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2015

Research articles in medicine & pharmacy

RĪGA STRADIŅŠ UNIVERSITY

Collection of Scientific Papers 2015

Research articles in medicine & pharmacy

Internal Medicine
Surgery
Medical Basic Sciences
Stomatology
Pharmacy

Rīga
RSU
2016

UDK 61(063)

Rīga Stradiņš University.
Collection of Scientific Papers: Research articles in medicine & pharmacy, 2015: Internal Medicine. Surgery. Medical Basic Sciences. Stomatology. Pharmacy. – Rīga: Rīga Stradiņš University, 2016. – 99 p.

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IPD Nr. 15-268

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Dzirciema iela 16, Rīga, LV-1007, LATVIA

ISBN 978-9984-793-84-9
ISSN 1691-497X

Contents

Digestive System Diseases as the most Common Disease Group among Patients with Extended-spectrum Beta-lactamase Producing Bacterial Infection

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Abstract

Extended spectrum beta-lactamase (ESBL) producing bacteria are associated with increased antimicrobial resistance.

The aim of this study was to characterise ESBL-producing bacterial infection cases by analysing all consecutive ESBL-producing bacteria isolation cases during 6-month period, using patient medical records and bacteriological material.

136 isolation cases were obtained from 110 hospitalisation episodes and 108 different patients. ESBL-producing *Enterobacteriaceae* were isolated from 52 (38.2%) female and 84 (61.8%) male patients with the mean age of 61.35 ± 16.92 years. ESBL-producing *Klebsiella pneumoniae* (48.5%) was mostly isolated from wound biomaterial (32.4%). Digestive system diseases were the most commonly found disease group, accounting for 49.26% cases. Patients spent an average of 56.80 ± 67.19 days at hospital, in 82 cases (60.29%) patients were admitted to the Intensive Care Unit (ICU) and spent there 24.35 ± 30.69 days. In 28 cases (20.6%) patients died as a hospitalisation outcome at the age of 70.43 ± 13.23 years. Higher risk for worse hospitalisation course and outcome in patients with ESBL-producing bacterial infection was observed in patients younger than 65 years, patients with injuries, musculoskeletal and infectious diseases.

The study allows for following conclusions:

1. In Latvia, the prevalence of *Kl. pneumoniae* is higher comparing to other European countries.
2. Longer hospital stay, more frequent admission to the ICU and longer ICU stay is associated with higher mortality in patient population with ESBL-producing bacterial infection.
3. Digestive system diseases are the most common disease group among patient population with ESBL-producing bacterial infection.

4. Risk factors associated with worse hospitalisation course and outcome in patient population with ESBL-producing bacterial infection are: patient age under 65 years, injuries, musculoskeletal and infectious diseases.

Keywords: extended spectrum beta-lactamases, *Klebsiella pneumoniae*, resistance, epidemiology, digestive system diseases, *Enterobacteriaceae*.

Introduction

Since the first outbreak in 1983 (Germany), extended-spectrum beta-lactamase (ESBL) producing Gram negative bacteria (*Enterobacteriaceae*) reports have increased due to the increasing consumption of antimicrobials and widespread gene mutation, providing bacterial resistance to extended spectrum penicillins and cephalosporins (Coque et al., 2008; Canton et al., 2008).

ESBL are mostly produced by *Enterobacteriaceae*, mainly by *Escherichia coli* and *Klebsiella pneumoniae* (Canton et al., 2008; Shaikh et al., 2015; Lee et al., 2012; Spanu et al., 2002). ESBL-producing bacteria predominate in cases of urinary tract infections and ventilator-associated pneumonia, but these bacteria can cause also a wide variety of other nosocomial and community acquired infections (Osthoff et al., 2015; Spadafino et al., 2014).

Risk factors for ESBL-producing bacterial infection acquisition include severe underlying diseases, immunosuppression, prior administration of antibiotics, long stay in hospital, nursing home, intensive care unit, presence of catheters and longer stay in the intensive care unit (Spadafino et al., 2014; Skippen et al., 2006; Tacconelli et al., 2014).

Antibacterial resistance differs from region to region and according to ECDC Surveillance 2013 report data more than 50% of *Kl. pneumoniae* and 10–25% of *E. coli* strains in Latvia produce ESBL and are resistant to third generation cephalosporins (Coque et al., 2008; Canton et al., 2008; Spanu et al., 2002; Weist et al., 2012).

Bacterial phenotypes and genotypes, which determine ESBL-producing bacteria prevalence and antimicrobial sensitivity vary widely depending on geographic location, hospital, ward, patient group or even type of infection (Canton et al., 2008; Shaikh et al., 2015; Lee et al., 2012; Hawkey et al., 2009).

Only a few studies concerning ESBL-producing bacterial infection molecular epidemiology have been conducted in the Baltic region (Lillo et al., 2014; Paberza et al., 2007). Most of them include Pauls Stradins Clinical University Hospital and cover the period before 2012, where the number of ESBL-producing bacteria isolation cases at Rīga Eastern Clinical University hospital (RECUH), the largest hospital in Latvia, is growing and have not been studied before.

Aim

The aim of the study was to obtain general characterisation (demographic, epidemiological, bacteriological, disease, hospitalisation course and outcome background) of patients with ESBL-producing bacterial infection and specify risk factors for worse hospitalisation course and outcome in ESBL-producing bacterial infection cases in RECUH – the biggest hospital in Latvia.

Material and Methods

A cross-sectional single centre study was conducted at Rīga Eastern Clinical University Hospital (RECUH), including all consecutive ESBL-producing bacteria isolation cases collected from Bacteriology laboratory over a 6-month period, dating from September 1, 2013 till March 1, 2014.

All patient cases regardless of patient age, gender and clinical severity with ESBL-producing bacteria found in the tested biomaterial were included in the study. All patient cases where ESBL-producing bacteria were not found in the biomaterial were excluded from the study. The number of ESBL-producing bacteria isolation

cases included in the study accounted for 43% of all ESBL-producing bacteria cases isolated during one-year period in RECUH.

Data sources included bacteriological biomaterial (wound biomaterial, urine, bronchoalveolar fluid, abdominal cavity biomaterial, blood, abscess, cerebrospinal fluid and sputum) for ESBL-producing *Enterobacteriaceae* strain identification and patient medical records for demographic, hospitalisation and disease data collection. Data were collected in a database using *MS Excel 2013* software, based on an originally designed study protocol and questionnaire, containing 106 parameter groups, including demographic, epidemiological, clinical, disease and bacteriological parameter groups. Statistical analysis was performed with SPSS version 20.0, using Spearman's correlation coefficient, Mann–Whitney U test and Pearson's Chi-square test.

Bacteriological analysis was performed according to the EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance, version 1.0, December 2013 and provided information about the biomaterial that was tested, as well as ESBL-producing *Enterobacteriaceae* strains identified during the study.

Information about the diseases was gathered from official patient discharge documentation and grouped according to ICD-10 (International Statistical Classification of Diseases) version 2015 (ICD-10, 2015). Each disease was encoded separately and summed up in total disease count for statistical analysis.

Hospitalisation course in patients was evaluated according to the length of the hospital stay, ICU stay and ICU admission. Hospitalisation outcome in patients was evaluated according to mortality.

The study was reviewed and accepted by the Rīga Eastern Clinical University Hospital Ethics committee.

Results

Epidemiological and demographic data

A total of 136 ESBL-producing bacteria cases were isolated from 110 hospitalisation episodes and 108 different patients. 98 ESBL-producing bacteria cases (72.1%) were isolated from Clinic "Gaiļezers", 17 cases (12.5%) – from Oncology Centre of Latvia and 21 cases (15.4%) – from Clinic "Bikernieki".

ESBL-producing *Enterobacteriaceae* in 52 (38.2%) cases were isolated from female and 84 (61.8%) cases – male patients with the mean age of 61.35 ± 16.92, ranging from 22 to 89 years.

Bacteriological testing data

ESBL-producing bacteria was mostly isolated from wound biomaterial (n = 44; 32.35%) (Figure 1).

In most cases ESBL-producing *Kl. pneumoniae* (n = 66, 48.53%) and *E. coli* (n = 36; 26.47%) were isolated (Figure 2).

Figure 1. Biomaterial for ESBL-producing *Enterobacteriaceae* isolation

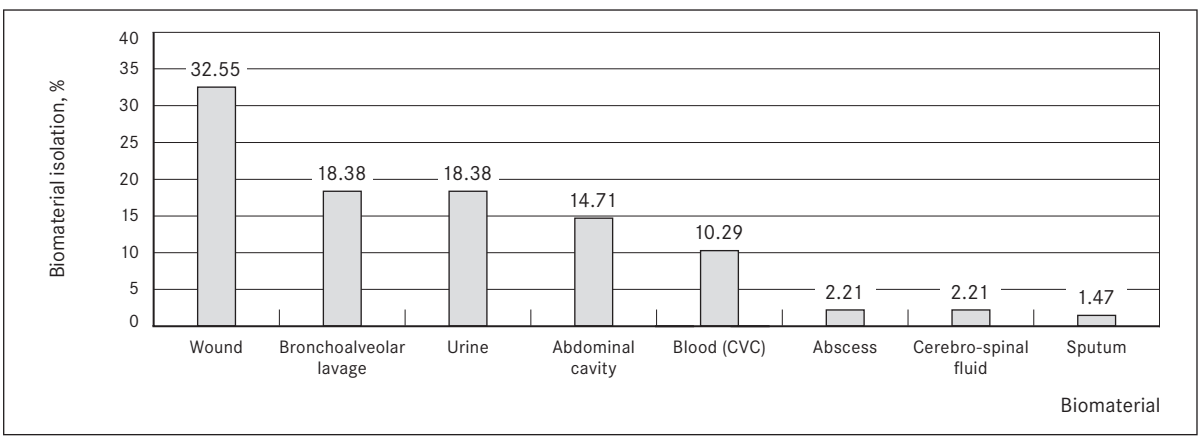
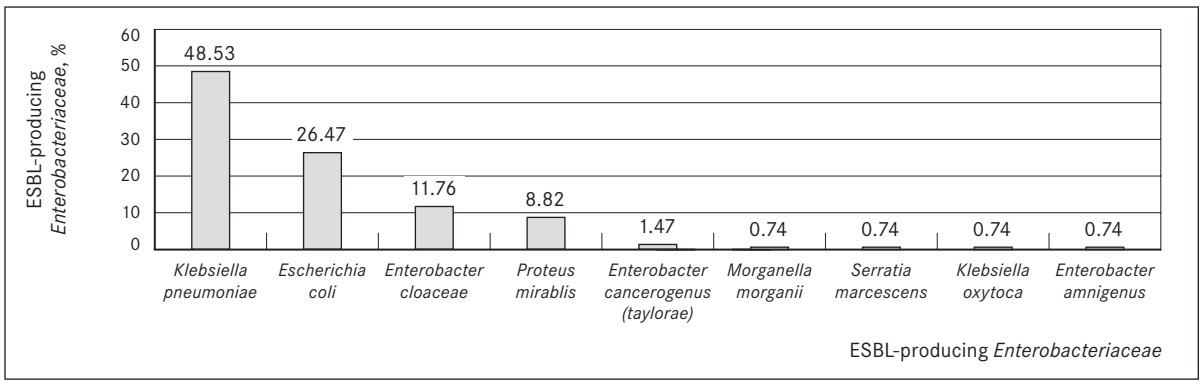


Figure 2. ESBL-producing *Enterobacteriaceae* isolated



Hospitalisation data

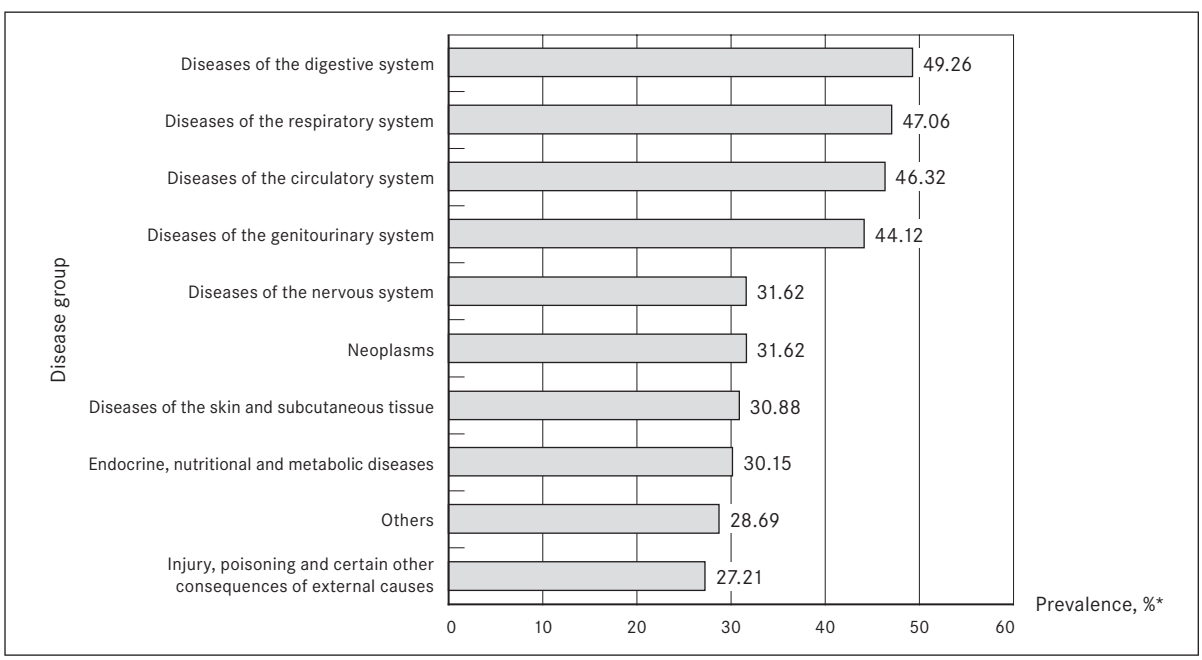
Patients spent an average of 56.80 ± 67.19 days at the hospital, ranging from 1 to 390 days during each hospitalisation period. In 82 ESBL-producing bacteria isolation cases patients (60.29%) were admitted to the ICU at least 1 time, ranging from 1 to 3 times each hospitalisation period and spent there an average of 24.35 ± 30.69 days, ranging from 1 to 141 days each hospitalisation period. Most of them (n = 64, 78.05%) were admitted to the ICU one time and 54 patients (39.71%) were never admitted to the ICU during their hospitalisation period.

In 28 ESBL-producing bacteria isolation cases (20.6%) patients died as a hospitalisation outcome at the mean age of 70.43 ± 13.23, ranging from 46 to 96 years.

Diseases

Patients in most ESBL-producing bacteria isolation cases had digestive system diseases (n = 67, 49.26%), respiratory system diseases (n = 64, 47.06%) and diseases of the circulatory system (n = 63, 46.36%) (Figure 3).

Figure 3. Disease groups (ICD-10) found in patients with ESBL-producing bacterial infection, %

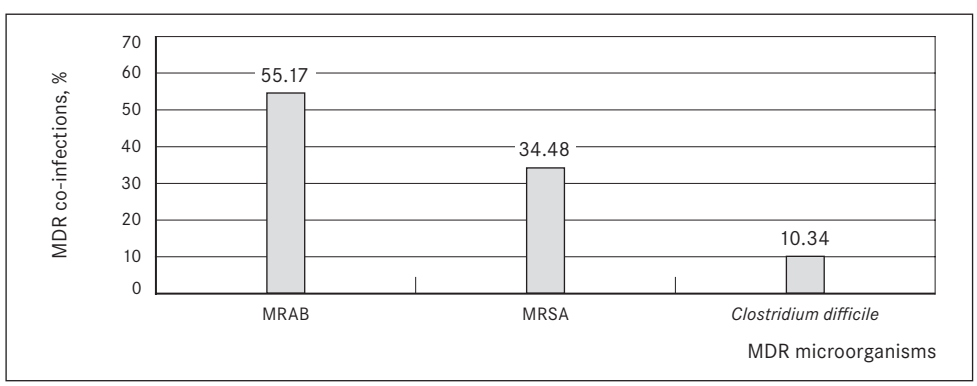


* Prevalence of each disease group was analysed within all ESBL-producing bacteria isolation cases.

The most common digestive system diseases (n = 101) observed in patient population with ESBL-producing bacterial infection were disorders of gallbladder, biliary tract, pancreas and spleen, diseases of small and large intestine and diseases of the peritoneum. Most common group of digestive diseases were chronic (n = 11; 10.9%) and acute (n = 15; 14.9%) pancreatitis with complications, including sub-diaphragmatic (n = 6; 5.9%) and retroperitoneal (n = 4; 4%) abscesses, acute necrotic collections (n = 3; 3%) and pancreatic fistula (n = 1; 1%). Frequently found disorders also included mechanical ileus (n = 11; 10.9%) with complications, including transverse colon perforation (n = 5; 5%), ileum perforation (n = 1; 1%) and intestinal necrosis (n = 1; 1%); acute cholecystitis (n = 5; 5%) and choledocholithiasis (n = 9; 8.9%) with complications, including mechanical icterus (n = 7; 6.9%) and cholangitis (n = 3; 3%); as well as intra-abdominal abscesses (n = 5; 5%). Other gastrointestinal diseases included ulcerative colitis, primary sclerosing cholangitis, diverticulosis, haemoperitoneum and their complications (n = 14; 14%).

In 29 ESBL-producing bacteria isolation cases (21.32%) patients had another multi-drug resistant (MDR) microorganism co-infection. In 16 cases (55.17%) patients were co-infected with multi-drug resistant *Acinetobacter baumannii* (MRAB), in 10 cases (34.48%) – methicillin-resistant *Staphylococcus aureus* (MRSA) and in 3 cases (10.34%) *Clostridium difficile* infection (Figure 4).

Figure 4. Multi-drug resistant (MDR) microorganism co-infections in patients with ESBL-producing bacterial infection, %



In 60 cases (44.12%), patients had at least one surgery within the studied hospitalisation period, ranging from one to four surgeries each hospitalisation period. In 60 cases (44.12%) patients had had at least one surgery in the past (before the studied hospitalisation period), ranging from one to six surgeries each hospitalisation period.

In 32 ESBL-producing bacteria isolation cases (23.53%) patients had sepsis, in 23 cases (16.91%) – MODS (multi organ dysfunction syndrome) and in 18 cases (13.24%) – shock, mostly septic and traumatic shock.

Disease association with epidemiologic data

Female patients had more diseases in general, including ESBL-producing bacterial infection and concomitant diseases, more circulatory system diseases, neurologic diseases and more endocrine diseases, including diabetes, where male patients had more neoplasms and more mental and behavioural disorders (Table 1).

Younger patients suffered more from mental and behavioural disorders, injuries and shock. Older patients had more neoplasms, circulatory system diseases, neurologic diseases, genitourinary system diseases, MDR infections and diabetes (Table 2).

More diseases at the same time, especially circulatory system diseases, respiratory system diseases, musculoskeletal system diseases and neurologic diseases, as well as shock, MODS and sepsis were associated with higher mortality. Whereas people with circulatory diseases, genitourinary system diseases and more diseases at the same time, including ESBL-producing bacterial infection cases and concomitant diseases died at an older age (Table 3).

Table 1. Comparison of the most common underlying conditions in female and male patients with ESBL-producing *Enterobacteriaceae* infection

Female patients	p value	Male patients	p value
More diseases	p = 0.017	Neoplasms	p = 0.013
Circulatory system diseases	p = 0.055	Mental and behavioural disorders	p = 0.033
Neurologic diseases	p = 0.045		
Endocrine diseases	p < 0.001		
Diabetes	p = 0.006		

Table 2. Comparison of the most common underlying conditions in the patient age groups with ESBL-producing *Enterobacteriaceae* infection

Patients younger than 65 years	p value	Patients older than 65 years	p value
Mental and behavioural disorders	p = 0.026	Neoplasms	p < 0.001
Injuries	p = 0.001	Circulatory system diseases	p < 0.001
Shock	p = 0.003	Neurologic diseases	p = 0.009
		Genitourinary system diseases	p < 0.001
		MDR infections	p = 0.003
		Diabetes	p = 0.014

Table 3. Medical conditions associated with mortality in patients with ESBL-producing *Enterobacteriaceae* infection

Conditions associated with increased mortality	p value	Conditions associated with later age of death	p value
More diseases	p = 0.002	More diseases	p = 0.018
Circulatory system diseases	p < 0.001	Circulatory system diseases	p = 0.003
Respiratory system diseases	p = 0.006	Genitourinary system diseases	p = 0.002
Musculoskeletal system diseases	p = 0.002		
Neurologic diseases	p = 0.003		
Shock	p = 0.039		
MODS	p < 0.001		
Sepsis	p = 0.001		

The most frequently found circulatory system diseases included arterial hypertension, coronary heart disease, myocardial infarction, atrial fibrillation, chronic heart disease, intra-cerebral haemorrhage, cerebral aneurysms, atherosclerosis and aneurysms of peripheral arteries. The most frequently found neurologic diseases included Parkinsonism, Parkinson's disease, vascular dementia, polyneuropathy, myelitis, cerebral abscesses, acute and chronic cerebral ischemia, encephalitis, meningitis and epilepsy. The most frequently found endocrine diseases, included type 1 and type 2 diabetes, Cushing's disease, adrenal insufficiency, colloidal goitre, autoimmune thyroiditis, hypothyroidism and adiposity. The most frequently found neoplasms included colorectal cancer, laryngeal cancer, breast cancer, prostate adenocarcinoma, anaplastic ependymoma, Hodgkin's lymphoma and uterine cancer. The most frequently found mental and behavioural disorders included delirium, psychosis and psychoorganic syndrome. The most frequently found injuries included multiple bone fractures, burns, cerebral contusions and commotions, subdural hematomas, epidural hematomas, subarachnoid haemorrhages, ruptured spleen and liver. The most frequently found genitourinary system diseases included chronic glomerulonephritis, pyelonephritis, cystitis, acute renal failure and nephrolithiasis. The most frequently found musculoskeletal system diseases included osteoarthritis, psoriatic polyarthritis, gout, spondylosis, spondyloarthritis, spinal stenosis, ankylosing spondyloarthritis, scoliosis, osteoporosis and osteomyelitis.

Disease association with hospitalisation course and outcome

Patients with more respiratory system diseases, infectious and parasitic diseases, injuries, poisoning and certain other consequences of external causes and additional MDR infection spent more days at hospital. In addition, patients with more surgeries and MODS spent more days at hospital. Patients with musculoskeletal system and connective tissue diseases and patients with genitourinary system diseases spent fewer days at hospital (Table 4).

Patients with more diseases were admitted to the ICU more frequently, including patients with digestive system diseases, infectious and parasitic diseases, sepsis, MODS and shock. Patients with diabetes were admitted to the ICU less frequently (Table 5).

Patients with multiple diseases spent more days in the ICU, including patients with respiratory system diseases, MDR infections, mental and behavioural disorders, sepsis, MODS, shock, as well as patients who underwent more surgeries during their hospital stay. Patients with diabetes, musculoskeletal system and connective tissue diseases, neoplasms and genitourinary system diseases spent less days in the ICU (Table 6).

Patients with injury, poisoning and certain other consequences of external causes were admitted to the ICU fewer times ($p = 0.022$), but spend more days there ($p < 0.001$).

Table 4. Medical conditions associated with the length of hospitalisation in patients with ESBL-producing *Enterobacteriaceae* infection

Longer hospitalisation period	p value	Shorter hospitalisation period	p value
Respiratory system diseases	p = 0.001	Musculoskeletal system diseases	p = 0.029
Infectious and parasitic diseases	p = 0.047	Genitourinary system diseases	p = 0.038
Injuries	p < 0.001		
Additional MDR infection	p < 0.001		
More surgeries	p = 0.012		
MODS	p < 0.025		

Table 5. Medical conditions associated with ICU admission in patients with ESBL-producing *Enterobacteriaceae* infection

More frequent admission to the ICU	p value	Less frequent admission to the ICU	p value
More diseases	p = 0.014	Diabetes	p = 0.027
Digestive system diseases	p = 0.002		
Infectious diseases	p = 0.047		
Sepsis	p < 0.001		
MODS	p = 0.006		
Shock	p = 0.003		

Table 6. Medical conditions associated with the length of the ICU stay in patients with ESBL- producing *Enterobacteriaceae* infection

Longer ICU stay	p value	Shorter ICU stay	p value
More diseases	p = 0.001	Diabetes	p = 0.027
Respiratory system diseases	p < 0.001	Musculoskeletal system diseases	p = 0.004
MDR infection	p < 0.001	Neoplasms	p = 0.003
Mental and behavioural disorders	p = 0.048	Genitourinary system diseases	p = 0.008
MODS	p < 0.001		
Shock	p < 0.001		
More surgeries	p = 0.025		

Discussion

Before analysing certain result categories in detail, it is important to note the common principles that could explain the results obtained in the study. The biomaterial in most of ESBL-producing bacteria isolation cases was gathered from surgical profile departments, where most of the diseases accounted for digestive system disease group (ICD-10). This, together with the fact that 15% of the biomaterial was gathered from the wound clinic, may have resulted in higher wound biomaterial prevalence for ESBL-producing bacteria isolation. The results could also be directly associated with common medical practice in Latvia – to obtain biomaterial and perform bacteriological testing mostly from surgical sites and wounds, opposed to other biomaterial – urine, blood and bronchoalveolar fluid, which is mostly bacteriologically tested in cases of serious infection or unfavourable course of treatment. Therefore, some of ESBL-producing bacteria isolation cases from wound biomaterial could be associated with ESBL-producing bacteria colonisation. In these cases hospitalisation course and outcome, including length of hospital stay, ICU stay, ICU admission and even mortality, could not be associated with ESBL-producing bacterial infection, but more with the main disease, determining the clinical severity.

On the other hand, many studies in literature also analyse just the consecutive ESBL-producing bacteria isolation cases and associate differences with geographical diversity of ESBL-producing bacteria. In addition, the fact that patient age and gender in this study is similar to patient populations with ESBL-producing bacterial infection researched in other studies allows us to speculate about geographical differences in ESBL-producing bacteria phenotype. Although we try to analyse and find geographical differences among ESBL-producing bacteria, we should always keep in mind that these geographical differences might also be explained by local common medical practice, which determines the indications for biomaterial collection and bacteriological testing.

Epidemiological, demographic and bacteriological testing data

Mean age and gender in the studied patient cohort with ESBL-producing bacterial infection corresponded to the data found in other literature sources. ESBL-producing *Enterobacteriaceae* were mostly isolated from male patients around 60 years of age (Skippen et al., 2006).

Kl. pneumoniae was the most frequently isolated ESBL-producing microorganism in this study, where *E. coli* was the most frequently isolated ESBL-producing strain in other studies (Moor et al., 2008; Sader et al., 2014).

This difference could be explained by different biomaterials and medical conditions researched in various studies. Wound biomaterial was the most frequently studied biomaterial in this study, where urine was the most common biomaterial for ESBL-producing bacteria isolation in other studies (Ruiz de Alegria et al., 2011; Kassakian et al., 2014).

Hospitalisation course and outcome data

Patients in this study stayed at hospital more than 2 times longer (Ruiz de Alegria et al., 2011), were admitted to the ICU at least 3 times more frequently (Mehrgan et al., 2008) and stayed in the ICU more than 2 times longer (Mehrgan et al., 2008) than in other ESBL-producing bacterial infection studies. In addition, mortality rates were higher than found in literature (Ruiz de Alegria et al., 2011).

These differences could be explained by the different periods taken into account when analysing the days spent at hospital. In this study all hospitalisation period from the day of admission until the day of discharge was taken into account, where in several other studies – only days after the ESBL-producing bacteria isolation was determined. The differences could also be explained by the fact that in many studies biomaterial was gathered only from the ICU, where in this study biomaterial was gathered from the whole hospital – including ICU, as well as regular therapy and surgery wards. The differences could also be explained by the variety of biomaterials used for ESBL-producing bacteria isolation and medical conditions associated with ESBL-producing bacterial infection. The authors of this study acknowledge in some cases

ESBL-producing bacterial infection was not always the main condition that determined the clinical severity and outcome for a patient, but acted as a serious risk factor for less favourable hospitalisation course and outcome due to a more challenging empirical antimicrobial therapy.

The mean age of death in patient population with ESBL-producing bacterial infection has never been described in literature before. As mentioned before, in this study the mean age of death was determined for both ESBL-producing bacterial infection and possible colonisation cases; therefore, in some cases other underlying disease may have influenced patients' age of death.

Disease data

The most frequently found disease group in patient cohort with ESBL-producing bacterial infection in this study was digestive system diseases. Some literature sources state biliary tract diseases (Ruiz de Alegria et al., 2011; Kassakian et al., 2014; Peralta et al., 2012; Kolar et al., 2006), liver cirrhosis (Moor et al., 2008; Peralta et al., 2012) and intra-abdominal infection (Ruiz de Alegria et al., 2011; Kassakian et al., 2014; Peralta et al., 2012) as conditions associated with ESBL-producing bacterial infection, but none of them have been described as frequent. The most common disease group associated with ESBL-producing bacterial infection and colonisation in literature was genitourinary tract diseases (Moor et al., 2008; Ruiz de Alegria et al., 2011; Kassakian et al., 2014; Peralta et al., 2012; Kolar et al., 2006). Respiratory tract diseases (Moor et al., 2008; Ruiz de Alegria et al., 2011; Kassakian et al., 2014; Peralta et al., 2012; Kolar et al., 2006), cardiovascular diseases (Moor et al., 2008; Ruiz de Alegria et al., 2011; Kassakian et al., 2014; Peralta et al., 2012; Kolar et al., 2006), neoplasms, diabetes, nervous system diseases, primary bacteremia (bloodstream infection) (Ruiz de Alegria et al., 2011; Kassakian et al., 2014; Peralta et al., 2012; Kolar et al., 2006) and sepsis (Kolar et al., 2006) were other frequently found diseases and conditions associated with ESBL-producing bacterial infection found both in this and other studied in the literature (Moor et al., 2008; Peralta et al., 2012). The higher digestive system disease prevalence and lower genitourinary system disease prevalence in our study could be explained by different biomaterial ESBL-producing *Enterobacteriaceae* were isolated from in various studies (Ruiz de Alegria et al., 2011; Kassakian et al., 2014).

Slightly more patients in this study were co-colonised or co-infected with other MDR bacteria (Meyer et al., 2011) than found in other literature sources. The co-infection rate with *Methicillin-resistant Staphylococcus aureus* (*MRSA*) found in this study was approximately the same as found in other literature sources (Meyer et al., 2011). Co-infection cases with *Pseudomonas aeruginosa* and *Vancomycin-resistant Enterococcus* (*VRE*) *faecium* have been reported in literature, but co-infection with these pathogens was not found in this study. On the other hand, co-infection with *Multi-resistant Acinetobacter baumannii* (MRAB) and *Clostridium difficile* was found in this study, but have not been described in literature before. The higher MDR infection prevalence in this study could be explained by the difference in biomaterial ESBL-producing *Enterobacteriaceae* were isolated from in this study (mostly wound material) on other studies (mostly urine material) (Ruiz de Alegria et al., 2011; Kassakian et al., 2014).

