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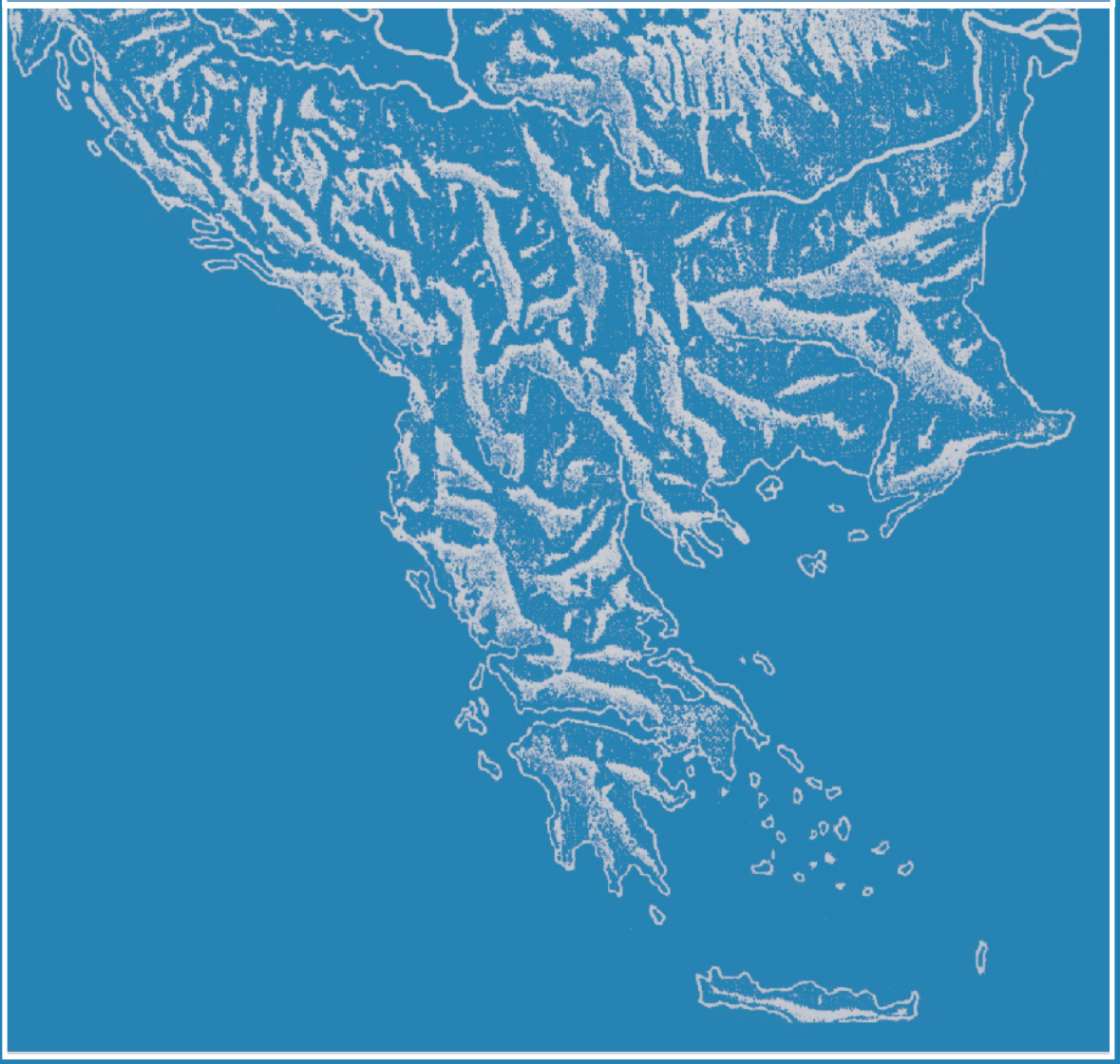

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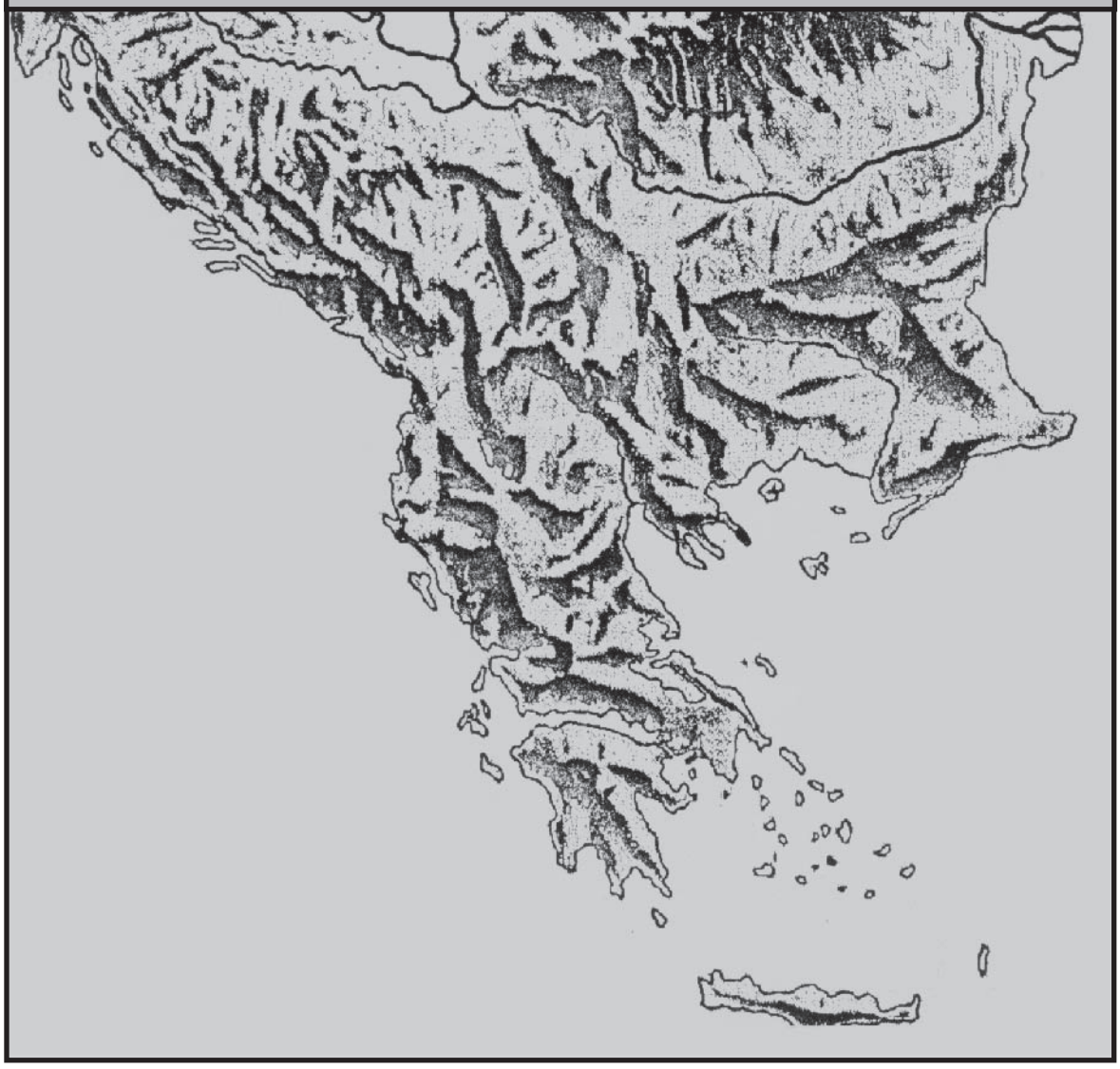

