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Crouzon Syndrome: a Comprehensive Review

SUMMARY

Crouzon syndrome is a rare genetic disorder with autosomal dominant inheritance. The underlying pathological process is premature synostosis of the cranial sutures with subsequent phenotypic alterations of the affected person. A review of the literature has been conducted in order to resume the overall characteristics of Crouzon syndrome such as cranio-maxillofacial malformations, clinical features, dentoalveolar characteristics, aesthetic impairments, and psychological background, as well as, the different therapeutic procedures, which combine surgical and orthodontic interventions. Facial and functional malformations in individuals with Crouzon syndrome could be significantly improved after a series of surgical and orthodontic procedures in almost all cases. A multidisciplinary treatment approach would provide the best outcomes in affected patients.

Key words: Crouzon, Crouzon Syndrome, Diagnosis, Multidisciplinary Approach, Orthodontic Management, Surgical Management

Introduction

The term “Crouzon syndrome” describes an autosomal disease, which results from hereditary mutations identified in specific genes in the human DNA chain. The molecular deformities most customarily occur in FGFR2 gene and, in rare instances, in the FGFR3 gene. Moreover, recent studies have identified mutations in FGFR1, MSX2, TWIST1, EFnb1, NELL1, GLI3 and TCF12 genes. These mutations result in premature synostosis of several sutures of the craniofacial complex. Specifically, the fusion of the sagittal and coronal sutures begins, in the most of the cases, during the first year of life leading to the growth inhibition at the affected sutures and compensative growth at other sutures.

It is one of the most frequent craniosynostosis syndromes, with estimated incidence of 1:60000 live births.

The aim of this study was to investigate the available information on the general characteristics, the therapeutic needs and the existing management protocols of patients with Crouzon syndrome.

Clinical features

Craniofacial and dentoalveolar characteristics

The syndromic patients are characterized by specific craniofacial characteristics. The main feature of Crouzon syndrome is the premature synostosis of craniofacial sutures, which leads to cranium deformity manifested as brachycephaly, scaphocephaly or oxycephaly with dolichocephalic growth pattern. Moreover, concave facial profile appears due to midfacial hypoplasia with implied proptosis, which is connected to shallow orbits. Part of the midfacial discrepancy includes the deviated nasal septum, which results in beaked nose.

The premature synostosis of several sutures of the middle third of the facial skeleton results in differentiation of the intraoral anatomy in Crouzon patients. The hypoplastic, narrow and high arched palate results in decreased upper dental arch dimensions. Also, severe crowding of the primary and secondary dentitions, anterior and posterior crossbite are usually diagnosed.
as well as malformed teeth, delayed dental eruption and impactions\textsuperscript{18-20}. High risk of maxillary canine and premolar impactions is evident in patients who present severe lack of space in the upper arch. In some cases, about 40% of the patients with Crouzon syndrome, ectopic dental eruption of the upper first permanent molars is observed and also dental agenesis, especially of the lower second premolars and upper lateral incisors\textsuperscript{21-23}. Class III malocclusion and Class III skeletal discrepancy is observed in all patients\textsuperscript{18-20}.

**Functional impairment**

The above mentioned characteristics result in some functional complications. Eye exposure, as a result of shallow orbits, can result in corneal abrasions, scarring, exposure keratitis or luxation of the eyeballs in acute cases\textsuperscript{24}. Additionally, increased intracranial pressure may lead to bilateral optic atrophy, which bring about nystagmus, strabismus or even blindness if the condition is not treated\textsuperscript{25,26}. Bilateral atresia of auditory meatus often causes the deafness\textsuperscript{1}. Factors contributing to the respiratory distress or obstructive sleep apnea syndrome would have included the retrusive location of the maxilla or a very long and fleshy soft palate, which is often observed in Crouzon syndrome\textsuperscript{28}. These situations are usually linked to hypertension and cardiac arrhythmia or cardiac arrest\textsuperscript{27}. As a consequence of the class III malocclusion, patients feel discomfort over the masseter region during eating.

**General findings**

Beside the functional problems, patients with Crouzon syndrome show some general clinical features such as cervical spine anomalies, acanthosis nigricans, increased intracranial pressure, hydrocephalus and occasionally mild mental retardation\textsuperscript{29-31}. Developmental delays can also occur.

**Diagnostic procedure**

Craniosynostosis can be suspected prenatally based on indirect signs visualized using two or three dimensional ultrasound, magnetic resonance imaging (MRI) or computed tomography (CT) scan\textsuperscript{32}. Prenatal 2D ultrasound diagnosis aims to visualize the cranium shape, allowing the calculation of the cephalic index\textsuperscript{33} [CI: Cephalic Index expresses the relationship between the biparietal and occipto-frontal diameters]. Abnormal values of the CI correspond to patients with brachycephaly, scaphocephaly or dolichocephaly. Regarding to the differentiated face morphology, common characteristics of Crouzon syndrome, such as hypertelorism, ocular proptosis and beaked nose can be identified by ultrasound scan. Nowadays, 3D ultrasound scan provides more accurate evaluation of fetal head, face and sutures, as well as detailed visualization of the whole fetal anatomy which contributes to the differential diagnosis between craniosynostosis syndromes\textsuperscript{38,39}. In addition, MRI can be considered complementary to the above mentioned methods and seems to have negative predictive value\textsuperscript{30}. The syndromic nature of craniosynostosis is, finally, confirmed by molecular diagnosis. Specifically, mutations in FGFR1, FGFR2 or FGFR3 genes are investigated by amniocenteses, chorionic villus sampling or pre-implantation diagnosis\textsuperscript{37}.

It is worth to mention that due to the less severe phenotype of Crouzon syndrome in relation to other craniosynostosis syndromes the prenatal diagnosis is confoundedly difficult\textsuperscript{38}. The postnatal diagnosis of the Crouzon syndrome requires extensive clinical and radiological examination. Cephalometric analysis of the anteroposterior and lateral radiographies and CT scans confirm clinical findings and shows the premature suture closure\textsuperscript{39,40}. Patients are usually examined by geneticists in order to detect the associate mutations.

**Treatment management**

Patients with Crouzon syndrome present complex abnormalities, which require early clinical management of multidisciplinary teams. These teams should consist of craniofacial surgeons, oral and maxillofacial surgeons, neurosurgeons, plastic surgeons, ENT (ear, nose and throat) surgeons, orthodontists, dentists, dental technicians, speech pathologists, psychologists, ophthalmologists, nurses, speech-language pathologists, anesthesiologists, geneticists, pediatricians and radiologists\textsuperscript{41,42}.

**Surgical intervention in the craniofacial region**

The surgical treatment of the syndromic patients consists of two phases. The first phase takes place in the first year of life and is limited to the correction of the cranial deformity and prevention of the cranial pressure increase and optic nerve damage\textsuperscript{43-45}. The midfacial advancement, the second phase, takes place in older age. To achieve the desired result a combination of Le Fort III osteotomy, or frontofacial Monobloc osteotomy with distraction osteogenesis is required\textsuperscript{46,47}. The use of distraction osteogenesis overcomes the complications of the osteotomies, such as increased operative time, relapse of midface protrusion, need for bone grafting and severe blood loss\textsuperscript{48,49}. In cases of severe sleep apnea, tracheostomy may be required as additional intervention\textsuperscript{50}. Interventions on the lid occlusal suture or tarsorrhaphy are part of the ophthalmologic management, while plastic surgery is usually performed in adulthood to restore patients appearance\textsuperscript{51}. 
Several protocols have established through the years. McCarthy et al.,51 published the parameters of care of craniosynostosis established in a multidisciplinary meeting titled “Craniosynostosis: Developing Parameters for Diagnosis, Treatment, and Management” in 2010. Generally, according to this protocol the surgical interventions are divided in six treatment periods. The first period includes early operative treatment for synostosis and for selected suture fusion, aiming to cranial vault decompression and suture release. Common surgical procedures for this stage are the cranietomy or cranietomy and fragmentation of the cranium, the anterior or posterior skull expansion, the endoscopic strip-cranietomy with external molding, spring, or distraction and the open cranial vault procedure which take place between birth and the first year of life. At the same time, airway and ophthalmologic management are strongly suggested. The second period (between 6 months to 4 years) aims to the fronto-orbital advancement and reshaping inclusive of cranial vault remodeling, strip cranietomy and midface or monobloc distraction osteogenesis. The correction of midface deformities, as well as secondary cranial vault procedures and adjunct procedures are made during the third or the fourth phase (between 4 to 12 years). Conventional monobloc or Le Fort III combined with distraction osteogenesis are suggested for the midfacial advancement. Hypertelorism correction is necessary in this period. Phase five (between 13 and 17 years) consists of adjunct procedures such as lateral canthopexy, rhinoplasty and cranioplasty and secondary – complementary procedures when needed. The last period begins after 17 years of living and includes orthognathic surgery for craniofacial dysmorphology or malocclusion for skeletally mature patients. It is noticeable that several of the aforementioned surgical procedures can be performed in different periods depending on the functional, aesthetic, and psychological needs of each patient.

Pagnoni et al.,44 suggested correction of craniosynostosis between the ages of three and six months and midfacial advancement with distraction procedures between the age of 4 to 5 years depending on the extremity of malocclusion, psychological factors and the existence of obstructive sleep apnea. According to this protocol, hypertelorism is corrected in conjunction with the midfacial advancement or separately between the ages of 4 and 6 years. Le Fort I or II combined with mandibular osteotomy is performed after full maturity to normalize appearance.

Kahnberg et al.,45 suggested the use of distraction osteogenesis by the age of 7 to 8 years in patients with severe form of deformity or psychological burden. In the rest of the cases the midfacial advancement took place between the ages of 16 and 18 years. The surgical procedure consisted of Le Fort I or II osteotomy for the correction of the midface retrusion (single jaw maxillary surgery) and the additional set-back of the mandible or segmental osteotomy of the mandible when needed (bimaxillary surgery). Rhinoplasty was performed in some cases to refine the result.

Won Lee et al.,52 treated midfacial hypoplasia using dual midfacial distraction osteogenesis in 6 patients with mean age 7.2 years. Le Fort III or frontofacial monobloc was performed in these patients and a rigid distraction device was placed.

In contrast with the above mentioned protocols, Mithlbauer et al.,53 suggested the correction of the midfacial hypoplasia alongside with the fixing of the cranium deformity in the first months of life in order to improve severe exorbitism, obstruction of nasal airways and implied functional problems. Additional advantages of this technique are the reduction of the psychological effect on the patient and the technical advantages of the surgery due to the elasticity of bones and tissues and the anatomical characteristics in infancy. Nowadays, it is generally accepted that early distraction of the hypoplastic midface usually requires secondary procedures in older age54, 55.

Orthodontic and Orthognathic procedures
Orthodontist specialists can evaluate and manage the patient’s growth pattern through various diagnostic and therapeutic procedures. In that way, they play an important role in the timing of craniofacial surgery. Thus, it is generally recommended for each patient to be monitored by an orthodontist from infancy to adulthood.

Orthodontic treatment can be, also, divided into two periods, the preoperative and postoperative period. In the preoperative period, early evaluation allows guidance of teeth eruption, expansion of the maxilla with suitable appliances, alignment of dentitions and preparation for orthognathic surgery52. The creation of interdental spaces for osteotomies is a common procedure56. Moreover, surgical prediction tracings and splints are created57, 58. After the orthodontic preparation, the midfacial advancement is performed in adolescence. The postoperative orthodontic procedure involves occlusion settlement and functional guidance, which are essential after the surgery. These are arranged using intermaxillary elastics or orthodontic appliances such as reverse activators59.

In cases where an additional orthognathic surgery is needed in adulthood, the orthodontist in cooperation with the maxillofacial surgeon will choose the appropriate time for the secondary surgery10.

Vargervik et al.,10 published the dental and orthodontic perspectives of craniosynostosis resulted from the multidisciplinary meeting mentioned above. The orthodontist, as stated by the participants of the meeting, is obligated to obtain and maintain photographic, radiographic and other imaging records from infancy to early adulthood. Additionally, growth monitoring and dental extractions coordination with pediatric dentists are necessary until the age of 7 years, as well as orthognathic procedures in coordination with surgeons. Between the ages of 7 and 12 years, the first phase of the orthodontic treatment is carried
out. Specifically, alignment of dentitions, active guidance of permanent teeth eruption, insertion of intraoral appliances and midface advancement planning, with cephalometric analysis prediction tracings, images and splints are necessary. The second phase of orthodontic treatment takes place between the ages of 12 and 21 years and includes orthodontic preparation for craniofacial and orthognathic surgery (midfacial advancement or distraction osteogenesis or Le Fort I osteotomy or facial bipartition procedure or 2-jaw surgical correction) using the same procedures mentioned in phase 1. In addition, management of dental impactions with surgical exposure and orthodontic assisted eruption, cooperation with other dental specialties for general dental treatment objectives (prosthodontics, periodontal care, extractions and preventive dental care) and intraoral soft tissue procedures are made at this stage. After surgery, the orthodontic treatment is completed, retention is placed and final records are obtained. Since the 21st year of life onwards a follow up assessment is obligatory at appropriate intervals.

Nurko et al.56 splitted orthodontic therapy in two periods: (1) orthodontic treatment during childhood and (2) orthodontic treatment during adolescence. During childhood, the orthodontic therapy aims to deal with ectopic eruptions, delayed tooth emerge, crowding and posterior crossbite in patients with mixed dentition (5 to 12 years old). Recommended methods are the extractions, the use of a lingual arch and/or fixed appliances and the palatal expansion. In patients with suture closure in the palate, segmental surgery in Le Fort I level is proposed as alternative to palatal expansion with orthodontic appliances. The preparation for midfacial advancement, which usually takes place between 6-10 years, requires extractions of the primary teeth, fixed orthodontic appliances for acrylic surgical splint holding and full dimension rectangular arch wires, while a removable retainer is suggested after surgery to prevent dental relapse.

Later, during adolescence, the patient is prepared for orthognathic surgery which requires full permanent dentitions and skeletal maturity (13-15 years for girls and 15-17 years for boys). The orthodontist aligns the teeth of each jaw without worrying about reverse overjet or dental occlusion using fixed appliances and arch compatibility is checked on dental casts. Full dimension rectangular arch wires are placed and final records should be taken (panoramic and lateral cephalogram radiographs, dental casts, facial and intraoral photos and posteroanterior cephalometric radiograph for asymmetry evaluation). Root separation (4 to 5mm) is required to facilitate osteotomies. Final, predictions and surgical splints are created. Post-surgical orthodontic therapy starts approximately 4 to 6 weeks after the surgery. Vertical elastics are used to settle occlusion and prevent relapse or shifts different from centric relation. This procedure lasts about 6 months and then a retainer is used full time for 12 months.

Kahlberg et al.43 used fixed orthodontic appliances in order to align dentitions preoperatively. Sectional orthodontic arches were put in position, 4 to 6 weeks before the operation to facilitate transverse maxillary expansion. A wafer was constructed for the final planning of the surgery. Postoperatively, minor corrections were made using intermaxillary elastics. The retention period duration varied between 6 and 24 months and removable orthodontic appliances with a grid to avoid tongue thrust were used during this period.

**Dental management**

Supervision by a pediatric dentist in early childhood is critical in order to ensure satisfactory oral hygiene of patients with syndromic craniosynostosis. Because of the anatomical discrepancies, these patients present decreased upper arch dimensions which lead to crowding, delayed and ectopic eruptions with implied propensity for periodontal disease60.

Furthermore, the morphological abnormalities of teeth, lower levels of calcium, phosphorus and magnesium in dentine in combination with the difficulties in maintaining the adequate oral hygiene, usually, lead to dental caries61,62. Fluoride supplementation, pit or fissure sealants, restorative treatment and oral hygiene guidance are recommended after caries risk assessment51. The early localization of missing teeth is an important part of general treatment planning. Therefore, the dentist is obligated to keep baseline diagnostic records such as full head CT scans, cephalograms, intraoral and facial/head photographs. The oral examination needs to be repeated every 6 months or as indicated by the risk status of each syndromic patient51.

Post-surgery patients with Crouzon syndrome refer to a prosthodontist in order to replace missing teeth and restore full anatomical dimension of peg or abnormally shaped teeth10. A life-long follow up evaluation is mandatory for all these patients. Critical knowledge and understanding of the craniofacial growth are required for the dental specialist in order to treat syndromic patients effectively.

**Conclusions**

Patients with Crouzon syndrome need long term management by scientists of various specialties. Facial and functional malformations in individuals with Crouzon syndrome could be significantly improved after a series of surgical and orthodontic procedures in almost all the cases. According to the above mentioned protocols, the surgical treatment of craniofacial hypoplasia is obligatory. However, the role of orthodontist is crucial both before and after surgery to reach the desired treatment plan goals.

**References**


Oral Alterations in Diabetes Mellitus

SUMMARY

Diabetes mellitus is one of the most common chronic diseases which continue to increase in number and significance. It presents the third most prevalent condition among medically compromised patients referring for dental treatment. Diabetes mellitus has been defined as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Hyperglycemia leads to widespread multisystem damage which has an effect on oral tissue. The present article summarizes current knowledge regarding the association between diabetes mellitus and oral and dental health.

Key words: Diabetes Mellitus, Oral Disease, Oral Health

Classification and epidemiology of diabetes mellitus

Diabetes mellitus is defined as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both according to American Diabetes Association. Although number of different classification of diabetes has been proposed, current classification is based upon the disease etiology. Type 1 diabetes, characterized by the lack of insulin production resulting in severe hyperglycemia and ketoacidosis and includes immune mediated diabetes and idiopathic diabetes (5-10% of diabetic populations).

Type 2 diabetes is characterized by insulin resistance mainly by altered insulin production but with certain capacity for insulin production without autoimmune destruction of β-cells (90-95% of diabetic population). Risk factors for this form of diabetes are overweight, obesity and age. It is more common in women with history of gestational diabetes mellitus and in those with hypertension or dyslipidemia. Type 2 diabetes commonly remains undiagnosed for a long time as hyperglycemia appears gradually and often without symptom, while ketoacidosis seldom occurs.

Gestational diabetes mellitus refers to glucose intolerance of variable severity which is identified during pregnancy for the first time, regardless of possibility that the glucose intolerance may antedate pregnancy. Gestational diabetes significantly increases the risk for later development of type 2 diabetes mellitus.

Other forms of diabetes mellitus are represented as genetic defects related to the β-cell or insulin action, exocrine pancreas diseases, diabetes secondary to autoimmune endocrinopathies, diabetes caused by drugs, chemicals, infections, rare forms of immune-mediated diabetes, and diabetes mellitus associated with genetic syndromes.

