	21	9.9	23	10.9

Table 3. WAI scores distribution in the control and intervention group

	WAI categories	First period					Second period				
		WAI (± SD)	min	max	n	%	WAI (± SD)	min	max	n	%
Control group	Poor	26.0 (1.4)	25.0	27.0	5	2.4	24.7 (1.2)	24.0	26.0	7	3.2
	Moderate	33.9 (2.0)	28.0	36.0	137	64.6	33.7 (2.1)	29.0	36.0	66	31.2
	Good	38.5 (1.6)	37.0	42.0	70	33.0	39.6 (2.0)	37.0	43.0	100	47.2
	Excellent	–	–	–	–	–	44.8 (1.0)	44.0	47.0	39	18.4
Intervention group	Poor	23.3 (2.5)	19.0	26.0	18	8.5	24.5 (3.4)	19.0	27.0	17	8.0
	Moderate	33.7 (2.3)	28.0	36.0	134	63.2	33.8 (1.9)	29.0	36.0	71	33.5
	Good	38.8 (2.0)	37.0	43.0	56	26.4	39.6 (2.0)	37.0	43.0	104	49.1
	Excellent	46.0 (2.8)	44.0	48.0	4	1.9	46.0 (1.9)	44.0	49.0	20	9.4

Table 4. WAI of the control and intervention group

Parameters	Control group, first period, WAI (± SD)	Control group, second period, WAI (± SD)	p	Intervention group, first period, WAI (± SD)	Intervention group, second period, WAI (± SD)	p
Age group:						
< 29	35.2 (4.8)	36.1 (5.8)	NS	33.8 (4.8)	38.8 (5.0)	p < 0.01
30–39	36.0 (4.4)	37.0 (6.1)	NS	35.6 (3.8)	37.3 (4.4)	NS
40–49	34.8 (3.6)	39.4 (4.2)	NS	35.2 (5.4)	38.2 (4.7)	NS
50–59	33.9 (3.0)	38.7 (4.4)	NS	33.6 (4.2)	36.1 (4.4)	NS
> 60	37.0 (1.4)	36.5 (6.5)	NS	34.0 (2.2)	37.7 (6.5)	NS
Marital status:						
unmarried	35.6 (4.0)	37.1 (6.1)	NS	32.6 (5.1)	37.6 (3.6)	p < 0.001
married	35.0 (4.2)	37.2 (5.3)	NS	35.0 (4.6)	37.0 (5.6)	p < 0.01
unmarried but co-habiting	35.6 (3.0)	38.0 (5.1)	NS	35.8 (3.4)	40.1 (5.0)	p < 0.001
separated	34.2 (4.1)	33.2 (9.0)	NS	–	–	
divorced	35.5 (2.7)	38.9 (5.6)	NS	32.3 (4.3)	36.6 (2.6)	p < 0.01
widow/widower	34.5 (0.7)	34.5 (6.4)	NS	34.0 (1.0)	40.3 (3.2)	p < 0.04
Education:						
secondary and special education	35.1 (4.5)	39.3 (4.5)	p < 0.03	34.8 (4.6)	38.6 (4.6)	p < 0.05
higher education	35.3 (3.3)	36.8 (4.8)	NS	34.5 (4.4)	37.1 (5.1)	p < 0.001
Work experience, years:						
< 9	35.4 (3.6)	37.0 (5.8)	NS	33.6 (4.7)	38.2 (5.2)	p < 0.001
10–19	34.9 (4.6)	38.0 (4.8)	NS	37.0 (3.3)	37.5 (5.4)	NS
20–29	35.6 (2.3)	39.5 (4.8)	NS	35.0 (1.8)	37.6 (4.8)	NS
30–39	35.5 (3.5)	31.0 (3.8)	NS	35.2 (5.0)	38.4 (4.6)	NS
40 and > 50	–	–		37.5 (2.8)	34.5 (2.1)	NS
Type of work:						
mental work	35.2 (3.6)	37.3 (5.3)	NS	34.4 (4.6)	37.9 (5.1)	NS
physical work	–	–	–	–	–	–
mixed work	35.5 (4.9)	37.0 (8.1)	NS	36.0 (2.8)	38.3 (4.1)	NS

NS – not statistically significant.

Stress factors. Analysis of QPS_{Nordic}, eight questions regarding leadership's attitude towards an employee as a cause of stress, showed that in between 80.6% and 93.5% of control group cases superiors encourage to participate in important decisions, help to develop employees' skills, tackle problems as soon as they surface, distribute work and treat workers fairly and impartially. However, superiors do not encourage participating in important decisions for 25.8% of employees and the relationship between superiors and employees may be the cause of stress for 29.3% of respondents. A similar situation was also observed in the second period. Also in the intervention group, leadership's attitude to employees does not cause stress both in the first and in the second period (before and after the ME® training). More than 85% (85.9%–97.0%) of employees believe that their leadership helps to build good working relationships. Before the training, 23% of employee's answers showed that the leadership of immediate superior did not encourage their participation in important decisions, while after the training the number increased to 25%. If before the training relationships with the leadership was the cause of stress for 22% of the employees, then after the training, it was the cause of stress for 26.8% of employees.