The higher surgical operation prevalence in this study comparing to other literature sources (Moor et al., 2008; Mehrgan et al., 2008) could be explained by the fact that more ESBL- producing *Enterobacteriaceae* were isolated from surgical profile patients. The difference also could be explained by the biomaterial source for ESBL-producing bacteria isolation – more wound and abdominal cavity material was used in this study, comparing to urine and bronchoalveolar biomaterial in other studies (Ruiz de Alegria et al., 2011; Kassakian et al., 2014; Mehrgan et al., 2008).

Hospitalisation course and outcome data association with epidemiologic, demographic and disease data

Relationship stating that mental and behavioural disorders, injury, poisoning and certain other consequences of external causes, neurologic diseases and genitourinary system diseases prevail in younger (< 65 years) patients, and neoplasms, as well as circulatory system diseases and MDR infection diseases are found in older (> 65 years) patients is also observed in the general population (NSDUH,

2013; Warner et al., 2005; MacDonald, 2000; Liu et al., 2012; National Cancer Institute, 2015; Thu Trang et al., 2013; Countries et al., 2010; Imai et al., 2009) and, therefore, cannot be considered as specific characteristics for patient population with ESBL-producing bacterial infection.

During this study certain risk factors associated with prolonged hospital stay, more frequent ICU admission, longer ICU stay and higher mortality were identified.

Risk factors associated with longer hospital stay included: (1) younger age (< 65 years), (2) more time spent in the ICU, (3) respiratory system diseases, (4) infectious and parasitic diseases, (5) injuries, poisoning and certain other consequences of external causes, (6) additional MDR infections, (7) more surgeries and (8) MODS.

Risk factors associated with more frequent ICU admission included: (1) older age (> 65 years), (2) more time spent at hospital, (3) more diseases, (4) digestive system diseases, (5) infectious and parasitic diseases, (6) sepsis, (7) MODS and (8) shock.

Risk factors associated with longer ICU stay included: (1) younger age (< 65 years), (2) more diseases, (3) injury, poisoning and certain other consequences of external causes, (4) respiratory system diseases, (5) mental and behavioural disorders, (6) additional MDR infections, (7) more surgeries, (8) sepsis, (9) MODS and (10) shock.

Risk factors that were associated with higher mortality rate as hospitalisation outcome were: (1) patients admitted to the ICU more frequently, (2) patients spending more days in the ICU, (3) more diseases, (4) circulatory system diseases, (5) respiratory system diseases, (6) musculoskeletal system diseases, (7) neurologic diseases, (8) sepsis, (9) MODS and (10) shock.

Some risk factors have been studied and found in the general population and, therefore, are not specific for patient population with ESBL-producing bacterial infection. Respiratory system diseases, mental and behavioural disorders, additional MDR infections, multiple diseases, more surgeries, sepsis, MODS and shock have been mentioned as risk factors for longer hospital and ICU stay in the general population (Kwizera et al., 2012; Du et al., 2013). Older patient age, longer time at hospital, more diseases, digestive system diseases, sepsis, MODS and shock have been described as reasons for more frequent ICU admission in studies concerning general population (Kwizera et al., 2012; Du et al., 2013). More frequent ICU admission rate, longer ICU stay, more diseases, circulatory system diseases, neurologic diseases, respiratory system diseases, sepsis, MODS and shock have been described as risk factors for higher mortality in general population before (Mayr et al., 2006).

Patients younger than 65 years, injuries, musculoskeletal and infectious diseases have never been described in literature before as risk factors associated with worse hospitalisation course and outcome in patient population with ESBL-producing bacterial infection. Although this might be a novel fining, the authors of this study note that ESBL-producing bacterial infection in many cases were found in severely injured patients with musculoskeletal diseases and infectious comorbidities – mostly young male patients. Therefore, these factors should be considered as risk factors for worse hospitalisation course and outcome in patient population with ESBL-producing bacterial infection all together as a risk factor combination.

Conclusions

1. In Latvia, the prevalence of ESBL-producing *Kl. pneumoniae* is higher comparing to other European countries.
2. Longer hospital stay, more frequent admission to the ICU and longer ICU stay is associated with higher mortality in the studied patient population with ESBL-producing bacterial infection.
3. Digestive system diseases are the most common disease group in the studied patient population with ESBL-producing bacterial infection.
4. Risk factors associated with worse hospitalisation course and outcome in the studied patient population with ESBL-producing bacterial infection include patients younger than 65 years, injuries, musculoskeletal and infectious diseases.

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Clinical Efficacy of Ceftriaxone in Cases of Early Forms of Syphilis

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Abstract

Syphilis (*lues*) is a chronic infectious sexually transmitted disease, caused by a spirochete bacterium *Treponema pallidum* affecting all human organs. Ceftriaxone has high anti-treponemal activity and nowadays has become one of the methods of choice in treatment of syphilis.

The aim of the study was to define the efficacy of ceftriaxone in cases of primary and secondary syphilis, collecting and analysing data of Rīga 1st Hospital, Clinical Centre of Skin and Sexually transmitted Diseases, and to provide evaluation and recommendations according to clinical serological observation results of syphilis treatment by ceftriaxone.

Analysis of treatment efficacy with ceftriaxone was performed in 120 patients (62 female and 58 male, age 16–66 years), who were treated at Rīga 1st Hospital, Clinical Centre of Skin and Sexually Transmitted Diseases, in period 2008 until 2013. Nevertheless, the existing tendency of epidemiological stabilisation of morbidity, serologic resistance to specific therapy of syphilis develops often.

The therapeutic results were evaluated taking into account time of regression of clinical signs of syphilis and negativisation of serologic reactions, clinical relapses, serologic relapses and frequency of development of serologic resistance. Negativisation of serologic reactions in patients with primary syphilis was achieved in all cases in a 6-months period. In 72.3% of *luess secundaria* and in 27.7% of *luess latens praecox* patients stable SR negativisation was achieved in 12 months after completion of treatment course. Slowed SR negativisation was recognised in 24.4% of *luess secundaria* and in 44.4% – of *luess latens praecox* patients treated with ceftriaxone. The efficacy of treatment correlates with the duration of illness.

Based on the results of this project, the possibility of increasing dosage of ceftriaxone up to 1000 mg per day and extending the duration of treatment until 20 days in cases when late symptoms of syphilis appear to be considered in order to avoid the development of clinical seroresistance.

Keywords: syphilis, ceftriaxone, serologic resistance, dosage.

Introduction

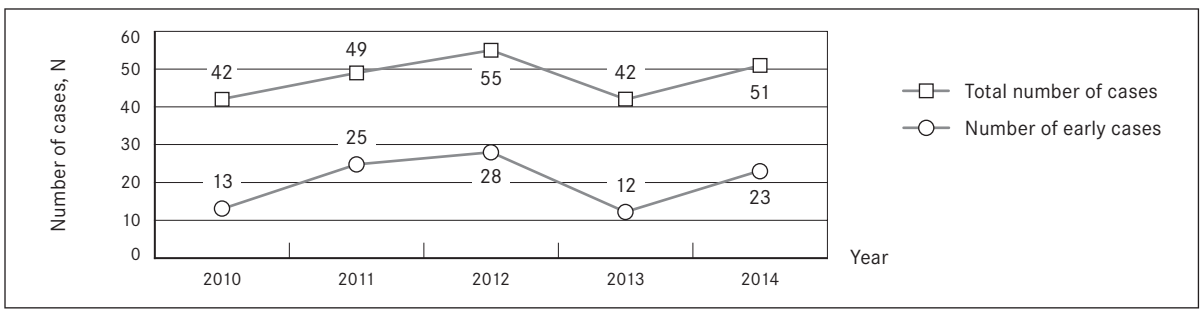
Syphilis is a highly contagious sexually transmitted chronic infectious disease, caused by *Treponema pallidum* (TP). It can affect all human organs. At the onset of disease, lesions of skin and mucous membranes are present proceeding into infectious changes in lymphatic system. Degenerative alterations in visceral organs and central nervous system develop in later stages of disease. Therefore, a topical problem is improvement of specific therapy of syphilis (Starm, 2010; Psomas et al., 2012).

According the statistical data of Rīga 1st Hospital, Clinical Centre of Skin and Sexually Transmitted Diseases, there is a tendency of stabilisation of morbidity of syphilis per year (Table 1, Figure 1).

Table 1. Epidemiological data (for the first time registered morbidity with syphilis – number of cases per year) at Rīga 1st Hospital, Clinical Centre of Skin and Sexually Transmitted Diseases

Year	Total number of cases, N	Number of early syphilis cases, n
2010	42	13
2011	49	25
2012	55	28
2013	42	12
2014	51	23

Figure 1. Epidemiological data tendencies (for the first time registered morbidity with syphilis – number of cases per year) at Rīga 1st Hospital, Clinical Centre of Skin and Sexually Transmitted Diseases



Early forms of syphilis – *luess primaria*, *luess secundaria* and *luess latens praecox* with the duration of disease more than one year are treated ambulatory. Nevertheless, the existing tendency of epidemiological stabilisation of morbidity, serologic resistance to specific therapy of syphilis develops often. There is an obvious change in the choice of methods and tactic of treatment of syphilis during the last 5 years. In clinical praxis, there is an increase in number of anti-syphilitic drugs and tendency to decrease the duration of treatment course and the number of administering of medicines on daily bases. Moreover, in the guidelines available (Goh et al., 2001; French et al., 2009; Janier et al., 2014; Hartmane et al., 2004), there are no definite recommendations, which particular method of treatment of syphilis is to be ordinated in different new forms of syphilis, to avoid the development of clinical serological resistance. Although the tendency of last years is the use of a wider spectrum and on more frequent basis of semi-synthetic 3rd generation cephalosporin antibiotic – ceftriaxone in cases of early forms of syphilis.

Ceftriaxone has high anti-treponemal activity (www.ema.europa.eu). Its efficacy is defined by ability to suppress the synthesis of cell membrane of TP inhibiting synthesis of mucopeptides. Ceftriaxone is resistant to β-lactamases produced by TP. The main advantage of this drug to other cephalosporins of 3rd generation is its 100% bioavailability and long period of half-life (t ½), stating the once a day regimen of administering of this drug (French et al., 2009). The treatment regimens and duration of administering of ceftriaxone differ in several available guidelines and methodological recommendations in cases of those forms of early syphilis (*luess secundaria* and *luess latents praecox*) where clinical and serological resistance develops more often (Hook et al., 2007).

Aim

The aims of this study were:

1) to define the efficacy of ceftriaxone in cases of primary and secondary syphilis, collecting and analysing data of Rīga 1st Hospital, Clinical Centre of Skin and Sexually Transmitted Disease;
2) to provide evaluation and recommendations regarding clinical serological observation results of syphilis treatment with ceftriaxone.

Material and Methods

Analysis of treatment efficacy with ceftriaxone was performed in 120 patients (62 female and 58 male, age 16–66 years), who were treated at Rīga 1st Hospital, Clinical Centre of Skin and Sexually Transmitted Diseases, starting from 2008 until 2013. *Luess primaria* was diagnosed in 7% of patients, *luess secundaria* – in 78% and *luess latens praecox* – in 15% of patients, respectively. Diagnosis was based on specific clinical symptoms of syphilis and confirmed by positive serologic results (*TPHA*, *IFA-IgG*, *IgM* and immunoblot *IgG, IgM* reactions). In 9% of secondary syphilis patients remaining clinical signs of primary syphilis period were still observed – epithelialised hard chancre and enlargement of regional lymph nodes). In case of secondary syphilis roseolas on the trunk skin were clinically observed in 28 patients (29.8%), papules on the body surface skin – in 12 patients (12.7%), papules on the palmar skin – in 17 patients (18%), papules on the plantar skin – in 18 patients (19.1%), erosive papules on the oral mucosa – in 24 patients (25.5%), specific erythematous tonsillitis – in 8 patients (8.5%), papules on the tongue – in 16 patients (17%), specific laryngitis – in 5 patients (5.3%), anogenital erosive papules – in 23 patients (24.4%) and condylomata lata – in 14 patients (14.8%).

In patients with condylomata lata also alopecia was recognised, which approves a belated stage of secondary syphilis. Similar to literature data, more frequent roseolas, seldom – alopecia and condylomata lata, were detected (French et al., 2009). Treatment of syphilis was administered by intramuscular injections of ceftriaxone solution 500 mg once a day for 10 days, according to the European guideline on the management of syphilis (Janier et al., 2014). Tolerability of drug was good, no allergic reactions were observed. The therapeutic results were evaluated taking into account time of regression of clinical signs of syphilis and negativisation of serologic reactions (SR), clinical relapses, serologic relapses and frequency of development of serologic resistance.

Results

Results of treatment of syphilis were evaluated in 120 patients, presenting with early forms of syphilis, one year after treatment course with ceftriaxone. The results obtained were compared with literature data of recent years. Analysing the regression time of clinical signs of secondary syphilis, (Table 2), it was recognised that body roseolas disappeared on day 3–12, palmar and plantar papules on day 7–15, erosive papules of oral cavity mucosae on day 3–10, erosive papules of anogenital region on day 6–15, and condylomata lata on day 11–20.

The results of treatment efficacy were evaluated according to observational data of clinical-serological investigation one year and longer after completion of ceftriaxone treatment course. SR control was done according to recommendations of dynamic observation (French et al., 2009; Janier et al., 2014; Hartmane et al., 2004) once in 3 months. Data obtained are reflected in Table 3 and Figure 2.

Table 2. Regression time of clinical signs of secondary syphilis

Clinical signs	Number of patients, n	Regression time, days (average)
Roseolas	28	3–12 (7.5)
Papules on the trunk skin	12	5–14 (9.5)
Papules on the palmar skin	17	7–15 (11)
Papules on the plantar skin	18	7–15 (11)
Erosive papules of oral cavity mucosae	24	3–10 (6.5)
Specific erythematous tonsillitis	8	3–10 (6.5)
Papules of lingual mucosae	16	8–12 (10)
Specific laryngitis	5	7–14 (10.5)
Erosive papules of anogenital region	23	6–15 (10.5)
Condylomata lata	14	11–20 (15.5)

Table 3. Results of clinical-serological observation after syphilis therapy with ceftriaxone

Results (total) (N = 120)	*Luess primaria* (n = 8)	*Luess secundaria* (n = 94)	*Luess latens praecox* (n = 18)
SR negativisation, n (%)	8 (100)	68 (72.3)	5 (27.7)
Time of negativisation, months	3–6 (average 3.6)	3–12 (average 7.5)	6–12 (average 9)
Decrease of SR positivity, n (%)	–	23 (24.4)	8 (44.4)
Clinical relapses, n	–	–	–
Serological relapses, n (%)	–	–	1 (5.55)
Seroresistance, n (%)	–	3 (3.19)	4 (22.2)

According to Table 3, negativisation of SR in patients with primary syphilis was achieved in all cases in a 6-month period. In 72.3% of *luess secundaria* and in 27.7% *of luess latens praecox* patients stable SR negativisation was achieved in 12 months after completion of treatment course. Slowed SR negativisation was recognised in 24.4% of *luess secundaria* and in 44.4% of *luess latens praecox* patients treated with ceftriaxone. The share of adverse results (clinical-serological relapses, seroresistance) was recognised in 3.19% cases of *luess secundaria* and in 27.7% cases *of luess latens praecox* (data are visualised in Figure 2). Seroresistance was confirmed in three patients with *luess secundaria* patients and in four of *luess latens praecox* patients; in one patient serological relapse developed (Table 4). In three *luess secundaria patients* who developed seroresistance after treatment, condylomata lata and alopecia symptoms were stated.

Figure 2. Results of clinical-serological observation after syphilis therapy with ceftriaxone

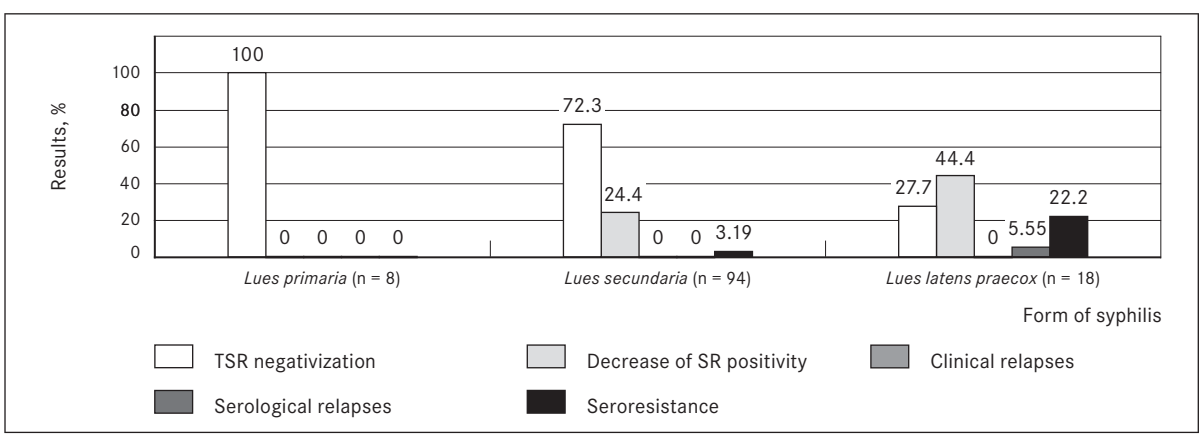


Table 4. Results of syphilis treatment with ceftriaxone

Form of syphilis	Number of patients, n	Serological relapse, n (%)	Seroresistance, n (%)
Luess primaria	8	–	–
Luess secundaria	94	–	3
Luess latens praecox	18	1	4
Total	120	1 (0.83)	7 (5.8)

Discussion

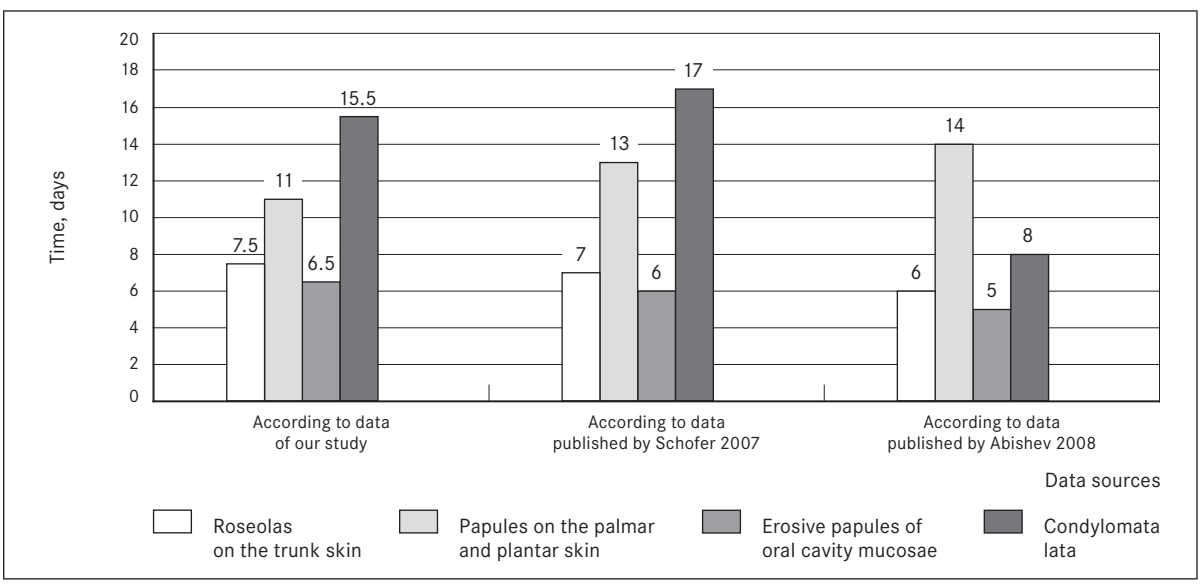
According to data published by Schofer et al. (2007), roseolas on the trunk skin regressed within 7 days from the beginning of treatment course, papules on the trunk skin within 11 days, palmar and plantar papulous lesions within 13 days, erosive papules of oral cavity and lingual mucosae within 6 days, erosive papules of anogenital region regressed within 8 days and condylomata lata persisted on average for 17 days (Figure 3).

According to data published by Abishev et al. (Абишев А. Т., 2008), the regression of syphilides occurred in the following periods: roseola – on average within 6 days, papules on the palmar and plantar skin – 13 days, erosive papules of oral cavity mucosae – 5 days and condylomata lata – 8 days (Figure 3).

The results of current study are comparable with data presented by other authors. The published data by Hook et al. (2007) and Schofer et al. (2007) report slowdown of SR negativisation for about 30% of *luess secundaria* patients and in 60% of *luess latens praecox* patients, as well as failures of therapy found in 3% of patients. Psomas et al. (2012) describe total negativisation of SR in all *luess secundaria* patients treated with ceftriaxone in duration 5–6 months. According to their report, the regression of clinical signs of syphilis occurs more slowly, comparing with other literature data (Psomas et al., 2012; Hook et al., 2007; Schofer et al., 2007). Slower negativisation of SR was observed in one of three *luess secundaria* patients and in more than a half of *luess latens praecox* patients treated with ceftriaxone.

According to the data of our study, seroresistance was more often observed in *luess secundaria* and *luess latens praecox* cases. The data obtained are comparable with data available in literature. Taking into account that pharmacokinetic resources for treatment of syphilis, using ceftriaxone is satisfactory (French et al., 2009; Janier et al., 2014; Hartmane et al., 2004), the reasons of insufficient clinical efficacy of treatment in these cases are obviously inadequate choice of single dose and too short duration of treatment course (French et al., 2009).

Figure 3. Regression time of clinical signs of secondary syphilis



Conclusions

Conclusions were based on the data, obtained by summarising and analysing clinical information about 120 cases of early syphilis forms in patients treated with ceftriaxone, as well as performing analysis and comparison of available literature.

1. Relatively faster regression of clinical symptoms and more prominent negativisation of SR were stated in patients with earlier forms of syphilis.
2. Clinical-serological resistance was confirmed in 5.8% of patients with *luess secundaria* and *luess latens praecox.*
3. Serological relapse and resistance were confirmed in patients who clinically presented condylomata lata and who started to develop symptoms of alopecia.
4. Based on the results of this project, the possibility to increase dosage of ceftriaxone up to 1000 mg per day and to increase the duration of treatment course until 20 days in cases when late symptoms of syphilis appear should be considered to avoid the development of clinical seroresistance.

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Brachiocephalic Vessel Duplex Sonography in Rheumatoid Arthritis Patients

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Abstract

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease affecting 1% of general adult population. The systemic inflammation associated with novel risk factors such as disease activity, and seropositivity could contribute to accelerated atherosclerosis. The latter correlates with a risk of morbidity and mortality due to cardiovascular diseases (CVD).

Therefore, the article reflects on the examination of brachiocephalic vessels of rheumatoid arthritis and control group patients with duplex ultrasound and focuses on the methods that are used for screening subclinical and already proved atherosclerosis. 20 patients with confirmed RA and 27 sex and age matched healthy controls (aged 25–82) were recruited for prognostication and prediction of real cardiovascular risks for these patients. Carotid arteries haemodynamic parameters, elastic properties, IMT (intima media thickness) and plaques were measured using high resolution B-mode, M-mode and Doppler-mode ultrasound to calculate arterial wall distensibility and β stiffness indices, blood flow velocities, maximal IMT, and the size of atherosclerotic plaques. Correlations between brachial arterial blood pressure, carotid haemodynamic, wall elastic indices and patient's age, activity of disease, inflammation markers were calculated separately and in comparison with controls.

Our preliminary observations indicated that IMT dx (dextra) could be a reliable marker correlating with the disease activity of rheumatoid arthritis apart from a patient's age. Duration of the disease did not correlate with IMT, β stiffness parameters and carotid plaques. In our study carotid plaques of ACC (*a. carotis communis*) and ACI (*a. carotis interna*) in control group and RA group were age dependent, except plaques in *a. subclavia dx* that had correlation with seropositivity – ACPA (anti CCP autoantibody).

Keywords: duplex sonography, rheumatoid arthritis, cardiovascular disease.

Introduction

Recent evidence supporting an inflammatory basis for atherosclerosis (Kerekes, 2008) has led many investigators to study relationship between systemic inflammatory conditions such as RA and risk of cardiovascular diseases. Systemic inflammation represents a mechanistic link between risk factors and vascular dysfunction in both microvasculature and large vessel territories. Changes in microcirculation may be important predictors of CVD (cardiovascular disease).

Endothelial dysfunction has been recently described in patients with RA (Kerekes, 2008). Maintenance of vascular homeostasis is largely dependent on endothelial lining of blood vessels. Endothelia cells release vasoactive mediators (e.g., nitric oxide, endothelin-1) and express cell surface

molecules (e.g., leukocyte adhesion molecules) which influence the vascular tone, leukocyte adherence, platelet activation, coagulation, and smooth muscle proliferation (Vaudo, 2004). Altered function of the arterial endothelium is currently considered the earliest stage of development of atherosclerosis, and therefore it is recognised as a promoter of cardiovascular events. As a result, the study of endothelial function in clinical research has emerged as an important end point that complements the imaging techniques for structural arterial diseases burden (such as carotid intima media thickness).

Ultrasound measures the thickness of intimal and media layers combined, the carotid IMT. Measurement of the intima-media thickness (IMT) of the far wall of the common carotid artery by high-resolution ultrasonography has been established as a clinically useful index for identifying early-stage atherosclerosis (Baltgaile, 2012). Commonly used the IMT measurements become the marker of early stage of decreased elasticity or increased stiffness of arterial wall. The IMT and carotid artery stiffness turned out to be useful predictors of risk of cerebrovascular and cardiovascular events (Schachinger, 2000).

Since no precise direct measurement method for the determination of arterial wall elasticity or stiffness has been suggested, several indirect methods such as calculation of arterial compliance, Young's modulus of elasticity, β stiffness index and arterial distensibility are commonly used (Baltgaile, 2012).

Aim

The aim of this study was to investigate the changes in arterial wall elastic properties, β stiffness index, intima-media thickness (IMT) and plaque formation with relation to age, disease duration, disease activity and inflammation markers in patients with RA and control group in order to detect and estimate cardiovascular and cerebrovascular risk factors.

Material and Methods

20 patients with confirmed RA (13 women, 65%, and 7 men, 35%, mean age 50 ± 17 years, range 25–82; all Caucasian) and 27 sex and age matched healthy controls (aged 27–82) were recruited.

RA patients were selected from consecutive patients at the Outpatient Clinic of Rheumatology at Health Centre 4, who had been diagnosed according to the revised criteria 1987 of the American College of Rheumatology (Arnett, 1988).

The control group consisted of patients attending the same out-patient department for vascular ultrasound check-up without clinical symptoms of RA, vascular disease or any other systemic disease.

The demographic data, including sex, age and disease duration were recorded at the time of the study. Comparison between RA patients and controls accounted for CV risk factors through specification of enrolment criteria, matching, or statistical adjustment. For this reason patients with prominent arterial hypertension (> 150/80 mmHg), significantly elevated cholesterol markers – LDH (low density cholesterol) (> 3.5 mmol/l) and Triglyceride (1.5 mmol/l), obesity and any other systemic disease, smokers, patients with previous MI (myocardial infarction) and stroke – were excluded from the analysis. The same exclusion criteria were applied for the control group. Data from 20 patients with RA and 27 sex, age and risk factors matched controls were collected. Correlations between brachial arterial blood pressure, carotid haemodynamic, wall elastic indices and patient's age, activity of rheumatoid arthritis, inflammation markers were calculated separately and compared with controls.

Resting blood pressure in the right arm was measured with sphygmomanometer at the time of ultrasound examination, after at least 15 minutes of supine rest. Three measurements were taken every 5 minutes, and the average result was recorded. Laboratory variables relevant to RA activity as erythrocyte sedimentation rate (ESR), white blood cell count, platelet cell count, C-reactive protein (CRP), rheumatoid factor (RF), anti citrullinated peptide autoantibodies (Anti CCP) as well as serum levels of total cholesterol, triglycerides and high-density lipoprotein (HDL) cholesterol, LDL cholesterol were measured by routine methods. A written informed consent was obtained from all study participants.

Carotid Ultrasonography. All study participants underwent carotid ultrasonography, which was performed by experienced research sonographers. In brief, the participants were examined in supine position with slight hyperextension of the neck. Both extra cranial carotid arterial systems were extensively scanned in multiple planes to optimise the identification of atherosclerosis, which was defined as discrete plaque protruding into the lumen at least 50% beyond the diameter of the surrounding wall. Intima-media thickness was measured from end-diastolic (minimum dimension) M-mode images of the far wall of the distal common carotid artery. The intima-media thickness was not measured in the location containing plaque. Mean values of right and left intima-media thickness were presented. Reproducibility of intima-media thickness and detection of plaque has been well documented (Kanters, 1997). Brachiocephalic ultrasonography studies were performed in the control group before 2014, whereas the examination of the patients with RA were performed from 2012 to 2014.

Carotid arteries haemodynamic parameters, elastic properties were measured using high resolution B-mode, M-mode and Doppler-mode ultrasound to calculate arterial wall distensibility and stiffness indices, blood flow velocities (Golemati, 2003). Carotid distensibility measured as changes in the arterial diameter or circumferential area in systole and diastole is a reflection of mechanical stress affecting the arterial wall during the cardiac cycle (Baltgaile, 2012).

The distensibility can be calculated as Ds – Dd, where Ds is end-systolic diameter of artery, Dd is the end-diastolic diameter.

$$\text{Distensibility or Wall Strain} = \frac{Ds - Dd}{Dd}$$

Since the distensibility of arterial wall is mainly blood pressure and volume dependent, the systolic and diastolic pressure ratio is included in most calculations of the vessel's elastic properties (Baltgaile, 2012). The wall stress can be defined as the difference in systolic and diastolic blood pressure:

$$\text{Pulse pressure (PP)} = Ps - Pd$$

$$\text{The stiffness index is calculated as } \beta = \ln \frac{Ps}{Pd} \times \text{Strain}$$

Statistical Analysis. Descriptive data of normal variables are expressed as the mean ± SD. Statistical analysis was carried out by independent 2-tailed t-test. Correlations between variables were determined using Pearson correlation analysis for normally distributed values and Spearmen correlation analysis as nonparametric test. R values of these correlations were determined and corresponding p values < 0.05 were considered significant.

Results

Statistically, the control and RA group patients' age did not differ, T test $p = 0.20$, Levene's test $p = 0.13$.

Arterial wall elastic properties, β stiffness index, correlation with seropositivity inflammation markers and duration of RA, disease activity DAS28 (das28).

No statistically proved correlations were found between β stiffness parameters and the duration of disease ($r_s = 0.061$, $p = 0.797$), as well as DAS28, seropositivity and CRP.

There was no statistically significant difference between the control and RA groups for β stiffness parameters.

Arterial wall intima media thickness correlation with age, inflammation markers and duration of RA, disease activity (DAS28).

Patients with RA had mean values of IMT dx higher than the healthy subjects (± SD 1.02 ± 0.21 vs. 0.88 ± 0.22, $t = 2.45$, $p = 0.19$) but between both groups IMT sin had no differences (1.01 ± 0.28 vs. 0.89 ± 0.23, $t = 2.47$, $p = 0.18$).

No statistically proved correlations were found between the IMT and the duration of disease IMT dx (r_s = −0.145, p = 0.541) and IMT sin (r_s = −0.365, p = 0.114). IMT sin had no statistically proved correlations with inflammation markers (CRP) and disease activity (DAS28); however, IMT dx had weak correlation with DAS28 (disease activity) and IMT dx (r_s = 0.467, p = 0.038).