Diabetes mellitus presents one of the most frequent chronic diseases which continually grows in prevalence and significance. According to World Health Organization 171 million people had diabetes in 2000 and it is predicted that this number will increase to 366 million by 2030. Other estimate by International Diabetes Federation suggested that the prevalence was 381 million in 2013, and that the increase in worldwide numbers will be 592 million by 2035. These data indicate that the prevalence and incidence of diabetes mellitus is increasing, which could be attributed to demographic changes such as population growth and aging, or lifestyle change related to urbanization, as well as to the extended lifespan due to generally improved health of diabetic patients.
Diabetes mellitus induced complications

Hyperglycemia due to diabetes mellitus lead to generalized damage that affects multiple systems, particularly microvascular changes (microangiopathy) with thickening of the capillary basement membrane (includes retinopathy and nephropathy), macrovascular disease (macroangiopathy) with accelerated arteriosclerosis (includes coronary artery disease, cerebrovascular disease, and peripheral vascular disease), neuropathy affecting somatic and autonomic nervous system, and oral disease. Beside these chronic complications, diabetes could cause acute complications such as hyperosmolar hyperglycemia, diabetes ketoacidosis, and acute infection.

Microvascular changes are hallmark of many diabetic complications. Sustained hyperglycemia induces production of advanced glycation end products (AGEs) from proteins and lipids. AGEs are considered to stand in the basis of a variety of diabetic complications. AGEs often form on collagen, which precipitates in large blood vessels walls, and cause accumulation of low-density lipoprotein, thus contributing to atherosclerotic changes (macrovascular complications). AGE-modified collagen also plays a role in thickening the basement membrane in the small blood vessels leading to alterations in homeostatic membrane transport. AGEs formation and microvascular complications have been associated with elevated level of vascular endothelial growth factor (VEGF). AGEs receptors (RAGE) have been found on the surfaces of smooth muscle cells, endothelial cells, fibroblasts, neurons, lymphocytes and monocytes/macrophage. Hyperglycemia increases expression of RAGE while AGE-RAGE interaction on monocytes induces an oxidative stress and the activation of nuclear factor kappa B (NF-kB) and consequently increases the secretion of proinflammatory cytokines including interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). Increased production of these mediators is recognized as critical in the inflammatory process. AGE-RAGE interaction on endothelium enhances vascular permeability and thrombus formation. Vascular problems cause increased risk for infection, as well as its severity and duration due to reduced oxygen diffusion across the capillary wall, neutrophil microbicidal suppression and failure in delivering humoral and cellular components of the immune system. Changes in collagen metabolism are common findings in diabetic. As a consequence, synthesized collagen is rapidly degraded by higher level of matrix metalloproteinase (MMP), while AGE-modified collagen accumulates in the tissue, altering wound healing. Diabetes has a negative impact on bone healing by reducing the expression of genes responsible for differentiation of the osteoblasts and by decreasing growth factor and extracellular matrix production.

Oral complications in diabetes mellitus

Salivary findings

Xerostomia, altered saliva secretion and composition, taste impairment, and burning sensation are common oral complications related to diabetic salivary glands.

Xerostomia

Xerostomia is defined as the subjective complaint of the oral dryness that implies change in the salivary composition and/or flow rate. Patients with type 1 and type 2 diabetes mellitus complain of xerostomia more frequently than healthy controls. Estimated global prevalence of xerostomia among diabetic patients ranges between 34%-51%. Symptoms of dry mouth in type 1 diabetic patients have been linked with peripheral neuropathy. Nevertheless, this association has not been confirmed among type 2 diabetes patients. Recent animal study provided evidence that alterations in nitric oxide-tetrahydrobiopterin production correlate with reduced salivation and increased water intake, a typical symptom of xerostomia. Higher rates of xerostomia were related to the female sex in type 1 diabetic patients, but to xerogenic medication, current use of cigarettes, and more frequent snacking behavior in type 1 diabetic patients. Glycemic control level seems to generally influence the susceptibility of type 2 diabetes to xerostomia.

Salivary flow

Findings of salivary flow rates among patients with diabetes are conflicting. Some studies have shown lower resting and/or stimulated salivary flow in both type 1 and type 2 diabetes mellitus, whereas other authors have found no differences between diabetic patients and controls. Decrease in salivary flow in type 2 diabetic patients is less severe compared to type 1 diabetes. Some authors contended that hyposalivation is related to elevated blood glucose concentration. Namely, dehydration linked to increased blood glucose may enhance osmotic gradients in gland and thus decrease salivary secretion. Besides, decreased salivary flow in type 1 or type 2 diabetes has been associated with poor control of diabetes, xerogenic medication, presence of peripheral neuropathy and obesity–insulin resistance.

Recently, it has been hypothesized that beside hyperglycemia, decreased salivary secretion might be induced by dyslipidemia and hyperinsulinemia which give rise to oxidative stress, inflammation, sympathetic activity, and after insulin signaling in the salivary gland, eventually resulting in gland degradation. Furthermore, findings of another recent publication suggest that salivary gland hypofunction in type 2 diabetes patients might be associated with genetic polymorphisms of chromogranin A, secretory glycoprotein which is supposed to give
Impairment in salivary findings observed with increasing age in type 1 diabetes may partially be ascribed to the increased duration of diabetes. On the other hand, it seems that duration of disease, age, and sex do not affect salivary flow rates in patients with type 2 diabetes. Decreased salivary gland function has been reported to be incriminated in the pathogenesis of candidiasis, and associated with the increased risk of dental caries and periodontitis.

**Sialosis**

In certain percent, patients with long history of diabetes may develop sialosis. This pathology comprises bilateral, painless, non-inflammatory, non-neoplastic, but degenerative glandular enlargement, which usually affects parotid glands. It is related to an alteration in the neuro-anatomic regulation of the gland due to demyelinization and consequent atrophy of the mioepithelial cells. Hystopathologically, increase in glandular size is characterized by fatty infiltration of the stroma, ductal dilatation, and reduced size of the acini. According to the more recent reports, benign parotid hypertrophy might be related to the enlargement of acinar cells, probably as a result of an interruption in the protein synthesis and release. It has been stated that the enlargement is accompanied by the salivary hypofunction, or that the salivary function is generally preserved.

**Salivary composition**

Composition of saliva has also been examined and certain changes in the quality have been observed in both type 1 and type 2 diabetics in comparison to the healthy controls. These disturbances include higher concentration of total sugars, glucose, α-amylase, urea, and acidic pH of resting saliva. In addition, resting and stimulated concentrations of salivary proteins, calcium, magnesium, and potassium ions were found to be increased, while zinc ions were decreased. Furthermore, concentrations of certain salivary elements varied in diabetics and healthy individuals regarding to the sex. It has been hypothesized that microvascular changes and neuropathy may play a role in these changes. Higher levels of glucose and decreased pH in saliva were linked to higher susceptibility of diabetics to the dental caries while more calcium and less zinc ions in the saliva may play a role in predisposition to the dental calculus formation and periodontitis in diabetic patients. Diabetes mellitus was associated with decrease in the secretion of immunoglobulin A suspected of influencing propensity to infections among diabetes patients.

**Taste impairment**

It has been previously reported that diabetic patients show altered taste sensations including hypoguesia for the primary tastes. Among the patients with diabetes or prediabetes, about 6% showed changes in sweet taste perception and about 9% showed salt taste disorder. Taste impairment may impede the maintenance of a proper diet, because of the loss of the sweet and salty taste sensation, with favoring sugary and/or salty food. These changes are generally tolerated without complaint, and significantly associated with the complications and duration of diabetes. Namely, consumption of the large amounts of sugar and sodium, may rapidly enhance blood glucose level and hypertension. A neuropathic mechanism, as well as the dryness of mucosa, decreased gustin production, zinc deficiency and coated tongue has been suggested to explain alteration in taste. Moreover, changes in sweet taste perception might be related to the general impairment in the sweet taste receptor, associated with low glucagon-like peptide 1 (GLP-1) secretion and enhanced glucose absorption.

**Burning sensation**

Diabetes mellitus, especially if uncontrolled, might be associated with the burning sensation in the oral mucosa. In addition, diabetes control results in the improvement of oral burning. Burning sensation in diabetic patients has been attributed to poor glycemic control, metabolic alterations in oral mucosa, angiopathy, candidiasis and regional neuropathy. The neuropathic pain in diabetic patients has been reported as burning, tingling, or even as electric shock or stabbing sensation and present one of the most disabling symptoms in patients suffering from the painful diabetic neuropathy. Painful diabetic neuropathy has been linked to the pain perception generated by a stimulus that does not usually provoke pain (allodynia) and increased response to painful stimuli (hyperalgesia). These pain sensations have a considerable and detrimental impact on the physical and psychological functioning, and correlate with the level of sleep disturbance, anxiety and depression.

Other oral symptoms observed in the poorly controlled diabetics related to the diabetic neuropathy, comprise pain related clinical conditions such as trigeminal pain and temporomandibular joint disorder.

**Orofacial Pain**

Available findings suggest that the oral nerve pain might be related to diabetic polyradiculopathy. Arap et al. explored the oral symptoms and facial somatosensory signs in patients with extraoral complications of painful diabetic neuropathy. According to their results, the majority (55 %) of the patients reported rhe orofacial pain, and the mean pain intensity was 5.6 by the visual analogue scale (VAS). Patients described the pain as fatigue (50%), throbbing, sensitive and queasy (about 43% each), bothering (about 36%), or twinge, pinch and aching (approximately 28% each). In addition, higher pain thresholds were observed in the facial areas innervated by the trigeminal nerve and they were positively correlated.
with the higher levels of glycated hemoglobin. Neuralgia in the orofacial region in conjunction with diabetes is rarely reported. However, in a diabetic woman, severe oral pain provoked by touch in the mandible area, tongue and gingival region have been observed, which led to the assumption of the involvement of the mandibular branch of the trigeminal nerve.

Although the data are scarce, there are certain indicators that suggest there is an association between diabetes and temporomandibular disorders (TMD). A study in Finnish population reported TMD in 27% of type 2 diabetic patients and in 16% of controls. In addition, peripheral and autonomic parasympathetic neuropathies were identified as independent risk factors for temporomandibular dysfunction. According to the results obtained in another study, the prevalence of TMD in the group of participants with diabetes mellitus type 2 and the neuropathic pain in peripheral diabetic neuropathy was similar to the values reported in general population, and the majority were classified as non-painful TMD diagnosis.

These findings need to be clarified in the future studies.

Periodontal disease

Diabetes has been described as a risk factor for periodontal disease. Although the majority of researches have focused on type 2 diabetes, type 1 diabetes appears to have an identical effect on periodontitis occurrence risk. Some young diabetic people develop periodontitis, but periodontal disease is much more common in diabetic adults. Diabetes may influence not only the prevalence but also severity and progression of periodontitis. Recent evidence reported increasing periodontitis prevalence with increasing levels of blood glucose in type 2 diabetes, namely, 19.2, 33.9, 46.3, 53.3, and 62.5% persons with periodontitis, apropos, in person with normal glucose level, prediabetes, newly detected diabetes, well-controlled (HbA1c<7.0%), and poorly-controlled (HbA1c≥7%) in addition, in uncontrolled diabetics both type 1 and type 2 periodontal destruction was more severe, with greater mean bone loss, attachment loss and tooth loss. However, there is no unequivocal dose-response relationship between glycemia and periodontitis.

There is a wide range of mechanisms by which diabetes adversely affects the periodontium. These mechanisms are not entirely understood but involve aspects of inflammation, immune functioning, neutrophil activity and cytokine biology. The microbial composition of the subgingival biofilm between nondiabetic patients and those with type 1 and type 2 diabetes exhibit subtle differences, but clinical relevance of this is not clear. The immune response to periodontal bacterial infection differs in diabetics by means that they do not develop antibodies to periodontitis associated pathogens. Both type 1 and type 2 diabetes are associated with elevated levels of systemic markers of inflammation. Diabetes increases inflammation in periodontal tissues and people with type 2 diabetes have higher levels of inflammatory mediators such as IL-1β, TNF-α, interferon-γ (IFNγ), osteoprotegrin (OPG), IL-17 and IL-23, but also exhibit a downregulation of IL-4. There is a possible role for type 2 diabetes in modulating the level of receptor activator of NF-κB ligand (RANKL)/(OPG) in chronic periodontitis. A limited number of studies have investigated the role of adipokines in periodontal disease and diabetes. Kardesler et al. found no effect of type 2 diabetes on serum leptin and adiponectin in chronic periodontitis, while in another study serum adiponectin was elevated in type 1 diabetes patients with chronic periodontitis. Pradeep et al. showed a possible association between pre-B-cell colony enhancing factor (Visfatin) and type 2 diabetes in chronic periodontitis patients. Also, neutrophil function is altered in the diabetic patients. Oxidative stress in diabetes may activate periodontium pro-inflammatory mechanisms which could influence diabetes.

On the other side, there is an evidence to support a negative impact of periodontal disease on diabetes and much emphasis on the two-way relationship between these two diseases has been given. While diabetes significantly impacts the periodontium, there is also evidence that periodontitis may promote development of type 2 diabetes. Also, periodontal disease adversely affects glycemic control. Ulcerated pocket epithelium could constitute a chronic source of systemic challenge for bacterial products and locally produced inflammatory mediators such as TNF-α, IL-6 and IL-1 due to predominance of gram-negative anaerobic bacteria in periodontal infection. All these important periodontal inflammation mediators are reported to antagonize insulin action. Increased insulin resistance and poor glycemic control may occur as a consequence of chronic gram-negative periodontal infections. In addition, periodontitis increase the risk for diabetic complication. The prevalence and severity of non-oral diabetes related complications such as retinopathy, diabetic neuropathy, proteinuria and cardiovascular complications are reported to be correlated with the severity of periodontitis. The classic complications of diabetes may be closely associated with the periodontal disease, which lends further credence to the concept that periodontal disease may be the sixth complication of the diabetes.

Intervention trials are of the most importance for determining the influence of the periodontal diseases on diabetes. The impact of periodontal therapy on glycemic control is often related to the changes in periodontal health after treatment. The changes in glycemic control may reflect changes in the gingival inflammation level. Recent systematic review and meta-analysis evaluating the effect of periodontal therapy on the outcome of diabetes in patients with type 2 observed moderate
reduction in HbA1c\textsuperscript{59}. Another review including patients with type 1 and type 2 diabetes showed that there is low evidence that the periodontal treatment by scaling and root planning improves glycemic control in people with diabetes, with a mean percentage reduction in HbA1c of 0.29% at 3–4 months\textsuperscript{50}. In addition, the authors found no evidence showing that one periodontal treatment is more effective than another in improving glycemic control\textsuperscript{59}. Therefore, larger, well conducted prospective studies are required to elucidate the effect of periodontal treatment on glycemic control of the patients with diabetes.

**Oral mucosa alterations**

Diabetes mellitus was associated with several specific oral mucosa alterations although these associations have not been found to be consistent in all subjects with diabetes\textsuperscript{51}. There are reports of higher incidence of development conditions, such as coated and fissured tongue, benign migratory glossitis, melanin pigmentation and varices\textsuperscript{52}. Also, diabetic patients are more prone to fungal infection\textsuperscript{53} and potentially malignant disorders including leukoplakia, erythroplakia\textsuperscript{53}, and lichen planus\textsuperscript{52}. Susceptibility of diabetic patients to alteration and infection in oral cavity is still debated but inadequate control of diabetes, immunological alteration, microcirculatory changes with reduction of blood supply, xerostomia and alteration in salivary flow and composition and smoking was mentioned\textsuperscript{4,54}.

**Dental tissue alterations**

**Dental pulp changes**

Chronic hyperglycemia may cause irreversible changes in pulp tissue leading to pulp necrosis. Histological studies showed reduction in the concentration of collagen and fibroblast, increased thickness of blood vessel basement membrane, angiopathy and calcification\textsuperscript{55,56}. Increased presence and activity of inflammatory components, such as kallikrein and myeloperoxidase, with progressive deterioration of the matrix components were observed in diabetic rats after 30 and 90 days of streptozocin treatment\textsuperscript{55}. In another study an increasing trend in inflammatory cells volume density was found in the diabetic rats at 1 and 3 months of diabetic induction, with necrosis areas after 3, 6, 9, and 12 months\textsuperscript{56}. Also, diabetes modifies the parameters of the antioxidant system in rat pulp tissue, by increasing catalase activity and reducing sialic acid concentration, which indicates that diabetic pulp could have impaired response to AGEs and reactive oxygen species\textsuperscript{57}. Increased level of BMP2 and VEGF in healthy teeth and their decreased level in teeth with indirect pulp capping were observed in diabetic human pulp tissue\textsuperscript{58}. Diabetes mellitus may interfere with dental pulp healing. Inhibition of dentin bridge formation and an increase in pulp inflammation was observed after pulp capping procedures in rats with streptozocin induced diabetes mellitus\textsuperscript{59}.

**Dental caries**

The findings regarding relationship between diabetes and coronal or root caries are not conclusive. In experimental models, prevalence and severity of dental caries is increased in diabetic animals and the poor metabolic control contributes to increased caries rate\textsuperscript{56}. Another study denies such correlation\textsuperscript{60}. Many cross-sectional clinical studies found higher prevalence of dental caries for subjects with diabetes both type 1\textsuperscript{59,61} and type 2\textsuperscript{62,63}. Also, positive correlation of caries with duration of diabetes\textsuperscript{62}, type of diabetes\textsuperscript{62} and metabolic control has been reported\textsuperscript{62,63}. On the contrary, a number of authors have not found the correlation between dental caries and diabetes, type of diabetes, duration of disease, metabolic control and the existence of diabetic complications\textsuperscript{64}. The mechanisms by which diabetes could affect prevalence and incidence of dental caries are still debated, but reduced salivary flow, increase of carbohydrate in the saliva, increased level of oral yeasts, *Mutans streptococci* and *Lactobacilli* are some of the factors considered to play a role\textsuperscript{17}.