Analysis of the control group's OSQ 33 stress characterising questions in the first period revealed that there are several factors (13) which may be the reason for stress at workplace (the score higher than 10%); 16.1% of employees consider their relationship with colleagues as being rather negative; not highly positive evaluation of the family regarding the job – 11%; the atmosphere at workplace is competitive and stressful – 29%; the workplace is not always encouraging and open to new ideas – 29%; sometimes there is psychological violence or bullying at workplace – 30%; there are difficult tasks to perform – 15.1%; there is a hurry to finish one's task – 41.3%; they must suspend carrying out an ongoing task – 41.9%; insufficient amount of discussion at a workplace in relation to aims and objectives of work tasks –16.1%; they can accidentally ruin some valuable equipment or work result – 23.3%; more than a half of employees have a mentally strenuous job – 60.2%; 41.9% experience stress at workplace, and the majority – 76.3% cannot relax after work.

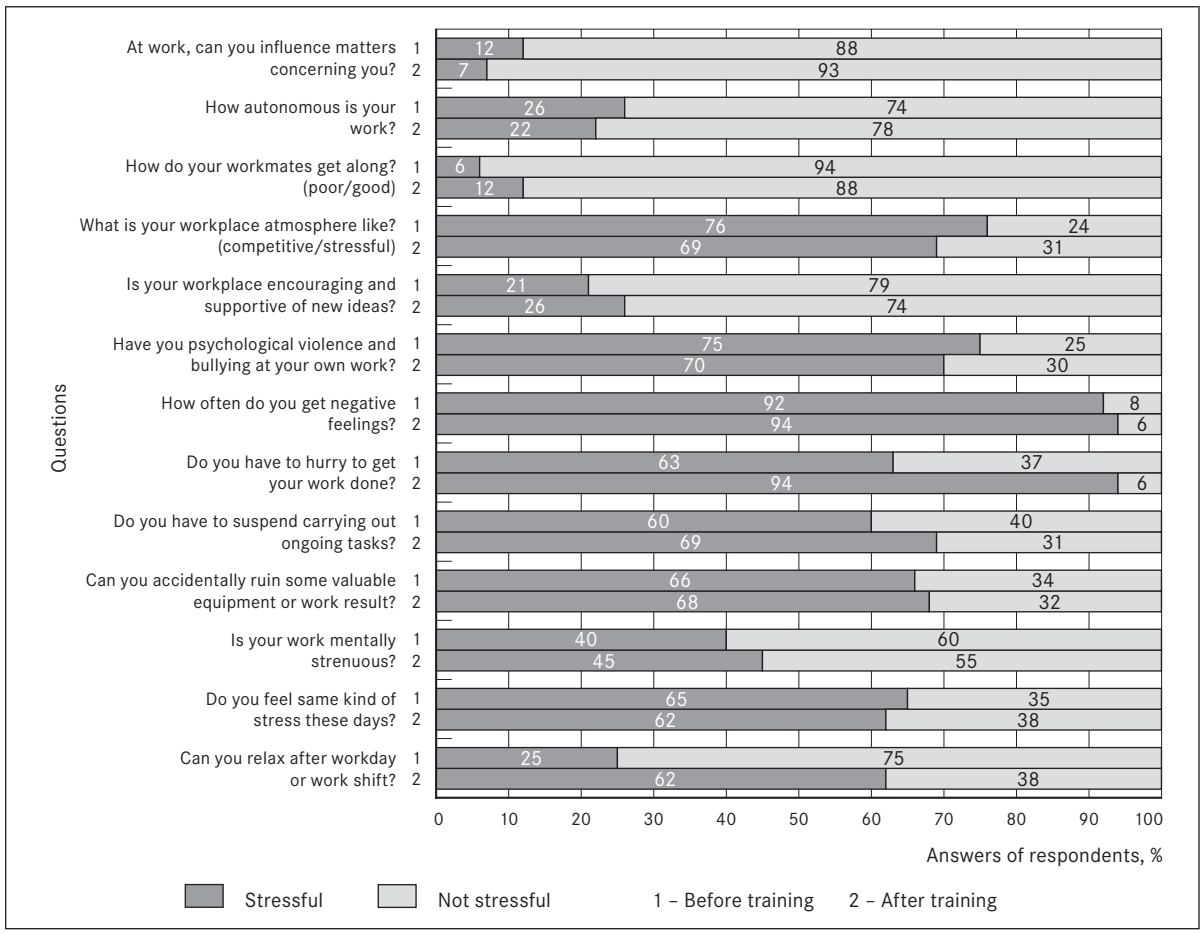
During the second period, the answers to questions regarding stress-causing factors improved in three cases: in relationships with colleagues (6.6%) and family (6.5%), and in question about strenuous workplace atmosphere (22.8%). However, some of the indicators declined: there is less possibility to influence the situation at workplace – 16.1%; decreased employee's autonomy – 25.8%; there are situations at workplace which cause indignation, fear, shame – 12.9%; there is insufficient amount of discussion regarding work tasks – 22.6%; there is a possibility to ruin some valuable equipment or work result – 32.2%.