Statistically proved correlation was found between IMT dx et sin of the control group and RA group with age IMT sin (r_s = 0.524, p = 0.018), except IMT dx of RA patient group, which did not correlate with patient's age respectively (r_s = 0.206, p = 0.383).

Atherosclerotic plaques indicate correlation with age, duration of disease, seropositivity, disease activity and inflammation markers.

The presence of carotid plaques correlates with age in both groups, especially the plaques in ACI dx (r_s = 0.852, p < 0.001), ACC dx bifurcation (r_s = 0.706, p = 0.002), ACI sin (r_s = 0.654, p = 0.021) and ACC sin bifurcation (r_s = 0.625, p = 0.010).

No statistically proved correlations were found between the carotid plaques and the duration of disease, CRP and DAS28. Brachiocephalic vessel plaques did not correlate with seropositivity (RF, Anti CCP), except the plaques of arteria Subclavia dx, which had statistically significant correlation with Anti CCP (r_s = 0.715, p = 0.002).

Discussion

In our small patient study we measured the wall elasticity or β stiffness of ACC (*arteria carotis communis*), usually not very frequently done; moreover, it is time consuming as well. We kept in mind that most similar researches of the arterial wall distensibility and elasticity have been performed on brachial arteries, but the recording of pressure ratio during the cardiac cycle in a brachial artery can provide only indirect information on pressure/strain ratio in carotid artery. Calculations of FMD (flow mediated dilatation), PWV (pulse wave velocity), and other stiffness parameters cannot be attributed to carotid artery properties only because brachial, femoral, aortic and internal carotid arterial segments differ in the proportion of elastin-collagen to smooth muscle as well as proportion of endothelium to media layer and neural control (Baltgaile, 2012).

Considering this argument, it seems logical to evaluate the carotid artery wall dynamics by ultrasound measurements of the arterial wall structure and movements in a strictly precised vascular area (Baltgaile, 2012).

A decrease of arterial distensibility (i.e. increase of arterial wall stiffness) seems to be a common pathological mechanism for many factors associated with cerebrovascular and cardiovascular diseases. The factors affecting the arterial wall motions depend mainly on the left ventricle, intra-arterial pressure and blood volume, endothelium function, smooth muscle tone and neural control mechanisms. Good reproducibility of carotid arteries diameters measured by 2D grayscale imaging, M-mode and A-mode (wall tracking) is proved (Baltgaile, 2012). However, it is also mentioned that very small changes in linear measurements of carotid diameters can have big effects.

In our study no statistically proved correlations between β stiffness parameters and duration of disease were found (r_s = 0.061, p = 0.797). Whereas the duration of the disease of RA for young people can be a couple of months to several years and, on the contrary, for older patients for some months. We calculated β stiffness by the formula described above, which includes logarithm of pulse pressure and strain. No statistically proved correlations were detected between β stiffness parameters DAS28, seropositivity and CRP. DAS28 and CRP are dependent on several IL (interleukin) releases that change all the time and are inconstant, as well as the autoantibody production that can be and cannot be present for every RA patient. In the study of Szekanecz et al., anti CCP production showed an insignificant association with more characteristic endothelial dysfunction (Kerekes, 2008).

Furthermore, the stiffness parameters of arterial wall in RA patients did not differ from the same indices of healthy controls. There was no statistically significant difference between control and RA groups for β stiffness parameters. It just means we had quite homogenous groups which did not show much difference in pulse pressure and wall strain on both sides. Another possibility is that β stiffness index is

not a very sensitive marker for small groups to show statistically significant differences for autoimmune and control group patients. Elasticity and β stiffness index mostly depends on the process of aging that impacts the degenerative process of elastic fibres, promoting fibrotic/sclerotic transformation of vessel walls. It approves that our small study group was more age dependent.

Our study is also a subject to potential limitations because of a small number of participants we could analyse, remembering that the decrease of stiffness and elasticity is found to people with atherosclerosis inducing factors (primary arterial hypertension, cholesterol, diabetes mellitus, hypertrophy of left ventricle). Therefore, we could not recruit many control and RA patients, taking into account all the risk factors mentioned above which increase with age and are widespread among people in Latvia. Prolonging the observation time to include more participants would likely increase the statistical power, allowing us to detect weaker associations that did not reach significance in the present analysis.

It is supposed that in rheumatic diseases ccIMT has become the most commonly used indicator of subclinical atherosclerosis. Numerous cross-sectional studies have demonstrated increased ccIMT in patients with RA, indicating accelerated atherosclerosis (Kerekes, 2012). Therefore, early determination of ccIMT in RA patients may be useful to assess patients with high cardiovascular and cerebrovascular risk.

Early carotid arterial wall disease is a useful predictor of risk of both ischemic stroke and coronary heart disease in asymptomatic population as well. However, ultrasound measures the thickness of intimal and media layers combined and the carotid IMT. It is important because the media layer is sensitive to increased pressure in the lumen, responding by hypertrophy (Baltgaile, 2012). Thus, the thickened IMT in the presence of hypertension may not reflect the presence of atheromatous plaque. That reduces the number of patients we could include, keeping in mind that older patients more frequently suffer from arterial hypertension, hypercholesterolemia. Furthermore, inhabitants of Latvia suffer from increased arterial hypertension and hypercholesterolemia.

No statistically proved correlations were found between IMT and duration of disease as well. In the study by Szekanecz et al. ccIMT (common carotid artery IMT) also correlated with age. What is more, in Szekanecz et al., study seropositivity (RF, anti CCP) and inflammatory markers as CRP showed an insignificant association with more pronounced atherosclerosis and endothelial dysfunction (Kerekes, 2008).

Current guidelines recommend the assessment only of the common carotid segment. Moreover, the assessment of ccIMT is considered to be a quantitative approach, but this technique does not allow the fine ultrastructural analysis of the whole extra cranial carotid system (Kerekes, 2012). In a comparative study, the ccIMT was reported to have only a moderate sensitivity in predicting in the future cardiovascular events. The presence of plaque in the carotid system or the determination of total plaque area are considered to be better predictors for future myocardial and cerebrovascular events than ccIMT (Kerekes, 2012).

Therefore, we examined brachiocephalic vessels, not only the branches of arteria carotis, which are mainly investigated with ultrasound and found something unexpected beside the things we could prognosticate like carotid plaque correlation with age. Plaques of *arteria subclavia dx*, had statistically significant correlation with anti CCP ($r_s = 0.715$, $p = 0.002$), with one of autoantibodies which plays a crucial role in the pathogenesis and progression of RA (Kerekes, 2008).

The presence of carotid plaques correlates with age in both groups especially the plaques in ACI dx ($r_s = 0.852$, $p < 0.001$), ACC dx bifurcation ($r_s = 0.706$, $p = 0.002$), ACI sin ($r_s = 0.654$, $p = 0.021$) and ACC sin bifurcation ($r_s = 0.625$, $p = 0.010$).

Statistically unproved correlations were found between carotid plaques and duration of disease, CRP and DAS28. Brachiocephalic vessel plaques did not correlate with seropositivity (RF, anti CCP), except plaques in *a. subclavia dx* that had correlation with seropositivity – ACPA (anti CCP autoantibody).

Conclusion

The past decade has experienced the emergence of two new paradigms in inflammatory disease: first, cardiovascular complications of atherosclerosis have markedly increased in patients with rheumatoid arthritis (RA) and second, inflammatory mechanisms are important in the pathogenesis of atherosclerosis (Shoenfeld, 2005). Therefore, there is a vital necessity to determine atherosclerosis at early

stages (Snow, 2005). Several non-invasive screening methods have been developed as a measurement of intima-media thickness (IMT) and carotid artery stiffness that are thought to be useful predictors of risk of cerebrovascular and cardiovascular events (Kumeda, 2002). The different parameters of carotid artery wall elasticity could be measured by high resolution B-mode and M-mode ultrasound using manual and automatic measurements as well as wall echo-tracking system (Baltgaile, 2012). The development of methods based on ultrasound RF signal, tissue Doppler imaging and other tracking systems help to increase the accuracy of automatic measurement of vascular wall properties such as IMT, arterial stiffness / distensibility and wall compliance, although even these methods are not absolutely perfect (Baltgaile, 2012). Good reproducibility of carotid arteries diameters measured by 2D grayscale imaging, M-mode and A-mode (wall tracking) has been proved. However, it is also mentioned that very small changes in linear measurements of carotid diameters can have big effects on estimates of arterial mechanical properties such as strain and Young's modulus (Baltgaile, 2012).

Our preliminary observations indicate that IMT dx could be a reliable marker correlating with disease activity of rheumatoid arthritis apart from a patient's age. The duration of disease does not correlate with the IMT, β stiffness parameters, and carotid plaques. In our study the carotid plaques of ACC and ACI in the control group and RA group were age dependent, except the plaques in *a. subclavia dx* which had correlation with seropositivity – ACPA (anti CCP autoantibody). In future we should pay attention and examine all brachiocephalic vessels not only the branches of *a. carotis* which are the most investigated ones, to reveal as much as we can about current patients atherosclerosis process. It could give us additional information of the whole atherosclerotic burden.

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Prevalence of Actinic Keratosis and Its Associated Risk Factors in Elderly Latvian People

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Abstract

Actinic keratosis (AK) is a frequent skin lesion that affects millions of patients worldwide and is becoming the most common *in situ* carcinoma in humans. Independent risk factors for AKs include older age, male gender, cumulative sun exposure, artificial UV radiation and Fitzpatrick skin types I and II. According to the World Health Organisation (WHO), the estimated prevalence by latitudinal band in Latvia could be 5–8% in men and 2.5–4% in women older than 60 years. The aim of this study was to determine the prevalence of actinic keratosis and its associated risk factors among elderly Latvian people. To achieve the aim, questionnaires from skin cancer screening campaign were gathered and analysed. Only patients above the age of 60 that had received full body examination were included in this study.

Actinic keratoses were diagnosed in 51.8% of study participants, with higher, but not statistically significant prevalence in men (62.5% *vs.* 47.4%, $p = 0.057$). The prevalence of UV related risk factors was high – 70.2% of participants had skin phototype I or II, almost half of the participants (46.6%) had had severe sunburns before the age of 18, 21.6% had had an outdoor job, 77.4% had spent more than two weeks per year relaxing in the sun, while only 6.9% of the participants always used sunscreens while sunbathing. The study also showed that actinic keratoses were more prevalent among participants that had had severe sunburns before the age of 18 (64.0% *vs.* 41.2%; $p < 0.01$) and among participants that had spent more than two weeks per year relaxing in the sun (56.5% *vs.* 37.2%; $p = 0.026$).

This study suggests that prevalence of actinic keratosis might be higher in Latvian population than previously expected and highlights the necessity to protect skin from UV radiation in order to diminish the prevalence of AK.

Keywords: actinic keratosis, skin cancer, UV radiation.

Introduction

Actinic keratoses (AK) are keratotic lesions occurring on chronically light-exposed adult skin (Berker, 2007). Historically AK have been defined as “precancerous” or “premalignant”, but more recent histopathologic and molecular studies support their current classification as the earliest stage of squamous cell carcinoma *in situ* (Röwert-Huber, 2007; Zalaudek, 2014; Ferrándiz, 2013). European guidelines of this disease concluded that AK are increasing in prevalence; it affects millions of patients worldwide and is becoming the most common *in situ* carcinoma in humans (Ferrándiz, 2013). Despite the fact that they are

frequent, they are not well recognised by public (MacKie, 2004). Independent risk factors for AKs include older age, male gender, cumulative sun exposure, artificial UV radiation and Fitzpatrick skin types I and II (Rosen, 2013, Ferrándiz, 2013). Recent study also indicates baldness in men as the strongest risk factor for the development of AKs (Flohil, 2013). There are not many prevalence studies in Europe especially in elderly population. The prevalence of AK in Latvian population has not been previously studied. According to the World Health Organisation (WHO), the estimated prevalence by latitudinal band in Latvia could be 5–8% in men and 2.5–4% in women older than 60 years (Lucas, 2006).

Aim

The aim of this study was to determine the prevalence of actinic keratosis and its associated risk factors among elderly Latvian people.

Material and Methods

To achieve the aim, questionnaires from skin cancer screening campaign, which took place from September 9 to October 6, 2013, were gathered and analysed. During this campaign doctors from nine cities representing different Latvian regions screened voluntary patients for all types of skin lesions and asked questions about previous sun exposure and sun protection habits. Only patients above the age of 60 that had received full body examination were included in this study. All questions were asked and skin examinations performed in health care centres in a single visit, and dermatoscopy was used to confirm the diagnosis. Questionnaires were filled with the following data: patient's gender, age, skin Fitzpatrick phototype and lifetime sun exposure. The later included such questions as whether the person had ever had an outdoor job; whether they had ever spent more than a year in a country with a higher UV index than Latvia; whether they had ever had severe sunburns before the age of 18 (sunburns with painful blisters or erythema lasting for more than 2 days); whether they used sunscreens while being in the sun for more than an hour and weather they used them while sunbathing; whether they had ever used a sunbed and what the average amount of weeks they spent in sun every year had been. Whenever possible it was asked to estimate the amount. Oral consent was obtained from all patients.

Statistical associations between the presence of actinic keratoses and risk factors were tested with Pearson's chi-square test, Fisher's Exact Test or Mann–Whitney U test. Results are described as numbers and percentages for categorical variables, means ± standard deviations for continuous variables. The level of significance was set at 0.05. All analyses were performed using IBM SPSS 20.0.

The study was approved by the Ethics Committee of Rīga Stradiņš University (permit issued on 28.11.2013.).

Results

A total number of 191 patients were included in this study. Female accounted for 70.7%, while male accounted for 29.3%. Their age ranged from 60 to 94 years (mean ± standard deviation: 74.0 ± 7.7 years).

Actinic keratoses were diagnosed in 51.8% of study participants, with higher, but not statistically significant prevalence in men (62.5% *vs.* 47.4%; p = 0.057, Pearson's chi-squared test). Prevalence of AKs rose with age in both genders (Figure 1) ranging from 29.5% in 60 to 69 year-old group, to 57.9% in 70–79 year-olds, to 68.5% in study participants more than 80 years old.

This study showed a high prevalence of most risk factors – 70.2% of all participants had skin phototype I or II, 21.6% had had an outdoor job, 46.6% had had severe sunburns before the age of 18, 10.6% had lived in a country with a higher average UV index than Latvia for more than a year, 77.4% had spent more than two weeks per year relaxing in the sun, 12.6% had a history of skin cancer and only 6.9% always used sunscreens while sunbathing and 2.6% while being outdoors for more than an hour. The least prevalent risk factor was use of sunbeds – only 5.8% of participants acknowledged ever using them.

In the group of participants that had had severe sunburns before being 18 years old, 64.0% had actinic keratoses, in comparison with 41.2% of those that had not had such sunburns. This association was statistically significant ($p < 0.01$, Pearson's chi-squared test). Severe sunburns before the age of 18 increased the risk of AK 2.55 fold (OR = 2.545, 95% CI: 1.417–4.570). In the group of participants that had had one to two severe sunburns before the age of 18, 42.9% had actinic keratosis in comparison with 78.2% of participants that had had three or more severe sunburns before the age of 18 ($p < 0.01$, Pearson's chi-squared test). The risk of actinic keratoses was 4.78 times higher in participants that had had 3 or more sunburns before the age of 18 in comparison with those that had fewer sunburns (OR = 4.778, 95% CI: 1.785–12.790).

Actinic keratoses were detected in 56.5% of participants that had spent more than two weeks per year relaxing in the sun and in 37.2% of participants that had spent less than that. This difference was statistically significant ($p = 0.026$, Pearson's chi-squared test). More than two weeks per year relaxing in the sun resulted in a twofold increased risk of AK (OR = 2.188, 95% CI: 1.088–4.403).

Skin phototype, outdoor job, more than a year spent in a country with a higher UV index than Latvia, use of sunscreens, use of sunbeds and skin cancer history was not significantly associated with increased risk for actinic keratoses. Associations between patient and UV risk factors and presence of AK are presented in Table 1.

Figure 1. Prevalence of actinic keratosis stratified by age group and gender

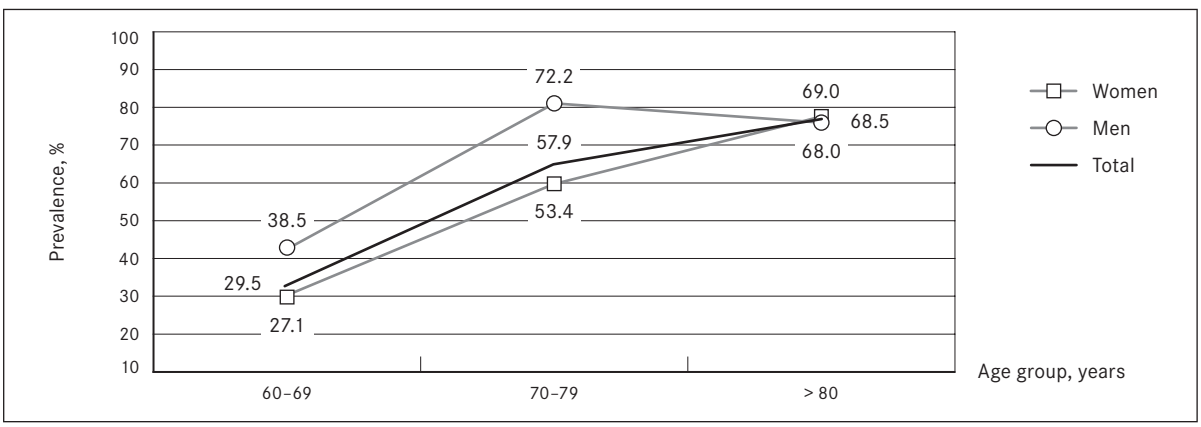

Table 1. Prevalence of AK according to patient and UV risk factors

Risk factor	From total study population, N (%)	With AK, n (%)	p-value	OR	95% CI
1	2	3	4	5	6
Gender					
Men	56 (29.3)	35 (62.5)	0.057	0.541	0.286–1.023
Women	135 (70.7)	64 (47.4)			
Skin phototype (in comparison to "phototype I")					
I	21 (11.0)	12 (57.1)	–	–	–
II	113 (59.2)	58 (51.3)	0.624	0.791	0.309–2.024
III	57 (29.8)	29 (50.9)	0.623	0.777	0.283–2.129
Outdoor job (in comparison to "no outdoor job")					
Total	41 (21.6)	17 (41.5)	0.123	0.579	0.287–1.166
< 1 year	4 (2.1)	2 (50.0)	1.000	0.817	0.112–5.956
2–5 years	11 (5.8)	4 (36.4)	0.348	0.467	0.131–1.663
6–10 years	6 (3.2)	4 (66.7)	0.693	1.634	0.290–9.197
> 10 years	20 (10.5)	7 (35.0)	0.092	0.440	0.166–1.165

(continued)

Table 1 (continued)

1	2	3	4	5	6
Severe sunburns before the age of 18					
	89 (46.6)	57 (64.0)	0.002*	2.545	1.417–4.570
Severe sunburns before the age of 18 ("1–2 times" in comparison to "3 or more times")					
1–2 times	28 (33.7)	12 (42.9)	0.001*	4.778	1.785–12.790
3 or more	55 (66.3)	43 (78.2)			
Sunscreen use while being outdoors > 1 hour (in comparison to "never")					
Always	5 (2.6)	3 (60.0)	1.000	1.319	0.214–8.115
Occasionally	29 (15.2)	13 (44.8)	0.407	0.715	0.322–1.585
Never	156 (81.7)	83 (53.2)	–	–	–
Sunscreen use while sunbathing (in comparison to "never")					
Always	13 (6.9)	5 (38.5)	0.387	0.543	0.169–1.751
Occasionally	47 (24.9)	24 (51.1)	0.776	0.907	0.465–1.770
Never	129 (68.3)	69 (53.5)	–	–	–
Residence in a country with a higher average UV index than Latvia					
	20 (10.6)	13 (65.0)	0.213	1.835	0.698–4.827
More than two weeks per year relaxing in the sun (in comparison to "less")					
	147 (77.4)	83 (56.5)	0.026*	2.188	1.088–4.403
Sunbed use					
	11 (5.8)	5 (45.5)	0.662	0.762	0.224–2.587
Skin cancer history					
	24 (12.6)	13 (54.2)	0.786	1.126	0.477–2.658

* Statistically significant.

Discussion

In this study we examined the prevalence of AK, individual and UV related risk factors and their associations in elderly population of Latvian. We found that more than a half (51.8%) of individuals after the age of 60 had one or more AK, with greater prevalence in men (62.5% vs. 47.4%). This result is much higher than expected and estimated by the WHO (Lucas, 2006) but the fact that study population consisted of participants in a skin cancer screening campaign has to be taken in consideration. The recently reported prevalence of actinic keratoses in other European countries is lower – ranging from 49% in men and 28.1% in women in Rotterdam study (age from 51 year) to 16.36% in men and 6.29% in women in study on German employees (age group 61–70 years) (Flohil, 2013; Schaefer, 2014). The greater prevalence in men is in accordance with other studies (Traianou, 2012).

This study also indicates severe sunburns with blister formation or erythema lasting for more than two days in the age group of younger than 18 years old and those spending more than two weeks per year relaxing in the sun, which account as two major risk factors for AK development. It also shows that risk of AK increases with the increasing number of such sunburns. The association between sunburns in childhood and AK is in accordance with previous studies (Frost, 1998; Kennedy, 2003; Traianou, 2012). Spending more than two weeks per year relaxing in the sun is a marker of high cumulative UV exposure that is considered the primary cause of AK as absorption of both ultraviolet A and B radiation produces disruptions in intracellular signalling, cytokine regulation, and protective apoptotic mechanisms (Berman, 2013). At the same time other markers of high cumulative UV exposure are outdoor job and time spent in countries with high UV index, but this study did not show an association between those and prevalence

of AK. It is in accordance with Rotterdam study that also found no such associations (apart from history of living in a sunny country for more than a year that had a protective effect on the development of 4 to 9 AK) (Flohil, 2013). At the same time in a study by Traianou et al., an outdoor occupation resulted in a fourfold increase of the risk for AK, and a residency in tropical countries resulted in more than threefold increase, if more than 10 years were spent (Traianou, 2012).

An association between skin type and presence of actinic keratoses was not found, although it has been shown in other studies (Traianou, 2012; Schaefer, 2014). The association was expected, but the difference might be explained by the fact that darker skin types (IV to VI) are rare and almost exclusively the first three phototypes are present in Latvian population and participants of this study.

This study also did not show an association between use of sunbeds and AK. Moreover, it showed that use of sunbeds is not prevalent among elderly population of Latvia and thus might not be a risk factor.

The results show that very few elderly people use sunscreens while being in the sun for more than an hour and even more surprising – while sunbathing. This together with the great prevalence of sunburns before the age of 18 suggests an inadequate protection from solar UV radiation and importance of information and education on sun-protection measures. The results from randomised controlled trials suggest that regular use of sunscreens prevents the development of AK and hastens the remission of existing lesions (Thompson, 1993; Werner, 2013; Iannacone, 2014). In this study sunscreen usage showed no protective effect on AK. Some other studies where sunscreen usage was low also did not show such effect (Naldi, 2006).

This study did not find an association between skin cancer history and presence of AK. Most of the cancers in patients' histories were basal cell carcinomas (91.7%), and other studies have found association (Naldi, 2006; Kaskel, 2015) and markedly higher risk of basal cell carcinomas in patients with AK (Khalesi, 2013). The reason why the association was expected is that ultraviolet radiation is the major etiologic agent in the pathogenesis of both basal cell carcinoma and actinic keratosis (Khalesi, 2013; Kennedy, 2003). Some of the reasons why the association was not found could be that patients with skin cancer history would most likely be interested in participation in a skin cancer screening campaign and that cancer history was asked in general without specifying the type, although several types of basal cell carcinoma are known and localisation.

This study has certain limitations. First, the population was not randomly selected. The screening was announced in mass media and everyone interested could participate and most often people with great amount of different skin lesions apply for such campaigns. Then, the majority of patients were female (70.7% *vs.* 29.3%), but the values are close to those provided by Central Statistical Bureau of Latvia on Latvian population distribution by age and gender – in year 2013, from total amount of permanent residents after the age of 60, 65.3% were female and 34.7% were male (Central Statistical Bureau of Latvia).

Conclusions

This study shows that the prevalence of actinic keratosis might be higher in Latvian population than previously expected. It also highlights severe sunburns before the age of 18 and more than two weeks per year spent relaxing in the sun as two most important risk factors for AK development. In relation to protection from UV radiation, this study indicates a very low sunscreen usage among elderly population of Latvia that might be one of the reasons for high prevalence of AK.

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Prospective Evaluation of Dexmedetomidine Sedation during Regional Anaesthesia and Correlation with Electroencephalogram Index and Clinical Effects

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Abstract

To evaluate dexmedetomidine sedation during regional anaesthesia and correlation with electroencephalogram index, clinical effects and the time and quality after awakening.

Prospective study involved 32 ASA I–II patients undergoing reconstructive surgeries and sedation with dexmedetomidine during regional anaesthesia. The loading dose of dexmedetomidine was 1 µg/kg over 10 min followed by infusion of 0.1–0.6 µg/kg/h. Depth of sedation was measured with electroencephalogram index. Time and quality of awakening were evaluated.

24/32 patients had a *plexus brachialis* block for hand reconstructive surgeries, 5/32 had spinal anaesthesia (SA) for leg reconstructive surgeries, 3/32 – SA and *plexus brachialis* block for free flap microvascular surgery. The mean duration of surgery was 89.38 ± 67.46 min, sedation – 102.81 ± 67.52 min.

After dexmedetomidine loading dose HR decreased by 8.44 ± 7.16 ×/min ($p = 0.000$), SBP decreased by 7.31 ± 12.03 mmHg ($p = 0.002$), DBP decreased by 4.75 ± 7.15 mmHg ($p = 0.001$). 2/32 patients had a bradycardia below 50 ×/min requiring a single dose of atropine, 5/32 had a temporary bradycardia that does not require treatment and in 25/32 cases sedation did not cause bradycardia. After loading dose SpO_2 decreased by 1.28 ± 2.37% ($p = 0.005$) and all patients had adequate spontaneous breathing without the need to use assisted ventilation or any airway device during sedation.

At the end of surgery all patients were promptly arousable with verbal stimulation without impaired cognitive abilities and psychomotor functions.

Loading dose of dexmedetomidine of 1 µg/kg intravenously over 10 min and a continuous infusion of 0.1–0.6 µg/kg/h provides safe management of sedation during regional anaesthesia under reconstructive surgery and correlates with electroencephalogram index of 50–70, provides fast and good quality of awakening and ensures a high patient satisfaction rate of sleep quality.

Keywords: dexmedetomidine, regional anaesthesia, sedation.

Introduction

Results of surgery under regional anaesthesia can be affected by fear and anxiety of a patient and discomfort from lying on the operating table [9]. In order to reduce a patient's stress of being awake during the surgery under regional anaesthesia and to increase surgeon's and anaesthetist's satisfaction of the surgery, sedation is widely used [5, 9, 15, 17]. For surgeries under regional anaesthesia midazolam and propofol are the most common sedatives [2, 11, 17].

Dexmedetomidine is a selective α-2 receptor agonist with an anxiolytic, sedative and analgesic effect, and is not associated with respiratory depression [1, 2, 11, 17, 21, 25]. Compared to sedatives we have used so far, dexmedetomidine causes the "natural sleep" through inhibition of neuronal firing in the locus coeruleus in the brain stem which means a patient is easily arousable on verbal stimulation without impaired cognitive abilities and psychomotor functions [1, 7, 8, 16, 17, 18]. Dose dependent bradycardia and hypotension are the most frequently reported adverse reactions of dexmedetomidine [4, 6, 11, 17, 18, 21, 25].

Aim

The aim of this study was to evaluate dexmedetomidine sedation during regional anaesthesia and correlation with electroencephalogram index, clinical effects and time and quality after awakening.

Material and Methods

32 ASA I–II patients over the age of 18 scheduled for reconstructive surgeries under regional anaesthesia (RA) with dexmedetomidine sedation were enrolled in a prospective study, after an ethical committee's approval and receiving written consent from all patients.

The following exclusion criteria were used:

- second or third degree heart block;
- bradycardia and arrhythmia;
- uncontrolled hypotension;
- mechanical ventilation;
- history of sleep apnea;
- liver failure;
- acute cerebrovascular accident;
- psychiatric disorder or currently being on psychotropic medication;
- pregnancy;
- coagulation disorders.

Anaesthesia methods. All patients received premedication with midazolam 7.5 mg before surgery.

For axillary brachial plexus blockade 20 ml 0.5% bupivacaine and 20 ml 1% lidocaine was used for hand reconstructive surgeries. For spinal anaesthesia (SA) 4 ml 0.5% levobupivacaine was used for leg reconstructive surgeries. Axillary brachial plexus blockade and spinal anaesthesia was done for free flap microvascular surgeries not exceeding the maximum recommended doses of total local anaesthetics.

Sedation method. After confirmation of successful regional anaesthesia, loading dose of dexmedetomidine 1 μg/kg over 10 min was administered IV followed by a continuous infusion of 0.1–0.6 μg/kg/h until the end of the surgery. The continuous infusion of dexmedetomidine was adapted by EEG index maintaining a definite target EEG index of 50–70 (complies with EEG stage C_2–D_1) providing an efficient sedation during surgery.

Monitoring. Standard monitoring was used – non-invasive systolic (SBP) and diastolic (DBP) blood pressure, heart rate (HR), respiratory rate (RR), peripheral oxygen saturation (SpO_2).

The respiratory depression was defined as oxygen saturation < 90% or RR under 12 breaths/min. HR < 50 beats/min for more than 5 minutes was considered to be bradycardia and patients received a solution of 0.5 mg atropine IV.

EEG monitoring with EEG monitor Narcotrend-Compact M was used during sedation. It reflects the depth of sedation by identifying brain waves and therefore regulates sedative dosage [3, 10]. After regional anaesthesia was performed, three self-adhesive disposable electrodes were placed on the forehead using electrode gel, the patient's leads were connected with Narcotrend-Compact M monitor and EEG recording was started to monitor the depth of sedation or hypnotic status of the patient during sedation. The monitor automatically classified EEG stages on a scale from stage A (conscious) to stage F (very deep sedation), this division refers explicitly to a range of EEG indexes: EEG stage A – awake (EEG index 95–100); EEG stage B, C – light sedation (EEG index 65–94); EEG stage D – moderate sedation (EEG index 37–64); EEG stage E, F – deep sedation (EEG index < 36) [3, 13].

Richmond Agitation Sedation Scale (RASS) was used to measure the level of sedation of the patients before and after loading dose and every 20 minutes until the end of surgery. Sedation was considered too deep when RASS was −4 or −5 (Table 1) [19].