**Apical periodontitis**

Animal studies showed more pronounced periapical inflammation, larger periradicular lesion and greater root and alveolar bone resorption in diabetic rats compared to nondiabetic\textsuperscript{65}. Human studies showed that the apical periodontitis was significantly higher in diabetic compared to nondiabetic individuals\textsuperscript{66,67}, and that diabetic patients exhibit four times higher risk of apical periodontitis\textsuperscript{68}. Furthermore, association between radiolucent periapical lesions and glycemic control of type 2 diabetic patients assessed by the mean HbA1c level was found\textsuperscript{67}. Other found no association of diabetes with higher prevalence of apical periodontitis\textsuperscript{68}. Recent systematic review was inconclusive regarding the relationship between diabetes mellitus and apical periodontitis prevalence\textsuperscript{69}. Concerning the influence of diabetes mellitus on prevalence of root canal treated teeth, Lopez-Lopez et al.\textsuperscript{68} found that the likelihood of having at least one root filled was twice in diabetic patients than in nondiabetic subjects. Other failed to find association between diabetes mellitus or glycemic control in diabetic patients with prevalence of root filled teeth\textsuperscript{67}. Several investigations studied the potential relationship between diabetes and root-filled teeth outcome and survival. The results of some studies showed that neither diabetes\textsuperscript{66} nor glycemic control in diabetics\textsuperscript{67} affected the endodontic outcome. On contrary, Fouad and Burleson\textsuperscript{60} found that patient with diabetes have a reduced likelihood of endodontic success in teeth with apical periodontitis comparing to controls, even after controlling for other risk factors. Significant difference in the prevalence of
root treated teeth with periapical radiolucencies between diabetics and controls have not been found, but males with type 2 diabetes presented higher risk of residual periapical lesions in the root filled teeth.87

Furthermore, diabetes contributes to decrease retention of root filled teeth. Recent systematic review and meta-analysis indicate significant association between diabetes and prevalence of periapical radiolucencies in root-filled teeth and recognize diabetes as an important pre-operative factor affecting the treatment outcome.71 Chronic inflammation, reduced tissue repair capacity, impaired immune response, impaired bone turnover and delayed wound healing that have been found in diabetes may be responsible for periapical status of root filled teeth in diabetic patients.71

Relationship between infections of dental origin and systemic health has been investigated predominantly in subjects with periodontal disease. However, apical periodontitis is also capable of potentiating systemic inflammatory changes. Astolfi et al. found that in non-diabetic rats apical periodontitis was able to cause alteration in the insulin signaling and insulin sensitivity, probably due to plasmatic TNF-α increase. Other found that oral infection and diabetes are associated to the changes in tryglyceride levels, and that oral inflammation affects glycemic condition and increases HbA1c levels in diabetic animals.74 Also, periapical and periodontal disease increase serum level of IL-17 in normoglycemic and diabetic rats, and levels of leucocytes, neutrophils and blood glucose concentration in the diabetic rats.75,76

Conclusions

Diabetes mellitus presents the third most prevalent condition among medically compromised patients referring for dental treatment. Diabetes causes multiple comorbidities and increase risk of systemic and oral complications. Various oral complications in diabetics might be dependent on type, duration and control of the disease. Thus, treatment goal is implementation of preventive and therapeutic measures for the management of both diabetes mellitus and oral complications. Concerning the fact that many individuals seeking dental care have undiagnosed diabetes and that early diagnosis is important in preventing or mitigating complications, oral findings may offer an opportunity for the identification of high-risk persons.

Namely, it has been shown that presence of 26% or more teeth with deep pockets (with probing depth ≥5mm) or 4 or more missing teeth identified prediabetes or diabetes in 72% of cases. In patients with an increased risk, a blood test can be applied (HbA1c) at first appointment, which increases correct identification to 87%. Thus, dental professionals have the opportunity not only to diagnose and cure diabetic complications, but also to take active role in identifying those with undiagnosed hyperglycemia.

References


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Presence of Different Candida Species at Denture Wearers With Type 2 Diabetes and Clinically Healthy Oral Mucosa-Pilot Study

SUMMARY

Background/Aim: The aim of this study was to examine prevalence of different Candida spp. at diabetics and nondiabetics wearing dentures without clinical signs of Denture Stomatitis (DS) and to study if some local and systematic factors are confounders for harboring Candida at these subjects.

Material and Methods: Total of 60 subjects wearing partial or complete upper acrylic denture having at least half of palatal mucosa covered by denture were selected and stratified into three experimental groups: systematically health subjects; patients with diagnosed Type 2 Diabetes (T2D) and good glycoregulation; and T2D subjects with poorly regulated blood sugar level. Cotton swab samples were obtained from each patient from hard palate mucosa and denture surface. Swab cultures were made on Sabouraud dextrose agar and ChromAgar Media for distinction of various Candida spp. Density growth was also measured.

Results: Frequency of Candida spp. findings were similar between groups. At healthy subjects, only C.albicans was detected. At diabetics, C.albicans was the most common isolated species, followed by C.glabrata and C.tropicalis. Negative finding of yeasts on palatal mucosa, but positive on denture surface were detected at all groups, with the highest frequency (33.4%) at diabetics with poor glycoregulation. Denture surface was heavier colonized than hard palate mucosa. Duration of diabetes in years were only independent predictors for harboring Candida spp. at denture surface (Exp B=1.186, CI=1.047-1.344, p=0.007).

Conclusions: Prosthesis of denture wearers without DS may serve as reservoir of Candida spp. Presence of more pathogenic and resistant non-albicans species are related to diabetics, even without clinical signs of DS.

Key words: Stomatitis, Denture; Candida albicans; Diabetes Mellitus Type 2; Denture, Complete; Denture, Partial, Removable

Introduction

Denture stomatitis (DS) is an inflammatory process of the mucosa located under partial or complete removable dentures. Prevalence of DS at denture wearers varies among studies from 14.3% up to 72% depending on study population. This condition is often asymptomatic and of multifactorial ethiology. Systematic illness (diabetes mellitus), vitamin A deficiency and cigarette smoking, gender (females) and age, are thought to be some of the systematic factors related to DS. Diabetes mellitus is considered to be the most important systematic factor related to Candida infection. Although precise mechanisms responsible for higher candida carriage/infections, as well as higher pathogenicity of yeasts at diabetic subjects are not defined, high salivary glucose level, low pH and low salivary flow rate are presumed to be some of them. Also, accumulation of glycated proteins in epithelial oral cells may facilitate adhesion of yeasts to these cells, which is considered as the essential first step for candida
colonization. Mentioned mechanisms, together with decreased phagocyte functions, may somewise explain influence of diabetes on Candida carriage/infections. Local factors are predominant in etiology of DS, and the commonest are amount of tissue covered with denture and constant denture wearing, poor denture hygiene, nocturnal dental wearing, low salivary/palatal mucosa pH, hyposalivation, and microorganisms. Among different microbes involved in pathogenesis of DS, Candida spp. are thought to be the most frequently isolated microorganism and also the commonest etiological factor responsible for DS. On the other hand, presence of Candida on palatal mucosa and/or denture surface of prosthesis wearers do not always mean DS. Also, cofactors for DS are not always same as for presence of Candida spp. Although DS or/and Candida presence are often asymptomatic, prosthesis may be a portal of entry for further infections, especially at immunocompromised subjects. According these facts, it is important to study prevalence and diversity of Candida spp. at denture wearers, and to explore potential cofactors facilitating colonisation of yeasts at palatal mucosa and dentures.

The aim of this study was to examine prevalence and variety of Candida spp. at denture wearers, and to explore potential cofactors facilitating colonisation of yeasts at palatal mucosa and dentures.

Material and Methods

This case-control observational study was approved by Ethical Committee of the School of Dental Medicine, University of Belgrade (Ethics Approval no. 36/8, 20th February 2013). Total of 60 subjects wearing partial or complete acrylic dentures without clinical signs of DS. Also, we studied if some local and systematic factors are risk factors for harboring Candida spp. at these subjects.

Informed consent was signed by every subject participated in the study.

Inclusion and exclusion criteria

The subjects included in the study wore upper denture more than a year, did not make modifications of denture in this period, and lacked any of the following criteria: presence of any disease except T2D and its chronic complication, aggressive periodontitis, presence of diseases or oral mucosa (Lichen planus etc.), clinical visible erythema of palatal mucosa, usage of medication that might affect presence of Candida spp., e.g. corticosteroids, antibiotics, antiseptics, usage of antiseptics for denture hygiene, pregnancy, lactation and periodontal treatment in the last 1.5 year.

Anamnestic data

Anamnestic data were done by a mean of medical questionnaire which included information about parameters that could potentially modify or predict clinical parameters of T2D or presence of Candida spp., but which could not be defined as exclusion criteria. This included identification of demographic and social data (gender, age, educational level), medical history (presence of T2D complications, xerostomia, glossodyniae and glossopyrosis, blood type (O vs. A+B+AB blood type), ill-fitting denture, number of years wearing denture, and habits (smoking habits defined as “smokers” and “non-smokers”, alcohol consumption, everyday intake of carbohydrates, nocturnal wearing of denture, continuous wearing of dentures, hygiene habits for dentures- usage of tooth brush, tooth paste). Patients who ceased smoking in period 6 months prior to study were not included in the study.

Clinical examination

Clinical examination included examination of oral mucosa and denture. Examination of oral mucosa were done by two trained doctors, according to WHO criteria. Lips, buccal mucosa, tongue, sublingual area, hard and soft palate were examined using appropriate dental light and dental mirror. Clinical variations of mucosa of tongue (fissured tongue, glossitis migrans etc.) were not exclusion criteria. Rhomboid glossitis and angular cheilitis, as common pathologic changes associated with DS, were exclusion criteria. Subjects with atrophic mucosa of dorsum of tongue or any clinical signs of candidiosis were excluded from the study. Present teeth were also examined at six sites per tooth (distobuccal, med-buccal, mesiobuccal, distolingual, med-lingual and mesiolingual) by two experienced and calibrated doctors, one performing the clinical measurements by Williams’ probe calibrated in millimeters (Hu-Friedy Chicago, IL) and another recording the results. Following periodontal
parameters were assessed: plaque index—Silness Loe (PI),
dichotomous bleeding on probing (BOP), probing pocket
depth (PPD) and clinical attachment level (CAL).

Dentures were also directly examined in terms of:
type of dentures, retention and stability, presence of
fractures, modifications, roughness or tooth abrasion.

Biochemical analysis

Biochemical analysis were done in order to measure
following parameters: fasting plasma glucose levels
(FPG), glycated hemoglobin (HbA1c), hematological
parameters (RBC, Hgb, HCT, MCV, MCH, MCHC, RDW)
and sedimentation rate.

Sample collection and cultivation

Samples were collected a day after clinical
examination. Patients were suggested not to brush their
teeth and to wear denture all night before the swabs were
taken. Swabs were collected by swabbing ten times from
the mucosa of hard palate or from palatal surface of
denture with the help of a dry sterile cotton stick. Swab
cultures were immediately inoculated on Sabouraud
dextrose agar (SDA) (Oxoid, Basingstoke, UK) at 37°C
for 48 h. After incubation, one calibrated microbiologist
counted the growth density. The yeast growth density was
defined as rare, medium or dense.

Further analysis were done using germ-tube
production test, carbohydrate assimilation test and by
using ChromAgar medium. Distinction of different
species of Candida genus by ChromAgar medium
were done according to colony color: green color for
C. albicans and C. dubliniensis, steel blue color for
C. tropicalis, white for C. parapsilosis and pink for
C. glabrata. Distinction between C. albicans and
C. dubliniensis were determined by carbohydrate
assimilation test. Microbiologist were blinded for any
clinical and anamnestic information.

Table 1. Demographic and clinical data of subjects

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male (%))</td>
<td>2 (28.6)</td>
<td>4 (50)</td>
<td>10 (66.7)</td>
<td>0.272</td>
</tr>
<tr>
<td>Age</td>
<td>53.14±4.221</td>
<td>50.25±8.844</td>
<td>47.93±6.216</td>
<td>0.243</td>
</tr>
<tr>
<td>Xerostomia in anamnesis</td>
<td>12 (28.6)</td>
<td>14 (50%)</td>
<td>28 (70%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Glossodyniae at glossopyrosis</td>
<td>1 (2.4)</td>
<td>3 (10.7)</td>
<td>4 (10)</td>
<td>0.298</td>
</tr>
<tr>
<td>Self-reported halithosis</td>
<td>14 (33.3)</td>
<td>11 (39.3)</td>
<td>10 (25%)</td>
<td>0.445</td>
</tr>
<tr>
<td>Presence of O blood type</td>
<td>8 (19.0)</td>
<td>6 (21.4)</td>
<td>4 (10.0)</td>
<td></td>
</tr>
<tr>
<td>Duration of denture wearing (years)</td>
<td>10±9.871</td>
<td>12.46±11.354</td>
<td>6.55±5.891</td>
<td>0.746</td>
</tr>
<tr>
<td>Smokers N (%)</td>
<td>16 (38.1)</td>
<td>9 (32.1)</td>
<td>10 (25.50)</td>
<td>0.445</td>
</tr>
</tbody>
</table>

*p*<sup>2</sup>, *b*ANOVA, *c*Kruskall-Wallis

Statistical analysis

Statistical analyses were carried out by using SPSS
18.0 software package for Windows (SPSS inc., Chicago,
USA). Descriptive data were presented as Mean ± SD
or the percentage for discrete measures. T-test and One
Way ANOVA were used for normally distributed data.
Non-parametric data were analyzed using the Kruskall
Wallis and Mann-Whitney test. Categorical variables
were compared using the Chi Square Test (*χ*<sup>2</sup>.
Spearman’s correlations were done in order to assess correlation
between presence of yeasts and measured parameters. The
linear regression model was used to determine predictors
of the presence of Candida spp. Subjects with missing
data were not included in the study. Differences were
considered significant when *p*-value was < 0.05.

Results

Groups were matched according to gender, age,
smoking status, ABO blood type and duration of denture
wearing in years. Also, presence of glossodyniae and
glossopyrosis and self-reported halitosis were similar
between the groups (Table 1).

Frequency of xerostomia in anamnesis were
higher at diabetics with poor glycoregulation than in
healthy subjects (*χ*<sup>2</sup>, *p*=0.000). None of
the patients reported presence of sialorrhea. Anamnestic
data about everyday consumption of carbo-hydrates,
showed that significant difference exists between group
A (N=8) and group B (N=0), (Chi, *p*=0.018). Groups of
patients with diabetes were similar according diabetes
duration (Independent sample T test, *p*=0.071) and the
diabetes treatment (Chi, *p*=0.194). These groups were
similar according to the presence of microvascular and
macrovascular chronic diabetic complications.
Presence of positive dental mirror stick test, denture wearing during the night, and presence of coated tongue were similar between the groups (Data not presented).

HbA1c and FPG levels were significantly different between all three groups (Bonferroni, p=0.000 for all comparisons). The most of hematological parameters were similar between the groups: Hgb (Kruskall-Wallis, p=0.998), Erythrocyte count (ANOVA, p=0.757), MCV (Kruskall-Wallis, p=0.960), MCH (Kruskall-Wallis, p=0.102). Only HCT differed between healthy control groups and two groups with diabetes (Mann-Whitney Test, p=0.000 for both comparisons).

Frequency of isolation of Candida spp. from palatal mucosa were similar between the groups (Fig. 1) (χ2=0.762). In group A, only C. albicans were isolated. In group B, two samples (2/16=12.50%) showed mixed isolates, while in group C, 20% of samples (6/30) exhibited mixed Candida species (presented in Fig 1) as 6.7%+13.3%. C. albicans+C. glabrata were only combination at diabetics with good glycoregulation. On the other hand, at diabetics with poorly regulated blood sugar level, 6.7% (2/30) of positive mixed isolates showed mentioned combination of yeasts, while 13.3% (4/30) exhibited C. albicans+C. tropicalis. Rare density of yeasts growth was present at all positive samples in groups A and B, and at 50% of isolates from group C. The rest of positive samples from group C exhibited medium (16.7%) and dense (33.3%) growth.

Positive finding of yeasts on the surface of the denture were 42.9% (6/14) at group A, 37.5% (6/16) at group B, and 66.7% (20/30) at group C (χ2=0.153) (Fig 1). Presence of multiple yeasts isolation were 12.5% (2/16) of positive sample at group B and 20% (6/30) at group C (also presented as 6.7%+13.3% in Fig.1). C. albicans+ C. glabrata were only isolated combination in group B, while in group C, this combination presented 13.3% (4/30). Another combination of yeasts in group C was C. albicans+C. tropicalis. Rare yeasts growth from denture surfaces was not detected. Medium growth was detected at 100% positive samples in groups A and B, and 80% of samples in group C.

Different finding of yeasts’ presence from swabs of palatal mucosa and denture surface existed. Negative finding of yeasts on palatal mucosa, but positive on denture surface were detected at 14.3% (2/14), 12.0% (2/16) and 33.4 (10/30) in groups A, B and C respectively. There were no samples with positive yeasts finding on palatal mucosa, but negative on denture surface.

Presence of yeasts on palatal mucosa correlate significantly (Spearman correlation) with HbA1c level neither at all patient (r=0.033, p=0.804) nor at diabetics (r=0.090,p=0.556). Correlations between positive finding of yeasts on denture and HbA1c also were not significant at all subjects (r=0.106, p=0.483) and at diabetics only (r=0.137, p=0.507).

Univariate regression models were done in order to assess if some of parameters (age, gender, xerostomia, glossodyniae at glossopyrosis, blood type, everyday consumption of carbohydrates, smoking, period of wearing denture measured in years, night wearing of dentures, ill-fitting dentures, duration of diabetes in years, dental mirror stick test, HbA1c, Hemoglobin level, Erythrocyte number, MCV, MCHC, MCH, Plaque Index)
can predict presence of Candida spp. on palatal mucosa or denture surface. None of the examined parameters were predictors of presence of Candida spp. on palatal mucosa. Presence of diabetes (Exp B= 1.243, CI=1.159-1.762, p=0.045), duration of diabetes in years (Exp B=1.169, CI=1.038-1.316, p=0.010) and Erythrocyte count (Exp B= 0.304, CI=0.103-0.901, p=0.032) were assessed as predictors of finding of Candida spp. on denture surface in univariate logistic regression. In multivariate logistic regression analysis, only duration of diabetes were predictor of Candida spp. presence on denture surface (Exp B=1.186, CI=1.047-1.344, p=0.007).

Discussion

Candida spp, commonly found yeast in humans, can be isolated in about 50% of healthy population without clinical signs of infection. In the case of diabetics, this prevalence is even higher.

Among oral Candida infections, Denture stomatitis is described as the commonest form of oral Candidiasis. Placement of a denture in the oral cavity leads to big changes of the environmental conditions because the denture, as non-shedding surface, becomes colonized with oral microorganisms and additionally cuts off the underlying mucosa from the mechanical cleaning of tongue and the flow of saliva. Colonization of acrylic surface of different roughness by yeasts cells and hyphae has been proven and is the initial step for the further accumulation of yeasts against supporting mucosa. Except this role in initial colonization, it is thought that dentures may keep microorganisms in long contact with the mucosa and permit the microbial metabolic products to initiate an inflammatory reaction. What is more, yeast may form biofilm, which is an essential strategy for their survival in oral milieus. Beside biofilm formation, this genus is able to produce exoenzymes, proteinases and metabolites in order to adhere and inhibit the function of polymorphonuclears.

In our study, we detected presence of different Candida spp. at patients with clinically health oral mucosa, including mucosa of hard palate. Unlike a lot of studies researching presence of yeasts at subjects with DS, there are limited number of researches dealing with yeasts at subject without signs of infections. At our study, frequency of Candida spp. finding was 36.6% at all subjects and 28.6%, 37.5% and 40.0% at groups A, B and C respectively. Overall prevalence is in agreement with findings of Figueiral et al. (27.2%) but is different from studies which revealed higher 51% or lower frequency (15.5%) at subjects without DS.