Comparison of answers regarding the stress-causing factors in first and second period in the control group shows that there is a statistically significant difference ($p < 0.05$) between answers to 17 questions.

Analysis of OSQ 33 stress questions in the intervention group in the first period (Figure 1) yielded similar to the control group results: 12 factors which may cause stress at workplace may include: there is no possibility to influence the situation at workplace – 12.1%; work is not autonomous – 26%; strenuous atmosphere at workplace – 24% and workplace is not encouraging and supportive of new ideas – 21%; there is psychological violence and bullying at workplace – 25%; there are difficult stages of work tasks – 14%; there is a need to hurry and carry out one's tasks – 37% and carrying out the ongoing task must be suspended – 40%; there is a possibility to accidentally ruin some valuable equipment or work result – 34%; more than half of respondents have mentally strenuous work – 60%; 35% experience stress at workplace, and the majority – 75% cannot relax after work.

During the second period, answers to seven questions regarding stress-causing factors improved: possibility to influence situation at workplace increased by 5.1%; work autonomy increased by 78.2%; more attention is paid to relationships with workmates – 11.9%; only 6% of employees consider that they could have better relationships with their workplace, and there is need to hurry in their job; and the ongoing task must be suspended – 30.7%; there is possibility to accidentally ruin some valuable equipment or work result – 31.6%; there was also a decrease in the number of employees whose job is mentally strenuous – 55.4%. While the atmosphere at a workplace became slightly more strenuous/competitive – 30.7%, the workplace is not so open and supportive of new ideas – 74.3%; psychological violence increased – 29.7% and employees experience stress – 38%, and cannot relax after work – 62%. The comparison of the answers to the questions regarding stress-causing factors in the intervention group in first and second period (before and after the ME® training) was found statistically significant ($p < 0.05$) in 14 questions.

Figure 1. Answers of respondents (%) on stress issues according to OSQ in the intervention group (answers were grouped in two sections: "not stressful" and "stressful")



For correlation analysis between the WAI and stressful factors, determination of Spearman's correlation coefficient was used. A statistically significant correlation was not found between influences of superiors upon important decision-making, assistance in the development of skills, work organisation, etc. (QPS_{Nordic} questionnaire) and work ability in both control and intervention groups in neither first nor second period.

The analysis of correlation between work ability and stress factors in the control group, according to OSQ, showed that there is a statistically significant correlation only in two cases in the first period, there was no correlation found after the second period (Table 5).

In the intervention group, statistically significant correlation was found between work ability and stress-causing factors, although it is weak (Table 6).

No correlation in the control and intervention group between work ability and relationships with immediate superior (8 questions) was found: does your immediate superior encourage to participate in important decisions; to speak up, when you have different opinions; help develop your skills; distribute work fairly and impartially; treat workers fairly and equally; is the realationship between you and your immediate superior a source of stress to you.

No correlation was found between work ability and OSQ stress questions about modifying factors/ resources at work (4 questions); social relations and esteem (5 questions); workplace atmosphere (2 questions); leadership and supervision (2 questions); perceived environment (7 questions); responsibility (hazards) and environment (2 questions); work strain, stress and wellbeing (5 questions); need for support and interventions in your job (1 question).

Table 5. Correlation between work ability and stress factors in first period in the control group

Questions*	Spearman correlation coefficient	p
Do workmates provide help and support when needed	0.22	0.03
How often do you, at your work, get into situations that invoke negative feelings such as indignation, hate, fear or shame in your mind?	0.26	0.10

* Only statistically significant values are shown in the table.

Table 6. Correlation between work ability and stress factors before and after ME® training in the intervention group

Questions*	Spearman correlation coefficient		p
	First period	Second period	
At work, can you influence matters concerning you?	–	– 0.21	0.04
How often do you, at your work, get into situations that invoke negative feelings such as indignation, hate, fear or shame in your mind?	–0.18	–	0.08
Do you have to suspend carrying out ongoing tasks because of other intervening or more urgent matters?	–0.24	–	0.02
Are you enthusiastic about your job?	0.21	–	0.04
What is your state of health compared with that of other people of your age?	–	0.27	0.01
How satisfied are you with your present job?	–	– 0.23	0.03

* Only statistically significant values are shown in the table.