Table 1. Richmond Agitation Sedation Scale

Score	Term	Description
+4	Combative	Overtly combative or violent, immediate danger to staff
+3	Very agitated	Pulls on or removes tubes or catheters or has aggressive behaviour towards staff
+2	Agitated	Frequent non-purposeful movements
+1	Restless	Anxious or apprehensive but movements not aggressive or vigorous
0	Alert and calm	–
−1	Drowsy	Not fully alert, but has sustained (more than 10 seconds) awakening, with eye contact/eye opening to voice
−2	Light sedation	Briefly (less than 10 seconds) awakens with eye contact to voice
−3	Moderate sedation	Any movement (but no eye contact) to voice
−4	Deep sedation	No response to voice, but any movement to physical stimulation
−5	Unarousable	No response to voice or physical stimulation

Time and quality of awakening were evaluated at the end of each surgery. In recovery room at 30 minutes patient's satisfaction with the quality of sleep was assessed by the use of handed out questionnaires.

Statistical analysis was performed with Microsoft Excel 2010 and SPSS (Statistical package for social sciences) 20. Data were evaluated with ANOVA (Analysis of variance) and Student's t-test. Results with p values of < 0.05 were considered statistically significant.

Results

Demographic data and surgical characteristics were similar in all patients (Table 2). Types of RA used: 24/32 had an axillary brachial plexus blockade (75.0%) for reconstructive surgeries in hand, 5/32 had a spinal anaesthesia (15.6%) for reconstructive surgeries in leg, 3/32 – SA with plexus blockade (9.4%) for free flap microvascular surgery.

Mean HR during sedation was 62.86 ± 7.90 beats/min. After dexmedetomidine loading dose, mean HR decreased by 8.44 ± 7.16 beats/min (p = 0.000) (before loading dose 73.75 ± 10.34 beats/min) (Table 3). We observed bradycardia below 50 beats/min requiring a single minimum dose of atropine in 2/32 patients (6.3%), 5/32 patients (15.6%) had a temporary bradycardia that does not require treatment and in 25/32 cases (78.1%) sedation with dexmedetomidine did not cause bradycardia (Figure 1). Loading dose bradycardia did not appear in any of the patients while EEG index was 20–80 (Figure 2).

Two patients with bradycardia requiring atropine had a Narcotrend EEG stage A – awake (EEG index of 95–100). Out of those two patients – one patient was a 28-year-old professional athlete, he had bradycardia during loading dose when EEG index was 98, the other patient was a 71-year-old man who had an acute surgery and bradycardia occurred 20 minutes after the start of continuous infusion at EEG index 96.

When comparing all types of RA used, HR was similar in all three RA groups after the loading dose (Figure 3).

Table 2. Demographic data and surgical characteristics

Gender, female/male, n (%)	14 (43.8 %) female	18 (56.2 %) male
Age, years	46.44 ± 16.88 (20 to 74)	
Weight, kg	75.00 ± 14.11 (50 and 120)	
Height, cm	172.85 ± 8.31 (163 and 185)	
Type of surgery	28 (87.5%) elective	4 (12.5%) acute
Duration of surgery, min	89.38 ± 67.46 (minimum 20, maximum 300)	
Duration of sedation, min	102.81 ± 67.52 (minimum 35, maximum 310)	

Table 3. Changes in heart rate (HR) during sedation

Heart rate	SM*	SSD**	Number of patients, n
HR before loading dose, b/min	**73.75**	10.336	32
HR after loading dose, b/min	**65.31**	9.107	32
HR 10 min after SCI***, b/min	63.78	6.568	32
HR 20 min after SCI, b/min	62.53	8.320	32
HR 30 min after SCI, b/min	61.39	7.013	31
HR 40 min after SCI, b/min	60.36	7.395	28
HR 50 min after SCI, b/min	59.62	7.180	21
HR 60 min after SCI, b/min	58.06	7.198	17
HR 1.5 h after SCI, b/min	58.33	8.818	9
HR 2 h after SCI, b/min	55.50	8.018	8
HR at the end of surgery, b/min	**60.34**	7.065	32

* SM – statistic mean;
** SSD – standard statistic deviation;
*** SCI – start of continuous infusion.

Figure 1. Incidence (%) of bradycardia during sedation

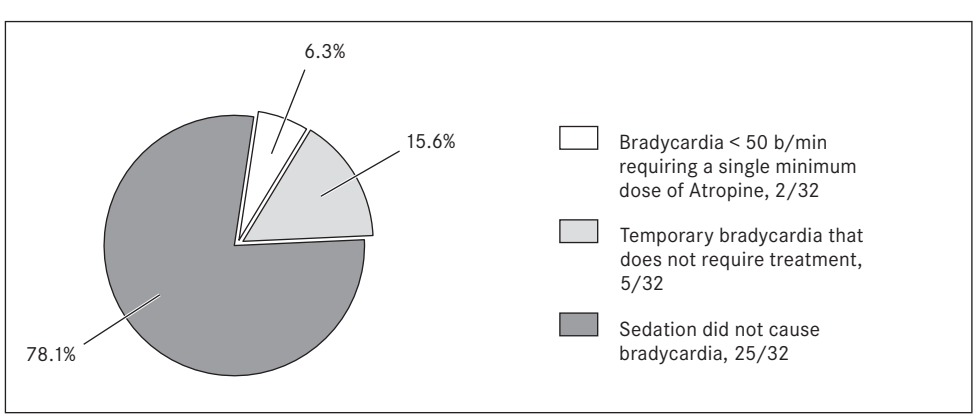
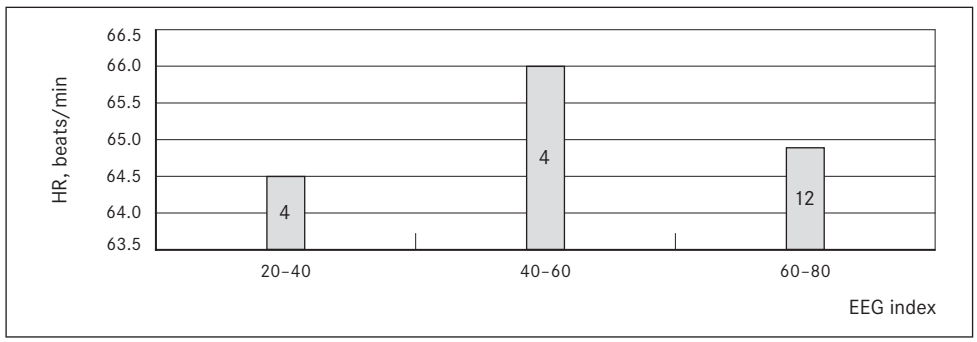
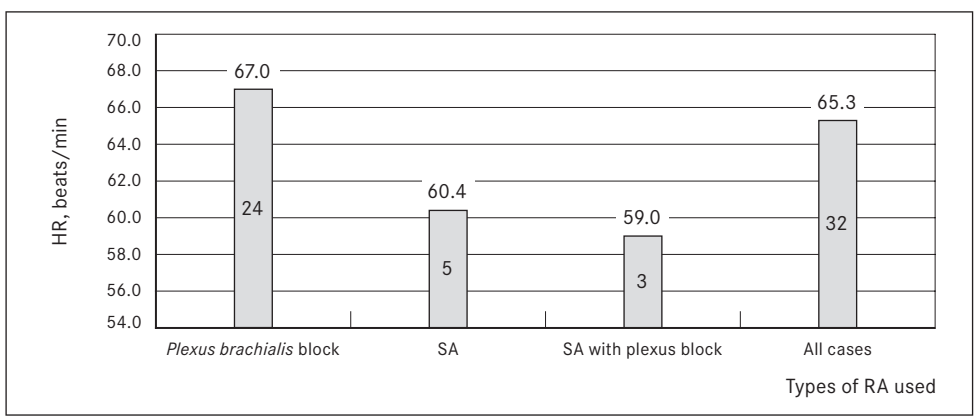


Figure 2. Changes of heart rate (HR) after loading dose according to electroencephalogram (EEG) index (20–80)*



* The number printed on bars indicates the number of patients in current group of EEG index.

Figure 3. Changes of heart rate (HR) after loading dose according to types of regional anaesthesia (RA) used*



* The number printed on bars indicates the number of patients in current group of RA used and the mean value of HR.

We did not observe any significant changes in SBP and DBP after dexmedetomidine loading dose was administered – mean SBP decreased by 7.31 ± 12.03 mmHg (p = 0.002) and mean DBP decreased by 4.75 ± 7.15 mmHg (p = 0.001). Mean SBP during the sedation was 119.39 ± 15.16 mmHg, mean DBP was 71.99 ± 9.83 mmHg (Table 4). Results showed that sedation with dexmedetomidine caused neither of the following in any of the patients: hypotensia, the need to stop the continuous infusion or add other sedatives.

We observed minimal decrease in SpO_2 levels after the loading dose (1.28 ± 2.37%, p = 0.005) without the need to use assisted ventilation or any airway device. All patients had adequate spontaneous breathing during their sedation.

Mean EEG index after loading dose decreased by 19.72 ± 23.85 (p = 0.000) indicating light to moderate sedation (Figure 4). During dexmedetomidine sedation, mean EEG index was 68.53 ± 21.70 which was within the target EEG index rage. The target level of sedation was reached 10 minutes after the start of continuous infusion. The mean lowest recorded EEG index was 53.10 ± 25.00 after a continuous infusion of 30 minutes. The environment had a significant negative impact to the quality of dexmedetomidine sedation. Increased noise levels rose EEG index during surgery; therefore, patients woke up. However, those patients were able to quickly fall back asleep. According to RASS, the level of sedation during surgery was from 0 to –3. We observed that at the end of surgery, all patients were promptly arousable with verbal stimulation without impaired cognitive abilities and psychomotor functions. According to answers from their questionnaires all patients were satisfied with the sedation they received.

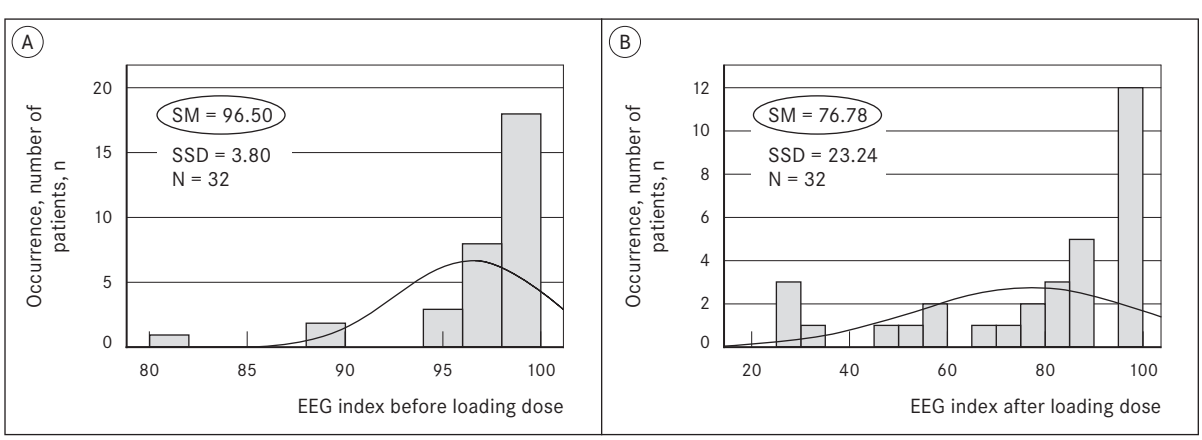
Table 4. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) values during sedation

Systolic blood pressure	SM*	Number of patients, n
SBP before loading dose, mmHg	**136.06**	32
SBP after loading dose, mmHg	**128.75**	32
SBP 10 min after SCI**, mmHg	123.22	32
SBP 20 min after SCI, mmHg	116.22	32
SBP 30 min after SCI, mmHg	114.68	31
SBP 40 min after SCI, mmHg	114.75	28
SBP 50 min after SCI, mmHg	110.62	21
SBP 60 min after SCI, mmHg	112.71	17
SBP 1.5 h after SCI, mmHg	112.44	9
SBP 2 h after SCI, mmHg	112.50	8
SBP at the end of surgery, mmHg	**114.34**	32

Diastolic blood pressure	SM*	Number of patients, n
DBP before loading dose, mmHg	**82.75**	32
DBP after loading dose, mmHg	78.00	32
DBP 10 min after SCI**, mmHg	73.94	32
DBP 20 min after SCI, mmHg	69.69	32
DBP 30 min after SCI, mmHg	68.23	31
DBP 40 min after SCI, mmHg	69.75	28
DBP 50 min after SCI, mmHg	66.00	21
DBP 60 min after SCI, mmHg	67.06	17
DBP 1.5 h after SCI, mmHg	68.33	9
DBP 2 h after SCI, mmHg	71.88	8
DBP at the end of surgery, mmHg	**68.75**	32

* SM – statistic mean;
** SCI – start of continuous infusion.

Figure 4. Changes of electroencephalogram (EEG) index before (A) and after (B) loading dose of dexmedetomidine



N – number of patients, n;
SM – statistic mean;
SSD – standard statistic deviation.

Discussion

Sedation is widely used to reduce anxiety and improve comfort during reconstructive surgery under regional anaesthesia [8]. A new sedative agent dexmedetomidine (highly selective α-2 adrenergic receptor agonist) has been used to provide sedation, analgesia, anxiolysis and to reduce opioid requirements. Because of dexmedetomidine properties to promote a sedative state similar to physiological sleep (stage 1 and 2 non-rapid eye movement sleep) without respiratory depression, it is described as an ideal sedative [8, 14]. Although, dose dependent bradycardia and hypotension are often reported, particularly with more rapid infusion and in patients with pre-existing cardiac problems [4, 6, 17, 20, 21]. We found that low doses of dexmedetomidine (loading dose 1 μg/kg/10 min, a continuous infusion 0.1–0.6 μg/kg/h) during reconstructive surgeries under RA sedation did not cause any significant haemodynamic instability and bradycardia was not seen while EEG index 20–80. Similar results were reported by Arain S. R. et al. and Kilic N. et al. using dexmedetomidine loading dose of 1 μg/kg over 10 minutes followed by a continuous infusion of 0.4–0.7 μg/kg/h and 0.2–0.7 μg/kg/h providing efficient sedation [10, 15]. Ok H. G. et al. study results showed that dexmedetomidine loading dose of 1 μg/kg over 10 minutes is sufficient for surgeries up to 60 minutes long. A continuous infusion of dexmedetomidine of 0.2 μg/kg/h is sufficient for surgeries up to 80 minutes long and a continuous infusion of 0.4 μg/kg/h provides efficient sedation for surgeries up to 120 minutes long under spinal anaesthesia [17].

The incidence of bradycardia and hypotension is the most frequently reported dexmedetomidine adverse haemodynamic response associated with increased dosage and concentration [4, 6, 11, 17, 21]. A study by Ok H. G. et al. reported that the frequency of bradycardia and hypotension does not increase when a low dose of dexmedetomidine is administered IV for sedation under spinal anaesthesia. In our study the incidence of bradycardia requiring atropine was low (2 out of 32 patients) in addition – hypotension was never recorded.

Authors report dexmedetomidine as a useful sedative for procedures because of its minimal effects on the respiratory system [15, 17]. Belleville J. P. et al. reported a study of examined ventilatory effects of a 2-minute intravenous four different dose level of dexmedetomidine infusion. Results showed that right after the maximum dose of 2.0 μg/kg irregular breathing with periods of apnea were noticed; however, there was no significant arterial oxygen desaturation below 90% [22].

In this study the level of sedation was assessed by RASS (the level was from 0 to −3 during sedation) and the depth of sedation or hypnotic status was measured by Narcotrend EEG monitor maintaining the pre-set target level EEG index between 50 and 70. Jiang Y. et al. reported that the use of a Narcotrend monitor for monitoring the hypnotic status may guide sedation according to the EEG index and, therefore, the dose of sedatives is more precise [10]. It is necessary to emphasise that there are some limitations using assessment scales like Richmond Agitation Sedation Scale, Ramsay Sedation Scale or Observer's Assessment of Alertness/Sedation scale. Assessment scales are subjective interpretations by the observers of patient's alertness, the quality of sedation is compromised because the assessments require a patient to be awaken every time an assessment is done [17]. Therefore, authors recommend using Bispectral Index System (BIS) or Narcotrend EEG monitoring for measuring the depth of sedation instead of assessment scales [17, 24]. BIS and Narcotrend EEG monitoring provide real time assessment and quality of sedation is not compromised by external stimulation [24]. Ekin A. et al. in a study measuring depth of sedation with BIS reported that environmental stimuli and application of tourniquet increase values of BIS, although it does not affect a patient's satisfaction with his sedation [5].

There are reports about dexmedetomidine's advantages of providing fast recovery after procedures, patients are easily arousable on verbal stimulation and able to perform psychomotor testing without impaired cognitive abilities and psychomotor functions [1, 12, 23]. In our study, at the end of each surgery, all patients were promptly arousable with verbal stimulation.

Conclusion

1. Loading dose of dexmedetomidine of 1 μg/kg administered intravenously over 10 minutes and a continuous infusion of 0.1–0.6 μg/kg/h provides safe management of sedation during regional anaesthesia under reconstructive surgery and correlates with electroencephalogram index of 50–70 and Richmond Agitation Sedation Scale 0 to −3.
2. After loading dose of dexmedetomidine of 1 μg/kg administered intravenously over 10 minutes while electroencephalogram index was 20–80, significant clinical effects did not appear – bradycardia and respiratory instability, all patients had adequate spontaneous breathing without the need to use assisted ventilation or any airway device during sedation.
3. The loading dose of dexmedetomidine of 1 μg/kg administered intravenously over 10 minutes and a continuous infusion of 0.1–0.6 μg/kg/h provides fast and good quality of awakening and ensures a high patient satisfaction rate of sleep quality during regional anaesthesia.

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Detection of *CDH1* Gene Variants in Patients with Hereditary Diffuse Gastric Cancer by Denaturing High Performance Liquid Chromatography

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Abstract

Germline variants in the *CDH1* gene are the major cause of hereditary diffuse gastric cancer (HDGC) and are identified in approximately 25–40% of families which fulfil strict criteria. Because these variants are spread over the entire gene, their detection requires sequencing of all 16 exons. For screening sequence variations in genes, rapid turnover time is of fundamental importance. Many of the current methods are time consuming and technically difficult to implement. Denaturing high performance liquid chromatography (DHPLC) has been shown to be a highly sensitive, time saving and economical method for variant screening.

In the present study DHPLC method was used to screen patients who fulfilled the hereditary gastric cancer criteria for variant in *CDH1* to confirm diagnosis.

DNA from 20 patients was screened for variant in the 16 exons of the *CDH1* gene, using DHPLC and approved using direct sequencing.

From 20 patients' families, three had at least two gastric cancer cases with one case of gastric cancer in a person younger than 50 years; 10 had multiple cases of gastric cancer diagnosed in person older than 50 years and seven patients were younger than 40 years. All gastric cancer patients were with poorly differentiated adenocarcinoma. DHPLC evaluation of the *CDH1* gene revealed sequence polymorphism (c.1937-13T>C, rs2276330) without clinical significance in 5 patients. No variation was identified in nine patients. Variant c.*54C>T was identified in six patients.

We have demonstrated the feasibility of DHPLC analysis as a sensitive and rapid method for the analysis of the *CDH1* gene in patients with hereditary diffuse gastric cancer.

Keywords: E-cadherin, hereditary diffuse gastric cancer, DHPLC.

Introduction

The E-cadherin is a member of the transmembrane glycoprotein family responsible for calcium-dependent, cell-to-cell adhesion and plays a fundamental role in maintenance of cell differentiation and normal architecture of epithelial tissues [1, *509*; 3, *74*; 5, *6*; 17, *1*]. The protein is encoded by the CDH1

gene which is located on chromosome 16q22.1 and consists of 16 exons. Variants in CDH1 gene are known to be associated with Hereditary Diffuse Gastric Cancer syndrome (HDGC) [4, *1*; 6, *436*; 7, *4086*; 8, *1705*].

The HDGC has been defined in 1999 by International Gastric Cancer Linkage Consortium (IGCLC) as two or more documented cases of diffuse gastric cancer (DGC) in first or second degree relatives, including at least one case of DGC diagnosed before the age of 50, three or more documented cases of DGC in first or second degree relatives diagnosed at any age, isolated individual diagnosed with DGC at less than 45 years of age, isolated individual diagnosed with both DGC and lobular breast cancer [9, *250*; 10, *1782*; 12, *2360*].

Among patients that fulfil the above clinical criteria about 25–40% of cases carry a pathogenic variant in the *CDH1* gene [14, *364*; 18, *2137*; 19, *646*]. Patients with germline variants in the *CDH1* have a high risk of developing diffuse gastric cancer and female carriers are at high risk of lobular breast cancer [20, *1349*].

Analysis of *CDH1* gene variants is important in patients fulfilling the HDGC criteria. Techniques for variant detection in disease related genes need to be sensitive and specific. Therefore the ideal method to use for variant analysis should be sensitive, non-hazardous, relatively inexpensive, and semi or fully automated to minimise labour and laboratory costs.

Denaturing high-performance liquid chromatography (DHPLC) had been shown to meet these criteria for variant screening. Denaturing high pressure liquid chromatography (DHPLC) is a relatively new technique, which uses heteroduplex formation between wild-type and mutated DNA strands to identify variants. Heteroduplex molecules are separated from homoduplex molecules by ion-pair, reverse-phase liquid chromatography on a special column matrix with partial heat denaturation of the DNA strands [13, *1735*; 21, *336*; 22, *956*].

DHPLC is potentially a very useful method for the screening of a large number of samples for variants. Numerous reports during the last few years have documented the high accuracy and excellent sensitivity of DHPLC (96–100%) in detecting variants in more than 250 genes [13, *1737*]. DHPLC appears to be a reliable method specifically for the analysis of large genes known to be highly polymorphic and with a large variety of pathogenic variants [13, *1737*; 22, *961*].

In the present study DHPLC method was used to screen patients who fulfilled the hereditary gastric cancer criteria for variants in *CDH1.*

Material and Methods

Patients. Twenty patients from families fulfilling the criteria of HDGC according to IGCLC guidelines were selected for analysis of *CDH1* gene. Three patients had at least two gastric cancer cases with one case of gastric cancer in a person younger than 50 years; ten had multiple cases of gastric cancer diagnosed in person older than 50 years and seven patients were younger than 40 years. All gastric cancer patients were with poorly differentiated adenocarcinoma.

PRC amplification conditions for DHPLC analysis of the *CDH1* gene. Samples used for variant screening were amplified with PCR, using Optimase polymerase (Transgenomic) according to manufacturer's guidelines. Primers for *CDH1* all 16 exons were as reported by Mullins et al. [16, *753*]. Reference sequence for *CDH1* is NM 004360.3.

DHPLC analysis. All samples after PCR were denatured at 95 °C and gradually cooled to 25 °C to promote heteroduplex formation. DHPLC analysis was carried out on Transgenomic Wave 4500 HT, system (Transgenomic) using partially denaturing conditions, using temperature and flow gradient as suggested by the system for each amplicon.

Fragments showing an abnormal DHPLC pattern were investigated for identification of sequence variants by direct sequencing using ABI3130 and SeqScape program (Applied Biosystems).

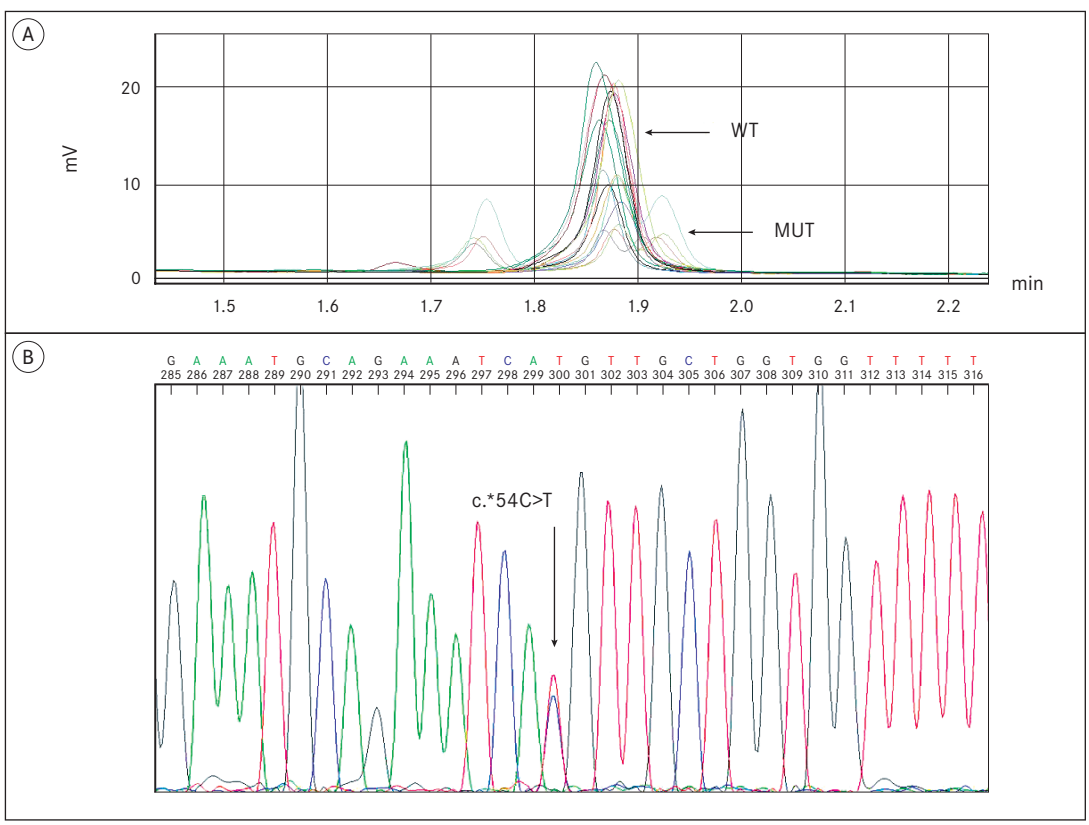
Results

The 16 exons of *CDH1* were screened for variant using DHPLC in 20 patients with hereditary gastric cancer. DHPLC evaluation revealed one sequence variation in six patients. They all had double peak in DHPLC chromatograms for exon 16 indicating heteroduplex formations (Figure 1a). Direct sequencing revealed c.*54C>T variant rs1801026 in all six patients (Figure 1b). Five patients had sequence polymorphism (c.1937-13T>C, rs2276330) without clinical significance. No genetic changes in *CDH1* were identified in nine patients.

Figure 1. DHPLC detection of E-cadherin germline mutations and confirmatory direct sequencing analysis. DHPLC and corresponding sequence chromatograms of fragments of the E-cadherin gene:

(A) The amplicons containing exon 16, showed a different elution profile compared to the wild type samples;

(B) Confirmatory direct sequencing of a new PCR-product identified the variant c.*54C>T



Discussion

CDH1 germline variants are associated with the development of the autosomal cancer syndrome namely Hereditary Diffuse Gastric Cancer (HDGC) [1, *508*; 2, *874*; 4, *1*; 9, *250*]. About 25–40% of families fulfilling the clinical criteria for HDGC established by the International Gastric Cancer Linkage Consortium (IGCLC) have constitutional alterations of the *CDH1* gene. The offspring of an affected individual has a 50% risk of also being affected.

The estimated cumulative risk of gastric cancer by the age of 80 is 67% for men and 83% for women. Women also have a 39% risk for lobular breast cancer [14, *364*; 18, *2137*]. The penetrance of *CDH1* gene variants is high. The lifetime risks of diffuse gastric cancer is 80% in both men and women by the age of 80, and lobular breast cancer is 60% in women by the age of 80.

Germline variants in *CDH1* gene were originally reported in three Maori families with aggregation of diffuse gastric cancer [1, *515*; 2, *874*; 9, *250*; 19, *649*]. Since this report, several studies have investigated the role of *CDH1* variants in gastric cancer in different ethnic groups [4, *2*; 19, *649*]. Different patterns of *CDH1* germline variants have been described as truncating, deletion, insertion, splice site, nonsense, silence, and at last, missense alterations [9, *253*].

Genetic testing should be initiated in affected patients. Many different methods have been used for identifying *CDH1* variants. As gold standard for variant detection has been direct sequence analysis. However, the cost and effort of DNA sequencing is often considerable, especially in genetically heterogeneous diseases [21, *335*]. In our study we applied DHPLC analysis to the detection of sequence variants in the *CDH1* gene. DHPLC provides information about whether a variant is present.

In order to determine the specific nature of the variant, however, PCR products in question need to be sequenced. DHPLC evaluation of the *CDH1* gene revealed sequence polymorphism (c.1937-13T>C, rs2276330) without clinical significance in 5 patients. Variant c.*54C>T was identified in six patients. Some clinical studies have demonstrated an association of variant c.*54C>T with tumour progression in HDGC, although the results are controversial [11, *993*; 15, *861*; 23, *26*]. No variation was identified in nine patients.

Variant detection by DHPLC, as it is presented in our study, is a high-throughput, time saving, and economical tool for variant screening.

Conclusion

The presented study demonstrates the feasibility of DHPLC analysis as a sensitive and rapid method for the examination of the *CDH1* gene in patients with hereditary gastric cancer. DHPLC may be cost-effective in the diagnosis of this disorder compared with other analytical methods as Single-strand conformation polymorphism (SSCP) and Denaturing gradient gel electrophoresis (DGGE) and a valuable alternative to the direct sequencing of all 16 exons of the *CDH1* gene.

Acknowledgments

We gratefully acknowledge the support from the National research programme "Biomedicine for Public Health", 2014–2017.

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Optimisation of Protocol for Isolation and Propagation of Cells from Human Breast Cancer Primary Tumour Core Biopsies

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Abstract

Breast malignant tumours are heterogeneous cell populations that can be characterised by immunocytochemical and molecular markers. Sometimes treatment is not effective against some populations of malignant cells. The identification of such cells could benefit treatment in future. Therefore, our aim was to elaborate protocol for isolation of various breast cancer cell populations from breast tumour core biopsy samples.

Mechanical and enzymatic disaggregation was performed and cells were seeded at various densities on collagen-coated plates.

This study presents data on cell counts, seeding efficiency, observed morphology and primary immunocytochemical results for the cells isolated from breast cancer core biopsies.

Isolated cell counts vary among biological samples obtained from different donors. Cultured cells represent mostly fibroblast-like and stromal-like cell morphology. They produce characteristic hormone receptors ER, PR and Her2 (ErbB2) and have a weak signal for cell differentiation marker CD24.

In future, the protocol will be advanced using specific media to improve isolation of epithelioid type cells.

Keywords: breast cancer core biopsies, cell cultures, immunocytochemistry.

Introduction

Breast cancer is the most common type of cancer among women in Latvia. According to current data of Disease Prevention and Control Centre of Latvia, 1133 new cases of breast cancer were registered in 2013, and in Latvia altogether there are 13,434 registered breast cancer patients (Smate et al., 2013). Breast cancer is a heterogeneous group of neoplastic cells originating from the epithelial cells that are lining breast milk ducts. There is a high degree of diversity between and within tumours, as well as among cancer patients (Polyak, 2011). Different strategies for therapy exist; however, many breast tumours are not responsive or eventually develop resistance to therapy. Therefore, it is essential to identify treatment-resistant breast cancer cells, to characterise them, and to determine differences between therapy-sensitive cells.

There is a variety of strategies for isolating primary cell cultures that depend on cellular properties, e.g., adhesion ability, differences in density, and the antibody binding capacity (Tomlinson et al., 2012). Another common method with a high efficiency rate (~ 50%) for primary culture establishment is explant

cultures. Explant cultures is a technique that has several significant advantages, for example, it maintains cellular migration and preserves the histotypic relationships among various cell types within an organ of origin without changing the tissue architecture, which is caused by enzymes (Pei et al., 2004).