A study conducted in Serbia, presented positive Candida finding at 36.5% systematically health subjects without DS, which is quite similar to frequency of yeasts at our group A. Furthermore, number of studies that considered different Candida spp. at subjects without DS is very low. Although results of frequency of isolation of Candida spp. are inconsistent, all studies describe unique results about most commonly isolated species- Candida albicans. The second common isolated species from palatal mucosa at our study was C. glabrata and afterward C. tropicalis, which is also in agreement with other studies. Recovery of non-albicans species is important because of its high level of resistance to some antifungal drugs. C. tropicalis was isolated only at T2D patients with poor glycoregulation at our sample, which is in agreement with fact that this species is commonly isolated from immunocompromised patients and not at healthy. Similarly, C. glabrata is also considered as second or third most common isolate of Candida spp., and is increasingly connected with mucosal and systemic infections, especially at diabetics.

It is well-known that principal site for Candida colonization is the surface of denture, and that colonization of prosthesis surface is heavier than colonization of supporting mucosa, as we showed in this study by measuring growth density of yeasts. Overall prevalence of yeasts at denture surface at our sample was 53.3%, and 42.9%, 37.5% -and 66.7% at groups A, B and C respectively. Some studies report even 68% or 78% of overall prevalence of Candida spp. on denture, but it should be noted that these studies included subjects with wider variety of diseases than our study, and more sensitive methodology for detection of yeasts. Distribution of different Candida spp. on surface of denture was same as on palatal mucosa- Candida albicans being the most frequently isolated, followed by C. glabrata and then C. tropicalis, which is in agreement with previous studies.

In contrast to some other studies, presence of C. glabrata or C. tropicalis at our sample were always associated with C. albicans. When comparing results of yeasts finding with other researches, variations of isolation of Candida species depending on the geographical region and/or patient group should be taken into consideration. It is proven that in some countries, even non-albicans forms could be more frequently isolated than C. albicans. In Serbia, there are not sufficient number of studies examining presence of different Candida species. A study in Serbia examining prevalence of C. albicans and non-albicans species at oral lesions and observing their extraoral prevalence showed also highest prevalence of C. albicans, followed by C. glabrata and C. tropicalis.

As it was expected, some of the subjects who exhibited yeasts on denture surface had negative finding on palatal mucosa. Although nonsignificant, percentage of such finding was higher at diabetics with poor glycoregulation. This could be explained by reliability of these two sampling methods (false negative results of swabs from palatal mucosa), or on the other hand,
dentures may be considered as potential reservoirs of yeasts. Last is particularly important, because although presence of yeasts at subjects without DS or even with DS are often asymptomatic, it may serve as a portal of entry for further infections, e.g. aspiration pneumonia. This is especially important at immunocompromised patients, such as diabetics, especially with poorly regulated blood sugar, who are anyhow more prone to yeasts colonisation/ infection. Relating to these facts and common findings of more pathogenic, and resistant, forms of Candida at such patients, screening of DS or even yeasts presence at denture wearers should be done regularly.

According that oral candida carriage may be influenced by many factors nonrelated to diabetes (gender, smoking, medications, saliva, site, blood type…), we considered as much as possible factors which can be potentially confounders for Candida carriage. Surprisingly, any of our examined factors were not predictor for carriage of Candida spp. on palatal mucosa. Independent predictors for candida carriage on denture were only presence and duration of diabetes, but with rather low risk ratio. On the other hand, it should be stressed that there are not a lot of studies which deal with regression models in order to access parameters which could facilitate presence of yeast.

Although glycoregulation at diabetics has often been correlated to presence of yeasts/candidiasis, at our sample we failed to show that. This can be explained by different definition of “good” and “poor” glycoregulation among studies. Some studies defines non-satisfactory glycemic control limit up to 12%, while others define “gap” in HbA1c value between subjects with satisfactory and poorly regulated blood sugar, what were not case in our study.

Unfortunatelly, usage of removable partial/complete dentures to rehabilitate partially/complete edentulous patients is the very common option due to the low cost. As is it mentoined, placement of denture, as non-shedding surface, causes changes in oral cavity. Oral cavity itself is a compound environment that is continually exposed to numerous opportunistic microbes, which are controlled by a vigorous arsenal of immune factors that maintain a healthy oral environment, prevent development or spreading of disease. In state of disturbed immunological state, as at diabetics, this is not always maintained successfully. As it is said before, diabetics and other immunocompromised denture wearers should be periodically examined for presence of DS or Candida carriage in order to prevent potential complications. It should be stressed that treatment of DS/yeast colonisation should be taken seriously, according that after antifungal therapy the infection often reestablish, because of resistens species or if predisposing factors persist.

Conclusions

Prosthesis of denture wearers without DS may serve as reservoir of Candida spp. Presence of more pathogenic and resistant non-albicans species are related to diabetics, even without clinical signs of DS.

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A Survey of Endodontic Irrigants Used by Dentists With Varying Years of Professional Experience

SUMMARY

Background/Aim: The aim of the present study was to describe the use of irrigants by dentists in Bulgaria in relation to their years of professional experience. Material and Methods: The data were collected with the help of a questionnaire. The survey included questions concerning frequency of irrigants applied, their respective concentrations, as well as spectrum of disinfectants used in endodontics. In addition, information about respondents’ age, years of professional experience, gender, and main areas of continuing education was collected. The statistical analysis was performed with the help of IBM SPSS Statistics 22.0. Results: 219 replies were analysed (response rate 27.3%). The majority of the respondents (31.1%) had 21 to 30 years of professional experience. 18.7% had over 30 years. Most of the practitioners reported their continuing education to be in the area of general dentistry – 52%, while about 1.2% had specialised in endodontics. Dentists with long-standing professional experience use predominantly H$_2$O$_2$ – 78%. Dentists with least experience use 17% EDTA – 53.6%. No significant differences were established for the use of sodium hypochlorite and 2% chlorhexidine. 82% of the respondents use conventional needle 27G for intracanal irrigation; 60% never use ultrasonic irrigation. Conclusions: The analysis of the usage of irrigants shows that many general dental practitioners do not follow the quality recommended protocols for endodontic irrigation protocols.

Key words: Endodontic Irrigants, Survey, Years of Professional Experience

Introduction

Endodontic irrigants play a crucial role in removing debris, microorganisms and smear layer. There are many endodontic solutions available which claim to help prepare and disinfect the root canal system$^{1,2,3}$. Only a few have evidence to support their clinical use.

The studies have observed that Sodium hypochlorite (NaOCl) in range from 0.5% to 5.25% was the most commonly used irrigating solution, because of its antibacterial activity and ability to dissolve vital and necrotic organic tissue. However, sodium hypochlorite has no effects on the inorganic components of the smear layer. Acid solutions (3-10-40% Citric acid) and chelating agents (15-17% EDTA, Smear clear (Sybron Endo, Orange, CA) are recommended for removing the smear layer during chemo-mechanical preparation and as a final irrigation solution$^{3,4}$. Chlorhexidine (CHX) is a potent antiseptic, which is used as endodontic irrigant in 2% concentration. CHX binds to hard dental tissue and remains antimicrobial up to 3 weeks. It has a broad-spectrum antibacterial action, sustained action and low toxicity. It is unable to kill all bacteria, cannot dissolve organic substances and necrotic tissue present in the root canal system, and cannot remove the smear layer$^{3,5}$. Several studies have revealed that the majority of dentists do not follow the formulated guidelines on the quality of root canal treatment$^{5,6,7,8,9}$. The aim of the present study was to describe the usage of irrigants by dentists in Bulgaria in relation to years of professional experience; the data were collected with the help of a questionnaire.
Material and Methods

The present study was conducted amongst dental practitioners in Bulgaria with the help of a questionnaire. The questionnaire contained multiple choices questions as well as free-text spaces for additional comments. The survey contained 16 questions about the most frequently used irrigants such as NaOCl, H₂O₂, CHX, the respective concentrations, and the spectrum of disinfectants used during the root canal treatment. In addition, information about respondents’ age, years of professional experience, gender, and main areas of continuing education was collected. Data were entered and processed with the statistical package IBM SPSS Statistics 22.0.

The following statistical methods were used: descriptive analysis, graphical analysis, alternative analysis, exactly test of Fisher, and χ² test.

Results

219 replies were evaluated. The majority of dentists (31.1%) had 21-30 years of professional experience. 18.7% had over 30 years (fig.1). Most of the practitioners reported that their continuing education covered mainly general dentistry – 52%, while about 1.2% had covered endodontics in particular.

![Figure 1. Distribution of survey respondents by years of professional experience](image)

Dentists with long-standing professional experience used more often Hydrogen peroxide – 78%. For other endodontic irrigants: 2.5% NaOCl, 2% CHX, 5.25% NaOCl and 10% Citric acid no significant correlations were found between the usage and the years of professional experience of the respondents. 2.5% sodium hypochlorite is more often used by respondents with professional experience between 21-30 years – 66.2%, while 5.25% sodium hypochlorite is applied by dentists with up to 10-year professional experience - 64.3%. 2% CHX is more often used by dentists with over 30 year professional experience. Finally 10% Citric acid is used by practitioners with up to 10 years of practice – 25% (tabl. 1 and fig 2). Dentists with less experience (less than 10 years of professional experience) used 17% EDTA – 53.6%.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3% hydrogen peroxide</td>
<td>N</td>
<td>16</td>
<td>17</td>
<td>19</td>
<td>43</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>57.1a</td>
<td>44.7a</td>
<td>61.3ac</td>
<td>63.2ac</td>
<td>78.0bc</td>
</tr>
<tr>
<td>2,5% sodium hypochlorite</td>
<td>N</td>
<td>15</td>
<td>25</td>
<td>13</td>
<td>45</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>53.6a</td>
<td>65.8a</td>
<td>41.9a</td>
<td>66.2a</td>
<td>61.0a</td>
</tr>
<tr>
<td>17% EDTA</td>
<td>N</td>
<td>15</td>
<td>7</td>
<td>6</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>53.6a</td>
<td>18.4b</td>
<td>19.4b</td>
<td>23.5b</td>
<td>17.1b</td>
</tr>
<tr>
<td>2% chlorhexidine</td>
<td>N</td>
<td>7</td>
<td>13</td>
<td>11</td>
<td>24</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>25.0a</td>
<td>34.2a</td>
<td>35.5a</td>
<td>35.3a</td>
<td>43.9a</td>
</tr>
<tr>
<td>5,25% sodium hypochlorite</td>
<td>N</td>
<td>18</td>
<td>20</td>
<td>17</td>
<td>38</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>64.3a</td>
<td>52.6a</td>
<td>54.8a</td>
<td>55.9a</td>
<td>46.3a</td>
</tr>
<tr>
<td>10% citric acid</td>
<td>N</td>
<td>7</td>
<td>7</td>
<td>5</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>25.0a</td>
<td>18.4a</td>
<td>16.1a</td>
<td>19.1a</td>
<td>9.8a</td>
</tr>
</tbody>
</table>

*the different letters show that there is a significant difference (p<0.05)
The use of ultrasonic irrigation is significantly lower among dentists with professional experience over 30 years -75.6% of them have never used ultrasonic irrigation. The highest percentage for usage – 51.6% is recorded for the group with 16-20 years professional experience. For other groups of respondents there was no clear downward trend in the percentage of usage of ultrasonic irrigation with the increase of professional experience (Table 3 and Figure 4).

Table 3. Analysis of the relation between use of ultrasonic irrigation and professional experience of respondents

<table>
<thead>
<tr>
<th>Ultrasonic irrigation</th>
<th>Professional experience (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 10</td>
</tr>
<tr>
<td>Yes</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>14</td>
</tr>
<tr>
<td>No</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>14</td>
</tr>
</tbody>
</table>

*the different letters show that there is a significant difference (p<0.05)

Table 2. Analysis of the relation between types of needles used for endodontic irrigation and professional experience of respondents

<table>
<thead>
<tr>
<th>Type of needles used for endodontic irrigation</th>
<th>Professional experience (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional needles 27G</td>
<td>N</td>
</tr>
<tr>
<td>%</td>
<td>82.1a</td>
</tr>
<tr>
<td>Endodontic needles</td>
<td>N</td>
</tr>
<tr>
<td>%</td>
<td>32.1a</td>
</tr>
</tbody>
</table>

*the different letters show that there is a significant difference (p<0.05)

The results presented in tabl. 2 and fig. 3 show that conventional 27G needles for endodontic irrigation are used most by respondents with professional experience up to 10 years - 82%, and least by those with 16-20 years professional experience. Conversely, endodontic needles are used most often by dentists having more experience (21-30 years) – 61.8%.

Discussion

The disinfectant has to fulfill various requirements. It has to be biocompatible and cause no damage to the tissues, it must have a long-lasting disinfecting property and it has to be removable. In many countries recommendations governing the use of possible irrigants exist for the dentists.

In the present study our results show that dentists with long-standing professional experience (over 30 years) use frequently hydrogen peroxide – 78%. Similar results are obtained by other authors. Willershausen et al.5 concluded that dentists with longer years of professional experience in Germany used H2O2 significantly more often than NaOCl. But in their survey they established that in general 3% sodium hypochlorite is the most frequently used irrigant. Tosic et al.7 in a survey performed in Serbia reported that the
most popular irrigant was Hydrogen peroxide, and it is used by all respondents regardless of their years of professional experience. For the respondents with professional experience from 6 to 20 years Hydrogen peroxide was the only irrigant for root canal treatment. Sodium hypochlorite was used as root canal irrigant only among dentists with 2-5 years of professional experience (28.5%). This was the result from their first survey. After a second survey (3 years later) NaOCl became the most popular irrigant in all groups and increased use of Chlorhexidine in the first group comprising young dentists.

In our study the results show that 2.5% sodium hypochlorite is more often used by respondents with professional experience between 21-30 years – 66.2%, while 5.25% sodium hypochlorite is applied by dentists with up to 10-year professional experience - 64.3%. The results were however not comparable to a survey performed in Australia which reported that 94% of dentists used sodium hypochlorite. In other studies 80% of respondents use sodium hypochlorite. Abtin H. et al. reported that in British Columbia 94.22% of the general dentists used sodium hypochlorite, EDTA-36.23% and Chlorhexidine 14.66%.

An alternative irrigant is 2% CHX. In the present study the results show that 2% CHX is more often used by dentists who have over 30 years professional experience-43.9%. The use of chlorhexidine as a primary irrigant was found to be low (25% up to 10 years of professional experience) amongst the practitioners. This was similar to the survey conducted in Himachal Pradesh.

The total removal of the smear layer is preferred in order to improve the adaptation of the obturating materials in the root canal dentin and facilitate the diffusion of the irrigant solutions and intracanal medications into the root canal dentin. Our data correlated with other studies in which more than 50% of the dentists were methodically removing the smear layer before obturation. In our study 10% Citric acid is used by 25% of the respondents and 53.6% used 17% EDTA (by practitioners up to 10 years).

In the present study 51.6% of the respondents with 16-20 years of professional experience use ultrasonic activation, while 75.6% of the dentists with professional experience of over 30 years have never used ultrasonic irrigation. The findings of the survey among the practising dentists in Chennai have shown that 40% of the respondents are using ultrasonic activation as an adjunct, as an aid to their irrigation technique.

We found that 82% of the respondents with up to 10 years professional experience used 27 gauge needle during mechanical activation. None of the respondents use negative pressure irrigation with system such as EndoVac.

In the present study, no question concerning the use of rubber dam was included. The review by Ahmad 2009 shows that rubber dam is not used as frequently as required in many general dental practices from various countries.

Conclusions

- Dentists with less professional experience up to 10 years used higher concentration 5.25% NaOCl. They used 17% EDTA, 10% Citric acid for smear layer removal.
- Dentists with more years of professional experience used H₂O₂ more often frequently compared to their younger colleagues.
- The most commonly used needles for endodontic irrigation are 27G, followed by endodontic needles.

The analysis of the responses concerning the usage of irrigants showed that most general dental practitioners do not follow quality recommended protocols for endodontic irrigation.

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Parents’ Knowledge about the Effects of Oral Hygiene, Proper Nutrition and Fluoride Prophylaxis on Oral Health in Early Childhood

SUMMARY

Background/Aim: Health education, as one of the important aspects of preventive dentistry, plays an important role in promoting and achieving good oral health. The aim of this study was to determine the influence of parents’ knowledge about the effects of oral hygiene, proper nutrition and fluoride prophylaxis on oral health in early childhood. Material and Methods: Parents were asked to fill a questionnaire which consisted of three sections, oral hygiene, fluoride prophylaxis and nutrition. The study included 136 parents of children, aged between 3-6 years. The survey was conducted in pedagogical-education institution – PE “Our happiness” - Leposavic, Kosovo and Metohia, Serbia. Results: More than 58% parents from urban areas and 63% parents from rural areas were informed that the teeth should be brushed at least twice a day (p=0.007). Only 31.11% of parents from urban and 15.22% of parents from rural areas were informed that a child should visit dentists for the first time, at the one year of age (p=0.083). The analysis of the questionnaire section regarding the nutrition and oral health, showed that parents from rural areas were better informed than parents from urban areas. Awareness about fluoride and their role in preventive dentistry was poor, as only 3% of children from urban and 1% of children from rural areas were using tablets based on fluoride while only 1% of children from urban and none from rural areas were using fluoride mouth rinses. Conclusions: The study showed that parents have the knowledge about the impact of oral hygiene, food and fluoride prophylaxis on the oral health but unfortunately they do not apply their knowledge in practice.

Key words: Oral health, Knowledge, Parents, Questionnaires, Oral hygiene, Proper nutrition, Fluoride prophylaxis, Child

Introduction

Oral health is an integral part of the general health and has an impact on the quality of life human population. Health education, as one of the important aspects of preventive dentistry, plays an important role in promoting and achieving good oral health. Proper oral hygiene, nutrition, regular visits to the dentist, fluoride prophylaxis have a significant impact on oral health.

The family provides the background for developing knowledge, attitudes and habits related to the children’s oral health. The parents, as the highest authority, have an important and crucial role in forming a personality and hygienic-dietary of the children in period of primary socialization, including their appropriate attitude to oral health. Gao et al. in their research established a relationship between parents’ knowledge, habits and oral health behaviors and oral health of their children. In addition to parents school and friends can modify the behavior and habits of children affected by process of secondary socialization.

Good oral health has an important role in the upbringing of children, contributes to their physical, mental and social development. The dentist has important...
The results of this study showed that most parents know when is the most important time to brush teeth (Figure 1). Of the total number of surveyed parents, 76% parents from urban and 60.87% parents from rural areas control their children in maintaining the oral hygiene on a daily basis (p=0.129).