Discussion

The concept of work ability relates to the capacity a worker has to perform his work tasks, given his work demands, health status, and physical and mental abilities and may be considered as a measure of functional aging [33]. Work, ability which is regarded as a dynamic process of human resources in relation to work, is influenced by a number of factors, which include socio-demographic characteristics, lifestyle, the aging process, and work demands [27]. Work ability varies in different factors of the population.

In the present study, 424 employees' answers about work abilities in 13 office companies were assessed; aged 19–74; ~ one-third were females. 66.8% of the respondents were co-habiting or married. 82.8% of the respondents had higher education. The majority of the employees (66.7%) were with little work experience – up to 9 years. Due to the nature of their jobs, 89% were engaged in mental work. The respondents were divided into two groups: the control group and the intervention group. Both groups were interviewed twice. Intervention group had a training course using Metal Age® method between surveys but the control group did not.

WAI was assessed during the first and second period.

According to WAI analysis, majority of our study population showed moderate or good work ability – in the control group average WAI = 35.3; in intervention group WAI = 34.5. The obtained WAI divided by class is as follows in control group: excellent – 0% of all respondents, good – 33.0%, moderate – 64.6%, poor – 2.4%; in intervention group: excellent – 1.9% of all respondents, good – 26.4%, moderate – 63.2%, poor – 8.5%. For those whose work ability index is moderate (score 28–36) improvement of work ability is recommended. Workers with good work ability index (score 37–43) should receive instructions on how to maintain their work ability. Those whose work ability is excellent (44–49) should also be informed which work and life style factors maintain work ability and which factors weaken it [4].

During the second period, WAI was determined after holiday period and average WAI increased in both groups – in the control group WAI = 38.6 (compared to WAI = 35.3 in the first period), in the intervention group WAI = 37.0 (compared to WAI = 34.5 in the first period). The WAI score difference in each group between two survey periods was still statistically significant ($p < 0.001$).

Distribution of respondents in WAI groups (poor, moderate, good, excellent) changed in the second survey period: in the control group varied from 24 to 47 (excellent – 18.4% of all respondents, good – 47.2%, moderate – 31.2%, poor – 3.2%); in the intervention group it ranged between 19 and 49 (excellent – 9.4% of all respondents, good – 49.1%, moderate – 33.5%, poor – 8.0%).

Changes could be associated with a decrease in the number of persons with moderate work ability and increase in the number of employees with good and excellent work ability. After the training, WAI increased in the age group up to 29 years of age, as well as depending on the marital status and education in the intervention group. The control group did not display such changes. The studies carried out in other countries show that office workers display excellent and good working abilities [3, 27]. According to Finnish researchers' data, mostly people of working age evaluate their work ability as good [10].

Results of the present study indicated that WAI score was moderate and good (WAI ~ 34–39) for employees with secondary and special education or higher education that is similar to research on nurses by Golubic et al. that suggests that respondents with higher educational levels have better work ability than their colleagues with lower educational level [34]. Young and well-educated people perceive their work ability to be better than those who are older or have less education. Moreover, widows and single or divorced men report more problems concerning work ability than those who are married, white-collar job workers report better work ability than blue-collar job employees. Good work ability is evident only among those with higher education, physically light work and good health [2, 8, 9, 10].

The present study identified the major groups of occupational stressors in the control and intervention groups. In the control group there was identified "perceived environment" – six stressors, "modifying factors/resources at work" two stressors, "social relations and esteem" – two stressors, "workplace atmosphere" – two stressors, "responsibility (hazards) and environment" – two stressors, "stress and wellbeing" – two stressors, "leadership and supervision" – 0 stressors. In the intervention group, slightly different results were obtained: "perceived environment" – three stressors, "modifying factors/resources at work" – two stressors, "workplace atmosphere" – two stressors, "responsibility (hazards) and environment" – two stressors, "stress and wellbeing" – two stressors, "social relations and esteem" – one stressor "leadership and supervision" – 0 stressors.

After ME® training, possibility to influence the situations at a workplace increased by 5.1% in the intervention group; work autonomy increased by 4.2%; increase in paying attention to relationship with workmates was 50%. There was an increase in the number of respondents who consider that the relationships at workplace should be improved; there is no need to hurry to finish their job and suspend carrying out ongoing tasks; decreased chance accidentally ruin some valuable equipment or the work result; there was also a decrease in the number of employees who considered their work as mentally strenuous and stressful.