Human breast cancer-derived cells is a widely used experimental tool that enable to gain specific, clinically important information. Attempts to establish breast cancer cell lines from primary tumours have always been a challenging task. Poor efficiency is often due to various factors: (1) the extraction of viable tumour cells from surrounding stroma; (2) slow proliferation rate of breast cancer *in vivo* which is maintained after transferring of cells into culture. Due to slow proliferation rate of primary breast cancer cells, it is essential to prevent overgrowth of normal stromal cells (e.g., fibroblasts) that can overtake breast cancer cells, therefore selective media is a prerequisite; (3) the different hormone and growth factor requirements of breast cancer cells from different patients and different breast cancer types and subtypes; (4) the importance of factors secreted by normal cells that negatively influence proliferation of breast cancer cells. These and other factors make primary cultures of breast cancer cells *in vitro* a much more difficult task than the culturing normal mammary epithelial cells (Ethier et al., 2000).

For successful cell propagation it is essential to adjust culture conditions that can vary in-between cell lines. Therefore, suitable cell culture media and supplements are of utmost importance. It is also important to note that most cells are substrate-dependent, and substrate is an important factor for optimal culture conditions (Liberio et al., 2014). To mimic *in vivo* environment and to promote cell adhesion, collagen coated surfaces can be used (Chandrasekaran et al., 1999). Within tissues, cells interact with each other and with the extracellular matrix. For maintenance of normal cell interaction and signalling, the key factor is seeding density as it may affect cell extracellular production, their metabolism and reduce viability (Issa et al., 2011).

This study presents data of pilot experiments to isolate primary cells from the breast cancer core biopsy samples. Different seeding density on collagen coating of the culturing plates was tested.

Aim

The aim of the study was to develop protocol for isolation and propagation of various cell populations from breast cancer core biopsy tissue samples.

Material and Methods

Reference cell cultures. Established human breast cancer cell lines MDA-MB-231 and MCF7 were used as reference cell lines for tumour cells (morphological and immunocytochemical characterisation). Dermal fibroblasts (a kind gift from Cell Transplantation centre, PSKUS) were used as fibroblast control.

Cell isolation from core biopsies. Breast cancer core biopsies were obtained from Pauls Stradins Clinical University Hospital, Centre of Breast Diseases. The study was approved by the Ethics Committee of the hospital. From each patient, who signed informed consent form, there were two core biopsy samples obtained: transversal (central) and tangential (peripheral) in relation to tumour node centre. Tissues were minced and enzymatically disaggregated using collagenase solutions. Type III collagenase (1.0–1.5 mg/ml) was used for enzymatic digestion of tissues from tumour peripheral samples. Type II collagenase/dispase (1 mg/ml/20 U/ml) was used for the central biopsy sample. Enzymatic treatment was performed overnight at room temperature and followed by incubation at 37°C until complete disaggregation of the sample. Samples were filtered and seeded on collagen-coated plates (6-well and 96-well) in DMEM:F12 (1 : 1) containing 10% fetal bovine serum (FBS), 1% 1x penicillin/streptomycin (10,000 U/ml), 0.002 mM L-glutamine and 2.5 mg/l Amphotericin B solution at 37 °C, 5% CO_2.

Development of explant cultures. One half of the tissues from peripheral sample of tumour was used to initiate explant cultures. Tissues were cut into small (approx. 1 mm^2) pieces that were placed on 24-well plate (1 explant/well). Fetal bovine serum was applied on the explant and incubated for 1–2 h at 37°C, 5% CO_2. DMEM:F12 (1 : 1) containing 10% FBS was added to the explants and they were

incubated overnight at 37 °C, 5% CO_2. After observation under microscope, the unattached explants were attached to the surface of the well using sterile scalpel. Media change was performed every 2–3 days.

Immunocytochemistry. Protein localisation was detected using AbCam Mouse and rabbit specific HRP detection IHC kit (Cat. No: ab93677). In brief, cells were seeded on 96-well plate with seeding density of 500–1000 cells/well. After reaching 70–80% confluence, they were fixed using ice-cold ethanol. None of the antibodies used in this research required antigen retrieval. Cells were washed, blocked with Protein block. Primary antibody was added (we used 1 : 200 dilution for all antibodies) and incubated for 1 h at the room temperature. Biotinylated secondary antibody was added and incubated for 10 min at room temperature. Cells were stained with DAB substrate kit (ab64238) and hematoxylin.

Statistical analysis. Statistical analysis was performed using IBM SPSS Statistics, Version 21. Exploratory statistical analysis was performed for various biopsy sample disaggregation protocols.

Results

Disaggregation of breast cancer core biopsy samples

During the study period, central and peripheral core biopsy samples from nine patients were received in the laboratory.

Central core biopsy samples from all nine patients were disaggregated enzymatically with collagenase II and dispase combination. Peripheral core biopsy samples from four patients were disaggregated enzymatically with collagenase III. Only half of the sample was used for enzymatic disaggregation (collagenase III for one sample, and collagenase II/dispase for four samples) for five peripheral core biopsy samples, the other half of the biopsy sample was used for initiation of primary explant cultures. The samples were exposed to enzyme solution until complete or almost complete disaggregation of sample and reaching relatively homogenous cell suspension, as determined visually.

The mean number of cells obtained from the biopsy samples after enzymatic disaggregation was highly variable: 2.33 ± 2.78, × 10^5 (range: 0.02–9.20, × 10^5) cells per biopsy, resulting in coefficient of variation of 119%. The numbers of cells obtained as a result of enzymatic disaggregation of biopsy samples for various isolation protocols are shown in Table 1.

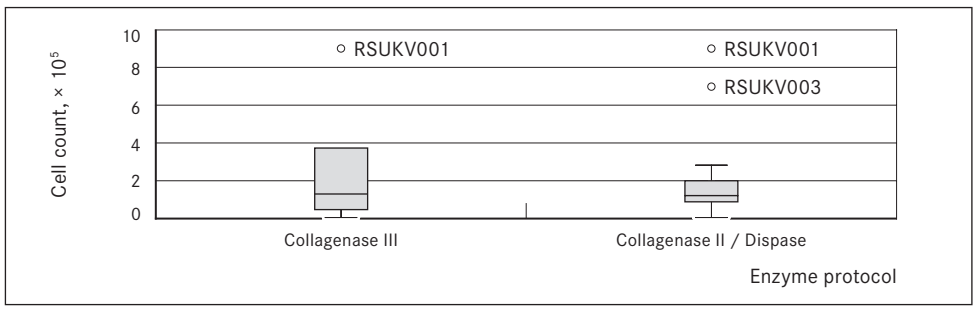
Table 1. Disaggregation of breast cancer core biopsy samples: enzymes used for disaggregation, length of disaggregation, and isolated cell counts

Protocol	Biopsy samples		
	n	Cell count, × 10^5 cells (Mean ± SEM)	Range
Enzymes			
Collagenase II + dispase	13	2.10 ± 0.68	0.02–8.80
Collagenase III	5	2.95 ± 1.68	0.06–9.20
Length of enzymatic disaggregation			
≤ 12 h	6	3.72 ± 1.68	0.50–9.20
> 12 h	12	1.64 ± 0.48	0.02–5.56

Enzymes for disaggregation of biopsy samples. Collagenase II in combination with dispase was used for disaggregation of 13 biopsy samples (9 central and 4 peripheral); collagenase III was used for disaggregation of five peripheral biopsy samples.

The average numbers of cells obtained after disaggregation with collagenase III and collagenase II / dispase did not differ significantly and were, respectively, 2.95 ± 0.61, × 10^5 (range: 0.06–9.20, × 10^5) and 2.10 ± 0.68, × 10^5 (range: 0.02–8.80, × 10^5) cells per biopsy sample (adjusting for material used for explant cultures) (Figure 1).

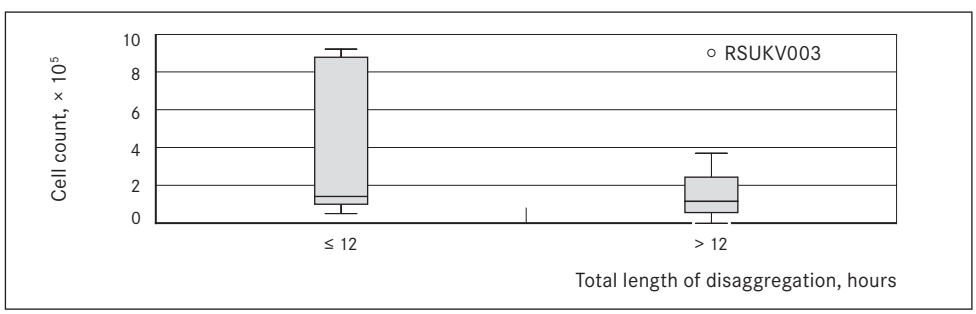
Figure 1. Boxplots of cell counts after tissue disaggregation with two different enzyme sets: collagenase III, and collagenase II/dispase



Length of enzymatic disaggregation. On average it took 2.33 ± 0.66 hours (range 2.50–43.80 hours) to completely or almost completely disaggregate a sample.

For biopsies exposed to enzymatic disaggregation for ≤ 12 hours and for > 12 hours the average numbers of cells obtained per biopsy were respectively 3.72 ± 1.7, × 10^5 (range: 0.50–9.20, × 10^5) and 1.64 ± 0.48, × 10^5 (range: 0.02–5.56, × 10^5) cells (Figure 2).

Figure 2. Boxplot of cell counts after different length of disaggregation: ≤ 12 hours or > 12 hours

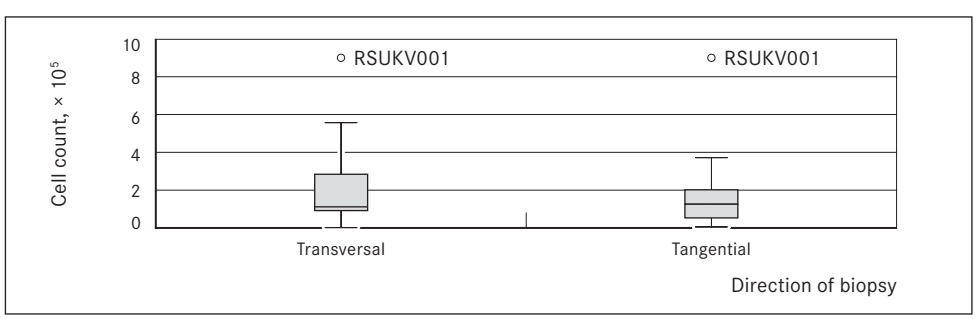


Cell counts for transversal and tangential biopsy samples did not differ significantly

The average number of cells obtained from the transversal and tangential biopsies after enzymatic disaggregation varied widely, and did not differ significantly.

The average number of cells (± standard error of mean) per biopsy (adjusting for material use for explant cultures) was 2.48 ± 0.96 × 10^5 (95% CI: 0.25 × 10^5, 4.70 × 10^5) for transversal biopsies (range: 0.02–8.80, × 10^5) and 2.2 ± 0.95 × 10^5 (95% CI: 0.01 × 10^5, 4.37 × 10^5) for tangential biopsies (range: 0.06–9.20, × 10^5) (Figure 3).

Figure 3. Boxplots of cell counts from tissues of transversal and tangential breast cancer core biopsies



Primary culture of cell populations after enzymatic disaggregation

After disaggregation of biopsy samples, cells were seeded on 96-well and 6-well plates. Seeding density on 96-well plates varied among biopsies. To observe clonogenic capacity of the cells, we seeded cells on 96-well plates with different densities: from 2 to 1900 cells/well, i.e., between 7–6550 cells cm^2, respectively. Cloning efficacy for cell seeding in densities from 2 to 60 cells per well (n = 10) in general was very low – cell attachment was observed for 20–30% of wells, but cells were nonviable and/or nonproliferating. Therefore, efficacy of clonogenic cell cultures very early in cell line isolation process in our experiments, using DMEM: F12 with 10% FBS and 1% glutamine as growth medium and collagen coating as adherent substrate, was close to 0%.

The results for cell seeding efficacy at higher cell densities – 1800 and 1900 cells per well, i.e., 6200 and 6550 cells/cm^2 – for representative transversal and tangential, respectively, biopsy samples are shown in Table 2.

Cell seeding efficacy was characterised by cell attachment on day 10 (number and proportion of wells with attached cells) and cell growth on day 30–35 (number and proportion of wells with various levels of cell confluence).

Table 2. Efficacy of cell seeding on 96-well plates in high density (1800–1900 cells/well) for representative transversal and tangential core biopsy sample cell populations

Characteristics of cell seeding efficacy	Transversal biopsy, number of wells		Tangential biopsy, number of wells	
	N = 480	%	N = 480	%
Cell attachment on day 10	228	47.50	25	5.21
Cell confluence on day 30–35, including:	232	48.33	24	5.00
< 1% – nonproliferating cells	80	16.67	6	1.25
1–20% – very low growth rate	64	13.33	2	0.42
21–40% – slow growth rate	26	5.42	8	1.67
41–60% – moderate growth rate	19	3.96	2	0.42
61–80% – high growth rate	17	3.54	4	0.83
> 81% – very fast growth rate	30	6.25	2	0.42

The recovery rates after seeding on 6well plates at the moment of publication were established for only some, but not all biopsies. Cells seeded in high or very high densities on 6-well plates gave 7.08% to 300% cell yield, demonstrating highly variable growth rate of different cell populations from different patients.

Primary culture of cell populations from tissue explants

To explore if enzymatic disaggregation has any impact on breast core biopsy cell population viability and growth, we performed also experiments to obtain primary cultures from tissue explants. For initiation of explant cultures, we used half of sample from five tangential breast cancer core biopsies. The efficacy of explant cultures as judged by cell population outgrowth from explant is shown in Table 3.

Efficacy of explant cultures differed among different patients, and was between 0.0–66.7%. For the explant cultures with near-confluent or confluent cell outgrowth (n = 3), we performed subcultivation, including cell count, as shown in Table 3.

Table 3. Explant cultures of breast cancer biopsy samples

Biopsy No.	Number of explants	Explants with cell outgrowth on day 30		Cell yield, × 10^5 cells
		n	%	
RSUKV005M	12	9	66.7	0.60
RSUKV006M	12	1	8.3	< 0.01
RSUKV007M	6	0	0.0	0.00
RSUKV008M	6	2	33.3	ND*
RSUKV009M	6	4	66.7	ND*

* ND – not determined: at the time of publication confluence of cell outgrowth is too low to perform subculture.

Isolated cells exhibited variable morphology

Cells were cultured on collagen-coated surfaces. Fibroblast-like phenotype was the most abundant among all samples (Figure 4A). Also in several cases sphere-forming-like cell aggregates (squamospheres) were observed (Figure 4B). During cultivation of isolated cells from one biopsy tissue material variable cell morphology was detected (Figure 4C and 4D). In some cases separate, dense cell colonies were observed (Figure 4 E). Rarely, epithelial-like cells were observed (Figure 4F).

Reference breast cancer cell line MCF7 has a cobblestone-like phenotype with a pronounced strong cell-cell adhesion (Figure 4G). Cells of another reference cell line MDA-MB-231 phenotypically appear as elongated spindle-shaped cells much a like to fibroblasts with a pronounced cellular scattering (Figure 4H). Fibroblasts appear as large in size, spindle-shaped cells (Figure 4I).

Cell outgrowth from the tissue sample explants was observed from the first week in culture. Most cells exhibited fibroblast-like morphology (Figure 5). Epithelial (or cobblestone) phenotype, characteristic for cancer cells was observed.

Immunocytochemical analysis of ER, PR, HER2 and CD24

Cells of reference cultures MCF7 and MDA-MB-231, dermal fibroblasts and central and peripheral biopsy tissues were stained for typical markers of breast cancer (ER, PR, HER2) and for CD24 (Figure 6).

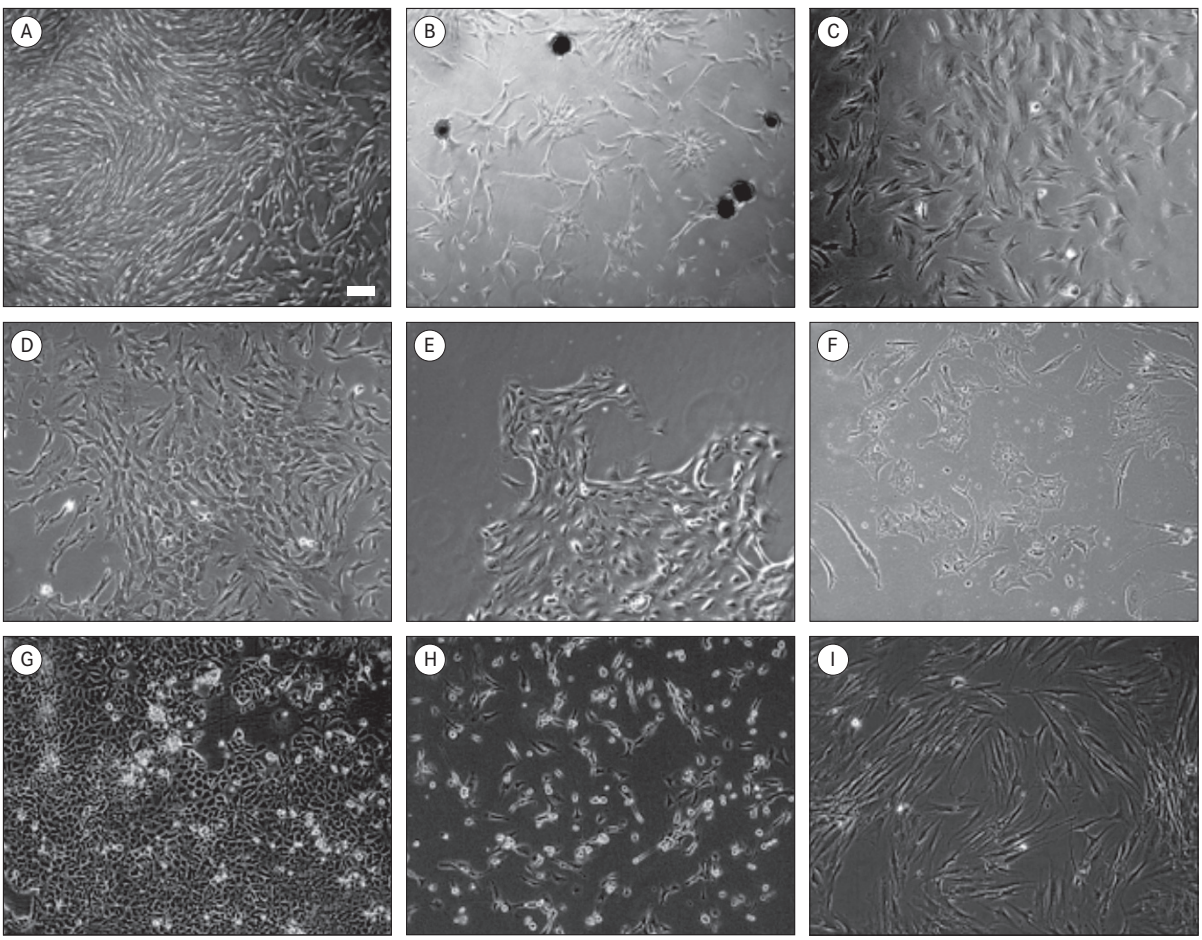
Oestrogen receptor (ER) staining was most abundant in the reference culture MCF7. Very weak staining was observed for another reference cell line of triple-negative breast cancer MDA-MB-231, whereas moderate ER staining was detected in both breast cancer cells, as well as in dermal fibroblasts (Figure 6, row 2, F–J).

Another typical marker of breast cancer cells – progesterone receptor (PR) was detected in all samples. Weaker staining was observed for reference cell line MDA-MB-231 (Figure 6, row 3, K–O).

Very strong staining of HER2 (ErbB2) was observed for MCF7 cells, whereas strong staining – for dermal fibroblasts, and weak staining was observed for cells isolated from representative biopsy and MDA-MB-231 cells (Figure 6, row 4, P–U).

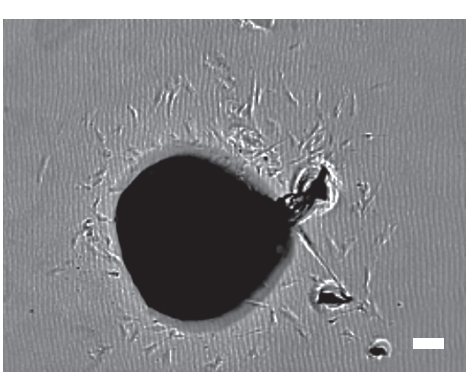
Staining with cell differentiation marker CD24 gave very weak signal in all cell samples tested (Figure 6, row 5, V–Z).

Figure 4. Representative morphologies of cells isolated and cultured from breast cancer core biopsy (**A**–**F**) and reference culture cells (**G**–**I**).* G – MCF7 cells, H – MDA-MB-231 cells, I – dermal fibroblasts



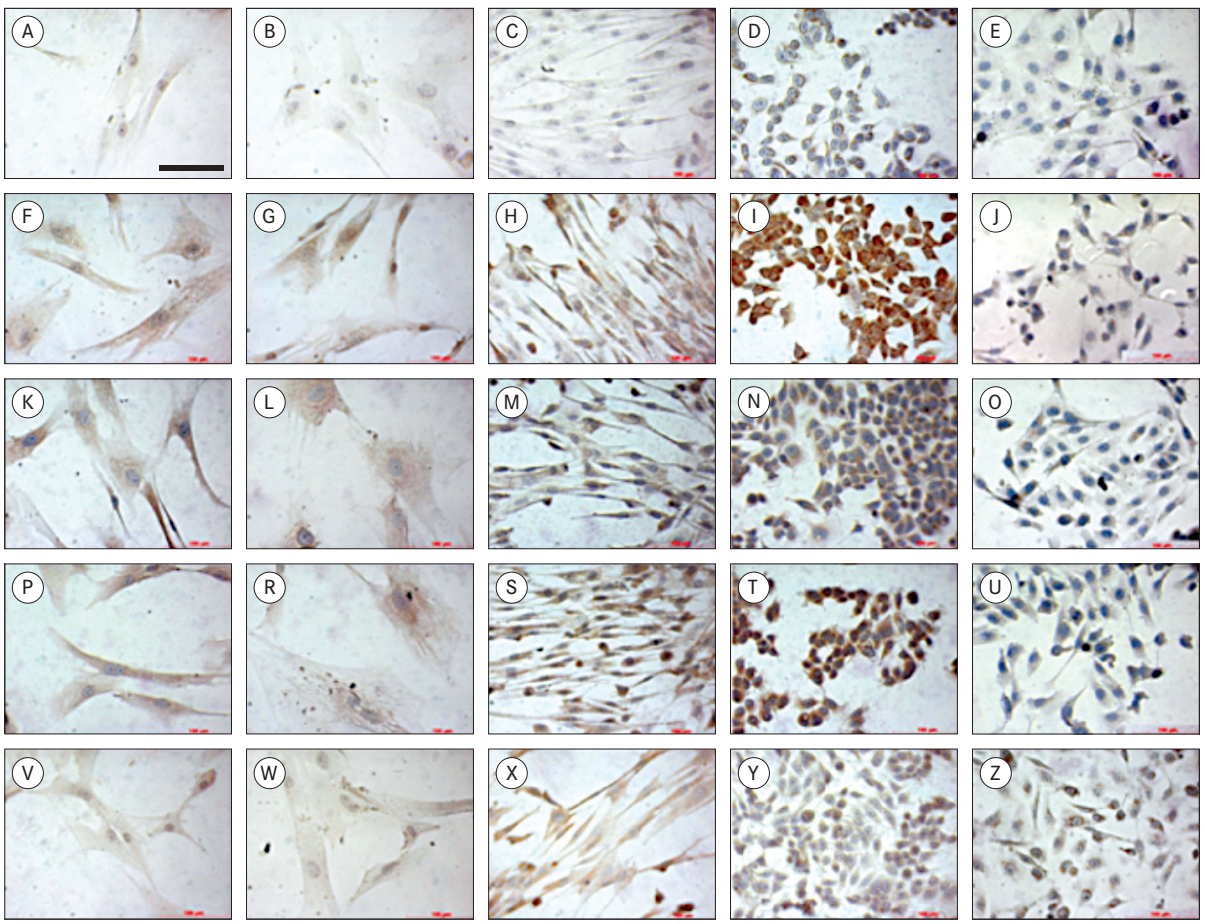
* Scale bar in section A, 100 µm

Figure 5. Representative image of cell outgrowth from the explant on day 7 after seeding.*



* Scale bar 100 µm

Figure 6. Immunocytochemical analysis of breast cancer markers*



* Scale bar in section A applies to all sections, 100 µm

Horizontally: row 1 (**A**–**E**) represents all negative controls; row 2 (**F**–**J**) staining with ER; row 3 (**K**–**O**) – cells stained with PR; row 4 (**P**–**U**) cells stained with HER2 and row 5 (**V**–**Z**) – cells stained with CD24.

Vertically: column 1 (**A**–**V**) – cells from the transversal tumour biopsy cultured for 5 passages; column 2 (**B**–**W**) – cells from the tangential tumour biopsy cultured for 5 passages; column 3 (**C**–**X**) – dermal fibroblasts; column 4 (**D**–**Y**) – MCF7 cells, and column 5 (**E**–**Z**) – MDA-MB-231 cells.

Discussion

Skilfully obtained (under the ultrasound control) breast cancer core biopsies contain representative cell populations that can serve as a good cell source for further investigation of heterogeneity of the tumour. To compare cell isolation strategies in different parts of the tumour, biopsies were collected from the peripheral and from the central part of the tumour (Oakes et al., 2014).

Collagenase type III was used for disaggregation of tissue from peripheral part of tumour (tangential biopsy sample) (Liu et al., 2007). Gentler approach was taken to disaggregate tissues from the central part of the tumour (transversal biopsy). Mix of different enzymes, usually collagenase and dispase or hyaluronidase has been described previously (Goldstein et al., 2011; Stingl et al., 2005). On average, there were no significant differences in cell counts after use of the two enzyme protocols (Figure 1). However, tissues disaggregated faster when collagenase II/dispase cocktail was used. Length of enzymatic disaggregation had no significant impact on number of cells obtained from the sample, although it showed slight trend towards lower number of cells with increase of length of digestion time (Figure 2). Also there were no significant differences observed in numbers of cells depending of tissue or

biopsy type (transversal *vs.* tangential biopsy); however, slight trend towards lower cell count in tangential biopsy samples in comparison to transversal biopsy samples was observed (Figure 3). This suggests that further development of protocol should include an enzyme cocktail consisting of collagenase and dispase or hyaluronidase, and shorter digestion times for both types of biopsies.

There are several recognised problem areas to solve to obtain efficient epithelial and cancer cell cultures. Nutrients required by cancer cells may differ from those required by equivalent normal cells, and removal of stromal elements and supportive cells may deprive tumour and epithelial cells of substrate (matrix), nutrients and growth factors necessary for their survival. Seeding of epithelial and / or tumour cells in low densities may dilute growth factors produced by adjacent similar (i.e., malignant) and supportive cells, and deprive cells of close contacts necessary for successful growth; therefore, usually closely interacting population is required (Freshney, 2010).

Similar difficulties and key factors have also been found to be specific for breast cancer cultures – cell density dependence for growth, slow proliferation rate *in vivo* which is maintained after transferring the cells into culture, different hormone and growth factor requirements of cells from different patients, and importance of factors secreted by normal cells (Ethier et al., 2000). These culture growth difficulties were observed also in our efforts to obtain clonogenic individual cell lines from primary cells. Even after seeding of cells in quite high densities, the attachment and growth of cells was comparatively low. Furthermore, at least 60–70% of cell populations with high or very high growth rates morphologically were fibroblast-type or mixed fibroblast and mesenchymal-type cells (Figure 4).

Results described in this report suggest that propagation of mixed cell populations in high-densities could be appropriate for early breast cancer cell line isolation stages due to density-dependent growth and slow-growth rates. Also, further research directions will include different growth media and supplements, as DMEM:F12 and fetal bovine serum as universal media does not meet specialised nutritional and growth factor requirements of various breast cancer cell populations, and various substrates, e.g., Matrigel (extracellular matrix) and feeder layers of fibroblasts or fetal intestinal cell lines, as more complete imitation of extracellular contacts needed for epithelial and breast cancer cells.

The explant cultures showed various efficacy (Table 3), but closer study of cell populations obtained after enzymatic and mechanical disaggregation of breast cancer core biopsy samples is still in progress, so at the moment of the publication we cannot make any conclusions whether enzymatic disaggregation exerts any selection pressure on cell populations obtained from the biopsy sample and decreases their heterogeneity, i.e., representativeness of cell line library obtained from the patient. Also, to explore improvement of efficacy of explant cultures, use of various cultivation media for cell outgrowth is planned in the nearest future.

Immunocytochemistry analysis was performed to do the primary characterisation of cultured cells from representative transversal biopsies and tangential biopsies. Although this method is not quantitative, it is still possible to compare the staining if they are performed simultaneously.

Breast cancer cell lines MCF7 and MDA-MB-231 were used as positive controls. Dermal fibroblasts were stained to obtain the information about marker expression in non-epithelial cells. After morphological analysis we already could predict that most of our cells exhibit fibroblast-like or stromal-like phenotype. Similarly, the expression of breast cancer markers was very close in intensity among representative isolated breast cancer cells and dermal fibroblasts. Whereas situation was different with reference cell line MCF7 that are of epithelial origin. According to literature, these cells have strong ER and PR expression (Subik et al., 2010) that is in line with our data. However, these MCF7 cells usually do not exhibit strong expression of HER2. It has been shown that elevated levels of HER2 in MCF7 can stimulate the activation of anti-apoptotic gene expression (Siddiqa et al., 2008); however, this direction was not further tested.

MDA-MB-231 is the reference cell line for triple negative type breast cancer, and the very weak hormone receptor staining is in line with overall known information (Subik et al., 2010).

CD24 is one of the cell differentiation markers that has been used in combination with CD44 as prospective cancer stem cell marker for basal/mesenchymal cell lines MCF7 and MDA-MB-231 (Al-Hajj et al., 2003; Sheridan et al., 2006). It has been shown that CD44+/CD24$^{low/negative}$ cancer cells exhibit enhanced invasive properties. The low expression of CD24 in MCF7 and MDA-MB-231 is in line with literature data (Siddiqa et al., 2008). CD24 is expressed by the range of cells (Fang et al., 2010) and is associated with mesenchymal stem cells (Wetzig et al., 2013). It has been previously described that dermal fibroblasts resemble several mesenchymal stem cell features, like cell plasticity by differentiation towards adipogenic, osteogenic and chondrogenic lineages (Chang and Guo, 2014). This similarity could explain CD24 expression in our dermal fibroblasts. Overall, the expression patterns of different breast cancer markers in our isolated cells were very similar to the ones observed for dermal fibroblasts. Nevertheless, morphologically they differ. More elaborate tests are needed to draw any conclusions about the origins of isolated cells.

Conclusions

Development of patient-specific representative breast cancer cell cultures from core biopsies is challenging mainly due to differences among patients, and the small volume of tissue sample (therefore small cell numbers). Shorter enzymatic disaggregation time, higher seeding density, surface features and cell type specific media could be more effective and will be tested in future experiments.