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Hereditary Defects of Tooth Dentin. Recent Progress on Genetic Aetiology Suggests for Modifications of the Existing Classification System

SUMMARY

Hereditary defects of dentin include dentinogenesis imperfecta (DGI) and dentin dysplasia (DD). They are characterized by abnormal dentin formation. Within the last 32 years, since the first classification system was proposed, significant advances have been made regarding their genetic aetiologies. In the classification system suggested by Shields et al (1973), dentinogenesis imperfecta type I (DGI-I) is associated with some types of osteogenesis imperfecta (OI), which is caused nearly in all cases by mutations of the genes encoding type I collagen (Col1A1 and Col1A2 genes). However, a more specific relationship between the type of OI, the genetic defect and the dental involvement can not been established. As far as isolated dentin defects are concerned, 10 mutations all occurring in different sites of the DSPP gene have been described to cause DGI-II, DGI-III and DD-II. No information about the gene defects in DD-I is currently available. Plenty of new evidence suggests that the existing classification system should be revised at least as far as the types of dentinogenesis imperfecta II and III are concerned.

Keywords: DSPP Gene; Mutations; Dentin Dysplasia; Dentinogenesis Imperfecta

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Introduction

2 main groups, Dentinogenesis imperfecta (DGI) and Dentin dysplasia (DD), have been identified under the term "dentin genetic diseases" by using clinical, radiographic and histopathologic features[1]. Both exhibit an autosomal dominant pattern of inheritance.

Dentinogenesis imperfecta (DGI) is the most prevalent human genetic disease affecting dentin formation with an estimated incidence between 1:6000 to 1:8000 in the United States[2]. Association of dentinogenesis imperfecta with autosomal recessive disorders is extremely rare[3-5]. DGI is further divided into 3 subgroups (types I-III). The dental defects associated with some forms of osteogenesis imperfecta (OI) has been defined as DGI type I (DGI-I). Osteogenesis imperfecta also known as "brittle bone disease" is an inherited clinical syndrome marked by skeletal fragility and other associated abnormalities, including dentinogenesis imperfecta[6]. According to the most frequently used classification, 4 main types of OI are recognized[6,7] (Tab. 1), the last type (IV) including all individuals who are not clearly part of the first 3 types. From this heterogeneous group, 3 separate clinical entities on the basis of distinct clinical and bone histological features have been identified more recently and termed types V, VI and VII[8-11]. The incidence of DGI is highest in OI types III and IV and less in type I[12], while there are no reports associating DGI with types V, VI, and VII[8].

DGI types II and III (DGI-II and DGI-III) occur as isolated traits. DGI-II, also called "hereditary opalescent dentin", presents almost complete penetration, a high expressivity and a low frequency of *de novo*

mutations[13,14]. DGI-III was first described in an isolated triracial population in southern Maryland known as the "Brandywine isolate"[2].

In all DGI types the teeth have a variable blue-grey to yellow brown discoloration that appears opalescent due to the defective, abnormally coloured dentin, shining through the translucent enamel. Enamel, although normal in structure, due to defective dentin frequently fractures from the teeth leading to rapid wear and attrition of the crowns. Radiographically, affected teeth show variable expression of bulbous crowns, cervical constrictions and short roots[1]. An important difference between the DGI types is that the teeth with DGI-III, in comparison with the other 2 types, radiographically show abnormally large pulp chambers and no pulpal obliteration. Dentine in all types of DGI appears histologically similar; having reduced numbers of tubules, irregular tubular morphology, immunoreactivity for type III collagen and poor mineralization[15,16].

Dentin dysplasia (DD) is a less frequent disease and it is subclassified into 2 types. In type I (DD-I) the teeth have a slight amber discoloration and are often mal-aligned. They appear radiographically short conical shaped roots with apical constrictions and pre-eruptive pulpal obliterations, which results in a crescent shaped pulp chamber. There are usually numerous periapical radiolucencies in DD-I that have essential diagnostic value for this disorder[1]. Type II (DD-II) presents normal-sized and shaped teeth that are again sometimes amber or translucent in colour. The primary teeth have pulpal obliterations, while the permanent teeth contain a hypertrophic dentin matrix giving the thistle tube appearance[1]. The description of DD-II as given by Shields et al[1] is consistent with most other reports, but that of DD-I is probably too limited[17] and the precise nature of the defect in DD-I has not been determined yet.

A number of systemic connective tissue disorders that can cause changes in dentin structure exist, such as OI, Ehlers-Danlos and Goldblatt syndrome[5]. One of the problems of the currently used classification system is that only OI is recognized. Another problem is that there are a number of reports on dentin dysplasias that do not fit into the previously created categories[18-21]. Moreover, great progress has been made over the last 6 years regarding the clarification of the genetic defects underlying dentin developmental malformations. This new knowledge provides new reasons to question whether the most frequently used classification of Shields et al[1] is the appropriate one for today. This paper offers a review of the molecular genetic basis of dentinogenesis imperfecta and dentin dysplasia, and discusses the implications that derive from it with respect to the classification system.

Genes and Related Proteins Candidates Dentin Diseases

Tooth development is a highly organized process involving complex interactions among a number of genes[22]. Disordered gene expression at the early stages of this process can arrest it. In contrast, genetic defects manifested at later stages (crown and root formation) and more specifically during matrix deposition of dentin are believed to result in malformations that occur exclusively within the tissue[23]. Consequently, candidate gene approaches to characterize the specific aetiologies of dentinogenesis imperfecta and dentin dysplasia focus on mutational analyses of the genes encoding dentin matrix proteins.

Dentin is a mineralized tissue whose composition and mode of formation are relatively similar to those of bone[24]. Bio-mineralization of dentin extra-cellular matrix requires complex interactions among several collagenous and non-collagenous molecules, secreted by the odontoblasts[25]. The bulk of the organic matrix of dentin (85-90%) consists of collagen. Most of the collagen is type I with minor amounts of type V and type I trimer[26]. Collagen fibrillogenesis is a precisely regulated process that ultimately defines the overall extra-cellular matrix (ECM) assembly and function, providing the structural scaffolding necessary for mineralization[27].