Material and Methods

The study included 136 parents, 90 from urban and 46 from rural areas (mean age of parents’ 33±4). The survey was conducted in pedagogical-education institution – PE “Our happiness” in Leposavic, Kosovo and Metohia, Serbia. Heads of educational institutions were informed about aim of study and gave their written consent to conduct this research in their institution. Parents’ level of information about proper nutrition, oral hygiene and fluoride prophylaxis was determined based on a questionnaire which consisted of three sections: oral hygiene, fluoride prophylaxis and nutrition.

Every part of the questionnaire contained a number of questions that analyzed demographic characteristics of children, habits, knowledge, attitudes of parents associated with oral health. The survey consisted of 32 questions in total, divided into three parts.

The first part of the questions included questions that examined parents’ knowledge about proper oral hygiene and demographic characteristics of parent and children. The second part of survey contained questions respecting the knowledge about fluoride prophylaxis and reasons for dental visits. The third group of questions included questions that examined Parents’ knowledge about nutrition. The surveys were anonymous and Parents’ were informed about the aim of the research and the method of implementation. Parents gave their written consent.

Data obtained in this study were processed by IBM SPSS Statistics 21 program. The results of the survey were analyzed using the χ² test.

Table 1. The analysis of the questionnaire section dealing regarding fluoride prophylaxis on oral health

<table>
<thead>
<tr>
<th>Question</th>
<th>Urban areas (%)</th>
<th>Rural areas (%)</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whether paste that you brush your teeth contains fluoride?</td>
<td>73.33%</td>
<td>73.91%</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>26.66%</td>
<td>26.08%</td>
<td>0.942</td>
<td></td>
</tr>
<tr>
<td>Whether daily use of tooth paste with fluoride has a role in oral prophylaxis?</td>
<td>72.22%</td>
<td>69.57%</td>
<td>0.105</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>27.78%</td>
<td>30.43%</td>
<td>0.746</td>
<td></td>
</tr>
<tr>
<td>Whether you use a fluoride tablets?</td>
<td>1.1%</td>
<td>0%</td>
<td>0.515</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>98.9%</td>
<td>100%</td>
<td>0.473</td>
<td></td>
</tr>
<tr>
<td>Whether your child use tablets with fluoride?</td>
<td>3.3%</td>
<td>2.2%</td>
<td>0.143</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>96.7%</td>
<td>97.8%</td>
<td>0.705</td>
<td></td>
</tr>
<tr>
<td>Since when your child use tablets with fluoride?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One year age</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Three year age</td>
<td>100%</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Six year age</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whether the child benefits mouth rinses with fluoride?</td>
<td>1.1%</td>
<td>0%</td>
<td>0.504</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>98.9%</td>
<td>100%</td>
<td>0.478</td>
<td></td>
</tr>
</tbody>
</table>

Results

The analysis of the questionnaire section regarding the oral hygiene showed that 58.89% parents from urban areas and 63.04% parents from rural areas were informed the teeth should be brushed at least twice a day (p=0.007). On question “How long it necessary to wash the teeth”, parents from urban (65.56%) and rural (63.04%) areas responded in an average of 1 to 3 minutes (p=0.081). Since the survey included parents whose children were 3 to 6 years old the questionnaire did not contain questions related to the usage of dental floss, mouthwash and interdental brushes.
Only 1.1% of parents from urban areas take fluoride tablets, contrary to none of the investigated parents in rural areas. Only 31.11% of parents from urban and 15.22% of parents from rural areas were informed that a child should visit the dentist for the first time, at one year of age (p=0.083). Results how often and when parents take their children to the dentists are presented in Figure 2.

![Figure 2. How often children visit the dentist?](image)

Of the total number of surveyed parents, 5.56% children from urban and 13.04% children from rural areas never visited the dentist.

The results of this study showed that 75.56% parents from urban and 65.22% parents from rural areas were informed that the primary teeth are equally important as permanent teeth (p=0.151). The main source of information regarding the oral health (urban areas- 82.22%) and (rural areas- 67.39%) was dentist. The results concerning the source of information about the oral health are presented in Figure 3.

![Figure 3. Educating parents about oral health](image)

The third group of questions included question that examined parents’ knowledge about nutrition. The analysis of the questionnaire section dealing with proper nutrition regarding on oral health, parents from urban (100%) and rural areas (98%) were informed that sodas contain harmful ingredients for oral health.

When it comes to food like chips or other “snacks”, 86.67% parents from urban and 91.30% parents from rural areas were informed that these types of food are harmful to oral health. All parents from both urban and rural areas were informed that diet and nutrition have an impact on oral health. Analysis of the questionnaire about the nutrition revealed that parents from rural areas were better informed than parents from urban areas (Table 2).

**Table 2. How often your child uses the following food?**

<table>
<thead>
<tr>
<th>Areas</th>
<th>Type of food</th>
<th>Never/ Rarely</th>
<th>Once a week</th>
<th>Several times a week</th>
<th>Once a day</th>
<th>Many times during the day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban/ Rural</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk, milk products</td>
<td>7%</td>
<td>9%</td>
<td>26%</td>
<td>30%</td>
<td>23%</td>
<td>24%</td>
</tr>
<tr>
<td>Fruits and vegetables</td>
<td>-</td>
<td>4%</td>
<td>41%</td>
<td>26%</td>
<td>13%</td>
<td>26%</td>
</tr>
<tr>
<td>Fruit juices</td>
<td>4%</td>
<td>11%</td>
<td>48%</td>
<td>37%</td>
<td>7%</td>
<td>25%</td>
</tr>
<tr>
<td>Sodas (Coca-cola)</td>
<td>99%</td>
<td>13%</td>
<td>40%</td>
<td>49%</td>
<td>27%</td>
<td>13%</td>
</tr>
<tr>
<td>Chocolate, cookies</td>
<td>8%</td>
<td>9%</td>
<td>40%</td>
<td>49%</td>
<td>27%</td>
<td>13%</td>
</tr>
<tr>
<td>Potato chips and other snacks</td>
<td>11%</td>
<td>15%</td>
<td>33%</td>
<td>51%</td>
<td>16%</td>
<td>10%</td>
</tr>
<tr>
<td>Candy, lollipops</td>
<td>24%</td>
<td>35%</td>
<td>50%</td>
<td>35%</td>
<td>4%</td>
<td>10%</td>
</tr>
</tbody>
</table>

**Discussion**

The aim of health education is to inform human population about the measures necessary to prevent the occurrence of oral diseases, combat causes of disease and methods of treatment. In addition, it is necessary to point out the necessity of proper nutrition, particularly in terms of controlled use of carbohydrates and a soft non-abrasive food. It is important to educate parents and children about the importance of conducting regular and proper oral hygiene, fluoride prophylaxis and regular visits at the dentist. Triangle, the parent, the child and the dentist plays an important role in the preservation and improvement of the oral health. Attempts to construct good oral health behaviors can affect the general health of individuals, as well. The adoption of good oral health habits in childhood often takes place with parents, especially with mothers.

Oral health of children is associated with oral health knowledge of their parents as oral health-related habits
are established during infancy and maintained throughout early childhood⁸. Parents are the primary social force influencing children development in the early childhood, so targeting parental oral health beliefs and practices may be important in the prevention of oral health problem such as bad oral hygiene and dental caries⁹. Results this study about role in supervision of oral hygiene of parents were encouraging. One study by Dye et al. showed a direct association between toothbrushing habits of the mother and her child¹⁰.

Gilinski et al. have shown that the effectiveness of health education intervention in the family depends on the initial attitudes and habits of parents regarding the oral hygiene and that education leads to improved knowledge about oral health¹². In this study, parents from urban and rural areas were well informed about the importance of oral hygiene. Only 2% of children from urban areas did not have toothbrush.

The notion that the “baby teeth don’t deserve care because you lose them anyway” has largely disappeared in western world. Most parents are unaware of the role a pediatric dentist play in their child’s life, and the importance of regular dental visits at an early age is underestimated as most believe that the primary teeth are going to exfoliate.

The first dental visit is an important milestone in the children life and a visit should be an essential part of the children general health care¹³. According to the American Academy of Pediatric Dentistry, every child must visit the dentist by the child first birthday⁶. In this study, only 31.11% of parents from urban and 15.22% of parents from rural areas knew when is necessary to take the child to the first dental examination. Of the total number of surveyed parents, 20% parents from urban areas and 17.39% of parents from rural areas do not know when it is necessary to visit the pediatric dentist for the first time. Most parents from urban (48.9%) and rural (67.4%) areas thought that the first dental visit should be after 3 year of age. There were parents (2%) who supplemented their answers, stating that they think there is no need to visit pediatric dentist if the child do not have toothache, and that the primary dentition is not important.

Alaa et al. reported that most parents thought that the first dental visit should be between 3-6 years of age, maybe because they believe that at one year of age all teeth were not erupted¹⁴. The results of Winier et al. showed that only 39% of the parents believe that the first dental visit should be at one year of age, while 21% reported to be as six months¹⁵. Since pediatricians see the children on a more regular basis for vaccination, it would be recommended they advise the patients about the importance of early dental visits. Hinze et al. noted that 40% of the pediatricians referred a one year old child to a dentist and only 29% believed it was important¹⁶.

With the facts that the caries is a widespread and common disease of teeth in children in the Republic of Serbia, agreed 64% of parents from urban and 63% of parents from rural areas. In this study, we asked parents to rate dental health of their children and get information that 46% children from urban and 28% children from rural areas have caries. Of the total number of survey parents, 97% parents from urban and 93% from rural areas considered regular visits to the dentist to be necessary. The fact is that a higher percentage of surveyed parents knows that the child should be taken to the dentist for regular checkups, but still they do not take them to the dentist on regular basis.

Research by Lalic et al. confirmed the hypothesis that the family affects the oral health of children¹⁷. It was found that the incidence of oral hygiene and parent’s daily control of the child in oral hygiene has a statistically significant impact on dental health status of the child¹⁷. In this study, 75% parents from urban and 61% from rural areas controlled daily oral hygiene of their children.

When it comes to awareness of fluoride usage, as one of the most powerful means in preventive dentistry, most parents from urban (99%) and rural (100%) areas did not use fluoride tablets or any other fluoride products. In this study awareness about fluoride and their role in preventive dentistry was poor, as only 3% of children from urban and 1% of children from rural areas were using tablets based on fluoride while only 1% of children from urban and none from rural areas were using fluoride mouth rinses. Of all the fluoride products, toothpaste with fluoride was used in 73.33% in urban and 73.91% rural areas. Parents whose children use fluoride tablets, although in low percentage, reported that their children do not have caries. Although parents have the information about fluoride prophylaxis in oral health, on the basis of these results, conclusion is that in practice very rarely or never use tablets or solutions based on fluoride. Results of this study were similar to the study by Jain et al.¹⁸, Moulana et al.¹⁹, Suresh et al.²⁰, as the knowledge regarding role of fluoride was poor. Contrary to this results were studies by Gussy et al.²¹ and Kamolmatyakul and Saoing²², who reported good knowledge about fluoride usage. Results showed that parents are informed about fluoride prophylaxis, but the information is very rarely applied in practice.

Latest guidelines on the use of fluoride do not recommend giving fluoride systematically to the whole population, only to children with a high caries risk. The use of toothpaste with fluoride is a basic caries preventive measure that is recommended to everyone and should be promoted²³. The author’s attitude on the use of fluoride tablets or any other fluoride products do not recommend giving systematically to every child, only to children with a high caries risk. It is necessary to inform parents more about fluoride tablets and other fluoride products and insist more on the use fluoride products in a dental office under the control of a dentist and after consultation with a dentist.

Regarding the source of information about the oral health, around 82.22% of parents from urban and 67.39
% parents from rural areas said that they got information about oral health from the dentist. Wyne et al. reported that 34.2% of the Saudi population gets the oral health information from dentist\textsuperscript{18}. The results of this study show that newspaper, TV media and internet have big influence on people in their everyday lives as the newspaper was a main source of information about oral health in 13.3% parents form urban and 21.7% rural areas. Also Jain et al. reported that newspaper and TV were the major source of information about the oral health\textsuperscript{18}. Strategies should be made to utilize media more effectively for oral health education.

Although parents were quite informed which food are harmful to the oral health, a large percentage of harmful ingredients were used several times a week, which is not encouraging. Parents have the knowledge about the impact of food on the oral health but unfortunately they do not apply their knowledge in practice.

**Conclusions**

Health education should raise the awareness about the importance of oral hygiene, proper nutrition and fluoride prophylaxis in the prevention of dental diseases. Parents should participate in the education processes, as educators and controllers of the implementation of the suggested preventive measures.

This study showed that parents from urban and rural areas have the knowledge about the impact of oral hygiene, proper nutrition on the oral health but unfortunately they do not apply their knowledge in practice. Parents have the information about fluoride prophylaxis in oral health, but it can be concluded that in practice very rarely or never use tablets or solutions based on fluoride. The result of this study cannot be extrapolated because the sample size was small.

**References**


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Adjunctive Subantimicrobial Dose Doxycycline in the Treatment of Chronic Periodontitis in Type 2 Diabetic Patients: A Unique Combination Therapy

**Introduction**

Type 2 diabetes mellitus (T2DM) and periodontal disease are common chronic diseases in adults. Both diseases are highly prevalent in the world population and constituting a global public health burden. Diabetes affects more than 150 million individuals worldwide and this incidence is increased annually\(^1\). By the year 2030, it was estimated that about 366 million people worldwide will have diabetes\(^2\). In addition, more than one in three people worldwide over 30 years of age will have periodontitis\(^3\). Diabetes mellitus and periodontal disease are assumed to share a common pathogenesis that involves an enhanced inflammatory host response\(^4\). Investigations have shown that diabetics with long history of diabetes mellitus and poor metabolic control are more likely to have severe periodontal destruction and alveolar bone loss than non-diabetics\(^5\).

Collagenases are considered the key mediators of inflammatory tissue destruction in periodontal diseases. Among collagenases, matrix metalloproteinase-8 (MMP-8) is the key enzyme in extracellular collagen matrix
and it is the major destructive collagenase that is present in large amounts in gingival crevicular fluid (GCF) of periodontally diseased subjects\(^6\). Patients with T2DM and chronic periodontitis have been shown to express higher levels of active MMP-8 in their GCF\(^8\). Specific immunoassays targeting MMP-8 were considered to be useful in monitoring the course of periodontitis in patients with diabetes\(^9\). Evidence based studies have indicated that control of periodontal infection has a positive effect on glycemic control evidenced by a reduction in glycated hemoglobin (HbA1c) levels\(^10,11\).

The current therapeutic strategies for the treatment of chronic periodontal disease in T2DM patients have been directed towards two different approaches: antimicrobial therapy and host modulation therapy (HMT)\(^12,13\). Antimicrobial therapy can be effective in decreasing the total bacterial count in periodontal infection with down-regulation of the inflammatory mediators leading to an improvement of glycemic control in T2DM subjects. However, in HMT excessive and pathologic host inflammatory responses adjusted to provide wound healing and periodontal stability without impairing normal defense mechanisms or inducing inflammation\(^12,14\).

Subantimicrobial dose doxycycline (SDD) is the only host modulation drug approved by the food and drug administration (FDA) which can inhibited the activity of Matrix metalloproteinases (MMPs) involved in the degradation of collagen in connective tissue as a result of periodontal disease\(^15\).

The aim of this clinical study was to evaluate the effects of combining systemically administrated SDD and locally delivered doxycycline gel (LD) as adjuncts to scaling and root planing (SRP) in the treatment of chronic periodontitis in T2DM patients by assessing the changes in the clinical and biochemical parameters.

### Material and Methods

#### Subjects

Forty patients with T2DM aged 35 to 60 years fulfilling the inclusion criteria were selected from the outpatient clinic of the Department of Oral Medicine, Periodontology, Oral Diagnosis and Oral Radiology, Faculty of Dentistry, Tanta University. The study was reviewed and approved by the University Review Board and the Research Ethical Committee, Faculty of Dentistry, Tanta University. The study was performed in accordance with the Helsinki Declaration of 1975 as revised in 2000.

The patients were given written explanations of the study, and they provided written informed consent.

#### Inclusion criteria

The inclusion criteria for selection were subjects with controlled T2DM that had been established diabetes mellitus at least one year before baseline examination, with at least two consecutive values of glycated hemoglobin (HbA1c) level of ≤ 7%, had been taking a stable dose of oral hypoglycemic medications and/or insulin for at least three months as assessed by their medical records and had been clinically diagnosed with chronic periodontitis. The patients had at least ≥ 10 teeth per dental arch, excluding the third molars and teeth indicated for extraction.

#### Exclusion criteria

Patients were excluded if they were received antibiotics within 3 months prior to the study, patients subjected to periodontal treatment in the six months period prior to the study, patients with other systemic conditions known to modify periodontal disease expression such as pregnancy, cardiovascular disease, and smokers. Patients in need for prophylactic antibiotics before treatment as well as those hypersensitive to doxycycline or any other drug in the tetracycline class were also excluded. Other exclusion criteria includes: renal impairment, severe liver disease, and grade 3 or 4 retinopathy.

#### Study design

At the first visit (screening visit), patients were screened for eligibility for the study and they were participated into the study if they met the inclusion/exclusion criteria outlined above. Patients were received oral hygiene instructions (OHI) and their ability to fully comply with OHI was evaluated. Eligible subjects were randomly divided (by tossing a coin) into two equal groups: test group (TG) and control group (CG) of twenty patients each.

At baseline visit, all patients of the TG and CG were received basic periodontal treatment including full mouth scaling and root planing (SRP) using hand and ultrasonic instrumentation followed by tooth polishing with a fluoride containing paste and their OHI was reinforced. Full mouth SRP was conducted in two visits (in two successive days). In each visit one side either right or left (upper and lower) were treated under local anesthesia when indicated. A comprehensive periodontal examination was performed for all patients, including measures of clinical parameters by a single examiner for bleeding on probing (BOP: Ainamo and Bay)\(^16\), PD, and the clinical attachment level (CAL: Ramfjord)\(^17\), using Williams periodontal probe, Hu-friedy, Chicago, IL. Assessments of clinical parameters were performed at baseline, 3, 6 and 9 months after treatment.

Subjects in the TG were treated by full mouth SRP and were received systemic SDD, (doxycycline 20 mg) twice per day at approximately 12 hours intervals, one hour before eating for 6 months. In addition, locally delivered doxycycline gel 10% [LD] (prepared at Pharmaceutical Technology Department, Faculty of...
Pharmacy, Tanta University) was applied using a special syringe with a blunt end that inserted into the periodontal pocket until the gel was extruded from the pocket at selected sites (sites with PD≥ 5mm, with bleeding and/or suppurative on probing). Subjects in the CG were treated by full mouth SRP only. For all subjects, HbA1c level assessments were carried out in a private laboratory to allow for monitoring of the overall glycemic control.