Acknowledgements

The present work is supported by the RSU project "Reconstruction of breast cancer genetic evolution based on proteomics in cancer cell cultures".

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Reduced Retinal Nerve Fibre Layer Prediction for Multiple Sclerosis Patients

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Abstract

Multiple sclerosis often affects afferent visual system. Recently it has been recommended to introduce investigation with optical coherence tomography as a part of routine monitoring in multiple sclerosis patients. However, not in every multiple sclerosis centre optical coherence tomography technique is available. The aim of our study was to develop a statistical model for predicting the risk of reduced retinal nerve fibre layer using simple structural and functional visual system tests.

The study recruited 76 multiple sclerosis patients and 28 normal controls. Routine opthtalmologic and radiologic tests were performed. Independent variables, believed to be associated with reduced retinal nerve fibre layer, were included in the study. Forward binary logistic regression was used to find significant variables and probabitity of retinal nerve fibre layer reduction.

The changes in visual evoked potential amplitude and latency, the number of demyelinating lesions in magnetic resonanse investigation and disease duration are the independent variables found significant to predict the risk of reduced retinal nerve fibre layer ($p < 0.05$).

Forward stepwise (likelihood ratio) binary logistic regression model is an accurate method to predict reduced retinal nerve fibre layer in the presence of reduced N75 / P100 amplitude, prolonged P100 latency with the high number of demyelinating lesions in magnetic resonanse investigation. Our model may be applied for identification of individuals who are at a high risk of reduced retinal nerve fibre layer in order to refer them to optical coherence tomography examination.

Keywords: multiple sclerosis, optical coherence tomography, visual evoked potentials.

Introduction

Afferent visual pathways are a suitable clinical model for investigation of multiple sclerosis (MS) and neuroprotective drugs (Costello, 2013). Significant advantage is that visual pathways are available for detailed and direct structural and functional studies. A recently published article suggests that OCT method should be considered as a part of routine monitoring of MS patients (Saidha and Calabresi, 2014). However, such an approach was later criticised and the usefulness of OCT questioned (Jenkins and

Toosy, 2014). The fact should be taken into account that not in all MS centres OCT method is available to date. The question arises whether changes detectable by OCT examination could be estimated by using other visual system tests, regardless of the history of optic neuritis (ON) episode.

The aim of our study was to develop a statistical model for predicting the risk of reduced retinal nerve fibre layer (RNFL) using simple structural and functional visual system tests for everyday use.

Material and Methods

Subjects. The cross-sectional study included 76 relapsing-remitting MS patients both with and without ON history. The control group included 28 age-matched healthy individuals.

MS patients were recruited from Pauls Stradins Clinical University Hospital, Multiple Sclerosis Center within the period from October 2011 to April 2014.

Inclusion criteria:

- relapsing remitting MS diagnosis, based on the 2010 McDonald criteria;
- for MS patients with history of ON ≥ 6 months after the unilateral ON episode;
- ≥ 30 days after corticosteroid therapy.

Exclusion criteria:

- acute ON clinical signs;
- refraction disorders exceeding ± 6 diopters;
- other neurological and ophthalmic diseases, which can affect the afferent visual system;
- inability to participate in the visual system examinations.

Tests. Following functional and structural visual system tests were performed for each eye separately.

Visual acuity determination with visual acuity test figures using Snellen chart located 6 metres from the patient's face was performed. Visual acuity measurement results were expressed as a decimal, recording the lowest line of the smaller figures, which the patient was able to name error-free. Whenever necessary, the corrected visual acuity was used.

The computerised visual field perimetry was performed. The 30-2 threshold programme was used, retinal sensitivity was measured at 54 points; the points tested were distributed 3° from the vertical or horizontal meridian. Each light stimulus was displayed for about 0.2 seconds, the test results were analysed using a decibel scale.

In order to inspect the colour vision, the Ishihara test was used, showing the polychromatic tables to a patient in daylight, from a distance of one metre, for five seconds. For patients who were unable to distinguish the hidden numbers or shapes, colour vision disorders were diagnosed.

For all of the study participants *pattern-reversal* VEP record was performed using the hardware "RETI port 21 ROLAND CONSULT". Individuals were placed 70 cm away from the screen, fixing their view on the red dot in the centre of the screen. If necessary, a full refractive correction was made. In order to establish the potential, vision was repeatedly stimulated in a monocular way with a black-white video monitor at 1.6 Hz frequency. Record of the potentials was made with the disc electrodes, placing them in the brain visual cortical projection areas of the International 10–20 system (Odom, Bach et al., 2010). The average potentials of action were filtered and analysed, repeatedly performing 100 re-stimulations twice for each eye. N75 / P100 amplitude measurements in microvolts (mcV), as well as P100 latency measurements in miliseconds (ms) have been made. N75 / P100 amplitude below 10.52 mkV was considered to be reduced, P100 latency, longer than 110.25 ms, was considered to be prolonged.

The investigation of the frontal part of the eye was carried out using a slit-lamp biomicroscopy. *Fundus oculi* examination was performed using a 90 diopter lens. In the *fundus oculi* examination the following parameters were evaluated: colour of the optic nerve disc, borders, level, as well as the temporal pallor of the optic nerve disc was diagnosed.

With the OCT method (Heidelberg Engineering SPECTRALIS), RNFL thickness was measured in six standard sectors (temporal, temporal upper, temporal lower, nasal, nasal upper and nasal lower), measurements expressed in micrometres (mcm). RNFL thickness results were evaluated according to the OCT apparatus normative database, where green-marked areas were classified as normal, but the red-marked areas were considered abnormally reduced. OCT images of unsatisfactory quality were rejected.

MRI examination of the brain was performed by means of MRI device with a 1.5 T magnetic field strength. FLAIR 3D with MPR reconstructions axially and/or sagittally, coronary, T1 3D (T1 3D IR) with MPR reconstructions axially and/or sagittally, coronary were performed. T2 axially 4 mm and T2 FS for optic nerves coronary 3 mm including *chiasma opticum* was conducted. When assessing the MRI results, the number and localisation of demyelinating lesions and active, contrast enchancing lesion existence were analysed.

Statystical analysis. Statisical analysis was carried out by means of IBM SPSS v.22. programme. Data were presented as mean (M) and standard deviation (SD) or median (Me) and interquartile range (IQR) for continuous variables, and counts and percentages (%) for categorical variables. Logistic regression modeling techniques were used to determine which patient factors were associated with the binary outcome. All tests were two-sided and considered statistically significant at $p < 0.05$.

Results

The study included 76 MS patients whose average age was 38.64 years (SD = 10.60). The control group included 28 healthy subjects aged 19 to 65 years and in this group the mean age was 35.78 years (SD = 12.14). On the basis of the analysis of variance (ANOVA), it was found that MS patients and the control group by the average ages were not statistically significantly different ($p = 0.12$).

Comparing the control group and MS patients' group through OCT analysis, it was concluded that RNFL set was statistically significantly different ($p < 0.05$) in all quadrants. While, on the basis of the ROC curve analysis, it was concluded that the biggest difference was detected in the temporal RNFL quadrant (accordingly AUC = 0.72; 95% CI: 0.64 to 0.79; $p < 0.01$). Therefore, in the forthcoming calculations, data for the temporal quadrant of RNFL (RNFLT) were analysed.

Demographic details and clinical details of MS patients and controls are represented in Table 1.

Table 1. Clinical and demographical characteristic of subjects

Characteristic	MS patients	Controls
Number	76	28
Age, years	38.64 (SD = 10.60)	35.78 (SD = 12.14)
Disease duration, months	39.56 (6–384)	N/A
EDSS Me, IQR	1.5 (1)	N/A
Visual acuity (corrected)	0.94 (SD = 0.16)	1.00
Mean N75 / P100 amplitude, mcV	9.69 (SD = 4.85)	14.51 (SD = 3.35)
Mean P100 latency, ms	118.53 (SD = 16.33)	101.81 (SD = 5.66)
Mean RNFLT, mcm	61.62 (SD = 14.33)	70.93 (SD = 9.50)
Colour vision disturbances (eyes), %	27 (17.8%)	N/A
Visual field defects (eyes), %	101 (66.4%)	N/A

Logistic regression model development. In total, assessing all the factors affecting RNFLT and basing on the logistic regression analysis method, the mathematical models were developed that further assisted in prediction of reduced RNFLT.

Out of the 11 independent variables, only four variables were found to be significant ($p < 0.05$) after applying binary logistic regression analysis to predict risk of reduced RNFLT.

A binary logistic regression analysis was computed to analyse the impact of those predictors on reduced RNFLT that proved to be significant in the univariate comparisons. Following regression models were developed.

The first model. In the first logistic regression model we placed the following parameters: reduced VEP N75 / P100 amplitude and prolonged P100 latency. In the model both the parameters and the constant were statistically significant ($p < 0.05$). Logistic regression equation coefficients and their credibility predicting reduced RNFLT are shown in Table 2.

Table 2. Predictors of the first regression model for classifying reduced RNFLT

Predictors	Beta	SE	Wald	p-value	OR (95% CI)
Prolonged P100 latency	1.83	0.42	18.90	< 0.001	6.23 (2.73–14.22)
Reduced N75 / P100 amplitude	1.41	0.46	9.50	0.002	4.12 (1.67–10.16)

The regression model explained 30% of the variance (Nagelkerke's $R^2 = 0.30$) and altogether classified 79.8% of the reduced RNFLT correctly.

The resulting logistic regression equation in the logit form is as follows:

Logit (for the reduced RNFLT) = – 2.53 + 1.31 × N75 / P100 amplitude + 2.36 × P100 latency.

In logistic regression model, we have computed the predicted risk (P) with the help of the following equation:

$$P \text{ (for the reduced RNFLT)} = \frac{1}{1 + \exp. - (2.53 + 1.31 \times N75 / P100 + 2.36 \times P100)}$$

Analysing the odds ratios from the calculated logistic regression model, it was found that for individuals, whose N75 / P100 amplitude was reduced, the chance to have a reduced RNFLT was 4.12 (95% CI: 1.67–10.16) times more than for those with normal amplitude. Conversely, for the individuals having prolonged P 100 latency, the chance to have a reduced RNFLT was 6.23 (95% CI: 2.73 to 14.22) times more than for patients with normal VEP latency.

The second model. In the next model, which characterises a reduced RNFLT, in addition to changed VEP parameters we have added also the patients' disease duration, and obtained the following picture (Table 3).

Table 3. Predictors of the second regression model for classifying reduced RNFLT

Predictors	Beta	SE	Wald	p-value	OR (95% CI)
Prolonged P100 latency	0.68	0.43	14.90	< 0.001	5.37 (2.28–12.63)
Reduced N75 / P100 amplitude	1.65	0.50	10.96	0.001	5.23 (1.96–13.92)
Disease duration	0.05	0.002	6.80	0.009	1.005 (1.001–1.01)

The second logistic model classifies 76.3% of the reduced RNFLT correctly and explains 35% of the variance (Nagelkerke's $R^2 = 0.35$).

The resulting second logistic regression equation in the logit form is as follows:

Logit (for reduced RNFLT) = – 3.23 + 1.65 × N75 / P100 amplitude + 1.68 × P100 latency + 0.05 × disease duration

Conversely, the equation useful for practice is as follows:

$$P \text{ (for reduced RNFLT)} = \frac{1}{1 + \exp. - (3.23 + 1.65 \times N75 / P100 + 1.68 \times P100 + 0.05 \times \textit{disease duration})}$$

As can be seen from the second equation, the chance to get reduced RNFLT increases by 5% with each year of the disease.

The third model. The third model also involves changes in the *fundus oculi*, this parameter was with p = 0.74, which means that this parameter only could potentially have a statistically significant effect on reduced RNFLT existence. In this model, Nagelkerke's R^2 increases by 2.7% to 37.4%, and as we have concluded, for individuals who are observed changes in *fundus oculi*, the chance to get reduced RNFL is 2.39 (95% CI: 0.92 to 6.25) times more than for patients with normal *fundus oculi* finding.

The fourth model. Logistic model, which has classified our patients in the best way in order to predict reduced RNFLT embodied prolonged P 100 latency, reduced N75 / P100 amplitude and the number of MRI demyelinating lesions (Table 4).

Table 4. Predictors of the third regression model for classifying reduced RNFLT

Predictors	Beta	SE	Wald	p-value	OR (95% CI)
Prolonged P100 latency	1.70	0.59	8.21	0.01	5.523 (1.71–17.77)
Reduced N75 / P100 amplitude	1.54	0.61	6.36	0.01	4.68 (1.41–15.54)
The total number of lesions in MRI	0.06	0.02	8.15	0.01	1.07 (1.02–1.12)

The fourth regression model has explained even 52% of the variance (Nagelkerke's R^2 = 0.52) and classified 84.4% of the reduced RNFLT correctly, which is a very high ratio.

Logit (for reduced RNFLT) = −4.01 + 1.54 × N75 / P100 amplitude + 1.70 × P100 latency + 0.06 × number of foci.

From the equation, it is clear that log odds have linear relationship. The predicted risk of RNFLT can be calculated by means of

$$\text{P (for reduced RNFLT)} = \frac{1}{1 + \exp. - (-4.01 + 1.54 \times \text{N75} / \text{P100} + 1.70 \times \text{P100} + 0.06 \times \text{number of foci}}$$

By increasing the number of lesions for one, the chance to get reduced RNFLT grows by 7%.

In the logistic regression model we have put also the rest of the signs, which by experience or logic could influence the reduced RNFLT, such as EDSS, age, visual acuity, colour vision and visual field changes as well as clinical signs of optic neuritis in history; however, these signs failed to provide a statistically significant model.

Discussion

For MS patients visual disturbances and changes in the retinal nerve fibre layer are often observed, which relatively recently have been offered as biological markers for monitoring disease process. Debate continues whether this examination is required for each MS patient.

It should be noted that not always OCT method is available and hardware purchase possible. Our results show that the reduced RNFLT prediction is possible by employing routine methods of vision investigation. There is not a big surprise in the fact that the reduced RNFLT is best to foresee by using altered VEP results. VEP examination was originally referred to as an additional criterion for PPMS diagnosis (McDonald, Compston et al., 2001; Polman, Reingold et al., 2005); however, in McDonald criteria revised in 2010 was not repeatedly included (Polman, Reingold et al., 2011) and VEP role in the diagnosis of MS is reduced. From experimental autoimmune ON model, it is known that VEP amplitude reduction indicates the axonal tissue lesion, but latency prolongation is an early sign of demyelination (You, Klistorner et al., 2011).

Similarly to previous studies (Trip, Schlottmann et al., 2005; Fisher, Jacobs et al., 2006; Klistorner, Arvind et al., 2008; Naismith, Tutlam et al., 2009; Talman, Bisker et al., 2010; You, Klistorner et al., 2011), our results confirm that both VEP and OCT measurements can provide additional information on the integrity of visual ways. In literature sources there is a correlation between RNFL and the average N75 / P100 amplitude, as well as the average P100 latency both in eyes after ON and ON unaffected

eyes (Naismith, Tutlam et al., 2009; Di Maggio, Santangelo et al., 2014). However, some authors approve isolated association of RNFLT with P100 latency (Fatehi, Shaygannejad et al., 2012), while others with VEP amplitude (Trip, Schlottmann et al., 2005). In studies, RNFLT has been established as a parameter with a relatively lower sensitivity compared to VEP changes. In publications describing the number of cases detected, VEP method has revealed abnormalities more frequently if compared to OCT (Naismith, Tutlam et al., 2009; Di Maggio, Santangelo et al., 2014). One of the possible explanations for the low sensitivity of RNFL is the fact that this test concerns only the beginning portion of the afferent visual ways, whereas the VEP indicates whole integrity of the visual system from the retina to the visual cortex. Therefore, if the circumstances allow to apply only one method, VEP examination is preferable; however, a combination of both methods increases the chance to diagnose the optic nerve damage. In addition, on the basis of the model developed in our study, it is possible to predict reduced RNFLT by using VEP parameters.

Although in previous studies colour vision disorders (Villoslada, Cuneo et al., 2012) and changes in visual fields (Costello, Hodge et al., 2008) statistically significantly correlated with RNFL, in our hands these figures fell outside the developed prediction models. Similarly, there are different studies on the EDSS step correlation with reduced RNFL (Grazioli, Zivadinov et al., 2008; Toledo, Sepulcre et al., 2008; Tatrai, Simo et al., 2012); however, our data show that using the EDSS step in the prediction of atrophic retinal changes is impossible.

Studies carried out confirmed the impact on the disease duration on RNFL thickness and estimated continuous decrease for about 3.7 mcm/year after ON episode (Costello, Coupland et al., 2006). However, there are also published studies claiming that the RNFL thickness is not affected by the duration of the illness (Serbecic, Aboul-Enein et al., 2010). According to our results, the chances to get reduced RNFLT increases by 5% with each year of the disease.

By analysing the MRI results, unfortunately, we took into account the total number of lesions in the brain, but did not precisely localise lesions in visual pathways. Interestingly, the atrophic changes in the MRI examination of the optic nerves did not fit into reduced RNFLT prediction model, but it should be noted that the optic nerve atrophy was determined approximately, without a precise optic nerve cross-sectional area measurements.

At present, the role of OCT in monitoring of MS progress is not clear; however, by employing the proposed regression models it is feasible to provide a selective sending of patients to tertiary visual investigation centres, where OCT device is available.

Conclusions

The best choice to predict a reduced RNFLT is by using reduced N75 / P100 amplitude, prolonged P100 latency and the number of demyelinating lesions in MRI examination. Our model may be applied for identification of individuals who are at high risk of reduced RNFLT and sending them to perform OCT to tertiary MS centres. Future studies are, however, required for further validation of its practical and diagnostic utility, as well as analysis of a greater number of patients and a more precise MRI diagnostics that would improve the prediction quality of the model.

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Peculiarities of Phonetic Adaptation of Patients after Oral Rehabilitation with Conventional Removable Dentures (Evidence-based Literature Review)

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Abstract

Speech distortions often appear after oral rehabilitation with removable dentures, in the course of time these changes become less evident or disappear completely. Adaptation potential in elderly patients is usually diminished and speech function restoration could be compromised. This phenomenon is not fully reflected in literature.

The aim of the study was to analyse the present knowledge on phonetic adaptation in patients with removable dentures.

Literature for the study was selected through search in PubMed and other databases, including papers published in the period of 1990–2015. All in all, 104 articles were identified, out of which 50 were selected for detailed assessment.

Removable dentures are perceived as irritants causing responsive reaction of compensatory-adaptive nature. The following review attempts to summarise and systematise possible factors contributing to patients' phonetics adaptation to removable dentures.

Phonetic adaptation to removable dentures is a complicated process depending on various factors, the most significant of which are the functional quality of denture and patient's motivation to use prosthesis. Denture adhesives should be used to improve retention of the denture thus facilitating patients' phonetic adaptation. While planning the design of complete removable or partial denture with acrylic base, special attention should be given to anterior palatal region; there is scientific evidence that incorporation of rugae improves patients' phonetic performance. In case of conventional partial dentures with metal connector the middle type of the major connector should be used whenever it is possible, avoiding coverage of the frontal and distal areas of the palate with artificial base. Mechanical or psychological trauma caused by removable dentures may prevent successful phonetic adaptation. Patients with removable dentures should be invited for check up on regular basis.

Keywords: speech, dental prosthesis, removable denture, phonetic, adaptation, ageing.

Introduction

Speech is a unique phenomenon which only human beings possess. It is an important form of communication in society which directly or indirectly affects patients' quality of life (Scott, 2001; Jindra, 2002; Ozbek, 2003; Papadaki, 2012). After tooth loss, patients undergo chronic limitations in fulfilling common function – especially mastication, speech functions as well as aesthetics are influenced. It can cause psychological and social problems (Fiske, 1998; Scott, 2001; Jindra, 2002; Papadaki, 2012). The goal of prosthetic rehabilitation is to replace missing teeth thus improving aesthetics and restoring impaired functions. In case of extensive or total tooth loss, it is possible to fabricate soft tissue supported conventional removable dentures (Bilhan, 2013).

For a long time conventional removable dentures presented the only means of compensation for tooth loss and restoring function in patients with extensive or total edentulism. Appearance of implant supported constructions considerably improved stability and retention of the dentures. However, there are many patients whose circumstances (medical, psychological or financial) make them keep to the conventional means of prosthetic modalities (Critchlow, 2009; Carlsson, 2010). In Latvia 60.4% of patients in the age group 65–74 years require at least one removable denture (Vidzis, 2012). Taking into account the amount of lost teeth and considering every patient's financial possibilities, 25% of patients are provided with conventional partial-prosthesis with acrylic base (Soboleva, 2006; Vidzis, 2012).

These constructions are known to have certain drawbacks – they are massive and take much space in oral cavity, their functional value is connected with the condition of denture bearing area. It has been established that grinding ability of a removable prosthesis is restored to only 25%, as compared to that of people with natural teeth (Roumanas, 2009; Carlsson, 2010). Despite the above-mentioned shortcomings, the degree of patient satisfaction with removable dentures is high (Carlsson, 2010; Critchlow, 2010; Vidzis, 2012).

It is a fact that insertion of removable dentures substantially changes oral cavity volume, interfering with exhaled air flow, as well as with articulation contacts between tongue and teeth, hard palate and alveolar ridge mucosa during speech sound production. It is well known that at the initial stage of patients' adaptation to removable denture speech distortions which decrease over time are often observed; however, in some cases they may become permanent (Dragobetskii, 1992; Ozbek, 2003; Rogrigues, 2010; Van Lierde, 2012; Knipfer 2012).

It should be taken into consideration that in elderly patients the ability for adaptation diminishes. It could be explained by age related alterations in both nervous system and muscular coordination and other ageing processes (Muller, 1995a; Helgeson, 2002; Critchlow, 2010; Mysore, 2012). With increasing life expectancy, demand for prosthetic rehabilitation for ageing population is growing. Muller studied different aspects of patients' physiological ageing process and their connection with the success of prosthetic rehabilitation. It was stressed that elderly patients present a heterogenic group where each person requires individual approach and dentists should be well acquainted with gerontological peculiaries of dental treatment (Muller, 1993; Muller, 1995a; Muller, 1995b). Fiske and coworkers studied the emotional aspect of tooth loss and its influence on a patients' adaptation process to prostheses (Fiske, 1998; Scott, 2001).

It should be pointed out that the factors influencing patients' phonetic adaptation are not fully reflected in literature and therefore practical recommendations for improvement of prosthetic rehabilitation results have not been elaborated yet.

Aim

The aim of the study was to analyse literature on phonetic adaptation in patients with removable dentures, to identify the factors contributing to the degree of speech adaptation.

Material and Methods

Literature was selected through search in PubMed (Medline), Science Direct, EBSCO, DynaMed and Cochrane Library. The search was restricted to papers published in English, Latvian and Russian, in the period 1990–2015.

Keywords used for search were speech, dental prosthesis, removable denture, phonetics, adaptation and ageing.

Additionally, a manual search in the major prosthetic journals was performed. In order to be precise in data collection and to obtain all available information, references to all selected articles were screened and, in case of relevance, included in current review.

Within the context of the aim of this review, the following questions were formulated:

- What is phonetic adaptation, its definition and what methods of its assessment are available in modern dentistry?
- What factors contribute to patients' phonetic adaptation after oral rehabilitation with conventional removable dentures?

Full text papers were obtained and assessed for the inclusion or exclusion criteria. Only articles on adult patients with partial or total tooth loss, rehabilitated with different kind of conventional removable dentures were included in the review. Papers dealing with rehabilitation of patients with congenital or acquired maxillary defects were excluded from the research due to difference in speech quality determining parameters. Papers on speech performance of patients with dental implants were also excluded due to difference in biomechanical properties of implants and soft tissue supported constructions.

Results

The digital literature search revealed 104 papers in English and Russian. After evaluation of inclusion criteria, only 28 papers were included in the review, out of which 3 were literature reviews. The remaining 76 papers did not suit the pre-established inclusion criteria. Manual search resulted in adding 23 papers, out of which 5 were literature reviews.

All in all, 50 scientific papers were included in the review: 7 literature reviews and 43 clinical researches.

Discussion

What is phonetic adaptation, its definition and what means of its evaluation are available in modern dentistry?

Removable dentures are perceived as irritants causing responsive reactions of compensatory-adaptive nature. Adaptation in general is a complex biological process with a very individual pace (Dragobetskii, 1992). This process is described and classified differently; most often three main stages are distinguished – straight after insertion of the removable dentures the first reaction appears, which is characterised by patients focusing attention on the new object in the mouth, physiologically it is manifested in hyper salivation, speech and chewing function transient disturbance and in some cases appearance of gagging reflex (Dragobetskii, 1992; Leles, 2003). After transitory period, patients acquire a neuromuscular control that compensates alterations in oral cavity (Leles, 2003). This stage is the so-called partial suppression stage during which salivation becomes physiological, speech function is gradually restored and the gagging reflex disappears (Dragobetskii, 1992). It is mentioned that patients' complete adaptation to removable dentures including speech quality improvement takes place in the total suppression stage (Dragobetskii, 1992; Muller, 1995a).

The term phonetic adaptation denotes the degree of restoration of speech, articulation and diction in patients after prosthetic rehabilitation; be it biological, psychological, physiological and neurological processes unity (Wada, 2011; Luraschi, 2013). Patients have to adapt themselves to the altered environment of oral cavity and to develop new stereotypes of speech sound production (Muller, 1995).

So far there is no universal methodology of speech quality evaluation after prosthetic treatment. In some papers, patients' speech analysis was performed by means of acoustic phonetic methods (Runte, 2001; Runte, 2002; Jindra, 2002; Stojcevic, 2004; Zaki Mahross, 2015) (spectrograms, spectral analysis were assessed).

In other researches experienced speech therapists were invited for cooperation (Ozbek, 2003; Rodrigues, 2010), whereas other authors recommend the use of palatogrammas for assessment of speech process and for further personalisation of removable dentures, resulting in improvement of phonetic adaptation (Farley, 1998; Kong, 2008).

Each of these methods has a certain drawback that is why other objective and rather independent methods of speech evaluation are needed. Lately there appeared several scientific papers where patients' speech quality was analysed with the help of autonomic, computer-based speech recognition systems usage (Inukai, 2006; Ando, 2006; Stelzle, 2010; Wada, 2011; Knipfer, 2012; Wada, 2014). These methods proved to be objective and rather simple to perform. However, due to technical limitations this kind of analysis is only available for certain languages – namely German (Stelzle, 2010; Knipfer, 2012) and Japanese (Ando, 2006; Inukai, 2006; Wada, 2011; Wada, 2014).

In several articles more than one method of speech quality evaluation was used; for example, in the study performed in the University of Cairo, Egypt, the effect of different denture adhesives on phonetic function of 15 patients was assessed using both perceptual and acoustic techniques (Aziz, 2010). In the research held in King Saud University, the influence of setting of artificial teeth in the neutral zone and its effect on speech was evaluated both with the help of objective methods, such as acoustic analysis and duration taken for recitation of certain religious text, as well as patients subjective evaluation was assessed (Adaki, 2013). Yet as it has been stressed by Knipfer et al, there is no universal method that allows for objective and independent assessment of speech worldwide. That is why the comparison of different studies is somehow complicated (Knipfer, 2012).

What factors contribute to patients' phonetic adaptation after oral rehabilitation with conventional removable dentures?

The main requirement for restoration of speech function is total completion of patients' adaptation process to the removable denture (Muller, 1995a). According to the data found in literature, between 10% and 16% of all edentulous patients cannot achieve complete adaptation to conventional dentures (Laurina, 2006; Critchlow, 2009). Critchlow performed an extensive literature review, aimed at identification of possible prognostic indicators for predicting the success of oral rehabilitation of patients with conventional dentures (Critchlow, 2009). But we could not find any literature review on predictive indicators of patients' phonetic adaptive ability to conventional prostheses published in scientific literature.

There are certain contradictory points of view on patients' phonetic adaptation (Critchlow, 2009; Carlsson, 2010). Many authors consider that restoration of speech function plays an important role in a patients' satisfaction with prosthetic rehabilitation (Celebić, 2003; Zlatarić, 2008). However, it is a fact that during denture fabrication speech function restoration is not paid much attention to as a rule (Kong, 2008). Another contradiction is related to the functional quality and technical characteristic of removable dentures. While some authors do not find any correlation between speech alterations and the technical quality of prosthesis (Rodrigues, 2010), others stress that even minimal changes in removable dentures construction will inevitably lead to alteration in speech performance (Ichikawa, 1995; Runte, 2001; Runte, 2002, Inukai, 2006; Wada, 2011; Adaki, 2013, Zaki Mahross, 2015).

A review of data found in literature made it possible to come to the conclusion that the factors contributing to patients' phonetic adaptation are closely interrelated. For convenience reasons, we attempted to divide them into two major groups – local factors related to the prosthetic field and removable denture itself and general factors, which are related to patients' psychoemotional state, motivation to use dentures and the degree of individual adaptive capacity.

Local factors

In their turn local factors can be divided into two main groups: biological factors such as anatomical and physiological conditions of the prosthetic field and the second group which could be defined as biotechnical factors, describing specific features of the removable dentures, their technical quality and functional value.

Biological factors describe anatomical and physiological condition of the prosthetic field. This factor should be taken into consideration as one of important prognostic indicators for the success of conventional removable dentures, but in most cases it cannot be improved, unless implant supported prosthesis are planned (Critchlow, 2009; van Lierde, 2012).

The degree of atrophy of alveolar ridge and the clinical conditions of denture supporting soft tissues are of great importance for patients' phonetic adaptation. The retention and stability of the conventional removable dentures to a great extent depends on the amount and condition of its supporting structures. A proper amount and quality of saliva is an important prerequisite for successful retention of the conventional dentures. It is considered that well formed alveolar ridge and technically correct dentures with good denture retention facilitate the adaptation process (Muller, 1995b; Critchlow, 2009). However, poorly formed alveolar ridge, namely in lower jaw is a negative factor for denture retention (Critchlow, 2009). Geriatric patients often have chronic diseases, in most cases they use some prescribed medicines and in some cases the side effect of medication could influence the condition of denture bearing mucosa and/or alter the salivation function (Dragobetskii, 1992; Helgeson, 2002). In this case dental implants could improve denture stability. Nevertheless, osseointegration significantly improves denture retention and stability, phonetic function and adaptation remain in the focus of scientific attention both for fixed and removable prosthesis based on dental implants (Van Lierde, 2012). If due to different circumstances dental implants are not an option for treatment planning, then it is recommended to use dental adhesive in order to improve retention and facilitate patients' phonetic adaptation. Fifteen completely edentulous patients with flat mandibular ridge were recruited in the study performed in Cairo University, the effect of three different denture adhesives on speech performance were assessed both with the help of perceptual and acoustic techniques, it was concluded that whenever possible denture adhesive should be used to improve denture retention and facilitate speech sound articulation process (Aziz, 2010).

Evaluating different complications in patients with existing complete dentures, Bilhan came to the conclusion that dentures related sore spots interfere with a patient's speech ability (Bilhan, 2013). Persistent pain and discomfort related to denture wearing are disturbing for a patient's phonetic adaptation. Thus, it is strictly recommended to control denture base border extensions, and to eliminate sharp edges (Dragobetskii, 1992; Muller, 1995; Mysore, 2012; Bilhan, 2013).