Poor communication with colleagues was one of the common stressors at work. Good communication can protect from harmful effects of other stressors and can contribute to better safety at work. The concept of work ability presumes a modern concept of human ability for work conditions to worker's abilities and capabilities. A worker's psychophysical abilities change with time [35].

Some studies show that stress at work can reduce safety of workers, and work ability becomes lower with age and working time [36]. The present study showed a statistically significant Spearman correlation in the intervention group between different stress-causing factors, e.g. "how often do you, at your work, get into situations that cause negative feeling"; "do you have to suspend carrying out ongoing tasks because of other intervention or more urgent matters" and work abilities prior to the training. However, this correlation was weak. Also after the training, there was a weak correlation between stress factors and work abilities: "at work, can you influence matters concerning you"; "state of health compared with that of other people"; "satisfaction with present job".

Conclusions

1. The Work Ability Index is a simple, cheap and effective method, which helps to assess the overall situation in an organisation, employees' work abilities and to become aware of the methods how to improve the situation.
2. Office workers display moderate and good work ability.
3. The best work ability is shown in the age group between 20 and 49; work ability decreases with age.
4. After ME® training, work ability improved in the age group up to 29 years, in the group with the working time up to nine years, as well as regardless of the family status and education.
5. Employees' stress-causing factors include bad relationship with their workmates; competitive and strenuous atmosphere at workplace; psychological violence or bullying at workplace; a workplace not always being supportive and open to new ideas; suspending carrying out ongoing task because of an urgent matter; insufficient amount of discussion at a workplace concerning the aims and tasks related to one's work; possibility to accidentally ruin some valuable equipment or work result. Approximately half of respondents consider their work being psychologically strenuous, and employees experience stress at their workplace; the majority of employees cannot relax after work.
6. After ME® training, some of stress-causing factors improved: possibility to influence the situation at workplace; work became more autonomous, more attention is paid to relationships with workmates at a workplace, there is less hurry to finish one's task, or suspend carrying out an ongoing task; decreased the number of employees who consider their job being psychologically strenuous, although the workplace atmosphere became slightly competitive, the workplace still was not supportive of new ideas and employees cannot relax after a working day.
7. There was found a weak correlation between work ability and stress-causing factors which characterise the situation in work, perceived environment, job satisfaction and health status. No correlation between work ability and relationships with immediate superior was detected.

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Mandibular Condyle Characteristics in Juvenile Idiopathic Arthritis Patients Compared with Control Group by Cone Beam Computed Tomography Assessment

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Abstract

Juvenile idiopathic arthritis (JIA) patients have a high risk of mandibular condyles being affected. Cone beam computed tomography (CBCT) has the advantage of providing 3D images allowing an accurate description of mandibular condyle features, morphology and disorders. Assessment of mandibular condyle structure in JIA patients using CBCT enables an understanding of the typical radiologic characteristics of morphological change in these JIA patients.

The aim of this study was to evaluate the mandibular condyle characteristics seen in CBCT and to compare them with the features observed in the control group of the same age.

A cross-sectional study analysing CBCTs of 65 (130 joints) patients with a confirmed JIA diagnosis and 30 (60 joints) control group – patients without JIA up to the age of 17. For the control group the inclusion criteria was an age of up to 17 years with an indication for CBCT scans such as impacted canines. Structural radiologic characteristics of the joint's osseous structures were assessed in the sagittal, coronal and axial planes.

The flattening of the condyles was significantly more often seen in JIA patients compared to the controls (82.8%) and more in females than in males. The surface erosion was seen only in the JIA group and affected 58.7% and 63.0% of the right and left side respectively in females. The osteophytes were a less frequently seen destruction characteristic and affected 39.1% and 30.4% of the right and left side respectively in JIA patients only.

Mandibular condyle destruction characteristics observed in CBCT images were frequent in the JIA group and occurred with less frequency in the control group.

Keywords: condyle, juvenile idiopathic arthritis (JIA), cone beam computed tomography (CBCT).

Introduction

Temporomandibular joint (TMJ) condyles are frequently affected in juvenile idiopathic arthritis (JIA) and lesions in or on the condyles appear early in life of a patient below the age of 16 [Cassidy, 1986]. Females are more often affected than males [Ferraz et al., 2012] and can be affected uni- or bilaterally [Te Veldhuis et al., 2014]. Despite how minimal the damage to the condyle is, craniofacial morphology may become severely disturbed [Billiau et al., 2007]. The consequence of this inflammation

and subsequent destruction during growth has a large variation in severity ranging from minor mandibular asymmetries with no clinical significance to major growth deviations of the mandible leading to disturbed mandibular function and growth [Billiau et al., 2007; Ringold et al., 2009; Arvidsson et al., 2010; Fjeld et al., 2010].