MMP-8 assay
Supragingival plaque was removed, and the area was isolated with cotton rolls and gently dried before sampling. GCF samples were collected from all patients from areas of deepest pocket depth by a single examiner using sterile prefabricated paper points prior to recording the clinical parameters at baseline, 3, 6, and 9 months. The absorbed GCF volume of each point was measured using a calibrated device (Periotron 8000, ProFlow Inc., NY, USA). All samples were then immediately placed in an Eppendorf tubes® containing 200 μl phosphate buffered saline (PBS pH 7.2), and stored at −20°C until analyzed®. MMP-8 levels in the GCF samples were determined by Enzyme-Linked Immunosorbent Assay (ELISA) test kit (Quantikine®, R&D Systems, Minneapolis, MN, USA) according to the instructions of the manufacturer. The specific kit can detect total MMP-8 (pro- and active forms of MMP-8) in saliva, GCF, serum and plasma. The levels of MMP-8 in each sample were calculated based on the dilutions, and the results were expressed as the concentration in GCF sample.

Statistical Analysis
The Student’s t-test (two tailed, independent) was used for comparison between the two groups (intergroup analysis) and a Student’s t-test (two tailed, dependent) was used to determine the significance of study parameters within each group (intragroup analysis). Chi-square and Fisher exact tests were used to test the significance of study parameters on a categorical scale. All statistical tests were conducted at a significance level of $p<0.05$. Statistical tests were performed using computer software program SPSS ver. 20.0 (SPSS Inc., Chicago, IL, USA).

Results
A total of 40 patients (26 males and 14 females) were included and completed the study in adherence to the prescribed protocol. Demographic data, clinical parameters, and GCF MMP-8 levels of the patients were summarized in (Table 1). At baseline, the TG and CG had similar mean values for age and gender distribution. The types and doses of hypoglycemic agents and/or insulin were constant during the 9 months study period as verified by the patients’ physician and their glycemic control were maintained throughout the study. For the TG, there were no adverse events reported during the study and the treatment appeared to be well tolerated.

Periodontal and biochemical parameters
As previously stated, all clinical and biochemical parameters were assessed in both groups at baseline and again at 3, 6 and 9 months after treatment. At baseline, there were no statistically significant differences among patients of both groups in clinical parameters and GCF MMP-8 levels (Table 1). However, both TG and CG showed significant improvement in clinical and biochemical parameters over the study period.

The mean PD at baseline in the TG was (3.97±0.64) and in the CG was (4.19±0.23) which was not statistically significant ($P=0.06$). During the study, a statistically significant reductions in PD compared to baseline were seen in both groups at 3, 6, and 9 months ($P<0.05$). Comparison between the TG and CG, showed a greater reduction in mean PD in the TG than in the CG at 3 months (TG 3.00±0.47, CG 3.71±0.22), 6 months (TG 3.04±0.39, CG 3.68±0.24) and 9 months (TG 2.95±0.40, CG 3.66±0.22) from baseline ($P<0.05$) (Table 2).

Compared to baseline, the CAL also showed significant reductions at 3, 6 and 9 months ($P<0.05$) in both groups. Comparison between the TG and CG, showed a greater reduction in CAL in the TG than in the CG at 3 months (TG 3.99±0.64, CG 2.99±0.39), 6 months (TG 3.69±0.62, CG 2.77±0.38) and 9 months (TG 3.51±0.52, CG 2.64±0.49) from baseline ($P<0.05$) (Table 2).

The mean BOP values of both TG and CG were significantly lower at 3, 6 and 9 months compared to baseline ($P<0.05$). The reduction was more significant in the TG compared to the CG at 3 months (TG 34.94±14.30, CG 57.21±06.96), 6 months (TG 27.41±14.29, CG 54.16±10.29) and 9 months (TG 26.62±13.70, CG 52.78±10.37) ($P<0.05$) (Table 2). From baseline to 9 months, GCF MMP-8 levels were significantly reduced in both groups ($P<0.05$). However, GCF MMP-8 levels in the TG was significantly lower than that of the CG at 3 months (TG 34.40±5.23, CG 52.80±8.26), 6 months (TG 25.00±7.72, CG 44.20±8.44), and 9 months (TG 18.60±8.26, CG 36.40±3.44) ($P<0.05$) (Table 2).

Table 1. Demographic data, clinical parameters, and MMP-8 levels of the Test group (TG), and control group (CG).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>TG (n=20)</th>
<th>CG (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43.33±4.34</td>
<td>44.12±5.20</td>
</tr>
<tr>
<td>Probing depth</td>
<td>3.97±0.64</td>
<td>4.19±0.23</td>
</tr>
<tr>
<td>Clinical attachment level</td>
<td>4.42±0.82</td>
<td>4.20±0.39</td>
</tr>
<tr>
<td>Bleeding on probing</td>
<td>74.22±22.88</td>
<td>77.61±13.94</td>
</tr>
<tr>
<td>MMP-8</td>
<td>65.80±6.56</td>
<td>67.20±9.13</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation.
There were no significant differences between groups ($P>0.05$).
Table 2. Results of periodontal and biochemical parameters at baseline and at 3, 6, and 9 months in the Test group (TG), and control group (CG)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TG (n=20)</th>
<th>CG (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probing depth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.97±0.64</td>
<td>4.19±0.23</td>
</tr>
<tr>
<td>3 months</td>
<td>3.00±0.47 a,b</td>
<td>3.71±0.22 a</td>
</tr>
<tr>
<td>6 months</td>
<td>3.04±0.39 a,b</td>
<td>3.68±0.24 a</td>
</tr>
<tr>
<td>9 months</td>
<td>2.95±0.40 a,b</td>
<td>3.66±0.22 a</td>
</tr>
<tr>
<td>Clinical attachment level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.42±0.82</td>
<td>4.20±0.39</td>
</tr>
<tr>
<td>3 months</td>
<td>3.99±0.64 a,b</td>
<td>2.99±0.39 a</td>
</tr>
<tr>
<td>6 months</td>
<td>3.69±0.62 a,b</td>
<td>2.77±0.38 a</td>
</tr>
<tr>
<td>9 months</td>
<td>3.51±0.52 a,b</td>
<td>2.64±0.49 a</td>
</tr>
<tr>
<td>Bleeding on probing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>74.22±22.88</td>
<td>77.61±13.94</td>
</tr>
<tr>
<td>3 months</td>
<td>34.94±14.30 a,b</td>
<td>57.21±06.96 a</td>
</tr>
<tr>
<td>6 months</td>
<td>27.41±14.29 a,b</td>
<td>54.16±10.29 a</td>
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<tr>
<td>9 months</td>
<td>26.62±13.70 a,b</td>
<td>52.78±10.37 a</td>
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<tr>
<td>MMP-8 levels</td>
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<tr>
<td>Baseline</td>
<td>65.80±6.56</td>
<td>67.20±9.13</td>
</tr>
<tr>
<td>3 months</td>
<td>34.40±5.23 a,b</td>
<td>52.80±8.26 a</td>
</tr>
<tr>
<td>6 months</td>
<td>25.00±7.72 a,b</td>
<td>44.20±8.44 a</td>
</tr>
<tr>
<td>9 months</td>
<td>18.60±8.26 a,b</td>
<td>36.40±3.44 a</td>
</tr>
</tbody>
</table>

*a)p<0.05 compared to the baseline,  b) P<0.05 compared to the control group.

Discussion

The present study was designed to evaluate the effectiveness of combining SDD and LD as adjuncts to SRP in the treatment of chronic periodontitis in T2DM patients. The severity of chronic periodontitis was similar in TG and CG at the beginning of the present study. Significant improvements in clinical and biochemical parameters were observed after treatment in both groups, patients were evaluated every 3 months until the end of the study at 9 months and improvements were maintained throughout the study period. The selected patients were all controlled T2DM patients with HbA1c levels of ≤7%.

A follow-up period of 9 months was selected for the evaluation of clinical, and biochemical responses to the treatment which was considered a sufficiently long period for observation of any possible relapse.

To the best of our knowledge no study has compared SRP alone to SRP in addition to SDD and LD in T2DM patients with chronic periodontitis. Our results of combination therapy with SRP, SDD and LD compared favorably to the use of SRP alone, SRP and SDD or SRP and LD. SRP has been considered the gold standard for non-surgical periodontal therapy. It induces the resolution of inflammatory processes and reduction of progression of the periodontal disease, hence it results in reduction of PD and gain of clinical attachment. In the present study, patients of the CG received only SRP, OHI and the results showed significant reduction in PD and gain of CAL beside the improvements of gingival inflammation at 3, 6, and 9 months compared to the baseline. This was expected as sufficient time was spent for thorough SRP in addition to adequate periodontal maintenance given to the patients at recall visits. These findings were consistent with the results of previously reported studies that have been evaluated the efficiency of conventional SRP in patients with T2DM and chronic periodontitis which has been revealed a significant improvement of clinical periodontal parameters including PD, CAL, and BOP.

Data from previous studies showed that the addition of SDD to SRP resulted in significant clinical improvement in terms of CAL gain and PPD reduction over SRP alone in T2DM patients with periodontal disease following 3 and 6 months of therapy. Similarly, the beneficial effects of combining LD to SRP have been supported by studies on different patient groups. In a clinical study evaluated the effect of application of a single dose LD as an adjunct to SRP in the treatment of periodontitis in type 1 diabetes mellitus patients (T1DM), Lima et al. have reported a statistically significant PD reduction and CAL gain which were maintained during the entire 12 months study period. Moreover, a study with 2 years follow up by Machion et al. the adjunctive use of LD to SRP in the periodontal treatment of non-diabetic smokers were evaluated and the results showed a significant PD reduction, CAL gain at 6, 18, and 24 months. In this study, the addition of SDD, LD to SRP in the treatment of chronic periodontitis in T2DM patients (TG) was provided a significant improvement of the clinical periodontal parameters (PPD, BOP reductions, and CAL gain) at 3 months and maintained at 6, 9 months beyond that obtained by SRP alone (CG). These results suggested that a synergy of action possibly exists between systemically administrated SDD and locally delivered LD which could be represented in their combined potential for modifying the local, systemic inflammation and tissue destruction.

In the present study, the levels of MMP-8 in GCF were measured by using ELIZA test and high concentrations of MMP-8 were reported at baseline in both study groups. The levels of GCF MMP-8 showed marked reductions following treatment in both groups which maintained throughout the study period but the results were statistically significant for the TG rather than CG. Hence decreased levels of MMP-8 in both groups might suggest the effectiveness of conventional periodontal therapy represented by full mouth SRP and OHI in reducing the bacterial loads in periodontal environment. Furthermore, these reductions could be related to the combined anticollagenase activity of SDD and antibacterial efficiency of LD for patients of TG. Previous study reported significant reduction.
in the levels of GCF MMP-8 in T2DM patients with chronic periodontitis after conventional SRP for 3 months treatment period\(^6\). Furthermore, in a randomized controlled double blind study by Gilowski et al.\(^7\), the effectiveness of short term SDD as an adjunct to SRP in patients with T2DM and chronic periodontitis were studied by evaluating MMP-8 levels in GCF. The results revealed statistically significant reduction of GCF MMP-8 levels only in the test group received SDD for 3 months. Our results of combination therapy of SRP, SDD, and LD support and further extend the findings of these studies with a different treatment protocol, for a different follow up period and in correlation with changes in the clinical and biochemical parameters.

In our study design, only controlled T2DM patients have been participated based on their consecutive assessments of HbA1c levels \(\leq 7\). Previous studies investigating the effect of periodontal treatment on metabolic control of T2DM patients and presented a conflicting results. A number of studies have shown positive effects of periodontal treatment on metabolic control\(^8\), however, other studies reported the contrary\(^12,27,28\). In a recent systematic review, Pérez-Losada et al.\(^9\) stated that there was no clear evidence of a relation between periodontal treatment and improved metabolic control in patients with T2DM.

In brief, the results of current study suggested that greater benefits for improving the periodontal status of T2DM patients may be achieved by a combination therapy of SRP, SDD and LD. These improvements represented a significant clinical benefit to T2DM patients and reduced the need for further treatment provided that the patients maintain their glycemic control and continued their oral hygiene measures.

**Conclusions**

In conclusion, combination therapy of SDD and LD has a synergistic effect as an adjunct to SRP in the treatment of chronic periodontitis in T2DM patients. It was effective in suppressing pathologically excessive MMP-8 associated with chronic periodontitis in patients with T2DM.

**References**

9. Correa FO, Gonçalves D, Figueredo CM, Gustafsson A, Orrico SR. The short term effectiveness of non-surgical treatment in reducing levels of Interleukin-1ß (IL-1ß) and proteases in gingival crevicular fluid from patients with type 2 diabetes mellitus and chronic periodontitis. J Periodontal, 2008;79:2143-2150.


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Second Root Canal in Mandibular Incisors: an Ex Vivo Cone-Beam Computed Tomography (CBCT) Study

SUMMARY

Background/Aim: To analyze the internal morphology of mandibular incisors with two root canals using cone-beam computed tomography (CBCT). Material and Methods: 289 (143 central and 146 lateral) extracted intact mandibular incisors were radiographed for detection of a second root canal. The teeth presenting a second root canal were imaged with CBCT and evaluated regarding: root canal type, the distance of the cementoenamel junction (CEJ) to the bifurcation of the canals and the distance of the canal fusion to the apical foramen (in teeth in which canals rejoined). Results: Out of 143 central and 146 lateral mandibular incisors, 41 (28.7%) and 44 (30.1%) teeth respectively showed a second root canal. Types II, III, V and an additional type to Vertucci’s classification were identified. Type III was the most prevalent and presented in 30 (73.2%) central and 34 (77.3%) lateral mandibular incisors with two root canals. The mean values of the distance of the CEJ to the canal bifurcation were 4.2 mm and 4.0 mm for central and lateral mandibular incisors respectively. The mean values of the distance of the canal fusion to the apical foramen 5.5 mm and 5.1 mm for central and lateral mandibular incisors respectively. Conclusions: Mandibular incisors with two root canals mainly present with Vertucci’s Type 3 canal configuration. The canal bifurcation was identified mostly at the coronal and middle thirds of the root, while the canal fusion occurred in the middle third of the root.

Key words: Root Canals, Mandibular Incisors, CBCT

Introduction

A comprehensive understanding of the complexity of the root canal system for each tooth type is fundamental to successful endodontic treatment. Mandibular incisors typically present with one root and one canal, although the variant of two root canals is also mentioned in the literature. The first study to investigate mandibular incisors with two root canals, by Rankine-Wilson & Henry\textsuperscript{1} roentgenographically examined 111 specimens and concluded that 40.5% of them showed canal bifurcation. This study was followed by numerous ex-vivo and in-vivo studies analyzing this morphologic variation, with prevalences ranging from 4.0-67.5% and 10.6-63.0% for the central and lateral mandibular incisors respectively\textsuperscript{2,3} (Table 1). The wide range noted in the literature could be attributed to different methods of investigations, differences in the sample size and donor ethnicity.

Early studies investigating the internal morphology of mandibular incisors employed radiography, sectioning, modeling and clearing techniques\textsuperscript{4-6}, while most recent studies employ computed tomography techniques. Cone-beam computed tomography (CBCT) is a technique that has been used in numerous in vivo morphologic studies as a modality providing three-dimensional (3D) anatomical information on teeth\textsuperscript{7,8}. 

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Balk J Dent Med, 2018;38-42
The majority of the studies mentioned in the extant literature focus on the number of root canals, without providing sufficient complementary information regarding the root canal path. The aim of the present study was to employ CBCT to evaluate the internal morphology of mandibular incisors with two root canals in detail by recording the exact point of bifurcation and fusion of the root canals.

Table 1. Comparison of Vertucci’s canal configurations in mandibular incisors in previous studies