Biotechnical factors are related to the removable denture itself and it can be to a great extent influenced both by dentist and by dental technician. It was stated previously that correct technical construction of removable dentures is an important indicator for overall success of the removable denture (Jindra, 2002; Critchlow, 2010). However, we did not manage to find literature review on how different denture constructive solutions effect patients' speech performance.

Conventional removable dentures form a wide group of denture prostheses that differ in form and ways of fixation in oral cavity. Still there are several features that unify this group – first all the dentures are removable – meaning that dentures can be inserted and removed but patients themselves.

Removable denture base and speech production process. In the removable dentures the base is an essential part of the construction; it connects all other elements together, provides retention, support and stability (Ozbek, 2003; Hassel, 2006; Kong, 2008; Wada, 2011; Zakkula, 2014); at the same time it covers the places of articulation of the tongue and alveolar ridge and could cause signifinant phonetic distortions. Therefore, the association between the removable denture base and speech production was studied extensively. One of the main prerequisits of success is that the denture base should be well adapted to oral cavity anatomy (Jindra, 2002; Laurina, 2006).

Due to great difference in biomehanics of various types of dentures, for further analysis we divided literature according to the type of denture evaluated in the research – forming the best evidence available for two main groups of dentures – the first one consisting of complete dentures and extensive partial removable dentures with acrylic base and the second group of different modalities of conventional partial dentures with a metal connector and their effect on speech performance.

Complete denture is the most often evaluated modality of removable denture and its impact on patients' speech production is widely described in literature. One of the most frequently discussed topics is the thickness of the denture base, as after insertion in oral cavity complete removable denture covers all the palate and significantly diminishes the volume of oral cavity. It was concluded that denture base should be as thin as possible in order to minimise alteration of oral cavity, as well as to minimise the distortion of tongue movement during speech sound production and at the same time it should be thick enough to withstand its deformation under function such as masticatory forces (Ichiwaka, 1995). But there is no scientific evidence yet as to exact thickness of complete dentures should be to facilitate patients' phonetic adaptation. A recent experimental study on evaluation of palatal plate thickness of maxillary prosthesis on phonation was performed in Sibar Institute of Dental Sciences in India; twelve subjects were provided with experimental acrylic plates of different thickness and its effect on the phonation of vowels were assessed. The author explained the selection of these sounds by the fact that they are the simplest sounds to be analysed and described acoustically; it was concluded that increasing the thickness of the palatal plate from 1 mm to 3 mm has not shown any significant effect on phonation. This conclusion is somehow very predictable, but it is possible that other research will follow from the same group of authors that could clarify the mechanism of patients' phonetic adaptation to the conventional removable dentures (Zakkula, 2014). Whereas research performed in Japan showed that for correct pronunciation of speech sounds the curvature of the palatal contour in complete dentures should be from 2–4 mm (Ando, 2006).

Another topic of interest in connection with phonetic dentistry is different ways of personalisation of palatal surface of complete dentures and of extensive partial removable dentures, which covers the anterior region of the alveolar ridge. In order to facilitate quicker phonetic adaptation different methodologies of palatogram were described. Actually a palatogram is a record of exact contact made between the hard palate, natural and artificial teeth during speech sound production. This method can be used both in wax try in stage or after delivery of the denture, as the assessment of phonetic function (Farley, 1998; Ando, 2006; Kong, 2008).

In his turn Hassel proposed an easy and quick way of personalisation of complete denture. In his pilot study fourteen patients with conventional complete maxillary dentures participated, and in order to improve the patients' phonetic adaptation the anterior part of palatal surface was sandblasted. This study revealed that the above mentioned alterations to some extent improved patients' speech performance. As it was stated by the author, this method is easy and effective, but if the desired outcome is not achieved, then it is always possible to polish denture's surface as no irreversible alterations to the denture took place (Hassel, 2006).

Rugae reproduction on the conventional removable denture with acrylic base is a subject of significant controversies (Krishna, 2012; Adaki, 2013; Zaki Mahross, 2015). On the one hand, adding rugae increases denture thinkness, but on the other hand anatomical structures such as rugae and papillae incisive are definitive landmarks for the tongue during speech sound production. A positive effect of additional rugae was proved by spectral analysis (Zaki Mahross, 2015). Adaki performed the study in order to evaluate the impact of rugae on phonetics. The effect of arbitrary rugae and customised rugae on patients' phonetic adaptation was compared and as a result of this study they also came to the conclusion that addition of personalised rugae to the palatal surface of the denture facilitates the patient's phonetic adaptation (Adaki, 2013). The authors proposed that slight modification in anterior part of palate, which requires minimal amount of time, gives a better result regarding pronunciation of speech sounds and as a result enhances patient's ability and willingness to communicate.

The same facts of personalisation and modification of palatal surface of the denture base that are discussed in connection with complete dentures could be used in case of extensive partial removable dentures with acrylic base. Researches came to conclusion that rationally fabricated prostheses do not

interfere with patients' phonetic adaptation. In case of comparison of speech performance of edentulous patients without conventional dentures and after adpatation to the removable prosthesis it could be concluded that technically correctly fabricated dentures increase the quality of speech performance (Stelzle, 2010).

Conventional partial removable denture with maxillary connector. It is logical to assume that conventional partial removable dentures with maxillary connector are more useful from the phonetic adaptation point of view, as in most cases where this type of prosthetic modalities is recommended, there is still a significant amount of natural teeth. As well as the constructional solution of this prosthesis requires less coverige of oral mucosa. We have found the scientific evidence on this topic. Two studies were performed by the same researcher in Japan, with the help of originally developed speech recognition system two types of experimental connectors were evaluated from the phonetic point of view. The effect of narrow (8 mm width) and wide (20 mm width) metal connectors were assessed, as well as different cross sectional shapes were analysed and it was concluded that the width and shape of the connectors have a limited effect on the articulation of consonants (Wada, 2014). In a previous study the same speech evaluation method was used in order to evaluate the effect of different location of the experimental metal connector on speech performance, and it was found that locating the major connector in the middle area of the palate resulted in smaller distubance than in other areas from the point of view of accuracy of speech sound production (Wada, 2011).

Two following topics relating to the phonetic adaptation after oral rehabilitation are in common for all kinds of conventional removable dentures, namely the position of artificial teeth and the functional value of removable dentures and will be discussed without dividing in to smaller groups.

Tooth loss and position of artificial teeth. The degree of the patient's speech distortions following tooth loss and consequent prosthetic rehabilitation depends on location and extent of edentulous span. In particular the changes in the maxillary front region could influence the mechanism of speech production. In this area 90% of Latvian consonants are formed. Stojcevic analysed formation mechanisms of Croatian dental and postalveolar group of sounds and removable denture impact on sound pronunciation accuracy, using spectral analysis method. The study showed that patients with conventional partial removable prosthesis have 50% less distortions as compared to the same patients' speech without dentures (Stojcevic, 2004). In his turn Ozbek studied formation of Turkish sounds after prosthetic rehabilitation. The speech quality of 15 patients was evaluated by three experienced speech pathologists. They came to the conclusion that some sound groups are influenced to a greater extent as compared to others. However, after a week of adaptation, all sound production was improved (Ozbek, 2003). Using the spectral analysis method Runte indicated that even minimal displacement of maxillary incisors causes sound /s/ distortion (Runte, 2001; Runte, 2002). It was also concluded that neuromuscular adaptation plays a more significant role as compared to simple aerodynamic changes (Runte, 2002; Cagna, 2010). It is recommended to place artificial teeth in the original position of the lost natural dentition (Runte, 2001; Runte, 2002). For artificial teeth arrangement, it is recommended to use anatomical landmarks – incisive papilla and first prominent rugae palatinae – this will ensure the position of teeth in agreement with phonetic and esthetic requirements (Runte, 2001; Runte, 2002; Laurina, 2006). It was suggested that assessment of quality of /s/ sound pronunciation during fabrication of the denture, namely in wax denture try in stage, would decrease the amount of adaptation required from patient, thus facilitating the phonetic adaptation process (Inukai, 2006). Production of /s/ sound is of great interest in phonetic dentistry. It is mentioned in literature that the /s/ sound phonetic test can be used in determining the vertical dimension of occlusion, this method was already described in 1952, but so far it has not lost its relevance, and these articles were recently republished (Silverman, 2001; Pound, 2006). During production of sibilant sound /s/ the lower jaw protrudes and takes the so-called "closest speaking space (CSS)", which means that a 1–2 mm gap is formed between the upper and lower incisors. With the advance of technology, new possibilities in assessment of phonetic adaptation arise. The use of kinesiographic instruments and jaw tracking software program gives objective data on patients' adaptation mechanism to removable dentures (Leles 2003).

Another significant phonetic element is /m/ sound, which could be used both in the fabrication process and as a mean for evaluation of the existing dentures (Rodrigues, 2003). /F/ and /v/ phonetic probes are generally recommended for assessment of the position and length of restored front teeth (Roumanas, 2009).

The aesthetic side of removable dentures plays an important role in the patient's acceptance of the denture and satisfaction with prosthetic result, improvement of dentures visual characteristics will facilitate the patient's phonetic adaptation (Zlatarić, 2008). Tooth loss dramatically influences facial appearance. Removable denture could partially restore both missing teeth and alveolar ridge, thus providing support for facial soft tissues (Laurina, 2006). An extensive literature review was published on historical and modern clinical techniques for physiological registration of artificial tooth position in the so-called neutral zone. It was stated that this position improves dentures stability and also contributes to the patient's facial expression (Cagna, 2010, Al-Magaleh, 2012). A detailed analysis of phonetic characteristics in 30 patients with different approaches to denture manufacturing techniques proved superiority of dentures fabricated within the neutral zone as compared with other methods of artificial teeth arrangement, They appeared to facilitate patients' phonetic adaptation and these findings were assessed both subjectively and objectively (Cagna, 2010; Al-Magaleh, 2012).

Functional value of removable dentures. It has been observed that patients' adaptation may be a reversible process and with a change of oral condition, the patients adaptation to the existing dentures can diminish (Knipfer, 2012; Bilhan, 2013). The most frequent alteration in oral cavity appears due to continuing resorption of residual alveolar bone resulting in decrease in denture stability and retention (Bilhan, 2013). To some extent loss of physiological retention is compensated by muscular control (Muller, 1995a). It is mentioned in literature that the tongue of patients wearing removable denture has multiple functions taking part in speech sound articulation and control of denture stability. Thus, in case of decreasing dentures functional value, the demand for denture control increases and this could possibly interfere with speech production process (Laurina, 2006). From phonetic adaptation point of view, routine recalls for conventional denture wearers seem to be a crucial factor for preventing complications (Dragobetskii, 1992).

General factors contributing to phonetic adaptations

Phonetic adaptation of a patient with removable dentures depends on various factors such as: patient's individual adaptation capacity, acuteness of hearing, psychoemotional characteristics, motivation, social needs and acceptance of removable prosthetic modalities. It is essential for the dentist to find out the patients motives for oral rehabilitation. If a patient has a social need connected with his professional activities, then his phonetic adaptation will be more effective (Dragobetskii, 1992; Papadaki, 2012). The patient should have realistic expectations towards the possible drawbacks of provided dentures. If due to psychological or functional limitations the patient is not able to accept the situation, then another kind of treatment should be used (Mysore, 2012).

It is usually taken for granted that if alterations in oral cavity exceed the patient's individual adaptive capacity, then speech distortion will persist (Laurina, 2006). However, there are no definite criteria for evaluation of patient's individual adaptation potential in prosthetics dentistry as yet (Muller, 1993). Extensive research on a patients' adaptation to conventional dentures was carried out by Muller and coworkers. An attempt was made to predict a patient's adaptation outcome by means of standardised psychological tests, mental concentration capacity evaluation and manual motor ability evaluation by means of the so-called pinhole board test. Additionally, the oral motor ability was tested by asking patients to assemble two halves of test pieces a mouth (Muller, 1993). Adaptation index presented in this paper is a very important contribution to further development of this field of knowledge (Muller, 1993). In consequent studies a more detailed analysis of a patient's adaptation capacity to removable dentures was presented (Muller, 1995a; Muller, 1995b). Tooth loss inevitably leads to reduction of periodontal ligaments and alterations in proprioception. One indicator of a patient's acceptance of removable dentures might be assessment of oral perception. It was assumed that patients with a high level of oral perception should be more sensitive to alterations in oral cavity, and as a result they might be more intolerant to limitations of removable dentures. It was proposed that patients with lower level of oral perception have higher

adaptation potential to removable dentures. However, to produce intelligible speech a very coordinated and precise positioning of the tongue during sound articulation is required (Aken, 1991). Theoretically oral stereognosis might help in everyday practice to identify patients with reduced phonetic adaptation potential. In clinical studies several attempts to use stereognosis were described; however, there is no universal protocol for patients' phonetic adaptation ability evaluation by means of oral stereognosis as yet (van Aken, 1991, Muller, 1995a; Muller, 1995b; Runte, 2001). Jacob and coworkers published an extensive literature review with detailed recommendations on oral stereognosis methodology; however, such approach has not been widely used, and so comparison of the results among different studies is hard to be made (Jacobs, 1998; Runte, 2001).

Acuteness of hearing plays an important role in phonetic adaptation process. In elderly patients, age related hearing weakness is quite common (Ichikawa, 1995; Mysore, 2012). This diminishes the rate and degree of complete speech restoration in this group of patients.

Adaptation to removable denture requires a patient's cooperation, understanding of provided treatment limitations and responsibility for his own health, including fulfilling his dentist's recommendations. Friske and co-authors studied the emotional effect of tooth loss in edentulous people. They compared the emotional effect of extensive tooth loss to the effect of bereavement and distinguished five stages of grief; denial, anger, depression, bargaining and finally acceptance (Friske, 1995; Scott, 2001). Correspondingly only patients who passed through all these stages and accepted the inevitability of removable dentures can successfully adapt themselves to prosthetic treatment, otherwise complete phonetic adaptation is doubtful (Mysore, 2012). Many attempts at classification of dental patients are published in textbooks, for example, classification suggested by House, who divides all patients into four types according to their mind – philosophical mind, exacting mind, hysterical mind and indifferent mind. And the type the patient belongs to allows predicting the patient's possible reaction to the treatment (Mysore, 2012). But according to Friske, it is necessary to conduct a detailed investigation of the patient's emotional state and psychological condition (Friske, 1995; Scott, 2001). Much attention has been paid lately to the analysis of patients' subjective evaluation of their oral health conditions; in order to assess changes in a patient's perception of treatment outcome it is essential to use a unified protocol before and after treatment. Several questionnaires have been suggested, but the most frequently used one is a short version of the so-called OHIP-14 (oral health impact profile). It consists of two items for each of the seven subscales in the source instrument (functional limitation, physical pain, psychological discomfort, physical, psychological and social disabilities and handicap). Each item asks about the presence of a functional or psychosocial impact associated with problems involving teeth, mouth and dentures. Last year the original English version of the OHIP-49 was translated into Latvian and Russian. It was recommended to be used as an instrument for assessing OHRQoL among adults in Latvia (Pugaca, 2014). This instrument gives us a possibility to use validated protocol for patients' subjective evaluation of treatment outcome during phonetic adaptation process.

Conclusion

1. Phonetic adaptation to removable dentures is a complicated process depending on various factors, the most significant of which are the functional quality of denture and patients' motivation to use the prosthesis.
2. In order to facilitate patients' phonetic adaptation to the conventional dentures, denture adhesives should be used to improve retention of the denture and to ease speech sound articulation whenever possible.
3. While planning the removable dentures design, patient's individual phonetic peculiarities should be taken into consideration.
4. While constructing conventional complete removable denture and partial prosthesis with acrylic base, special attention should be given to anterior palatal region of the prosthesis. There is scientific evidence that personalisation such as incorporation of rugae of this area

improves patients' phonetic performance. In case of conventional partial dentures with metal connector the middle type of the major connector should be used whenever it is possible, avoiding coverage of frontal and distal areas of the palate with artificial base.

5. Mechanical or psychological trauma caused by removable dentures may prevent successful phonetic adaptation. Patients with removable dentures should be invited for check up on a regular basis.

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Fibrin Sealant in Maxillofacial Surgery (Literature Review)

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Abstract

The use of fibrin in oral and maxillofacial surgery is becoming increasingly relevant. Fibrin glue, also known as Fibrin sealant, demonstrates adhesive and haemostatic opportunities that are vital in surgery. Fibrin sealant has numerous applications in the field of maxillofacial surgery. It is a safe, cost-effective, and clinically proven method of providing haemostasis, securing or gluing hard and soft tissue, as well as sealing of difficult-to-reach tissues. However, some scientists disagree on fibrin sealants clinical efficacy.

The aim of this study is to summarise and analyse the most recent literature about fibrin sealant clinical usage in oral and maxillofacial surgery, as well as to ascertain the efficacy of fibrin sealants by reviewing several clinical studies.

A literature search was conducted electronically in databases PubMed and EBSCO. The search was limited to articles published in the English language between 1990 and 2015.

In all, 75 articles were found, 37 of which met the aforementioned requirements and thus were included and analysed in the study. 38 scientific papers could not be included in the literature review due to having been written prior to 1990. This article comprises and analyses materials about fibrin sealant application in oral and maxillofacial surgery.

The fibrin sealant has many applications in oral and maxillofacial surgery: for maxillary sinus floor augmentation, in bone surgery when mixed with bioceramic granules, in treatment of peri-implant defects, in facelift surgery to avoid the formation of hematomas or seromas, as well as for inducing wound healing. Furthermore, fibrin sealant is used to control bleeding in complicated situations such as difficult tooth extractions and treating patients with haemophilia.

Keywords: fibrin sealant, fibrin glue, bone surgery, facelift surgery, bleeding disorders.

Introduction

Fibronectin Sealing System was first used in 1909 by Bergel in the medical field for haemostatic purposes (Arnaud, 2000). However, the Food and Drug Administration (FDA) banned fibrin glue in 1978 due to reported cases of transmission of hepatitis. It was approved by the FDA again in 1998 after implementation of rigorous virus elimination methods (Fattahi, 2004).

Fibrin sealants are non-cytotoxic, fully resorbable biological matrices that contain fibrinogen, factor XIII, thrombin, and aprotinin. The components are extracted from human plasma, except for antifibrinolytic agent and calcium chloride. Mixing fibrinogen and thrombin simulates the last stages

of the natural coagulation cascade to form a structured fibrin clot similar to a physiological clot. This clot is naturally degraded by proteolytic enzymes from the fibrinolytic system such as plasmin (Reiss, 1996).

Traditionally, fibrin sealant is used as tissue adhesive and as a haemostatic agent, as well as in new and creative ways, such as for cellular growth stimulation in tissue engineering (Yücel, 2003).

Surgeons have long sought a material that could act as both a tissue adhesive and a haemostatic agent. Presently, fibrin sealant demonstrates the best equilibrium between both properties (Choukroun, 2006).

Fibrin sealant has numerous applications in the field of maxillofacial surgery. It is a safe, cost-effective, and clinically proven method of providing haemostasis, securing or gluing hard and soft tissue, as well as sealing or difficult-to-reach tissues (Diaz, 1994).

Aim

The aim of this study is to summarise and analyse the most recent literature about fibrin sealant clinical usage in oral and maxillofacial surgery, as well as to ascertain the efficacy of fibrin sealants by reviewing several clinical studies.

Material and Methods

A literature search was conducted electronically in databases PubMed and EBSCO (http://www.ncbi.nlm.nih.gov/pubmed/) using keywords “fibrin glue”, “fibrin sealants” and “maxillofacial surgery”. The search was limited to articles published in the English language between 1990 and 2015. After selection of the literature 37 articles were used: 21 controlled studies and 16 literature reviews.

Results

In all, 75 articles were found, 37 of which met the aforementioned requirements and thus were included and analysed in the study. 38 scientific papers could not be included in the literature review due to having been written prior to 1990 and contained outdated information and descriptions of obsolete methods.

Analysis of the literature made it clear that the usage of fibrin sealants in maxillofacial surgery is very extensive. This article summarises the most popular and available application methods of fibrin sealant in modern maxillofacial clinical practice.

Bone surgery

Considering their adhesive and haemostatic properties, fibrin sealants have been widely used in bone surgery. However, the role of fibrin sealants in bone healing or bone tissue response is controversial. Le Guéhennec (2004) has reported that fibrin sealant has a negative effect on bone healing. In another study, however, the fibrin sealant has a positive effect on bone healing (Le Nihouannen, 2007).

Currently, bone surgeons have several different possibilities when it comes to replacing bone. There are bone substitutes available that can overcome limitations of autologous bone due to their osteoconductive properties and biocompatibility (Bauer, 2000). The most frequently used alloplastic materials are based on calcium phosphate bioceramics such as hydroxyapatite and/or β-tricalcium phosphate (Suzuki, 2006).

Micro-macroporous biphasic calcium phosphate (MBCP) is a bioactive bone substitute material approved for bone filling. The porosity of the scaffold plays an important role to permit adequate tissue ingrowth. Microporosity enlarges the scaffold surface and enables osteoblasts, as well as precursor cells to adherence (Habibovic, 2005).

The combination of MBCP and fibrin sealant has shown stimulating properties in bone formation in maxillofacial surgery. The osseointegration results of the substitute material was reported as successful, and the newly formed bone was characterised by a mature, solid structure (Bagot d’Arc, 2003).

Fibrin scaffold has a double function in tissue engineering. Firstly, it is used as scaffold for incorporation of cells, proteins and other biological and pharmaceutical agents; and secondly as immobilizer of different substances in other biomaterials to provide long-term retention in site of clinical necessity and controlled release.

The biological properties may be enhanced due to fibrin which plays a positive role in vascularisation and blood vessel growth in bone defects. Fibrin mediates platelet and endothelial cell spreading, fibroblast proliferation and capillary tube formation (Mosesson, 2005).

Maxillary sinus floor augmentation

Maxillary sinus floor augmentation is a standard surgical procedure to increase bone height in the atrophic posterior maxilla for dental implant placement (Kaufman, 2003).

Micro-macroporous biphasic calcium phosphate bioceramic, combined with fibrin sealant, has been investigated in maxillofacial bone filling, including sinus floor augmentation, and it has proven to be biocompatible and osteoconductive in animal models as well as in clinical studies (Le Nihouannen, 2007).

Treatment of peri-implant bone defects with Platelet-Rich Fibrin (PRF)

Considering the high survival rate and the predictability of the procedure/practice, replacing missing teeth with dental implants has become a popular procedure among patients and clinicians (Weber, 2009). Long-term follow-up studies confirm that peri-implant complications are common and that implant survival does not necessarily indicate a successful implantation (Misch Ce, 2001).

Peri-implant defect treatment with Platelet Rich Fibrin (PRF) is clinically more effective than access flap surgery alone. It has been clinically proven in a study that took place in Oral and Maxillofacial Surgery and Periodontology of the Baskent University School of Dentistry in Ankara (Hamzacebi, 2015).

Treatment protocol: access to the implant surface and the inflammatory tissue removing, decontamination (citric acid for three minutes or tetracycline hydrochloride solution), infrabony defect filling with the PRF membranes.

PRF promoted probing depth reduction and lower distance between the restoration margin and peri-implant mucosal margin (Esposito, 2012).

Facelift surgery

Fibrin sealant is probably the most popular autologous blood product in plastic surgery practice. Fibrin sealants based on clot table plasma proteins have several potential advantages in improving the outcome of facelift surgery (Hamilton, 2001). The use of fibrin sealants strongly hinders the formation of hematomas and seromas, providing a faster recovery and return to daily activities, thus ensuring higher satisfactory rates among postoperative patients (Matarasso, 2005). The fibrin network is proven to reduce the amount of postoperative bleeding by sealing capillary vessels and making raw surfaces adhere to one another, thus closing the dead space (Zoumalan, 2008).

A prospective study was conducted in 20 patients (14 women and 6 men) undergoing a facelift surgery from June to October, 2010. The mean age of the patients was 56 (range 43–72) years. Comparisons were made considering hematoma and seroma rates, degree of induration, oedema, and ecchymosis, pain levels, as well as patient satisfaction.

Twenty four hours after surgery, no hematomas had occurred in 19 patients. The only exception was a significant hematoma on the right side in one patient. The bleeding was most probably due to a sudden rise in blood pressure during the immediate postoperative period (Botti, 2007).

Aerosolised fibrin sealant offers several potential advantages in improving the outcome of facelift surgery (Fezza, 2002). A number of studies have investigated the effectiveness of fibrin sealants in reducing formation of hematoma, seroma and ecchymosis. In addition, fibrin sealants reduce postoperative wound drainage which is important as it greatly influences patient comfort and reduces recovery time (Marchac, 2005).

Preoperative administration of autologous fibrin can be useful for enhancing the viability of skin flaps. Facelift certainly is a procedure that requires a proper preoperative planning, appropriate knowledge of the anatomy and correct surgical execution. It is also clear that fibrin glue application may not prevent possible complications such as skin necrosis. This may be practical for patients who have major risks for flap survival and are not willing to have additional delay surgery (Eppley, 2006).

Bleeding control

Patients with bleeding disorders who undergo a dental extraction are at risk of prolonged or excessive bleeding. Fibrin sealant has been used as an effective operative sealant in surgery for more than 10 years. It has also been used to control bleeding in difficult situations, such as dental extraction or surgery in patients with haemophilia or other coagulopathies, without the use of blood replacement (Rakocz, 1993).

After a tooth extraction with minimal trauma to the surrounding bone and soft tissue, the socket should be curetted and covered with fibrin gel which is based on fibrin sealant (Suwannuraks, 1993). Local homeostasis at the extraction site was achieved with a fabricated fibrin sealant. After the fibrin gel had formed, the soft tissue at the margin of the socket was sutured with non-absorbable silk, and the fibrin sealant was reapplied on top of the socket (Martinowitz, 1995).

After application at the extraction site, thrombin converts fibrinogen into an unstable fibrin clot, factor XIII stabilises the fibrin clot and aprotinin prevents clot degradation. This combined method is a safe, cost-effective procedure for dental extraction without replacement therapy in patients with bleeding disorders (Sigaud-Eiks, 2002).

Wound healing

Normal wound healing involves activation of blood clotting, fibrinolysis, kinin and complement cascades, followed by inflammation, granulation tissue deposition and remodelling (Baum, 2005). Activation of biological cascades leads to an influx of inflammatory cells into the wound and fibrin deposition. Inflammatory cells release cytokines that are involved in removal of dead tissues and deposition of macromolecules associated with the repair response (Kamolz, 2014). The inflammatory phase is followed by proliferation of capillaries and fibroblasts that lay down granulation tissue (Hosgood, 2006). During remodelling the delicate collagen fibrils deposited during granulation are replaced by larger diameter fibres that form the basis of scar tissue. Fibrin is an essential component of the wound healing process. It is the product of blood coagulation cascade (Jabs, 1992).

Fibrin is reported to stimulate the formation of granulation tissue, including increased deposition of collagen. Fibrin has also been used in skin-grafting for patients with malignant melanoma and is useful in areas where skin is stretched, such as the deltoid region (Kamolz, 2014). The wounds treated with fibrin sealant demonstrated neither oedema nor bleeding, whereas wounds that were not treated with fibrin glue exhibited oedema and bleeding as well as perivascular cell infiltration under the graft (Lundquist, 2008).

Fibrin sealant has been used to eliminate “dead space” beneath skin-grafts and to promote healing as it increases the probability that the graft will be vascularised. It is also useful in decreasing the number of sutures required in cosmetic and reconstructive surgery, thereby limiting the possibility of scarring (Silver, 1995).

Conclusions

The fibrin sealant has many applications in oral and maxillofacial surgery – for maxillary sinus floor augmentation, in bone surgery mixing with bioceramic granules, in treatment of peri-implant defects, in facelift surgery to avoid the formation of hematomas and seromas, as well as for inducing wound healing. Furthermore, fibrin sealant is used to control bleeding in complicated situations such as difficult tooth extractions and treating patients with haemophilia. Origin of fibrin is an important issue – to use allogenic commercial materials as fibrin sealant or autologous fibrin derived from the patient blood.

It is proven that fibrin sealant has many positive features, and therefore it is increasingly used in oral and maxillofacial surgery and clinical practices. However, not all results can be trusted. Fibrin sealant usage in treatment of peri-implantitis is questionable considering the lack of clinical studies dedicated to this subject and the insufficient number of proving results. A number of clinical studies are yet to be conducted in order to verify the efficacy of this method.

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Precise Assessment of Incisor Root Resorption Caused by Maxillary Impacted Canines

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Abstract

The objective of this study was with cone beam computed tomography (CBCT) to assess resorption of the lateral and the central incisors caused by upper maxillary impacted canines.

One hundred twenty six impacted maxillary canines in 104 patients were analysed using CBCT images in Osirix software. The severity of root resorption was assessed according to studies by Ericson and Kurol. Enlarged dental follicle and its association with the root resorption of the lateral and central incisors were evaluated.

In contact less than 0.5 mm with at least one adjacent tooth were 87.3% of all impacted canines. In contact with the lateral incisor were 83.3% of the impacted canines and with the central incisor – 24.6% impacted canines. Our reported rate of root resorption was 76.1% in the lateral incisors and 21.4% in the central incisors.

The results of our study show that root resorption of the lateral and the central incisor was common in patients, mostly affecting the lateral incisor to a mild degree. A wide dental follicle of impacted canine was not associated with a higher incidence of external root resorption of the lateral or the central incisors.

Keywords: impacted, canine, CBCT, resorption, incisors.

Introduction

Permanent maxillary canines are the second most frequently impacted teeth, and the prevalence of their impaction is 1–3% in general population. (Ericson, 1986; Aydin, 2004) Maxillary canines are important aesthetically and functionally, and impactions of these teeth are difficult and time consuming to treat. Moreover, the impaction of canines can lead to resorptions of neighbouring teeth, particularly the lateral incisors (Liu, 2008; Ericson, 2000).

The management of impacted canines requires accurate diagnosis and precise location of the teeth and their relation to the surrounding structures (Walker, 2005).

Diagnosis and treatment planning sometimes can be challenging with conventional radiographic methods, because of the two-dimensional (2D) representation of the three-dimensional structures (3D) that often are positioned at various angles (Walker, 2005; Tamimi, 2009).

Cone beam CT has become an increasingly popular diagnostic modality for impacted canines (Tamimi, 2009). And compared to conventional radiography, treatment plans are being altered after gaining additional information from 3D images (Bjerklin, 2006). Assessing root resorption and changes

in the root surface typically requires 3D information and reported rate of resorption to adjacent teeth have been markedly increased compared to conventional radiography (Liu, 2008; Yan, 2012; da Silva Santos, 2014; Mason, 2001; Alqerban, 2009).

Aim

The objective of this study was to assess resorption of the lateral and central incisors caused by upper maxillary impacted canines.

Material and Methods

The CBCT image database collected at the Institute of Stomatology, Rīga Stradiņš University, from March 2008 to December 2012 was reviewed on the subject of impacted maxillary canines. The study was approved by the Ethics Committee of Rīga Stradiņš University, Rīga, Latvia.

All CBCT scans were taken with an ICAT Scanner (Imaging Sciences International, Hatfield, Pa) as part of the initial orthodontic diagnostic records for the patients. The CBCT images were taken according to a standard protocol with the subject seated in a chair with the following parameters: 120 kV, 5 mA, 0.4 mm voxel, and scan time of 20 seconds. Orthodontic indication for CBCT other than palatinally impacted canines were excluded from this study (e.g. orthognathic or cleft patients).