The non-collagenous molecules can be subdivided into several broad categories: phosphoproteins, non-phosphorylated matrix proteins, proteoglycans, growth factors, amelogenin 5-7 kDa, growth factors, metalloproteinases, serum-derived proteins and phospholipids[28]. Although the exact mechanisms in dentinogenesis are not yet elucidated, the experiments so far indicate that the non-collagenous proteins (NCPs) have a central role in orchestrating this process. The phosphoproteins are of particular interest, as they appear to promote actively the mineralization of collagen fibres and crystal growth[25,29] within pre-dentin when this tissue is converted to dentin. One category of the NCPs is termed the SIBLING (Small Integrin-Binding LIgand, N-linked Glycoprotein) family[30], which includes bone sialoprotein (BSP), osteopontin or secreted phosphoprotein-1 (OPN or SPP1), dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP), and consequently DPP and DSP, matrix extra-cellular phosphoglycoprotein (MEPE/OF45) and others. All members share common features at the protein and gene level[24,30-32] .

The genes coding for these proteins have been mapped to human chromosome 4q21 within a gene cluster[33-37], in the overlapping region of the DD-II, DGI-

II and DGI-III critical loci[38-41] (Fig. 1), and therefore they were considered candidate genes for dentin diseases. Up to date, strong evidence for a causative role exists only for the DSPP gene and studies have shown the association of mutations in this gene with DGI and DD-II. DMP1 is located between DSPP and BSP (IBSP) gene (Fig. 1) and was appointed as candidate gene for DD-II, DGI-II and DGI-III. However, mutational analyses up to date exclude this gene from a causative role in the pathogenesis of DGI-II and DGI-III, at least within the families studied[32-33]. BSP and OPN were also strong candidates for DGI-II since linkage analysis using 2 large families with DGI-II demonstrated no recombination events with this disease[33]. However, mutation search in both OPN and BSP in individuals with DGI-II yielded negative results[34,44]. MEPE/OF45 is located between BSP and OPN and thus was indicated as another possible candidate gene for dentin diseases[37]. To date, no mutational analysis of MEPE/OF45 has been reported to support this hypothesis.

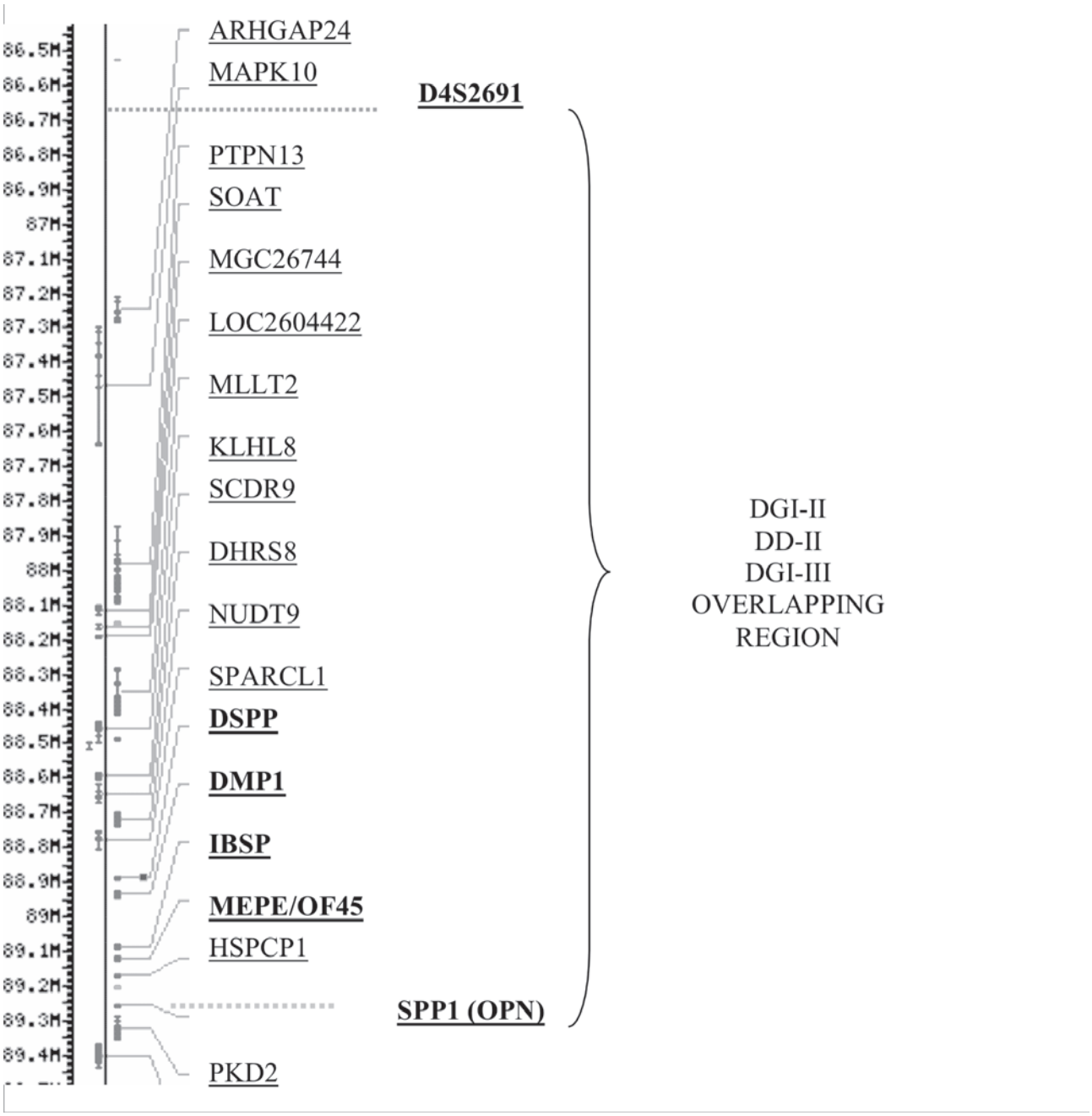


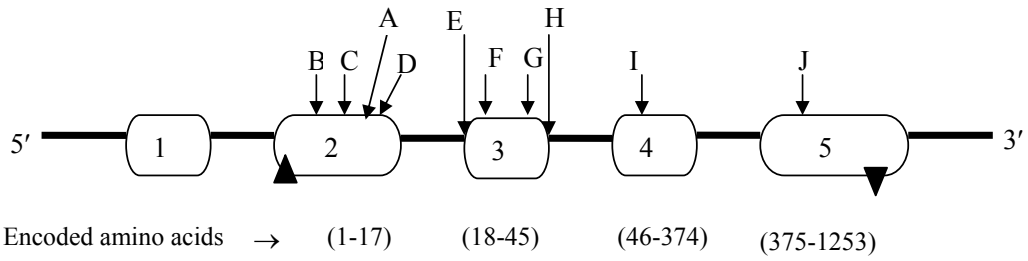
Figure 1. High resolution mapping of the overlapping region (between markers SSP1 and D4S2691) of the DD-II, DGI-II and DGI-III critical loci in human chromosome 4(4q21). Candidate genes for dentinogenesis imperfecta and dentin dysplasia are shown in bold

Dentin Diseases and Genetic Background

Mutations in the DSPP Gene; Defective Gene Expression and Its Association with Isolated Dentin Defects

The DSPP gene in all species studied to date[45-48], is organized into 5 exons and 4 introns. The human DSPP contains an open reading frame of 3759 bp encoding for a polypeptide with 1253 deduced amino acid residues. The 3′ portion of exon 2 together with exon 3, the 4 and 5′ portion of exon 5 encodes for DSP and DSP-DPP linker region (283-462 amino acids), while the remainder of exon 5 encodes for DPP[46-48] (Fig. 2).