Detecting these destructive signs early in the course of the disease clinically and radiographically is critical. Early diagnosis of the disease and being aware of the potential outcomes of the disease on the condyles facilitates the formulation of a treatment plan compensating for possible future condylar destruction, hence decreasing the impact on mandibular growth and facial appearance [Twilt et al., 2004; Pedersen et al., 2008]. Diagnosis of TMJ arthritis includes clinical examination and imaging of the joint [Müller et al., 2009].

Conventional radiographic methods can only show gross osseous changes [Cassidy, 1986] and panoramic radiology is inadequate in identifying small osseous lesions on the surface of the condyle [Dahlstrom et al., 1996; Petersson et al., 2010]. The advantage of using cone beam computed tomography (CBCT) is the possibility for acquiring 3D images, which enable an accurate description of the underlying osseous structure, morphology and disorders of the TMJ whilst having a significantly lower radiation dose than conventional computer tomography [Swennen et al., 2006; Alexiou et al., 2009; Garagiola et al., 2013]. Magnetic resonance imaging (MRI) is still the gold standard for diagnosing TMJ arthritis as it can show arthritic signs before the appearance of hard tissue changes [Swennen et al., 2006; Pedersen et al., 2008; Müller et al., 2009]. However, CBCT is more adequate in detecting changes in shape (flattening, erosion, osteophyte) than in MRI, which may be because MRI has a larger slice thickness (more than 3 mm compared to 0.4 mm in CBCT) in clinical use, as well as the possible presence of fibrous tissues inside the TMJ and the close proximity of the lateral pterygoid muscle to the articular surface of the condyle which are factors that could decrease the precision in the MRI analysis [Alkhader et al., 2010].

Aim

The aim of this study was to assess the presence of osseous destruction characteristics by CBCT of the TMJ mandibular condyles because of JIA and to compare these characteristics with a group of patients without JIA.

Material and methods

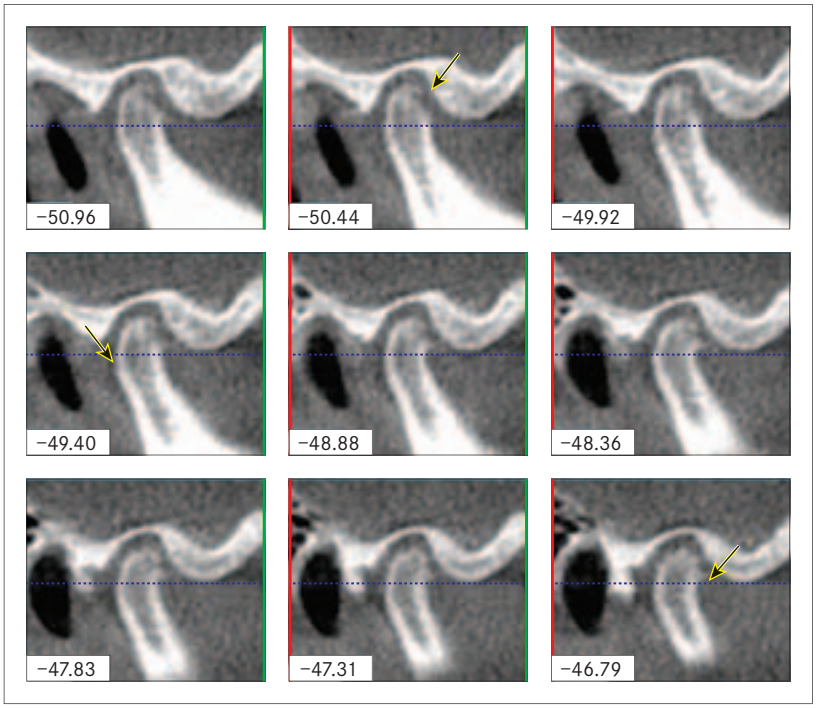
In this cross-sectional observational study, whereby CBCTs of 65 (130 joints) patients with a confirmed JIA diagnosis (referred from the Paediatric Clinical University Hospital's Rheumatology department) and 30 (60 joints) control group i.e. patients without JIA (obtained from the Orthodontic department of the Stomatology Institute) were evaluated by the author.

The inclusion criteria were children up to the age of 17 and with a confirmed diagnosis of JIA. For the control group the inclusion criteria were also the age up to 17 years and having an indication for a CBCT such as impacted canines. The JIA group had 45 females and 20 males and the control group had 24 females and 6 males. The mean age of the JIA group was 14.2 years (range 9–17), the mean age of the control group was 13.67 years (range 10–17). CBCT was used for the examination and evaluation of the mandibular condyle and verifying whether there was any radiographic evidence of disorders or abnormalities in these condyles.