The patients' records were revised and only those patients who came for the second consultation were included in the study. 136 patients were retrieved from the database and after exclusion criteria were applied, the study sample consisted of 104 patients (72 female, 32 male). The age range was 11 to 44 years with the mean age of 16.61 ± 5.96 years. A total of 126 impacted maxillary canines were studied, including 22 bilateral impactions, 42 left unilateral impactions, and 40 right unilateral impactions.

The cone beam generated DICOM files were imported into freeware Osirix software (v.5.7 32-bit) allowing assessment of the position of the canine and adjacent teeth in the multiplanar reconstruction (MPR) planes. MPR planes allow us precise assessment of the position of impacted maxillary canine and adjacent teeth in the three-dimensional space.

Proximity of the impacted canine to the adjacent teeth and resorption were assessed, and contact between the teeth was defined if the distance was less than 0.5 mm (Walker, 2005).

Resorption was graded in 5 slightly modified categories, based on the grading system suggested by Ericson and Kurol and also tried on CBCT by Liu et al. (Ericson, 2000; Liu, 2008):

- no contact (0);
- in contact, but intact root surfaces (no resorption) (1);
- mild resorption: resorption midway to the pulp or more, the pulp lining being unbroken (2);
- moderate resorption: the pulp is exposed by the resorption, the involved length of the root is less than one third of the entire root (3);
- severe resorption: the pulp is exposed by the resorption, and the involved length is more than one third of the root (4).

Each size of follicle was measured at the widest area and recorded. If dimension was greater than 2 mm, it was considered as enlarged follicle (Walker, 2005). Every measurement was screenshot for reference purposes.

Results

Most patients in this study with impacted canines were female (69%), and there was no significant difference between left and right impactions (42 left, 40 right, 22 bilateral impactions).

Statistical analysis was conducted using SPSS software (version 22.0). An intraclass correlation (ICCs) was used to assess the consistency of reproducibility of linear measurements. The coefficient of intraclass correlation (ICCs R value) was very high for reproducibility of linear measurements (ICC ranged from 0.992 to 0.999).

Of the 126 impacted canines in contact less than 0.5 mm were 110 canines (87.3%). In contact with the lateral incisor were 105 impacted canines (83.3%), and with the central incisor 31 – impacted canines (24.6%).

In total, 96 canines resorbed lateral incisor (76.1%) and 27 canines resorbed central incisor (21.4%) (Categories 2–4).

More detailed relationships between resorption categories and impacted canines have been represented in table 1.

107 impacted canines from 126 totally (84.9%) caused mild or worse resorption with at least one adjacent tooth (category 2–4).

Table 1. Contact and resorption relationship between impacted canine and incisors

Categories	Lateral incisor		Central incisor	
	N	%	N	%
0 (no contact)	24	19.05	94	74.60
1 (contact, no resorption)	4	3.17	4	3.17
2 (mild resorption)	78	61.90	25	19.85
3 (moderate resorption)	10	7.94	2	1.59
4 (severe resorption)	10	7.94	1	0.79

Discussion

To our knowledge, there are articles regarding three-dimensional impacted canine position and root resorption of adjacent teeth each with very different incidence of resorptions (Liu, 2008; Almuhtaseb, 2014; da Silva Santos, 2014; Walker, 2005; Oberoi, 2012). Adding a third-dimension to the radiographic information notably alters the prevalence of root resorption as it increases at least twice compared to studies with conventional x-rays (OPT, periapicals, occlusal x-rays or lateral cephalograms) (Ericson, 1987; Mason, 2001).

Osirix software allows precise and reproducible assessment of the impacted canine position and adjacent teeth root resorption by using linear measurements less than 0.1 mm which coincides with previous reports (Kim, 2012). That is more precise than in article mentioned by Walker et al. where they measured distances to the nearest 0.5 mm (Walker, 2005).

Most patients in this study with impacted canines were female (69%), and there was no significant difference between left and right impactions (42 left, 40 right, 22 bilateral impactions), which coincides with previous studies (Liu, 2008; Bjerklin, 2006; Rimes, 1997).

In our study most impacted canines (87.3%) were in contact (less than 0.5 mm) with at least one adjacent tooth. Mild, moderate or severe resorption (category 2–4) were observed in 76.1% of all lateral incisors and 21.4% of all central incisors. Although mostly mild resorption occurred in lateral and central incisors, the incidence is higher compared to other recent studies involving CBCT (Liu, 2008; Walker, 2005; da Silva Santos, 2014; Almuhtaseb, 2014; Oberoi, 2012; Oana 2013). Rate of resorption so high could be explained that we looked into palatally impacted canines where incidence of resorption is rated higher than buccally placed canines.

The mechanism of root resorption following impaction and the factors involved in the process are not yet clear. Ericson and Kurol mentioned the role of physical pressure due to the movement of the maxillary canine (Ericson, 2000). In our study, from 105 impacted canines in contact with lateral incisors, resorption occurred in 96 lateral incisors and from 31 impacted canines in contact with central incisors, 27 were resorbed. These findings support that resorption significantly correlated with contact between the adjacent tooth and impacted canine and coincides with previous studies (Ericson, 2000; Walker, 2005; Liu, 2008). During measurements, we noticed root dilacerations in impacted canines. Recently there was a CBCT study,

where reported root dilacerations were almost 60% (da Silva Santos, 2014). But it is still unclear why in some cases resorption occurs, and what the other factors are that influence resorption generation/creation after the contact of impacted canine with adjacent teeth has been established.

We did not find any association between follicle size and resorption and it coincides with previous studies (Ericson, 2002; Liu, 2008; Alqerban, 2009). In 8 cases (6.34%), follicles of the impacted canines were in contact with the adjacent teeth and four of them had a moderate or severe resorption.

The main weakness of the study is the relatively small sample size in specific resorption groups which could affect prevalence of specific resorption categories.

In conclusion, resorption of the incisors is very difficult to diagnose. Early diagnosis of impacted canine and root resorption might have further reduced complications during treatment and the presence or absence of root resorption will determine the treatment plan. Every dentist should palpate the maxillary permanent canines by the age of 9–10 and take necessary x-rays, if needed. The severity of lateral or central incisor root resorption cannot be accurately judged from two dimensional radiographs alone, as they fail to detect the exact localisation of the canines or any potential early or mild root resorption (Bjerklin, 2006; Mason, 2001; Ericson, 1987; Alqerban, 2009). Applying 'ALARA' (as low as reasonably available) principle to radiation dosage, field of view and assessing cost/risk/benefit ratio, a CBCT could be a reliable tool for diagnosing the position of the impacted canine and distance from adjacent structures to aid in further treatment planning.

Conclusions

The results of our study show that root resorption of the lateral and the central incisor was common in patients, mostly affecting the lateral incisor to a mild degree. In contact less than 0.5 mm with at least one adjacent tooth were 87.3% of all impacted canines. In contact with the lateral incisor were 83.3% of the impacted canines, and with the central incisor 24.6% impacted canines. Our reported rate of root resorption were 76.1% in the lateral incisors and 21.4% in the central incisors. A wide dental follicle of impacted canine was not associated with a higher incidence of external root resorption of the lateral or the central incisors.

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Assessment of Osseous Structure Disorders of TMJ in Class II and Class III Patients with Different Mandibular Rotation using CBCT

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Abstract

Temporomandibular joint (TMJ) osseous structures disorders (OSD) were evaluated in cone beam computer tomography images of 117 consecutive patients with Class II and Class III dentofacial deformities according to research diagnostic criteria related to the maxillary-mandibular plane (MM) angle. The distribution of the number and percentage of affected joints with the OSD signs in Class II was markedly different in groups divided according to the MM angle. Statistically significant increase was found in the percentage of joints affected with the OSD separately for each side, i.e., right ($p = 0.001$), left ($p = 0.04$) and both together ($p = 0.0001$), in Class II patient group, and an increased MM angle indicated backward rotation of the mandible. In Class III patients, there were no statistically significant differences in the number of joints affected by the OSD. The presence of the changes was asymmetrical between the left and right joints in both Class II and Class III patient groups.

In conclusion, the OSD signs are more common in patients with Class II skeletal dentofacial deformities with backward rotation of the mandible.

Keywords: TMJ, degenerative osteoarthritis, mandibular vertical rotation, CBCT.

Introduction

An important factor that should be considered when planning treatment for dentofacial deformities is existing relapses caused by destruction of the osseous tissue of the TMJ. Destruction of the osseous tissue into the joint is frequently associated with inflammatory or degenerative changes that can result in mandibular morphological and functional changes (Dworkin, 1992).

Although the TMJ is anatomically characterised as load-receiving, its structure can change through excessive or unbalanced functional loading to produce TMJ dysfunctional remodelling, which in the majority of cases passes asymptomatically and affects the function of the joint and occlusal stability, i.e., the volume of the osseous tissue changes, and the length of the mandibular condylar head and mandibular ramus decreases. Reduced mandibular growth in children and adolescents in addition to progressive mandibular backward rotation in adults can develop in such cases (Arnett, 1996).

Various controversial beliefs exist regarding changes of the TMJ osseous structure depending on the type deformation and its manifestation determined using two-dimensional (2D) radiological investigation methods (Kurita, 2000; Honda, 2001; Katsavrias, 2005, 2006; Hussain, 2008; Vitral, 2004, 2011).

In maxillofacial radiology, cone beam computed tomography (CBCT) provides a three-dimensional (3D) image and provides a more qualitative evaluation of the TMJ osseous tissue structure than conventional CT; it also offers the advantages of low radiation and the ability to be used in orthodontic practice (Lubele, 2009; Davies, 2012).

Evaluation of morphological features and quality of TMJ articulating surfaces in 3D CBCT reconstruction images allows precise judgements regarding osseous structure. Evaluation of these osseous changes using 3D CBCT is thus helpful for investigating in greater depth the morphology of the TMJ and for recognising the risk of occlusal stability.

Aim

The aim of the current study was to determine the relationship between the vertical rotation of the mandible and of the OSD of the TMJ in patients with dentofacial deformities Class II and Class III using CBCT images.

Material and Methods

The study included 117 orthognathic surgery patients with dentofacial deformities and without complaints related to the TMJ: 56 skeletal Angle Class II patients (42 patients in Class II/1 and 14 patients in Class II/2) and 61 skeletal Angle Class III patients before orthodontic treatment. The mean age of the patients was 20.58 ± 4.27 years, and the study groups included 55 or 47% males and 62 or 53% females, respectively. The exclusion criteria for the study were as follows: congenital dentofacial syndromes (including labial and/or palatal cleft), clinically visible skeletal facial asymmetry, rheumatoid or other types of arthritis, trauma in the maxillofacial area in the patient history, complaints regarding temporomandibular disorders, pain in the maxillofacial area, pronounced noise in the temporomandibular joint and previous orthodontic treatment with functional devices and/or fixed appliance.

Class II, Class III of the dentofacial deformity was determined from data obtained from cephalometric analyses of CBCT images in the sagittal plane based on SNA and SNB angles, ANB angle and Wits appraisal values.

Vertical relationship of jaws were analysed based on the cephalometric horizontal planes of the face, i.e., the cranial base, Frankfurt, palatal and mandibular planes. To classify the study groups, were used the maxillary and mandibular plane angle (MM angle) to determine the rotation of mandible. Based on the values of the MM angle, the patients of Class II and Class III were divided into study groups (Table 1). A neutral vertical relationship of the jaws was characterised by the MM angle of 22–32° or mean angle of 27 ± 5°; an angle smaller than 22° indicated forward rotation, whereas an angle larger than 32° indicated backward rotation of the mandible.

In all of the included patients, diagnosis and treatment planning were performed using cone beam computed tomography (CBCT) equipment CBCT (iCAT New Generation, Imaging Sciences International, Inc. Hatfield, PA, USA) before the orthodontic treatment was started.

During the examination, each patient was in a sitting position, with the head in a natural position to ensure maximum intercuspidation. A standardised protocol was used for the equipment (voltage, 120 KV; current, 38 mA; field of view (FOV), 17 cm; resolution, 0.4 voxels; approximate dose of radiation, 36 μSv).

Table 1. Distribution of patients by MM angle in study groups

MM Angle	Class II		Class III	
	N	%	N	%
22–32°	18	32	34	56
≥ 32°	22	39	15	24
≤ 22°	16	29	12	20
Total	56	100	61	100

For the cephalometric analyses of CBCT data, the Dolphin programme, version 11.0 was used (Dolphin imaging, CA, USA). Analysis of all of the CBCT images was performed by the authors.

The acquired examination data were processed and analysed by applying the software supplied with the iCAT Vision equipment. The presence of OSD was assessed in the coronary and sagittal planes (Fig. 1, Fig. 2) according to the research diagnostic criteria for temporomandibular disorders (RDC/TMD) of the osseous structures Axis I established by Dworkin (1992) and Ahmad (2009). This analysis included a description of the disorder signs of the structural quality and quantity of the articular condyle and articular fossa/eminence complex in the TMJ with regard to condylar hypoplasia, condylar hyperplasia, articular surface flattening, subcortical sclerosis, subcortical cysts, surface erosion, osteophytes, generalised sclerosis, loose joint body, deviation in form and ankylosis. The RDC/TMD scoring was used to determine the severity of the OSD in TMJ: no osteoarthritis, indeterminate osteoarthritis (IOA) and osteoarthritis (OA). In further detail, no osteoarthritis included normal relative size of the condylar head, no subcortical sclerosis or articular surface flattening and no deformation caused by subcortical cysts, surface erosion, osteophytes or generalised sclerosis. IOA included normal relative size of the condylar head or articular surface flattening with/without subcortical sclerosis and no deformation caused subcortical cysts, surface erosion, osteophytes, or generalised sclerosis. OA included deformation caused by subcortical cysts, surface erosion, osteophytes or generalised sclerosis. The prevalence was calculated separately for each joint; therefore, both TMJs of the same patient received different diagnoses.

The aim of the statistical data analysis was to evaluate the distribution of the OSD in TMJ (in condyle and fossa) between study groups. Data on the presence of descriptive signs were entered into the database, which later was converted into the database format of the statistical software SPSS (Inc., USA). All calculations were performed using this software. After at least a two-week interval, 71 selectively chosen patients (i.e., 50% of all included patients) were re-evaluated. Dahlberg`s approach was used for the calculation of measurement error (Dahlberg, 1940), and an error less than 1 was regarded as tolerable. Mean values and standard deviations were calculated. Distribution frequencies / prevalence were assessed. Pearson's Chi-squared test and Fisher's exact test were used to evaluate the statistical significance of differences in prevalence among the groups.

Figure 1. Image of the condyle in coronary plane

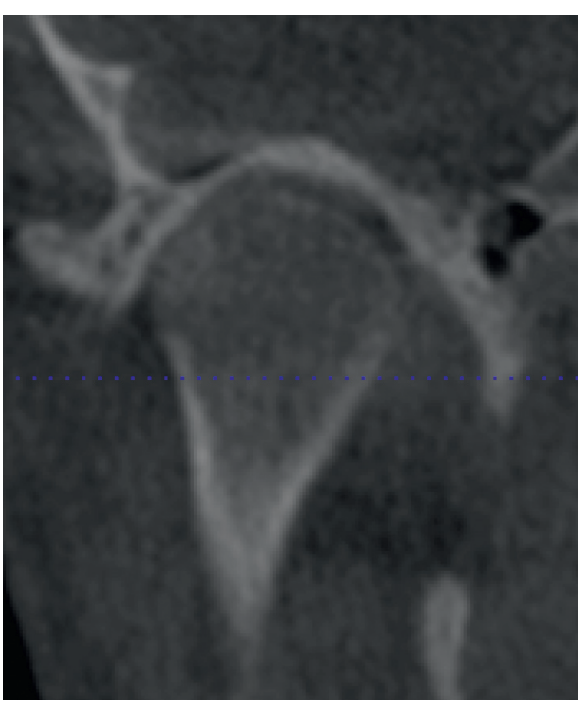
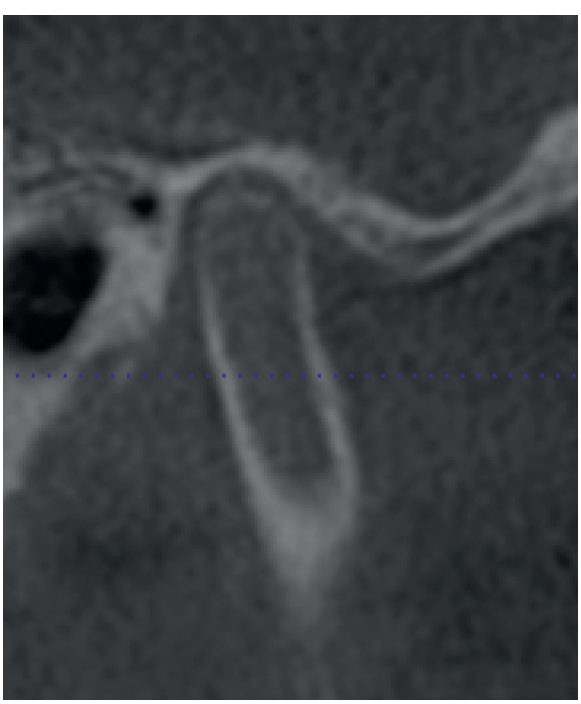


Figure 2. Image of the condyle in sagittal plane



The difference of means between groups was assessed using t-tests for paired data and for non-paired data. For comparison of means among more than two groups, an analysis of variance (one-way ANOVA) with Bonferroni correction was used. The level of statistical significance of $p \leq 0.05$ was used in all cases.

The study was approved by the permission of the Ethics Committee of Rīga Stradiņš University (Decision accepted on April 19, 2007), with the principles laid down in the Declaration of Helsinki.

Results

Analysis of prevalence of OSD signs in TMJ revealed a statistically significant difference ($p = 0.014$) between Class II and Class III patients. One or several signs were determined in 38.4% of Class II group and 28.7% of Class III group.

Class II/1 and Class II/2 patients presented no statistically significant differences in the prevalence of joints affected by OSD signs between Class II subdivisions (Tables 2 and 3). The number of joints, in which no signs of OSD were found and those in which at least one sign was found, was almost equal; therefore, these patients were analysed within the same group.

Table 2. Presence of OSD signs in Class II subdivisions on the left side

Presence of signs	Subdivision 1		Subdivision 2		Total
	Number of joints	%	Number of joints	%	
No	22	52.4	7	50	29
Yes	20	47.6	7	50	27
Total	42	100	14	100	56

Results non-significant ($p = 0.88$)

Table 3. Presence of OSD signs in Class II subdivisions on the right side

Presence of signs	Subdivision 1		Subdivision 2		Total
	Number of joints	%	Number of joints	%	
No	24	57.14	11	78.57	35
Yes	18	42.86	3	24.43	21
Total	42	100	14	100	56

Results non-significant ($p = 0.16$)

The number of affected joints with OSD in Class II was markedly different in groups that were classified based on the MM angle into backward and forward rotation; increased MM angle indicated backward rotation of the mandible. Statistically significant differences were found in the number of affected joints separately for each side: 54.5% ($p = 0.001$) on the right and 59.1% ($p = 0.04$) on the left (Tables 4 and 5) sides, and 56.8 % ($p = 0.0001$) in both (Table 6) patient groups.

In Class III patients, there was no statistically significant difference in the percentage of joints affected with and the type of mandibular rotation related to the MM angle (Tables 7–9). The presence of OSD was asymmetrical between the left and right joints in both Class II and Class III patient groups.

A detailed analysis of the prevalence of each sign of OSD in the condyle and fossa eminence in Class II patient group indicated that condylar hypoplasia, flattening, sub-sclerosis, osteophytes, deviation in form and the fossa flattening were present. Subcortical cyst, generalised sclerosis, loose joint body and ankylosis were not found. A higher percentage of signs was observed in the right and left sides of Class II patients with mandibular backward rotation than in the groups with neutral position or forward rotation.

Table 4. Number of joints with OSD signs related to the MM angle of the right side in Class II

MM Angle	No signs		Presence of signs		Total	
	Number of joints	%	Number of joints	%	Number of joints	%
22–32°	9	50.0	9	50.0	18	100
≥ 32°	10	45.5	12	54.5	22	100
≤ 22°	16	100	0	0	16	100

Results with statistical significance (p = 0.001)

Table 5. Number of joints with OSD signs related to the MM angle of the left side in Class II

MM Angle	No signs		Presence of signs		Total	
	Number of joints	%	Number of joints	%	Number of joints	%
22°–32°	10	55.6	8	44.6	18	100
≥ 32°	9	40.9	13	59.1	22	100
≤ 22°	15	93.8	1	6.3	16	100

Results with statistical significance (p = 0.04)

Table 6. Number of joints with OSD signs related to the MM angle on both sides in Class II

MM angle	No signs		Presence of signs		Total	
	Number of joints	%	Number of joints	%	Number of joints	%
22°–32°	19	52.8	17	47.2	36	100
≥ 32°	19	43.2	25	56.8	44	100
≤ 22°	31	96.9	1	3.1	32	100

Results with statistical significance (p = 0.0001)

Table 7. Number of joints with OSD signs related to the MM angle of the right side (in Class III)

MM Angle	No signs		Presence of signs		Total	
	Number of joints	%	Number of joints	%	Number of joints	%
22–32°	27	79.4	7	20.6	34	100
≥ 32°	12	80.0	3	20.0	15	100
≤ 22°	10	83.3	2	16.7	12	100

Results non-significant (p = 0.95)

Table 8. Number of joints with OSD signs related to MM angle on the left side in Class III

MM Angle	No signs		Presence of signs		Total	
	Number of joints	%	Number of joints	%	Number of joints	%
22–32°	23	67.6	11	32.4	34	100
≥ 32°	9	60.0	6	40.0	15	100
≤ 22°	6	50.0	6	50.0	12	100

Results non-significant (p = 0.54)

Table 9. Number of joints with OSD signs related to MM angle of both sides (in Class III)

MM Angle	No signs		Presence of signs		Total	
	Number of joints	%	Number of joints	%	Number of joints	%
22–32°	50	73.5	18	26.5	68	100
≥ 32°	21	70.0	9	30.0	30	100
≤ 22°	16	66.7	8	33.3	24	100

Results non-significant (p = 0.8)

The most common sign of OSD in patients of Class III group was flattening of the condyle. There was no difference in the prevalence of signs related to mandibular rotation. The percentage of joints affected by disorders was not significantly different among MM angle groups in Class II and Class III patient groups. The prevalence of OSD signs between the right and left sides was asymmetrical (Tables 10–13).

Table 10. OSD signs related to MM angle of the right side (in Class II)

Signs of TMD	MM Angle						Total	
	22–32°		≥ 32°		≤ 22°			
	N	%	N	%	N	%	N	%
Condylar hypoplasia	4	22.2	3	13.6	0	0	7	12.5
Flattening	4	22.2	6	27.3	0	0	10	17.9
Sub- sclerosis	1	5.6	1	4.5	0	0	2	3.6
Erosion	0	0	2	9.1	0	0	2	3.6
Osteophyte	0		3	13.6	0	0	3	5.4
Deviation	2	11.1	2	9.1	0	0	4	7.1
Ankylosis	0	0	0	0	0	0	0	0
Fossa flattening	0	0	2	9.1	0	0	2	3.6
Total	18	100	22	100	16	100	56	100

Results non-significant (p = 0.44)

Table 11. OSD signs related to the MM angle of the left side in Class II

Signs of TMD	MM Angle						Total	
	22–32°		≥ 32°		≤ 22°			
	N	%	N	%	N	%	N	%
Condylar hypoplasia	3	16.7	3	13.6	0	0	6	10.7
Flattening	4	22.2	9	40.9	0	0	13	23.2
Sub-sclerosis	2	11.1	2	9.1	1	6.3	5	8.9
Erosion	0	0	2	9.1	0	0	2	3.6
Osteophyte	2	11.1	5	22.7	0	0	7	12.5
Deviation	2	11.1	1	4.5	0	0	3	5.4
Ankylosis	0	0	0	0	0	0	0	0
Fossa flattening	1	5.6	3	13.6	0	0	4	7.1
Total	18	100	22	100	16	100	56	100

Results non-significant (p = 0.53)

Table 12. OSD signs related to MM angle of the right side in Class III

Signs of TMD	MM Angle						Total	
	22–32°		≥ 32°		≤ 22°			
	N	%	N	%	N	%	N	%
Condylar hypoplasia	0	0	1	6.7	0	0	1	1.6
Flattening	4	11.8	2	13.3	2	16.7	8	13.1
Osteophyte	1	2.9	0	0	0	0	1	1.6
Deviation	2	5.9	0	0	0	0	2	3.3
Fossa flattening	1	2.9	0	0	0	0	1	1.6
Total	34	100	15	100	12	100	61	100

Results non-significant (p = 0.59)

Table 13. OSD signs related to MM angle of the left side in Class III

Sign	MM Angle						Total	
	22–32°		≥ 32°		≤ 22°			
	N	%	N	%	N	%	N	%
Condylar hypoplasia	0	0	0	0	1	8.3	1	1.6
Flattening	9	26.5	4	26.7	3	25.0	16	26.2
Sub- sclerosis	1	2.9	0	0	1	8.3	2	3.3
Osteophyte	2	5.9	1	6.7	0	0	3	4.9
Deviation	0	0	1	6.7	1	8.3	2	3.3
Fossa flattening	0	0	1	6.7	0	0	1	1.6
Total	34	100	15	100	12	100	61	100

Results non-significant (p = 0.43)

Osteoarthritis was diagnosed according to quantification of the RDC/TMD of osseous components in relation to the MM angle in joints on both sides in Class II patients, and statistically significant indeterminate osteoarthritis and osteoarthritis in the TMJ was observed in 18.2% and 11.4%, respectively, of the backward rotation group. In the mandible neutral position group, the prevalence of osteoarthritis in both sides and only on one side was 38.9% and 8.3%, respectively (Table 14). The distribution of the diagnoses of indeterminate osteoarthritis and osteoarthritis was not significantly related to the MM angle in Class III patients (Table 15).

Table 14. Distribution of the diagnosis of TMJ osteoarthritis with respect to the MM angle in both sides in Class II

Diagnosis	22–32°		≥ 32°		≤ 22°	
	Number of joints	%	Number of joints	%	Number of joints	%
No osteoarthritis	19	52.8	19	43.2	31	96.9
Indeterminate osteoarthritis on both sides	0	0	8	18.2	0	0
Indeterminate osteoarthritis on only one side	0	0	5	11.4	1	3.1
Osteoarthritis on both sides	14	38.9	0	0	0	0
Osteoarthritis on only one side	3	8.3	2	4.5	0	0
Total	36	100	44*	100	32	100

Results with statistical significance (p = 0.001)

* Five patients had indeterminate osteoarthritis in one joint and osteoarthritis in other joint simultaneously (number of joints = 10).

Table 15. Distribution of diagnosis TMJ osteoarthritis related to the MM angle MM in Class III

Diagnosis	22°–32°		≥ 32°		≤ 22°	
	Number of joints	%	Number of joints	%	Number of joints	%
No osteoarthritis	50	73.5	21	70.0	16	66.7
Indeterminate osteoarthritis on both sides	8	11.8	4	13.3	2	25.0
Indeterminate osteoarthritis on only one side	8	11.8	5	16.7	6	8.3
Osteoarthritis on only one side	2	2.9	0	0	0	0
Total	68	100	30	100	24	100

Results non-significant (p = 0.67)

Discussion

This study of CBCT images evaluated mandibular condyle and articular/fossa structure disorders in RDC/TMD axis I related to mandibular rotation in patients with skeletal Class II and Class III dentofacial deformities and found a statistically significant relationship between the radiographic features of the disorders and mandibular backward rotation in Class II patients but not Class III patients.

Our results suggest different prevalence of osseous disorders signs in different study groups; more frequent in Class II patients than in Class III patients, which indicates the role of skeletal discrepancy of the jaws in the development of TMJ osseous destruction. The study groups were classified according to jaw skeletal discrepancy as determined by the MM rotation angle, which represents the vertical jaw rotation. The number of joints affected with OSD signs were identified in the groups classified according to the MM angle. The number of affected joints was highest in Class II patients with an increased MM angle. The signs were observed ***asymmetrically*** between the left and right joints, although these patients had no clinically visible jaw asymmetry. A previous study observed that TMJ changes had wide inter-individual variation even in patients with clinically similar malocclusions (Krisjane, 2012).

The most frequently occurring signs of OSD of TMJ in Class II and Class III study groups with mandibular backward rotation were flattening of articular surface of the condyle and fossa eminence. In literature, flattening of the articular surface of the TMJ was described to result from remodelling (Kurita, 2000), which can be radiologically defined as minor changes in the shape of the bone (Honda, 2001). Such flattening may present in both symptomatic and asymptomatic joints as a sign of indeterminate osteoarthritis (Ahmad, 2009), and it can be considered as a functional adaptation (Brooks, 1992). The other most common signs of OSD in TMJ for Class II and Class III patients with mandibular backward rotation were erosion and osteophytes of the condyle. The presence of erosion in joint surfaces characterises the initial stage of degenerative changes in osteoarthritis and indicates the likely instability of the TMJ osseous structure (Ahmad, 2009) accompanied by the risk of altered the area of the joint surfaces, which can cause occlusal changes (Hussain, 2008). The presence of osteophytes in the joint together with other signs is an important criterion for radiological diagnosis of osteoarthritis; in contrast to erosions, osteophytes occur in the late stage of degenerative changes during adaptation (van der Kraan, 2007).

All of Dworkin's (Dworkin, 1992) criteria for assessment of changes in the TMJ can be categorised in the following groups: 1) the result of chronic adaption or active on-going changes; 2) changes that have an impact on function of the joint and quality of life, or asymptomatic; 3) changes that are reversible or irreversible. Only part of the mentioned diagnostic system was used in the study, and results describe the prevalence of signs in osseous structures. Based on the RCD/TMD criteria used in the study, the temporomandibular joints were radiologically assessed as a whole with regard to structural changes of osseous tissue quality. The signs and prevalence of disorders were evaluated according to the scoring for indeterminate osteoarthritis and osteoarthritis.

The manifestations of condylar destruction allow further considerations regarding the course of degenerative joint destruction and function of the joint, and occlusion is characterised by progressive mandibular backward rotation in adults (Arnett, 1996; Alexiou, 2009; Cevidanes, 2010) and is the reason for mandibular growth deviations in childhood and adolescence (Pirttiniemi, 2009). The studied literature indicates that bone loss at the mandibular condyle may result from the dysfunctional remodelling by orthognathic surgery, systemic and local arthritis, post-traumatic remodelling and hormonal imbalance (Arnett, 1992; Gunson, 2012).

An opinion exists that considerations regarding on-going processes in the TMJ can be based on established changes before orthodontic and orthognathic surgical treatment.

A cephalometric study of mandibular advancement surgery in Class II patients demonstrated that high-angle patients were associated with both a higher frequency and a greater magnitude of horizontal relapse (Mobarak, 2001).

Conclusion

The most common OSD of the TMJ related to skeletal Class II patients is mandibular backward rotation.

Long-term observation allows the understanding of whether mandibular rotation is the cause or consequence of TMJ destruction in orthodontic and orthognathic surgery, and aids in determining instability in patients with dentofacial deformities.

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