Following the cloning and characterization of the human DSPP gene[46], mutational analyses, conducted in families with inherited dentin defects, have identified 10 different disease-causing mutations. All these mutations are inherited in a dominant way and can be divided into 4 categories: missense, nonsense, splice-site and compound mutations (Fig. 2). During all these studies, the researchers had to face a common problem related to the fact that the DSPP gene has a narrow pattern of expression, and thus there were no tissues that could be easily biopsied to gain DSPP mRNA from the affected individuals. Consequently, the sequence of the mutated DSPP transcripts could not be determined.



	Genomic sequence	*Protein mutated*	*Mutation type*	*Associated dentin defect*	*Mode of inheritance*	*Reference*
A	g. 49C>T	p.P17S	Missense	DGI-II	Autosomal Dominant (AD)	Zhang et al 2007
B	g.16T>G	p.Y6D	Missense	DD-II		Rajpar et al 2002
C	g.44C>T	p.A15V	Missense	DGI-II		Malmgren et al 2004
D	g. 49C>A	p.P17T	Missense	DGI-II		Xiao et al 2001,
E	g.1188C>G	p.V18-Q45del	Splice-site	DGI-II		Kim et al 2004b
F	g. 52G>T	p.V18F	Splice-site	DGI-II, DGI-III		Xiao et al 2001 Kim et al 2004a Song et al 2006
G	g. 133C>T	p.Q45X	Nonsense	DGI-II		Zhang et al 2001, Song et al 2006
H	g.1275G>A		Splice-site	DGI-II		Xiao et al 2001
I	g. 1474A>T	p.R68W	Missense	DGI-II		Malmgren et al 2004
J	g.3595 ins18bp 3479del36bp	Truncation	Compound	DGI-III		Dong et al 2005

Figure 2. Human DSPP gene its encoding protein and reported mutations. The intron-exon structure of the human DSPP gene (Gu et al, 2000). The numbered cylinders represent the exons, the line the introns. Translation of the signal sequence initiates in exon 2 (view shape ▲ in diagram) and translation stop codon is at exon 5 (view shape ▼ in diagram). Vertical arrows show the location of the mutations, while the branched boxes include the genomic DNA (upper row) and the mutated protein (lower row). The associated dentin defect with each mutation as well as the references where each mutation is described, are depicted below the gene representation.

*Abbreviations: **SP**: signal peptide, **DSP**: dentine sialoprotein, **DPP**: dentine phosphoprotein, **DSPP**: dentine sialophosphoprotein*

The first mutation in the DSPP gene was described by Zhang et al[49] in a Chinese family. It was a nonsense mutation at nucleotide 3658 (g.1272C→T), which introduced a premature stop codon in exon 3. This premature stop codon was predicted to result in the absence of DPP and a greatly shortened DSP - only 29 amino acids (Fig. 2). The same year, Xiao et al[50] reported 3 more disease-specific mutations. Their study included 3 Chinese families diagnosed with DGI-II. Furthermore, the affected individuals in 2 of them additionally presented progressive sensory-neural high-frequency hearing loss (Fig. 2). In the first family (the one without hearing loss) they detected a G→A transition at the donor-splicing site of intron 3 (g.1275G→A), a mutation predicted to result in the skipping of exon 3, which encodes part of DSP protein. In the second family, all the affected members carried a missense mutation at codon 17 (g.49C→A) predicted to result in a substitution of Pro by Thr, while in the third family all affected members carried a G→T transversion at codon 18 (g.1191G→T) predicted to result in a substitution of Val by Phe (splice site mutation). These 2 mutations were considered responsible for the DGI-II phenotype as well as for the progressive high-frequency hearing loss present in the affected family members. At the protein level, it was suggested that these 2 mutations might have interfered with the cleavage of the signal peptide. Given that these 2 mutations occurred in the DSP portion of DSPP, they concluded that they predominantly affected the function of the DSP protein. However, one cannot exclude that changes in the expression pattern or/ and the localization of DPP protein might have occurred as well.

Rajpar et al[51] reported a missense mutation at nucleotide 16 (g.16T→G) that resulted in the substitution of the amino acid Tyr by Asp within the hydrophobic core of the DSPP signal peptide domain (Fig. 2). They suggested that this mutation might have interfered with the translocation of DSPP to the endoplasmic reticulum during protein translation and impeded the secretion of the protein, so that roughly half of the normal amount of the protein was present in the dentin extra-cellular matrix. This mutation caused a DD-II phenotype where the permanent teeth were normal in colour, but radiographically displayed thistle-shaped pulp chambers containing pulp stones.

Malmgren et al[52] in their study investigated the genotype and phenotype of 2 unrelated Caucasian families diagnosed with DGI-II. These 2 families differed both clinically and radiographically, with the affected members in family B being more severely affected. Furthermore, the distinct phenotypes of the families were accompanied by different mutations in the DSPP gene. The affected members in family A carried a missense mutation in codon 68 (g.1474A→T) in exon 4 that resulted in the substitution of the amino acid Arg by Trp (Fig. 2). The affected members of family B carried a missense mutation in codon 15 (g.44C→T) in exon 2 that resulted in the substitution of the last residue of the signal peptide from Ala to Val (Fig. 2). The authors suggested that the mutation in exon 2 caused a great reduction in the amount of the secreted DSP and DPP proteins in the matrix, and the most severe phenotype present in the members of family B was attributed to this[52].

Kim et al[23] studying a Korean family that presented with clinical manifestations of DGI-II and progressive high-frequency hearing loss, identified a C→G transition (g.1188C→G) in intron 2 - splice-site mutation (Fig. 2); it was suggested that this mutation was responsible for both the DGI-II phenotype and the hearing loss. This mutation most probably affects mRNA splicing in such a way that some transcripts deriving from the mutant DSPP allele would retain intron 2 with its multiple upstream in-frame translation termination codons, while in other transcripts this would result in the skipping of exon 3. The latter would result in a DSP product shortened by 28 amino acids. This is the third mutation in the DSPP gene that has been associated with progressive high frequency hearing loss[23,50]. However, their exact correlation is not clear yet. DSPP expression is an important factor for proper inner ear formation but it appears that other factors contribute to the maintenance of full hearing as well[53-54]. A more detailed knowledge on DSPP's role in bone formation is needed in order to understand how ear deformities can result from mutations in the DSPP gene and how they may contribute to the pathology of progressive high frequency hearing loss.