Structural radiologic characteristics of the condyles were assessed in the sagittal, coronal and axial planes. The CBCT images assessed the condyles for presence or absence of the following; hypoplasia, flattening, subcortical sclerosis, subcortical cyst, surface erosion, osteophyte, generalised sclerosis, deviation in form, and bony ankylosis (Figure 1 for a sagittal view of a condyle with erosion, cyst, and osteophyte).

This study was conducted in the Orthodontic Department of Rīga Stradiņš University's Stomatology Institute, the Paediatric Clinical University Hospital's Rheumatology Department in referring the JIA patients in order to carry out a full assessment of the TMJ and obtain CBCT scans.

Figure 1. Sagittal view of condyle with erosion, cyst, and osteophyte (highlighted by the arrows)



For the CBCT images, the data was processed and analysed with I-CAT Vision equipment (Imaging Sciences International, Inc. Hatfield PA, USA). The equipment used standardised protocol: voltage: 120 KV, current: 38 mA, field of view: 17 cm, resolution: 0.4 voxels, radiation dose 36 μSv. Statistical data analysis was performed to evaluate the distribution of the radiologic osseous structural changes in the two study groups. Data was entered into MS Excel and processed by SPSS (version 20.0, SPSS Inc., Chicago, Il, USA). Chi-squared and Fisher's exact tests were used to calculate statistical significances between changes in the condyles of healthy individuals and JIA patients, as well as in frequencies of affected condyles in different groups.

This study was approved by the Ethics Committee of Rīga Stradiņš University (Decision accepted with the principles laid down in the Declaration of Helsinki).

Results

The prevalence of mandibular condyles with evident radiologic destruction characteristics, and a comparison between the JIA and control group for the right and left sides are shown in Tables 1 and 2 (Table 1 for females and Table 2 for males). The flattening of the condyles was seen significantly more often in JIA patients compared to the controls (82.8%) and more in females than in males. The surface erosion of condyles was seen only in the JIA group and affected 58.7% and in 63.0% of the right and left side, respectively, in females. The osteophytes were a less frequently seen destruction characteristic and affected 39.1% and 30.4% of the right and left side, respectively, in JIA patients only. Other destruction characteristics were observed less frequently (Table 1 and Table 2).

Table 1. Comparison of frequency of radiologic characteristics of mandibular condyle between JIA group and control group for the right and left sides in females

Characteristic (condylar head)	Right Side			Left side		
	JIA	Control	P value	JIA	Control	P value
Hypoplasia	32.6	28.3	0.038*	8.3	4.2	0.025*
Surface flattening	87.0	91.3	< 0.0001*	4.2	8.3	< 0.0001*
Subcortical sclerosis	28.3	23.9	0.003*	0	0	0.012*
Subcortical cyst	17.4	19.6	0.044*	0	0	0.023*
Surface erosion	58.7	63.0	< 0.0001*	0	0	< 0.0001*
Osteophyte	39.1	30.4	< 0.0001*	0	0	0.001*
Generalised sclerosis	2.2	2.2	1.000	0	0	1.000
Deviation in form	10.9	13.0	–	0	0	–
Bony ankylosis	4.3	0	0.157	0	0	0.087

* With statistical significance ($p < 0.05$).

Table 2. Comparison of frequency of radiologic characteristics of mandibular condyle between JIA group and control group for the right and left sides in males

Characteristic (condylar head)	Right Side			Left side		
	JIA	Control	P value	JIA	Control	P value
Hypoplasia	21.1	0	0.540	21.1	0	0.540
Surface flattening	68.4	0	0.005*	89.5	0	< 0.0001*
Subcortical sclerosis	26.3	0	0.289	5.3	0	1.000
Subcortical cyst	15.8	0	0.554	15.8	0	0.554
Surface erosion	42.1	0	0.129	47.4	0	0.057
Osteophyte	5.3	0	1.000	21.1	0	0.540
Generalised sclerosis	0	0	–	0	0	–
Deviation in form	0	0	–	0	0	–
Bony ankylosis	0	0	1.000	0	0	–

* With statistical significance ($p < 0.05$).

Discussion

A limitation of this study is that there is a time lapse between the diagnosis and the taking of the CBCT, which could have affected the degree of severity of the radiologic characteristics observed. Unfortunately, due to the design of this study, the examiner (Hadeel Al-Shwaikh) was aware of the diagnosis of the patients, therefore some bias could have occurred. The examiner was not a qualified radiologist; however, she was calibrated with an experienced radiologist before conducting this study. The clinical symptoms of the JIA patients were not taken into account since this was not the aim of the study. To achieve more informative and reliable results, a more selective group of JIA patients, or the severity and time elapsed between the taking of the CBCT and diagnosis have to be considered.