<table>
<thead>
<tr>
<th>References</th>
<th>Method</th>
<th>Sample Type</th>
<th>Teeth Group</th>
<th>Vertucci’s Classification (%)</th>
<th>Other types (%)</th>
<th>Teeth with 2 r.c. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertucci19, 1974</td>
<td>Clearing</td>
<td>central</td>
<td>I</td>
<td>100 central 70 5 22 3 - - - - -</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(ex vivo)</td>
<td>lateral</td>
<td>II</td>
<td>100 lateral 75 5 18 2 - - - - -</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kartal &amp; Yanikoglu6, 1992</td>
<td>Clearing</td>
<td>incisors</td>
<td>III</td>
<td>100 incisors 55 16 20 4 3 - - - -</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Caliskan et al11, 1995</td>
<td>Clearing</td>
<td>central</td>
<td>IV</td>
<td>100 central 68.6 13.7 13.7 - 1.96 - - - - -</td>
<td>1.96</td>
<td>31.4</td>
</tr>
<tr>
<td>Gomes et al3, 1996</td>
<td>Modelling</td>
<td>central</td>
<td>V</td>
<td>58 central 63.8 22.4 1.7 - 5.2 5.2 1.7 - -</td>
<td>-</td>
<td>36.2</td>
</tr>
<tr>
<td>Uma et al4, 2004</td>
<td>Radiography</td>
<td>incisors</td>
<td>VI</td>
<td>50 incisors 44 2 52 - - - - - - 2 -</td>
<td>-</td>
<td>56</td>
</tr>
<tr>
<td>Sert et al3, 2004</td>
<td>Clearing</td>
<td>central</td>
<td>VII</td>
<td>200 central 32.5 27 26 9 0.5 - - - 1 4</td>
<td>67.5</td>
<td>63</td>
</tr>
<tr>
<td>Al-Qudah &amp; Awawdeh21, 2006</td>
<td>Clearing</td>
<td>incisors</td>
<td>III</td>
<td>450 incisors 73.8 10.9 6.7 5.1 3.6 - - - - - -</td>
<td>-</td>
<td>26.2</td>
</tr>
<tr>
<td>Boruah &amp; Bhyum22, 2011</td>
<td>Clearing</td>
<td>incisors</td>
<td>V</td>
<td>480 incisors 63.8 7.1 22.9 6.3 - - - - - -</td>
<td>-</td>
<td>36.2</td>
</tr>
<tr>
<td>Al-Fouzan et al12, 2012</td>
<td>Clearing</td>
<td>central</td>
<td>I</td>
<td>40 central 70 - 30 - - - - - -</td>
<td>-</td>
<td>30</td>
</tr>
<tr>
<td>Rahimi et al23, 2013</td>
<td>Clearing</td>
<td>central</td>
<td>II</td>
<td>186 central 64.5 18.3 16.7 0.5 - - - - - -</td>
<td>-</td>
<td>35.5</td>
</tr>
<tr>
<td>Aminsohban et al13, 2013</td>
<td>CBCT</td>
<td>central</td>
<td>III</td>
<td>632 central 70.6 7.1 3.7 15.4 3.2 - - - - -</td>
<td>-</td>
<td>29.4</td>
</tr>
<tr>
<td>Altunsoy et al24, 2014</td>
<td>CBCT</td>
<td>central</td>
<td>IV</td>
<td>614 central 71.8 10.3 2.8 12.8 2.3 - - - - -</td>
<td>-</td>
<td>28.2</td>
</tr>
<tr>
<td>Han et al19, 2014</td>
<td>CBCT</td>
<td>central</td>
<td>V</td>
<td>1286 central 84.3 3.4 6.5 1.2 3.9 - 0.3 - 0.4</td>
<td>15.7</td>
<td>-</td>
</tr>
<tr>
<td>Leoni et al15, 2014</td>
<td>µCT</td>
<td>central</td>
<td>VI</td>
<td>50 central 50.0 - 28.0 - - - 4.0 - 18.0</td>
<td>50.0</td>
<td>-</td>
</tr>
<tr>
<td>Lin et al20, 2014</td>
<td>CBCT</td>
<td>central</td>
<td>VII</td>
<td>706 central 89.1 2.4 6.2 1.7 0.6 - - - -</td>
<td>-</td>
<td>8.9</td>
</tr>
<tr>
<td>Liu et al7, 2014</td>
<td>CBCT</td>
<td>central</td>
<td>I</td>
<td>706 central 74.5 3.7 19.3 2.1 0.4 - - - - -</td>
<td>-</td>
<td>25.5</td>
</tr>
<tr>
<td>Zhao et al16, 2014</td>
<td>CBCT</td>
<td>central</td>
<td>II</td>
<td>785 central 82.5 3.9 10.4 2.8 0.3 - - - - -</td>
<td>-</td>
<td>17.5</td>
</tr>
<tr>
<td>Arslan et al17, 2015</td>
<td>CBCT</td>
<td>central</td>
<td>III</td>
<td>1566 central 93.3 - 5.68 - 1.0 - - - - -</td>
<td>-</td>
<td>6.7</td>
</tr>
<tr>
<td>Muhammad et al23, 2015</td>
<td>Clearing</td>
<td>central</td>
<td>IV</td>
<td>1566 central 82.6 - 15.5 - 1.9 - 0.1 0.1</td>
<td>17.4</td>
<td>-</td>
</tr>
<tr>
<td>Da Silva et al18, 2016</td>
<td>CBCT</td>
<td>central</td>
<td>V</td>
<td>184 central 51.9 4.3 41.6 - 0.5 - - - 1.6</td>
<td>48.1</td>
<td>-</td>
</tr>
<tr>
<td>Kamtane &amp; Ghodke26, 2016</td>
<td>CBCT</td>
<td>incisors</td>
<td>VI</td>
<td>102 incisors 64.7 23.5 8.8 2.9 - - - - -</td>
<td>-</td>
<td>35.3</td>
</tr>
<tr>
<td>Zhengyan et al2, 2016</td>
<td>CBCT</td>
<td>central</td>
<td>VII</td>
<td>3375 central 96 0.2 2.8 0.1 0.8 - - - 0.1</td>
<td>4.0</td>
<td>10.6</td>
</tr>
</tbody>
</table>
Material and Methods

This study was approved by the research committee of the Aristotle University of Thessaloniki, Greece (protocol no. 27/03-02-2016). The sample initially comprised 289 (143 central and 146 lateral) mandibular incisors collected from the Department of Dental Surgery, Implantology and Radiology of the School of Dentistry, Aristotle University of Thessaloniki, Greece and dental clinics of Thessaloniki. Data regarding the donor age, sex, or race were not recorded. Teeth without fully formed apices as well as those with root cracks or resorption, endodontic treatment, or crown loss were excluded from the sample. The teeth were stored in 0.1% thymol, rinsed with normal saline, and dried. Contact radiographs were obtained from the facial lingual aspect and mesial distal direction of the samples.

The specimens presenting a second root canal were placed on low-density bases (sponges) in groups of four and scanned using a high-resolution imaging protocol (voxel size, 0.1 mm) with a CBCT scanner (Newtom VGI EVO, QR Verona, Verona, Italy; 110 kVp; 3.00 mA; exposure time 4.3 s). Image acquisition was performed in accordance with standardized procedures by a trained radiology technician. The acquired volumetric data were analyzed in the axial, coronal, and sagittal planes using the proprietary software supplied with the CBCT scanner to determine specific morphological features of the included teeth.

The following parameters were evaluated by analysis of axial images of the teeth:

- Root canal type according to Vertucci’s classification9 (Figure 1),
- Bifurcation distance; recorded from the most apical part of the cementoenamel junction (CEJ) to the canal bifurcation,
- Fusion distance; recorded from the canal fusion to the main apical foramen in samples where the root canals rejoined after the initial bifurcation.

The Bifurcation and Fusion distances were measured as follows. Each scan comprised 501 axial sections numbered on the upper left corner. Individual images were acquired at 0.1 mm intervals (voxel resolution, 0.1 mm). The Bifurcation distance for each tooth was calculated by subtracting the numbers of axial images of the CEJ and bifurcation point and multiplying the result by 0.1 mm. The same procedure was followed for the Fusion distance, by subtracting the numbers of axial images of the canal fusion and the main apical foramen and multiplying the result by 0.1 mm.

Three-dimensional models of the external and internal morphology of the teeth were reconstructed using OsiriX lite (Pixmeo SARL, Bernex, Switzerland) for visualization of the diverse canal configurations of the samples.

Results

Out of 143 central and 146 lateral mandibular incisors, 41 (28.7%) and 44 (30.1%) teeth respectively showed a second root canal in the lingual aspect of the root when radiographed in the mesio distal direction and these were then scanned using CBCT.

![Diagrammatic representation of Vertucci's canal configurations](image)

Table 2. Incidence of different root canal morphologies of permanent mandibular incisors

<table>
<thead>
<tr>
<th>Mandibular incisors</th>
<th>Teeth with 2 r.c.</th>
<th>Vertucci’s classification (%)</th>
<th>Type 1-2-1-2-1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incisors</td>
<td>85</td>
<td>14 (16.5)</td>
<td>5 (5.9)</td>
</tr>
<tr>
<td>Central</td>
<td>41</td>
<td>6 (14.6)</td>
<td>30 (73.2)</td>
</tr>
<tr>
<td>Lateral</td>
<td>44</td>
<td>8 (18.2)</td>
<td>34 (77.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Root canal type according to Vertucci’s classification: Table 2 presents the incidence of different root canal morphologies of mandibular incisors with two root canals. An additional root canal type (1-2-1-2-1) was found in 3 central and 2 lateral mandibular incisors (Figure 2).

Bifurcation distance: The distance of the CEJ to the root canal bifurcation was measured for 30 central and 34 lateral mandibular incisors, which belonged to Type III. Table 3 shows minimum, maximum, mean values and standard deviations.

Fusion distance: The distance of the canal fusion to the main apical foramen was measured for 30 central and 34 lateral mandibular incisors which belonged to Type III. Table 3 shows minimum, maximum, mean values and standard deviations.

Table 3. Minimum, maximum, mean values and standard deviations of the Bifurcation and Fusion distance for central and lateral mandibular incisors

<table>
<thead>
<tr>
<th>Mandibular incisors</th>
<th>Bifurcation distance (mm)</th>
<th>Fusion distance (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>Central</td>
<td>3.0</td>
<td>6.4</td>
</tr>
<tr>
<td>Lateral</td>
<td>3.0</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Discussion

The frequency of bifurcated root canals in mandibular incisors has previously been investigated by many research groups. In the present paper 28.7% of central and 30.1% of the lateral mandibular incisors examined demonstrated two canals; something in accordance with other papers. The main aim of this study was, in addition to investigating the frequency of this morphologic variation, to analyze some anatomic features of these teeth with critical clinical implications.

For this purpose, the study employed CBCT, a diagnostic imaging modality providing high quality, 3D representations of anatomic structures. It is a non-invasive technique providing large amounts of information in the form of axial, sagittal and coronal sections. In order to accurately evaluate the anatomic aspects examined, the specimens were scanned using a high-resolution protocol that allowed the observation of morphological details such as lateral root canals of minor diameter, isthmi, intracanal ramifications and apical deltas. However, these small anatomic details have not been included in the results of the present study, as they could be examined more accurately with micro computed tomography (μCT) providing even higher resolution and image quality.

The majority of both central and lateral mandibular incisors with two root canals belonged to Type III, with no specimens belonging in Type IV, something also remarked by other studies. Mandibular incisors have been shown to have complex canal configurations transcending Vertucci’s classification. Those variations could be attributed to the great deal of istmi, dentin islands and intracanal ramifications of these teeth. In the present study, Type 1-2-1-2-1 was identified in 5.9% of central and 7.3% of lateral mandibular incisors. This canal configuration was also recorded by Leoni et al. in 4% of central and 2% of lateral mandibular incisors.

According to the mean values of the Bifurcation distance, the canal bifurcation usually occurs at the transition from the coronal to the middle third of the root. The range of the measurements indicates that bifurcation could occur even at 6.4 mm from the CEJ. Such cases require special attention to detect the existence of the lingual canal. The results of the present study are in accordance with other studies which performed similar measurements regarding the bifurcation point. On the other hand, the Fusion distance of the teeth belonging to Type III has not been recorded so far in the literature. While the canal fusion would normally be expected to occur apically, the present findings indicate that it usually occurs at the middle third of the roots, and the existence of outliers for this measurement indicates that it could occur even in the coronal third in rare cases.

Consequently, when dental clinicians have to perform root canal therapy on mandibular incisors, they should take into account that one in three mandibular incisors present with a second root canal lingually, and the possibility of any of the morphologic variations mentioned in the literature so far has to be considered.

Conclusions

Mandibular incisors with two root canals mainly present with Vertucci’s Type III canal configuration. The canal bifurcation was identified mostly at the coronal and middle thirds of the root, while the canal fusion occurred in the middle third of the root.
References


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Primary Lymphoepithelial-Like Carcinoma of the Parotid Gland- Case Presentation

SUMMARY

Background/Aim: Primary Lymphoepithelial carcinoma (PLEC) is a rare subtype of salivary gland cancers, which comprises only 0.4% of salivary malignant neoplasms and only a few cases have been presented previously. Case report: A patient with PLEC of the parotid gland, its management and the available literature are presented. A 53-year-old woman with initial lesion of a lump in the region of the right parotid received treatment with antibiotics which did not lead to improvement. MRI was performed that recorded the presence of a tumor in the right parotid gland and the patient subsequently underwent excision biopsy. The histopathological evaluation together with additional immunohistochemical positive staining of EMA+, EGFR+, p63+, CK 5/6+, AE1/AE3+ established the diagnosis of PLEC. A PET-CT scanning has shown nor primary mucosal source neither skin lesion to account for any possible metastatic disease, consequently a course of adjuvant post-operative radiotherapy to the region of the right parotid gland was performed. Conclusions: In the differential diagnosis of a parotid gland lump should be included the possibility of a rare salivary gland neoplasm such as PLEC. Surgical excision and radiotherapy have been proposed for the treatment of early and advanced neoplasm stages. Ages of the patient, stage of the neoplasm as well as type of therapy are significant and individual variables for the prediction of the prognosis.

Key words: Lymphoepithelial Carcinoma, Salivary Gland Neoplasms, Parotid Neoplasms, Histopathology, Immunohistochemistry, Prognosis, Treatment.

Introduction

Primary Lymphoepithelial carcinoma (PLEC) is a very unusual subtype of salivary gland cancers, which involves mainly the parotid gland1,2. Furthermore for the salivary PLEC have been used different terms as: undifferentiated carcinoma with lymphoid stroma, malignant lymphoepithelial lesion, lymphoepithelioma-like carcinoma, undifferentiated carcinoma and carcinoma ex lymphoepithelial lesion1. PLEC is analogous and exhibits identical histopathological characteristics as non-keratinizing, undifferentiated nasopharyngeal carcinomas (NPC)3. However PLEC arises in organs other than nasopharynx, such as larynx4, tonsils5, lung6, thymus7, stomach and duodenum8, breast9, renal pelvis and urinary bladder10, uterine cervix11, endometrium12, ovary13, vulva and vagina14. The experience in PLEC is restricted to a small number of case reports and series, most of which describe female patients in specific geographic regions such as Arctic Circle, Greenland and Southern China, with documented association of Epstein-Barr virus (EBV) implication. Consequently the above mentioned case series proposed a racial, gender, and geographic predilection to the disease2,15-23. The scientific evidence presenting cases of PLEC in other regions are restricted with significant geographic variations15,16,23-25.

Case report

A 53-year-old Caucasian woman was referred to our department for the management of recently enlarging...
lesions: a lump in the submandibular area and a separate lump in the region of the right parotid (Figure 1). Her medical and family history revised extensively and proved as ordinary, without smoking habit. Initially a panoramic radiograph was performed by her dentist, and the patient received treatment with antibiotics (amoxicillin and clavulanate potassium) that did not lead to improvement. Afterwards the lower right wisdom teeth were extracted and the patient received treatment with a different antibiotic (clindamycin) that diminished the submandibular lesion whereas led to no improvement in the parotid lump. The neurological evaluation of the patient confirmed that bilateral facial nerve function was unimpaired in any way. Again her medical history was reviewed in detail and confirmed that the patient had no history of skin cancer or other type of malignancy in the head and neck region. An MRI was performed and there was recorded the presence of a malignant growth in the right parotid gland. A scintigraphy (i.v. Tc99m) in the salivary gland recorded two lumps in the lower part of right parotid gland that took on no Tc99m, so they were not parts of the parenchyma of the gland (Figure 2). Afterwards a Fine Needle Aspiration (FNA) for diagnostic cytology was conducted, which confirmed the malignant nature of the lesions.
Total parotidectomy with nerve preservation and unilateral upper neck dissection was performed, and the histopathological evaluation with H&E routine staining of the specimens (Figure 3), together with additional immunohistochemical positive staining of EMA+, EGFR+, p63+, CK 5/6+, AE1/AE3+ (Figure 4), and negative of LCA-, CA15-3-, TTF1-, S100-, SMA-, CK7-, CK20- established the final diagnosis of PLEC.

A PET-CT scanning has shown nor primary mucosal source neither skin lesion to account for any possible metastatic disease, consequently the patient received a course of adjuvant post-operative irradiation to the right parotid bed and the right neck including levels 1b, 2, 3, 4 and 5. She has received a post-operative dose of 60Gy in 30 sessions to each of these areas. No concomitant chemotherapy was used. Six years later, there was no metastasis neither in the head and neck nor in the lymph nodes. Excessive bone loss and highly increased DMFT were observed in the area which the radiotherapy was previously performed.

![Figure 4. Positive immunostaining of PLEC: AE1/AE3 highlighting the epithelial neoplastic component (Figure 4A AE1/AE3 immunohistochemistry stain, X 100), cytokeratin 5/6 reveals staining in the majority of the tumour cells (Figure 4B Cytokeratin 5/6 immunohistochemistry stain, X 100), EMA reveals staining in many of the tumour cells (Figure 4C EMA immunohistochemistry, stain X 100), p63 reveals intense nuclear staining in many of the tumour cells (Figure 4D p63 immunohistochemistry, stain X 100).](image)

**Discussion**

Primary Lymphoepithelial carcinoma (PLEC) of the major salivary glands is extremely uncommon neoplasm which constitutes 0.4% of the total salivary gland malignancies in non-endemic regions. Medical/dental personnel has available inadequate scientific information limited to disperse case series derived from endemic regions in order to decide alternative ways of treatment and to advise patients with PLEC.

Previous literature recorded elevated frequencies of PLEC in specific geographic regions such as in Arctic Cycle (Greenland, Canada, Alaska), Southeastern China, and Japan. In these areas PLEC usually involves the parotid gland with female predilection and exhibits a higher invasive course. In a recent study with two hundred and thirty-eight cases of PLEC with most of the patients of Caucasian origin (81.2%), the median age at diagnosis was 62 years with higher prevalence in ages 50–70, without gender preference.

The clinical features of salivary PLEC are consistent of a salivary gland mass/swelling, usually located in parotid gland. The onset of the lesion has been described as of considerable diversity, which in a percentage of the cases may appeared from 7 days to 20 years before observing their initial symptoms. In some cases patients is possible to encounter an accelerated
augmentation in neoplasm size, however the reported data presented an extensive variation (15.9–75%)\textsuperscript{29,30}. Furthermore it has been reported that infrequent symptoms are pain or tenderness (10.1–25%)\textsuperscript{29,30}, and facial paresis (1.4–20%)\textsuperscript{23,29,30}. 

The diagnostic procedure of PLEC includes mainly radiological techniques (CT, MRI), and fine-needle aspiration biopsy. A previous study evaluated the diagnostic efficiency of fine-needle aspiration cytology method which proved to be sensitive in 78.6% of 14 PLEC cases\textsuperscript{31}. CT and MRI radiological techniques have been considered as useful supplementary investigations for the diagnosis, preoperative assessments and biopsy guidance in PLEC cases, although these tumors in a study for their imaging characteristics on CT and MRI revealed nonspecific attenuation and signaling\textsuperscript{32}. In addition is required to rule out a possible regional metastasis from the nasopharynx, because this event may demand a completely distinct therapeutic approach. Furthermore due to histological similarity of non-keratinizing, undifferentiated nasopharyngeal carcinoma (NPC) to salivary PLEC, it has been recommended in such cases, endoscopy and random biopsy of smooth-appearing nasopharyngeal mucosa in order to confirm the diagnosis\textsuperscript{30}.

The diagnosis of PLEC is based predominantly on histological characteristics, which display well-circumscribed nodules, including anaplastic cells with prominent cosinophilic nuclei arranged in nests, sheets, and cords of syncytial-like growth pattern, and surrounded by moderate to heavy lymphocyte infiltrates\textsuperscript{33}. Additional immunohistochemical evaluation may be required, with positive staining of neoplastic cells for cytokeratin, epithelial membrane antigen, and variable expression of EBV and of lymphoid cells for CD20 and CD3 markers suggestive of B-cell and T-cell presence, in order to confirm the initial histological diagnosis\textsuperscript{34}.

In most PLEC tumors of salivary glands was reported the increased infiltration of CD20+CD3+ T lymphocytes in the tumor cell nests and the surrounding stroma and germinal centers. The above mentioned infiltration perhaps provoked a strong host immune response, which is possible to guide PLEC to an approximately better prognosis\textsuperscript{33}.

Differential diagnosis of PLEC in histological level includes squamous and mucoepidermoid carcinoma with abundant lymphocytic infiltration, poorly differentiated large cell carcinoma of salivary origin and nasopharyngeal carcinoma with parotid extension\textsuperscript{15}. The nasopharyngeal carcinoma as mentioned previously may required a challenging differential diagnosis to distinguished and is possible to demand a detailed clinical, imaging, histological and immunohistochemical investigation\textsuperscript{1}.