Dong et al[43] investigated the possible roles of DMP1 and DSPP in the pathogenesis of DGI-III, in a family located in the Brandywine region of southern Maryland and previously diagnosed with DGI-III. Their study indicated that no mutations within DMP1 were associated with DGI-III. In contrast, they found that a rare compound mutation in the DPP portion of the DSPP gene was responsible for the observed phenotype in the affected family members. The first alteration was a 36 bp deletion (3599_3634del GT GAC AGC AGT GAC AGC AGC GAC AGC AGT GAC AGC A) while the second alteration was a 18 bp insertion (3715_3716ins GC GAT AGC AGT GAC AGC A) (Fig. 2). This compound mutation resulted in an in-frame truncation of the DPP domain by only 6 amino acids near the highly conserved carboxyl terminus. These mutations altered the length of the repetitive segments (DSS) within the DPP domain, affecting the overall function of the DPP protein. The authors suggested that the mechanism of this mutation may be similar to that observed in neurological diseases caused by trinucleotide repeats mutations[43,55].

Kim et al[56] in a study that included a Korean and a Caucasian family identified a G→T substitution at the first nucleotide of exon 3 of the DSPP gene. The same mutation was previously described by Xiao et al[50] in a Chinese family. The clinical and radiographic features of these 2 families included the classic phenotypes associated with

both DGI-II and DGI-III. At the protein level, the authors suggested that the mutation might impede the secretion of the protein or interfere with the cleavage of the signal peptide. Alternatively, this substitution might alter or destroy the function of the secreted protein by affecting the tertiary and or its quaternary structure.

Song et al[57] in the study which included members from 2 Chinese families diagnosed with DGI-II identified 2 previously described mutations[49,50] as causing the disease - a nonsense mutation (c.133 C→T) in family 1 and a missense mutation (c.52G→T) in family 2. The affected members of the families in this study showed a remarkably different phenotype[57], in comparison with the families reported in other studies having the same mutations[49,50,56]. It was therefore suggested that the c.133C→T and c.52G→T could be 2 mutation hotspots, causative for different clinical phenotypes in multiple unrelated DGI families. More recently, Zhang et al[58] studying a 4-generation Chinese family diagnosed with DGI-II identified a novel missense mutation in exon 2 (c.49C→T) of the DSPP gene, that resulted in the substitution of the Pro17 residue by Ser (Fig. 2). The mutation was identified in all the affected individuals, but not in normal family members and 100 controls.

The identification of several mutations in the DSPP gene in families diagnosed with DGI and DD-II does not necessarily pre-exclude that other genes are also involved in the aetiology of dentin diseases. Namely, in a recent study[59], it was shown that in 3 out of 4 families with DGI-II, not any mutation was identified in the DSPP gene. This suggests that at least in some cases of DGI-II the responsible defective gene is other than the DSPP, or these cases are caused by mutations located in untranslated regions or introns of the DSPP gene and that could affect DSPP gene function.

Spectrum of Dental Aberrations in Osteogenesis Imperfecta

Biochemical and molecular genetic studies have shown that the vast majority of individuals (>90%) affected with OI types I-IV, have mutations in either the Col1A1 or Col1A2 genes that encode the pro-α1 and pro-α2 chains of type I collagen (Tab. 1). It is believed that DGI-I, when present in patients with OI (Types I-IV), most likely reflects the fact that type I collagen is the main organic component in both dentin and bone. Numerous studies have shown that there is a clear relationship between the degree of dentin dysplasia and the type and form of OI[12,60]. Thus, teeth from patients with OI type III have a higher degree of dysplasia than teeth from patients with other types, and an increasing severity of the disease is associated with an increasing degree of dysplasia[12]. This indicates a strong correlation between the genotype in OI and the presence or absence of DGI. Recently, Pallos et al[61], in a Brazilian family with OI type IV, have identified a Gly559Cys mutation in exon 32 of the Col1A1 gene present in all DGI-affected members but not in the individuals without DGI-I. This finding supports the notion that DGI-I might be associated with specific mutations in the Col1A1 and Col1A2 genes.

In the case of OI, the resulting phenotype can vary from very mild to lethal depending on which of the 2 α chains is affected, the position in the triple helix at which the substitution arises and which is the substituted amino acid[62]. Although it is not known how odontoblasts process mutated gene products or how much mutated protein is secreted and whether this is incorporated into the organic matrix, it has been proposed that α-chain stoichiometry[63] does not affect the teeth and aberrations in the α2(I) chain are more important for the development of DI than those in the α1(I) chain, which may be substantiated by the more frequent involvement of the α2(I) than the α1(I) chain in DI[64].

Table 1. Osteogenesis imperfecta types, genetic aetiology and dental aberrations

Osteogenesis imperfecta type	Association with dentinogenesis imperfecta	Mutations in collagen genes (Col1A1 and Col1A2)*	References
I	+	+	Sillence (1988)
II	-	+	
III	+	+	
IV	+	+	
V	-	Unknown genetic defect. No evidence for collagen type I abnormality	Glorieux et al. (2000)
VI	-	Unknown genetic defect. No evidence for collagen type I abnormality	Glorieux et al. (2002)
VII	-	Unknown genetic defect. No evidence for collagen type I abnormality	Glorieux et al. (2002)

* **Col1A1**: Collagen I alpha1 gene **Col1A2**: Collagen I alpha2 gene

The genetic defect underlying OI types V-VII remains to be elucidated as it does not appear to be associated with collagen type I (Tab. 1). A recent publication describing a deletion in the gene sphingomyelin phosphodiesterase 3 (Smpd3) that resulted in osteogenesis and dentinogenesis in mice indicates that sphingomyelinases are deeply involved in bone and dentin mineralization and, on the other hand, provides evidence that different etiopathological mechanisms are involved in non-collagenous OI[65].

Discussion

Our knowledge on the aetiology of isolated dentin defects has been promoted after the description of mutations in the DSPP causing DD-II, DGI-II and DGI-III. So far, no disease-causing mutations outside of the DSPP have been identified.

Overall, the data from mutational studies strongly suggest that the classification system should be revised as phenotypic differences that have been taken into account for this purpose[1] do not appear to have a correlation with the differences in the genetic background. More specifically, the fact that firstly DD-II, DGI-II and DGI-III are caused by mutations in the same gene, secondly that DD-II overlaps the critical regions for DGI-II and DGI-III[64], thirdly they appear phenotypic similarities, all the above indicate that DD-II, DGI-II and DGI-III are allelic and represent a spectrum rather than distinct entities[52]. This is further supported by the recent study of Kim et al[56], in which it was shown that a single mutation (g.1191G→T) is underlying both DGI-II and DGI-III phenotypes. Moreover, Song et al[58] found that 2 mutations previously described and causative for a DGI-II phenotype in the families they studied, were causative of a phenotype close to that of DGI-III. It was therefore suggested, that these 2 types should be recognized as phenotypic variations of a single disease, with differences in expressivity and severity and the term "Hereditary opalescent dentin" should be used to describe both the DGI-II and DGI-III types.

With respect to the severity of the phenotype, there is evidence suggesting that an important determinant of it is the locus of mutation in the DSPP gene, since this affects the amount of functional DSP and DPP proteins present in the dentin matrix. Thus, mutations that cause major variations in the amount of functional DSP and DPP (e.g. the p.Y16D mutation) will result in more severe phenotypes than mutations that cause subtle changes in the amount of one or both of them. The study of Malmgren et al[52] is representative of this assumption. The more severe phenotype in the members of family B was attributed to the great reduction in the amount of DSP and DPP caused by the mutation in exon 2. The phenotype of this family was similar to the DD-II phenotype reported by Rajpar et al[51]. There is a possibility, that mutations interfering with function of the signal peptide or its cleavage result in a DD-II or similar phenotype, whereas mutations in the central region or the carboxyl terminus result in a DGI-II or a DGI-III phenotype. However, relatively few mutations have been published so far and the establishment of genotype-phenotype correlations remains a difficult task.