To be able to comprehend the age specificities of the mandibular condyle, it is necessary to evaluate the CBCT images from a group of patients without JIA. A normal condyle is defined as being oval and rounded in shape in the axial plane and being convex, round, or flat in the coronal plane [Yale et al., 1966]. In the sagittal plane, the condyle should be round with the cortical outline intact, smooth, and even in thickness [Brooks et al., 1992; Loubele et al., 2009].

We observed that in our control group, condyle hypoplasia condyle surface flattening were present. The presence of these morphological features seen in CBCT could be due to the fact that condyles and the TMJ in general, undergo continuous functional remodeling as the child is growing and, therefore, condylar surface flattening and some perceived condylar surface erosions might in fact be nothing more than a normal physiological process [Brooks et al., 1992]. Karlo et al. suggested that the condyle in childhood changes form round to oval and until the age of 7, the condyle will have formed to 80% [Karlo et al., 2010]. Thus, physiological growth may explain the number of patients with these characteristics within the control group.

The involvement of the TMJ in JIA patients is described as containing heterogeneous deformities, which may be a result of local growth disturbances, remodeling, or healed destructive processes [Bache, 1964; Larheim et al., 1981; Arvidsson et al., 2010]. The most prevalent radiologic characteristic observed in our study concerning the structure and morphology of the JIA condyles were surface flattening, surface erosion, and osteophytes, which is corroborated by existing literature [Bache, 1964; Larheim et al., 1981; Arvidsson et al., 2010].

Arvidsson et al. in 2010 described the long-term radiologic findings, which were assessed by a different approach in terms of the methodology; by MRI and conventional computed tomography (CT) in adults with long-standing JIA. Concerning the CT findings, they looked for abnormal shape or size of the TMJ condyle cortical bone defects such as superior condylar concavity, cortical defects with and without a sclerotic margin and anterior condyle position in a closed mouth position. They also found bifid condyles, hyperplasia of condyles and fossa eminences as well as subchondral cysts of the condyle [Arvidsson et al., 2010]. These results are explained by the fact that adult patients were studied and so we cannot accurately compare those findings of hyperplasia and bifid condyles with our JIA sample.

A study conducted by Sidiropoulou-Chatzigianni et al. (2008) evaluated the presence of condylar destruction and lesions in orthopantomograms (OPG) of 66 children with JIA diagnosis. They also determined whether destructions were found uni- or bilaterally. The study showed that 50% of the children had some form of condylar destruction, and if they were present unilaterally, they seemed to favour the right TMJ condyle, which was to some degree in accordance with the results of this research since condylar hypoplasia, subcortical sclerosis and bony ankylosis were features, which seemed to favour the right condyle. However, the authors did not further classify these “lesions and destructions”; they simply indicated if there was any presence of any form of destruction [Sidiropoulou-Chatzigianni et al., 2008]. Again, the radiologic method differs from our study.

Hu et al. (1995) observed condyle surface erosion on conventional CT images of JIA affected children thus supporting our findings. One year later, Hu et al. published another report using CT and found that bony abnormalities of the TMJ in children with JIA occurred in almost two-thirds of children and they had “variable stages” of condyle degeneration, the type of degeneration was not mentioned [Hu et al., 1996]. Kitai et al. used CT and MRI to observe the TMJ and they reported similar results [Kitai et al., 2002] to Hu et al. Scolozzi et al. performed a study using both CT and MRI to lead to diagnosis of JIA and noticed multiple articular bony changes [Scolozzi et al., 2005], consolidating the findings of this study.

Huntjens et al. (2008) inspected CBCT images of JIA patients and observed several condylar destruction characteristics ranging from minor erosions of condyle to virtually complete deformation of condyle [Huntjens et al., 2008]. In 2010, Farronato et al. used CBCT to quantify the TMJ osseous destruction in JIA affected patients and concluded that with the advent of CBCT a more accurate visualisation of early morphologic changes in the TMJ is attainable [Farronato et al., 2010].

The present study has shown that CBCT is an effective tool for evaluating the type(s) of osseous destruction of the TMJ mandibular condyle because of JIA. As the reviewed literature has proven, it has become increasingly apparent that there is insufficiently published data regarding the type of the mandibular condyle osseous destruction in children because of JIA by using CBCT as well as a comparison of this group of patients to the control group.

Conclusions

CBCT images clearly showed that in the JIA group the most prevalent osseous destruction characteristics of the mandibular condyle were condylar surface flattening, followed by surface erosion and osteophyte. It seems that there is no pattern of the destruction for both sides symmetrically. In some control patients, mild condyle surface flattening and hypoplasia were observed, which could be due to physiological growth and development of TMJ.

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