Salivary PLEC regularly metastasizes to the regional lymph nodes (45.1%), usually with extensive lymph node involvement (69.1% N2 out of all lymph nodes positive infiltration) whereas in the same recent non-endemic region study, the incidence of distant metastasis in salivary PLEC, has been reported less than 10%, and the authors stated that routine radiological investigations in remote areas of the body are not always justified\textsuperscript{29}. In the above mentioned study was also revealed that in comparison to the parotid glands, submandibular gland PLEC displayed an increased percentage of occurrences in the American Indian, Aleutian, Eskimo, Asian population, presented with T3-T4 disease and regional metastasis. The previous observation was not significantly modified the survival rate in total of PLEC cases in parotid and submandibular gland sites\textsuperscript{28}.

The origin and pathogenesis of PLEC has been proposed to related with malignant transformation of a myoepithelial island or alternatively with malignant transformation of glandular and ductal insertions in intraparotid lymph node\textsuperscript{15}. The implication with EBV, since EBV was isolated in >90% of endemic PLEC patients, has proposed a possible function for the virus in the etiopathogenesis of PLEC but the association remains in dispute, taking consideration that EBV usually was undetected in the non-endemic PLEC cases\textsuperscript{27}. Additional research papers investigating PLEC neoplasms, reported viral presence in clonal episcopal form and viral LMP-1 oncoprotein expression, supporting EBV role in PLEC tumorogenesis\textsuperscript{35, 36}. Moreover in support of PLEC and EBV association, it has been suggested that EBV is possible to play a role in younger patients in Asian populations, because it was previously reported that primary infection of EBV in children of families with lower socioeconomic status was an early event\textsuperscript{37}. Furthermore it is well documented that primary EBV infection usually involves children of younger ages, causing approximately 90% seropositivity in early adulthood. Subsequently EBV persists in the host for life associated with low grade infections in the oropharynx, salivary ductal cells, and dormant in B-lymphocytes.

Nevertheless the findings of a study from a non-endemic region created the probability of a separate disease mechanism and etiopathogenesis in a Caucasian predominant population\textsuperscript{28}. Taking consideration that adult populations worldwide are seropositive for EBV\textsuperscript{38} it is possible that alternative viral immune control functions operate in distinctive geographic regions and ethnicities. The above mentioned aspects contribute to a supplementary level of complication in PLEC etiopathogenesis, part of which is associated mainly with EBV in specific geographic regions\textsuperscript{28}. Furthermore considering the histopathological similarity between nasopharyngeal carcinoma and PLEC and their interrelation with EBV, it has been proposed a hypothesis of a potential correlation between the two, which may influenced by common risk factors such as diets containing high quantities of nitrites\textsuperscript{28}. 


A previous clinicopathologic study, investigating the immunohistochemical characteristics of PLEC pointed out that lower level, or complete loss of expression, of p53, c-erb B-2 oncoproteins, and low rate of EGFR mutation were significantly correlated to a more favorable prognosis in PLECs of salivary glands. Furthermore, in the same research paper was detected positive bcl-2 immunostaining with moderate to strong intensity in the majority of the cases (20 in 21). This finding suggested that increased expression of bcl-2 protein created a specific growth enhancement to the EBV-infected B cells, and proposed simultaneously that the bcl-2 over-expression it is possible to be associated with a favorable prognosis.

The treatment for the salivary gland tumors remains challenging for most of the cases, especially for salivary PLEC; surgical excision with postsurgical radiotherapy together with neck dissection in case of cervical metastasis, were considered as the treatment of choice. In a recent study with most of the cases of Caucasian origin, the occurrence of cervical metastasis did not significantly affect the survival expectancy, in addition it has been reported a total survival difference, especially in early disease, in patients who performed both surgical interventions and radiotherapy. Whereas in a select cohort of 22 patients with Stage III PLEC who performed neck dissection, a significantly better survival was recorded. In the above mentioned study was also reported that in cases which performed postsurgical radiotherapy, was recorded an increased period of time free of recurrence, however no significant difference was observed in overall survival. Five year and ten year survival is 77% and 56% respectively whereas older age and advanced stage are supplementary significant and individual factors of worst survival.

Conclusions

In conclusion in the differential diagnosis of a parotid gland lump should be included the possibility of a rare salivary gland neoplasm such as PLEC. Consequently, the medical/dental personnel must be informed for the diagnostic sequence, the management and the available literature of such lesion. Surgical excision and radiotherapy have been proposed for the treatment of early and advanced neoplasm stages. Ages of the patient, stage of the neoplasm as well as type of therapy are significant and individual variables for the prediction of the prognosis.

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Oral Lipoma Located at the Left Lower Vestibule-Report of a Case and a Brief Review of the Literature

**SUMMARY**

**Background/Aim:** The present paper focuses on examining a case report of an oral lipoma located at the left lower vestibule. **Case report:** The patient's clinical state was thoroughly studied, along with the findings of histopathological examinations. The surgical treatment and postoperative course are also within the scope of this report. Numerous histogenesis theories and the appropriate tumor treatment are mentioned within the article, being always in accordance with the relative literature. **Conclusions:** Oral lipoma is a benign not very rare neoplasm, which occurs most commonly in adult males. The surgical excision is the treatment of choice. The diagnosis must always be established by histological examination. **Key words:** Oral Benign Neoplasm, Oral Lipoma, Oral Mucosal Swelling

**Introduction**

The oral lipoma is a benign neoplasm, probably of mesenchymal origin, composed of the mature adipocytes, usually covered by a fibrous capsule. Roux was the first who described this oral lesion in 1848, when he referred lipoma as yellowish epulis. The tumor is rarely located on the head and neck area (15–20% of the cases). Among the head and neck cases, merely 1-5% of the lesions are located intraorally, while only a few cases of malignancy have been reported in the literature.

A greater incidence of lipomas is reported in adult patients. According to sex distribution, the referred data are controversial. The ratio between male and female patients is reported to be either equal, or there is a strong male predilection, or a slight female prevalence is noted, with a female to male ratio of 1:2:1, especially between the fourth and the sixth decade of their lives. Most cases come from countries of the western world.

Regarding the site predilection, it is probably correlated to the availability of adipose tissue, which is high in the buccal mucosa due to the proximity of buccal fat pad and very low in the palate. The lesion is most frequently located at the buccal mucosa, followed by the lips, tongue, palate, vestibule, mandible, the floor of the mouth and retromolar area. Conversely, salivary glands and gingivobuccal fold, parotid masseteric region and neck, and pharynx/larynx are involved less frequently.

Clinically, oral lipoma appears to be a well circumscribed, painless, soft, slow growing tumor with either a sessile or a pedunculated base. The oral lipoma’s color ranges from yellow to pinkish, depending on the depth of the lesion. Regarding the size of oral lipoma, it varies greatly, but most of these lesions are about 10mm in diameter. When they occur as multiple lesions they may be correlated with syndromes such as neurofibromatosis, Gardner’s syndrome, Dercum’s disease, familial multiple lipomatosis, Proteus syndrome or Pai syndrome.

**Case report**

A 78-years-old male patient was referred to our Clinic by his dentist, who detected a swelling located at the buccal mucosa of the mandible, in the periapical region nearby the second left lower premolar. His medical history revealed that he has been under medical treatment of hypertension and moderate heart failure.
The clinical examination revealed a tumor located in the aforementioned region. The swelling was soft and painless in palpation, well defined, and covered by pinkish mucosa. The vitality test for the adjacent teeth was positive; so periapical inflammation was excluded. Extraoral examination revealed no swollen lymph nodes. The initial clinical diagnosis included oral lipoma or other benign neoplasm. The lesion was surgically excised in toto under local anesthesia. The yellowish color of the excised lesion was a supportive element to the diagnosis of lipoma (Figures 1 & 2).

The histopathological examination revealed mature adipocytes, without lipoblasts. Additionally, the lesion was surrounded by a fine fibrous capsule. A mature lipoma was the final diagnosis (Figures 3 & 4).

The patient’s postoperative period was uneventful and no recurrence was reported two years after the surgical excision.

**Discussion**

We describe a case of an oral lipoma, located at the left vestibule of the mandible, in the periapical region near the second left lower premolar. The treatment of choice was the surgical excision in toto and the histological examination confirmed the clinical diagnosis of lipoma.

Histologically, oral lipomas are divided into subtypes based on the matrix and the properties of tumor cells: simple lipoma, fibrolipoma, spindle cell lipoma, intramuscular lipoma, chondrolipoma, pleiomorphic lipoma, myxoid lipoma or myxolipoma, angiolipoma, osteolipoma, angioxyloolipoma, sialolipoma, infiltrating lipoma, perineural lipoma, intraneural lipoma and atypical lipoma. Despite the fact that the etiology and pathogenesis of the tumor are not clear, two main theories have been claimed: (i) The Hypertrophy theory, which correlates obesity and inadvertent growth of adipose tissue with the formation of oral lipoma. This theory lacks explaining the reason why lesions occur in areas without pre-existing adipose tissue, and (ii) the Metaplasia theory according which the lipomatous development occurs due to aberrant differentiation of mesenchymal cells in lipoblasts. Factors like endocrine disorders, inflammation, hypercholesterolaemia and obesity,
radiation, chronic irritation, spontaneous development, metaplasia of muscle cells and fatty degeneration, trauma as well as chromosomal abnormalities, have also been considered\(^1,2,20\). Furthermore, a number of authors have proposed diabetes mellitus and nutritional problems as possible causative factors\(^3,21\).

The clinical diagnosis of oral lipoma is easy due to its yellowish color, and its usual location superficially near the mucosa\(^1\). Extra caution is needed during differential diagnosis between oral lipoma and other types of tumor such as fibroma, sarcoma, dermoid cyst, minor salivary gland tumors, malignant lymphoma hemangioma or neuroma\(^1,9,22\). Some authors believe that oral lipoma located at the buccal mucosa is not a true tumor but, rather, herniation of the buccal fat pad through the buccinator muscle\(^9\). Such cases may occur following a local trauma in young children or a surgical excision of third molars in older patients\(^9\).

Most tumors are relatively asymptomatic and grow to a large size before patients seek medical care\(^8\), due to concerns regarding growth, cosmetic aspects or symptoms as a result of the compression of local structures\(^7\). Surgical excision is the treatment of choice\(^1\).

No recurrence has been reported, although it may occur in infiltrating lipomas mainly because of an inadequate excision combined with a non-encapsulated lesion\(^1\). More specifically, well-encapsulated lipomas are easily excised with no chance of recurrence or damage to the surrounding structures\(^9\).

In any case histological examination ought to confirm the clinical diagnosis. Special attention must be paid to avoid confusion with histological features of liposarcoma\(^5\).

Conclusions

The lipoma of the oral cavity is a rare benign tumor, usually growing in the buccal mucosa. The surgical excision of the tumor constitutes the treatment of choice. The histological examination confirms the diagnosis. Extra caution is needed through differential diagnosis between oral lipoma and malignant neoplasms.

References


The Rieger Syndrome: a Case Report with Unusual Dental Findings

Introduction

Rieger syndrome is characterized by hypodontia and primary mesodermal dysgenesis of the anterior chamber of the eye. The ocular component is usually bilateral and manifests partial or complete hypoplasia of the anterior stromal leaf of the iris, anterior iris synechiae and iridogoniodysgenesis. The main oral feature is oligodontia, particularly in the maxillary anterior segment, of the deciduous and/or the permanent dentitions, which varies from a single missing tooth to multiple missing teeth. Other dental defects reported are microdontia, barrel or conical crown form, taurodontism, shortened roots and eruption disturbances. Craniofacial abnormalities represent another constant feature of the syndrome. Deficient maxillary growth and oligodontia result in a mildly prognathic profile, a shortened philtrum, a relatively protruding upper lip and loss of vertical height. Subsequently, the middle part of the face is flattened and broad, flat nasal bridge may be observed. The cranial base also exhibits defects; an enlargement of the sella turcica has been reported in patients affected by the syndrome, although pituitary gland function may or may not be disturbed. Dental and craniofacial defects help to distinguish the Rieger syndrome from other anterior chamber malformations (Axenfeld’s syndrome, Peters’ anomaly) or other syndromes in which goniodysgenesis is a component (goniodysgenesis associated with juvenile glaucoma, anal atresia and goniodysgenesis, arachnodactyly and goniodysgenesis, deafness and goniodysgenesis, myopathy and goniodysgenesis, and short stature and goniodysgenesis). Apart from the aforementioned anomalies, a great variety of other developmental abnormalities have been observed. The only consistent among them is failure of the periumbilical skin to involute.

The purpose of this report is to present a case of the Rieger syndrome associated with bilateral cleft lip and palate and severe open bite, features not usually reported in association with this condition.

Case report

A 16-year-old boy was referred to the Department of Oral and Maxillofacial Surgery for closure of alveolar...
fistulae due to bilateral cleft lip and palate, evaluation and management of the concomitant dentoskeletal disharmony. He was the only child of unrelated parents with no significant family history. He was born after a 32-week gestation with normal vaginal delivery and his birth weight was 2150 gr. The mother reported having problems during pregnancy and received medication. However, it could not be determined which specific drugs she had taken. At birth the patient was found to have bilateral cleft lip and palate, cryptorchidism, obstruction of left lacrimal duct and congenital glaucoma at the left eye. He also suffered from jaundice of the newborn. Repair of the lip deformity was performed at the age of 6 months and closure of the cleft palate was done at the age of 3 years. At approximately the same period the patient was operated for dilation of the left lacrimal duct and at the age of 5 years for the cryptorchidism. At clinical examination the proband exhibited microcephaly, pronounced frontal suture and low set ears (Figure 1). The nose was pear-shaped, and hair curly and blonde. In addition, unilateral left microphthalmia with concomitant ptosis, as well as an iris defect were observed. Ophthalmologic examination disclosed, in the left eye, microcornea, dyscoria (Figure 2), central opacity and iridiogoniodygenesis. His visual acuity was 10/10 in the right eye with glasses -6.5-1.0/35° and 1/20 in the left eye with glasses -6.0-0.5/10°. In the abdomen failure of the periumbilical skin to involute was noted.

Oral investigation revealed bilateral oronasal fistulae, hypodontia (22, 32, 41, 42, 43 and 47 were missing), rotated and malpositioned teeth, eruption delay of 12 and pronounced microdontia and hypoplasia of the existing incisors. On the orthopantomogram, the roots appeared to be spiked (Figure 3). A severe anterior open bite was evident (Figure 4) (only the last molar teeth on each side were in occlusion), as well as a skeletal Class III relationship.

Based on orofacial characteristics, and particularly the dental and ocular findings, the diagnosis of the Rieger syndrome was set. After orthodontic preparation, repair of the alveolar clefts and closure of the orofacial fistulae was made.
Discussion

The Rieger syndrome is a rare, autosomal dominant, phenotypically heterogeneous disorder, characterized by structural defects of the anterior chamber of the eye (iridogoniodygenesis) coincident with missing or misshapen teeth\(^1\)-\(^3\). The essential ocular findings include hypoplasia of the iris stroma with adhesions to the posterior embryotoxon\(^6\),\(^12\),\(^20\),\(^21\). Iridocorneal adhesions obstructing the outflow of the aqueous humor cause increased intraocular pressure, resulting in glaucoma, in more than 50% of the patients. As this condition is resistant to therapy, it can lead to optic nerve damage and progressive visual loss\(^6\),\(^7\). Additional ocular manifestations may be microcornea, pupillary anomaly, cataract, glaucoma, corneal opacity, ectopia lentis, aniridia, optic atrophy and ptosis\(^12\),\(^20\).

As malformations of the anterior chamber of the eye have been reported in various conditions exhibiting overlapping features, it has been proposed that Rieger anomaly, Axenfeld anomaly and Rieger syndrome represent a single condition termed Axenfeld-Rieger syndrome\(^22\). On the other hand, other researchers have postulated that Rieger anomaly and Axenfeld anomaly are genetically different from the typical Rieger syndrome\(^12\),\(^23\).

Genetic linkage analysis, in spite of opposing evidence\(^23\),\(^24\)-\(^25\), had previously mapped the locus of the responsible gene to the 4q25-4q26 regions and suggested a tight connection to epidermal growth factor (EGF) supporting its role as a candidate gene\(^26\),\(^27\). In 1996, the EGF was excluded as a candidate gene\(^28\) and a gene causing Rieger syndrome was identified\(^29\). The researchers observed that in mouse embryos Rieg mRNA, the murine homologue of RIEG, was localized in the periciliary mesenchyme, maxillary and mandibular epithelia and umbilicus, all consistent with Rieger syndrome abnormalities\(^29\). The RIEG gene was also shown to be homologous to the mouse homeobox-containing gene Otx2 and expressed in the epithelium of the developing teeth. Another study showed that the expression of Otx2 in branchial arch epithelium starts very early already before tooth initiation is seen morphologically\(^30\). The RIEG/Otx2 gene appears to be involved in epithelial-mesenchymal interactions\(^31\). Later, Saadi and co-workers\(^32\) described the first dominant negative missense mutation, in the PITX2 bicoid-like homeobox gene, causing Rieger syndrome and suggested that such a finding supports a model that may partially explain the phenotypic variation within Rieger syndrome.

Other evidence has suggested genetic heterogeneity of Rieger syndrome. Legius and coworkers\(^23\) failed to find linkage to 4q25 in one pedigree. Deletion of 13q14 was described in 2 cases\(^33\),\(^34\). Phillips and coworkers identified a second locus on 13q14 by linkage analysis of a large four-generation pedigree\(^35\).

The aforementioned data is consistent with the current theory of a developmental arrest in the third trimester of tissues derived from neural crest epithelium\(^6\),\(^20\),\(^36\). Differentiation and migration of neural crest cells are responsible for the development of normal orofacial and ocular structures, hence a developmental disturbance of the neural crest may account for the phenotypic characteristics of the Rieger syndrome.

In the case presented, orofacial findings (hypodontia and microdontia), as well as ocular manifestations (congenital glaucoma, iridogoniodygenesis, dyscoria) and failure of involution of the periumbilical skin are part of the Rieger syndrome. In addition, the patient exhibits bilateral cleft lip and palate, severe anterior open bite, obstruction of the lacrimal duct and cryptorchidism, features not usually reported in association with this syndrome. From the literature, only one case of cleft palate is reported\(^12\) and another case of obstructed lacrimal duct\(^37\).

Conclusions

Consequently, the Rieger syndrome is a rare condition of particular dental interest as ocular anomalies are coincident with unusual dental and maxillofacial manifestations. Thus, the dentist may have significant contribution in diagnosing and referring such cases so as to minimize ocular complications. Moreover, such cases should always be accompanied by investigation of the relatives of the patient and referral to geneticists for proper and timely genetic counseling.

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