Conclusions and Future Directions

Mutations in the DSPP have been shown to cause DD-II and "hereditary opalescent dentin". However, it is possible that genes other than DSPP might be involved in the aetiology of some cases. DMP1 and MEPE/OF45 remain candidate genes for dentin diseases, but their involvement in the pathogenesis of these diseases has yet to be proven. The role of MEPE/OF45 in dentinogenesis and consequently in dentinogenesis imperfecta is far from clear. Towards this direction, the study of the tooth phenotype in MEPE null-mice will help establishing possible relationships (if any) between this protein and bio-mineralization of dentin.

The genetic defect behind DD-I is currently unknown, but it is believed to be different from that of DD-II. Future genetic research might also determine whether DD-I has various expressions or needs sub-classification. DGI-I, as originally described, is associated with some forms of OI (III, IV and I types). Currently, a more definite relation between mutations in the collagen genes and DGI-I cannot be established, something that would substantiate the higher incidence of DGI-I in certain OI types. As more mutations regarding OI and DGI-I are described, the correlation between clinical and molecular data may be better understood. A future goal could be the development of a classification system for dentin genetic diseases according to their genetic background.

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Influence of Skeletal Developmental Pattern on Evolution of Lower Third Molars. Statistic Comparative Study on Hypodivergent/ Hyperdivergent Cases

SUMMARY

The study of third molar evolution is one of the foremost topics in the orthodontic speciality literature, based principally on its ontogenetic and phylogenic characteristics and those of the dento-maxillary system. The rationale of this research was to evaluate lower third molars in relation not only to the dimensional characteristics, but also to the rotation pattern of the mandible.

2 samples of patients were selected, based on the mandibular development criteria: 25 patients with mandibular prognathia (Angle class III) and 30 patients with mandibular micrognathia (Angle class II). A comparative study of the available space for the third molar eruption was conducted between hypodivergent versus hyperdivergent sub-samples in each sample, and also between the groups of patients with the same rotational pattern in the 2 samples.

The space corresponding to the lower third molar did not display a statistically significant difference between the 2 samples as each sample contained subjects with /without adequate space available for lower third molars, despite the presence of mandibular macrognathia/micrognathia. In conclusion, the required space for third molar eruption depends not only on the dimension of the mandibular skeletal base, but also on other factors - dental and alveolar perimeter, the alveolar base/skeletal base relation, and the mandibular rotational pattern, among others.

Keywords: Lower Third Molars; Development; Eruption

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Introduction

As for any anatomical structure of the human body, growth and development of the third molar is determined not only by its intrinsic characteristics, but also by particularities of the area where it develops, the maxilla and mandible, neighbouring teeth and muscles having major influences[1,2]. The present research intended to study potential correlations between the skeletal growth characteristics of the maxilla and the mandible and the developmental dynamics of the third molar.

As regards skeletal development, previous literature describes the fact that macrognathia favours the eruption and alignment of the third molar, and micrognathia creates space deficits, including in the third molar area, generating crowding and third molar inclusion[3-7].

Accepting the premises that the type of facial skeletal development influences the evolution of the third molar, we explored its evolution within the main facial growth patterns. We aimed to realize a comparative study regarding the situation of the lower third molar among subjects with the same type of facial growth pattern and between subjects with opposed facial growth patterns.

Material and Method

Two samples of patients were selected, based on mandibular development criteria, to investigate the lower third molar:

- Group A - 25 patients with mandibular prognathia (Angle class III), aged 13-17 years (mean age 14.6 years), 18 with hyperdivergent and 7 with hypodivergent mandibular growth pattern;
- Group B - 30 patients with mandibular micrognathia (Angle class II), aged 13-20 years (mean age 14.8 years), 15 with hyperdivergent and 15 with hypodivergent mandibular growth pattern.

The samples were randomly chosen, using mandibular dimensions and type of malocclusion as criteria. Clinical observation and analysis of the orthopantomogram, lateral skull radiography and study models were done. The following parameters were investigated in order to diagnose the facial growth pattern and type of skeletal disharmony (Fig. 1):

- angular parameters - FMA, SNA, SNB, ANB;
- linear skeletal parameters - mandible base length (measured between points Xi-Pg), ramus height (CF-Go; CF=the point where the line perpendicular to the Frankfort horizontal is angent to the posterior contour of the pterigoid process), distances Go-Ar and Go-Me, mandible length (Co-Go).

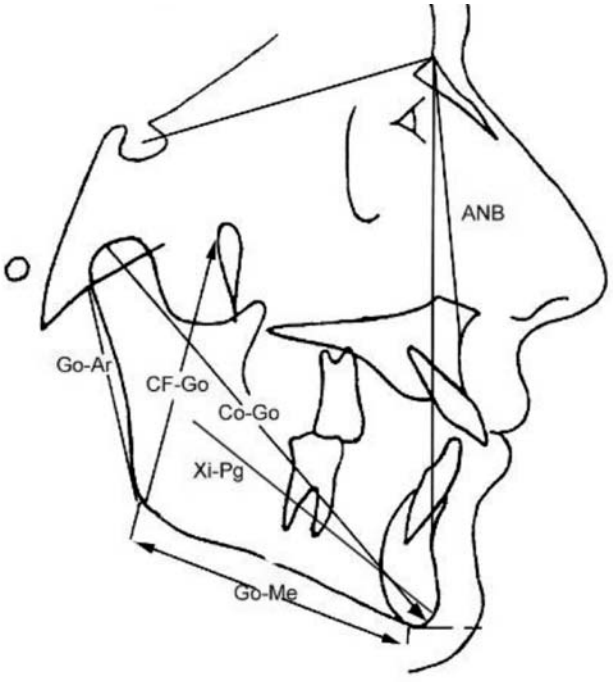

Figure 1. Parameters explored

The space corresponding to the lower third molar was measured on the lateral skull radiography through the distance to the distal aspect of the first molar and the anterior border of the ramus (Fig. 2).

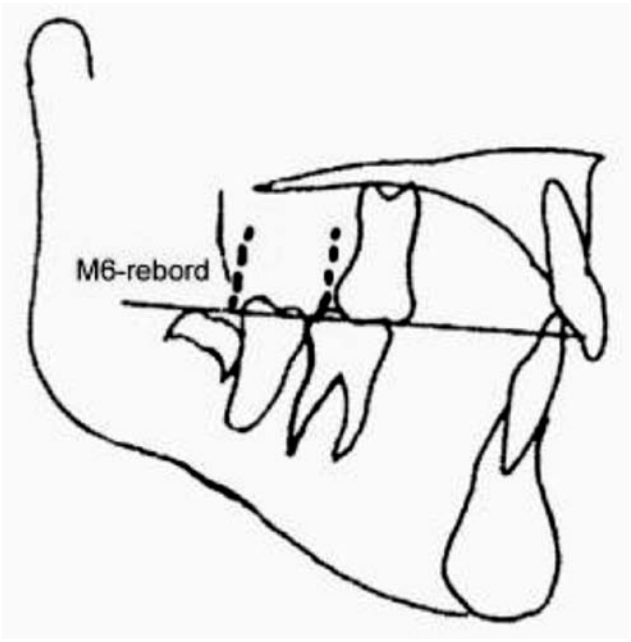

Figure 2. Measurement of the distances M1-ram, M2-ram on the lateral skull radiography, along the occlusal plane

Also, the space corresponding to the lower third molar was measured on the orthopantomogram, at the level of the occlusal plane (constructed between the tip of the highest cusp of the first premolar and the tip of the mesio-buccal cusp of the second molar). The following parameters were measured (Fig. 3):

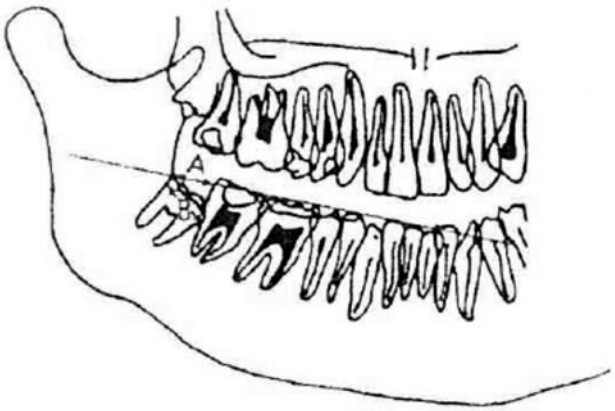

Figure 3. Measuring distances M1-ram, M2-ram on the orthopantomogram, along the occlusal plane (after Niedzielska8)

- distance from the distal side of the first molar to the anterior border of the mandibular ramus along the occlusal plane (M1-ramOPG);
- distance from the distal side of the second molar to the anterior border of the mandibular ramus along the occlusal plane (M2-ramOPG).

Both distances were measured on each side of the mandible.

To eliminate the error factor that could arise from the orthopantomogram, for each side we calculated an error coefficient (f_{M1}, f_{M2}) for the 2 distances measured: the ratio of the maximum diameter of the first/second molar measured on the model, and the maximum mesio-distal diameter of the first/second molar measured on the orthopantomogram:

f_{M1}= ø max M_1model/ø max M_{1OPG};

f_{M2}= ø max M_2model/ø max M_{2OPG}.

Each of the distances M_1-ram, M_2-ram, right/left, measured on the orthopantomogram, was multiplied with the corresponding error coefficient to obtain the actual distance values.

We also measured, on the study models, the real dental perimeter of the each upper and lower arch, using anthropometric callipers. The sum of the maximum mesio-distal diameters of all teeth present on the arch, except the wisdom teeth and any extractions or anodontia, represents the real dental perimeter for each arch: PD sup. - PD inf.

For each variable of interest, we statistically compared the 2 samples of patients, seeking factors which can explain the differences between them. The following statistical tests were used: ANOVA, Kruskal Wallis, and Mann Whitney.

Results

Results of the statistical analysis for the parameters investigated are presented in table 1.The significance of the parameter differences in groups A and B had a p value <0.05 for the following parameters:

- SNB
- ANB
- Xi –Pg: mandible base length
- Co-Go: mandible length

Table 1. Comparative statistical analysis for groups A and B through statistical tests ANOVA, Kruskal Wallis and Mann Whitney

Parameters	ANOVA Test		Kruskal Wallis Test		Mann Whitney Test	
	Significance	Test value	Significance	Significance	Test value	Significance
SNA	p=0.60	p > 0.05	p=0.84	p>0.05	p=0.87	p>0.05
SNB	p=0.01*	p < 0.05	p=0.01*	p<0.05	p=0.01*	p<0.05
ANB	p=0.0002*	p < 0.05	p=0.0003*	p<0.05	p=0.0003*	p<0.05
Cf-Go	p=0.40	p > 0.05	p=0.35	p>0.05	p=0.39	p>0.05
Xi-Pg	p=0.006*	p < 0.05	p=0.010*	p<0.05	p=0.011*	p<0.05
Go-Ar	p=0.081*	p < 0.05	p=0.71	p>0.05	p=0.74	p>0.05
Go-Me	p=0.18	p>0.05	p=0.16	p>0.05	p=0.18	p>0.05
Co-Gn	p=0.07	p>0.05	p=0.03*	p<0.05	p=0.03*	p<0.05
M1-ramus right	p=0.46	p>0.05	p=0.38	p>0.05	p=0.40	p>0.05
M1-ramus left	p=0.06	p>0.05	p=0.10	p>0.05	p=0.11	p>0.05
M2-ramus right	p= 0.19	p>0.05	p= 0.23	p>0.05	p=0.24	p>0.05
M2-ramus left	p= 0.01*	p<0.05	p= 0.02*	p<0.05	p=0.03*	p<0.05
PD upper	p= 0.6	p>0.05	p=0.78	p>0.05	p=0.81	p>0.05
PD lower	p=0.92	p>0.05	p=0.94	p>0.05	p=0.97	p>0.05

*- Statistically significant

The 2 groups statistically differed on their maxillary and mandibular development characteristics (the values of SNB and ANB angles that differentiate Angle class II from Angle class III malocclusions), and on the values of Xi-Pg and Co-Go parameters, which are different for micrognathia and macrognathia. On the other hand, the groups did not differ statistically on the FMA parameter, because we included both hyper- and hypo-divergent cases in both samples. With the exception of the M_2-ramus left, all the distances M_1-ramus, M_2-ramus, measured on the orthopantomogram, did not statistically differ since both groups included subjects whose third molars had enough space for eruption and alignment, and subjects whose third molars did not have adequate space.

Comparing patients whose third molar had sufficient space for eruption and alignment with patients whose teeth did not, ANOVA, Kruskal Wallis, and Mann Whitney were used to compare parameters within groups. The group A, consisting of patients with mandibular prognathia and skeletal class III malocclusion, showed significant difference for only one parameter: PD upper (Tab. 2, according to the Kruskal Wallis test), indicating that dental dimension of the complete arch is one of the essential factors in deciding the eruption space for the third molar. Within group B, composed of patients with mandibular micrognathia and skeletal class II malocclusion, similar finding has been noticed. Statistical significance was found for only 2 parameters: SNA and SNB, which can be linked to the hypo- or hyper-divergent pattern of growth, which modifies the position of point B (Tab. 3).

Clinical Cases

A few clinical cases illustrate the situation described.

The patient P.D., undergoing orthodontic treatment for mandibular prognathism, dental and skeletal Angle class III malocclusion, was examined at age of 14 years (treatment start) and 15 years and 6 months, during an intermediate treatment stage. Her situation was typical for the third molars display in class III: lower third molars had enough space for eruption and alignment and, would probably be conserved in occlusion with the upper second molars. However, extraction was advisable for the upper third molars, which did not have occlusal contact (Fig. 4).

Table 2. Statistical tests ANOVA, Kruskal Wallis and Mann Whitney for group A, comparing patients with/without space available for the lower third molar

Parameters	ANOVA Test		Kruskal Wallis Test		Mann Whitney Test	
	Test value	Significance	Test value	Significance	Test value	Significance
SNA	p=0.28	p > 0.05	p=0.48	p>0.05	p=0.28	p>0.05
SNB	p=0.29	p > 0.05	p=0.46	p>0.05	p=0.52	p>0.05
ANB	p=0.57	p >0.05	p=0.46	p>0.05	p=0.52	p>0.05
Cf-Go	p=0.69	p > 0.05	p=0.80	p>0.05	p=0.90	p>0.05
Xi-Pg	p=0.65	p > 0.05	p=0.92	p>0.05	p=1	p>0.05
Go-Ar	p=0.70	p >0.05	p=0.41	p>0.05	p=0.46	p>0.05
Go-Me	p=0.61	p>0.05	p=0.52	p>0.05	p=0.58	p>0.05
Co-Gn	p=0.26	p>0.05	p=0.27	p>0.05	p=0.31	p>0.05
M1-ramus right	p=0.65	p>0.05	p=0.85	p>0.05	p=0.92	p>0.05
M1-ramus left	p=0.53	p>0.05	p= 0.71	p>0.05	p=0.78	p>0.05
M2-ramus right	p= 0.65	p>0.05	p= 0.85	p>0.05	p=0.92	p>0.05
M2-ramus left	P=0.31	p>0.05	p= 0.23	p>0.05	p=0.27	p>0.05
PD upper	p= 0.07	p>0.05	p=0.52	p>0.05	p=0.06	p>0.05
PD lower	p=0.38	p>0.05	p=0.05	p=0.05	p=0.58	p>0.05

Table 3. Statistical tests ANOVA, Kruskal Wallis and Mann Whitney for group B, comparing patients with/without space available for the lower third molar

Parameters	ANOVA Test		Kruskal Wallis Test		Mann Whitney Test	
	Test value	Significance	Test value	Significance	Test value	Significance
SNA	p=0.04*	p<0.05	p=0.13	p>0.05	p=0.18	p>0.05
SNB	p=0.03*	p<0.05	p=0.05	p=0.05	p=0.05	p=0.05
ANB	p=0.86	p>0.05	p=0.72	p>0.05	p=0.85	p>0.05
Cf-Go	p=0.80	p>0.05	p=0.64	p>0.05	p=0.81	p>0.05
Xi-Pg	p=0.95	p>0.05	p=0.88	p>0.05	p=1	p>0.05
Go-Ar	p=0.33	p>0.05	p=0.23	p>0.05	p=0.29	p>0.05
Go-Me	p=0.87	p>0.05	p=1	p>0.05	p=0.88	p>0.05
Co-Gn	p=0.68	p>0.05	p=0.55	p>0.05	p=0.65	p>0.05
M1-ramus right	p=0.80	p>0.05	p=0.76	p>0.05	p=0.88	p>0.05
M1-ramus left	p=0.72	p>0.05	p= 0.76	p>0.05	p=0.88	p>0.05
M2-ramus right	p= 0.50	p>0.05	p= 0.65	p>0.05	p=0.76	p>0.05
M2-ramus left	P=0.43	p>0.05	p= 0.55	p>0.05	p=0.65	p>0.05
PD upper	p= 0.35	p>0.05	p=0.30	p>0.05	p=0.36	p>0.05
PD lower	p=0.26	p>0.05	p=0.23	p>0.05	p=0.29	p>0.05

*- Statistically significant

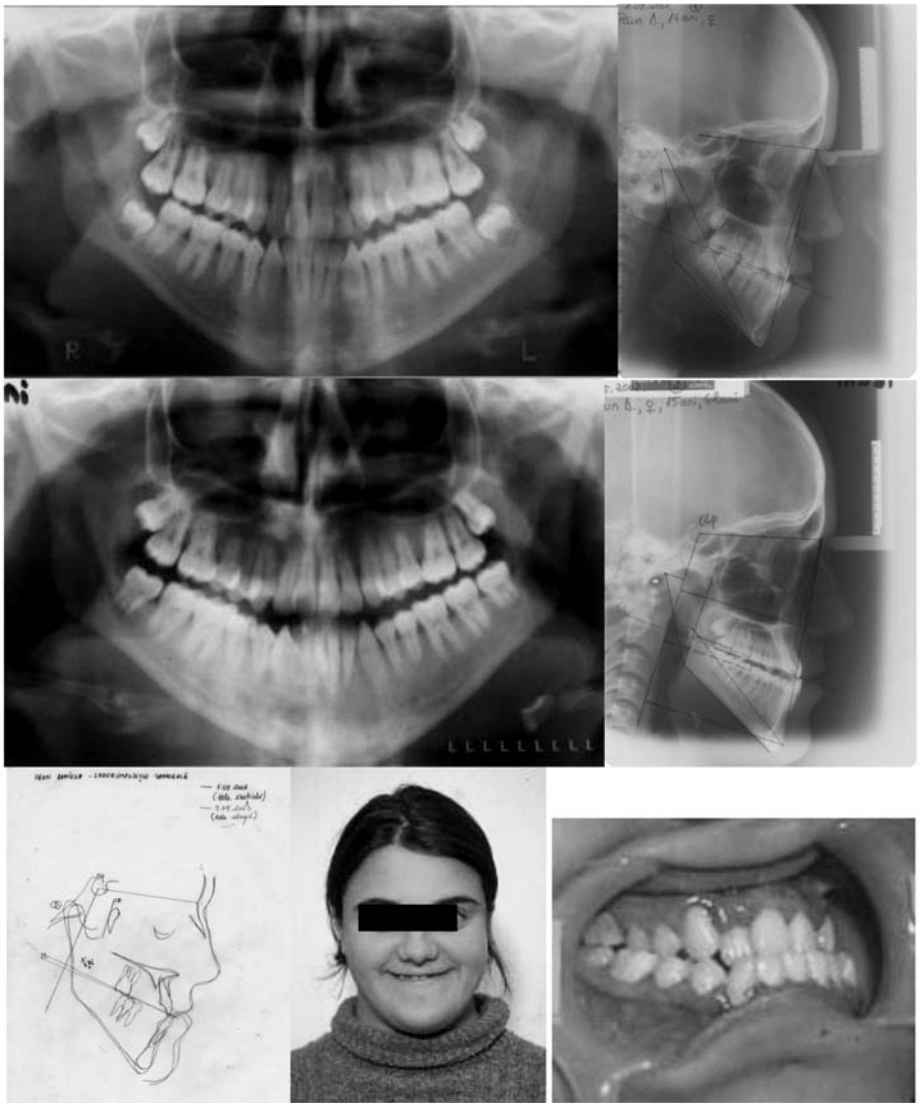

Figure 4. Patient P. D., female, orthopantomograms and lateral skull radiographs at the ages of 14 years, 15 years and 6 months; tracings superimposition, facial and dental aspects

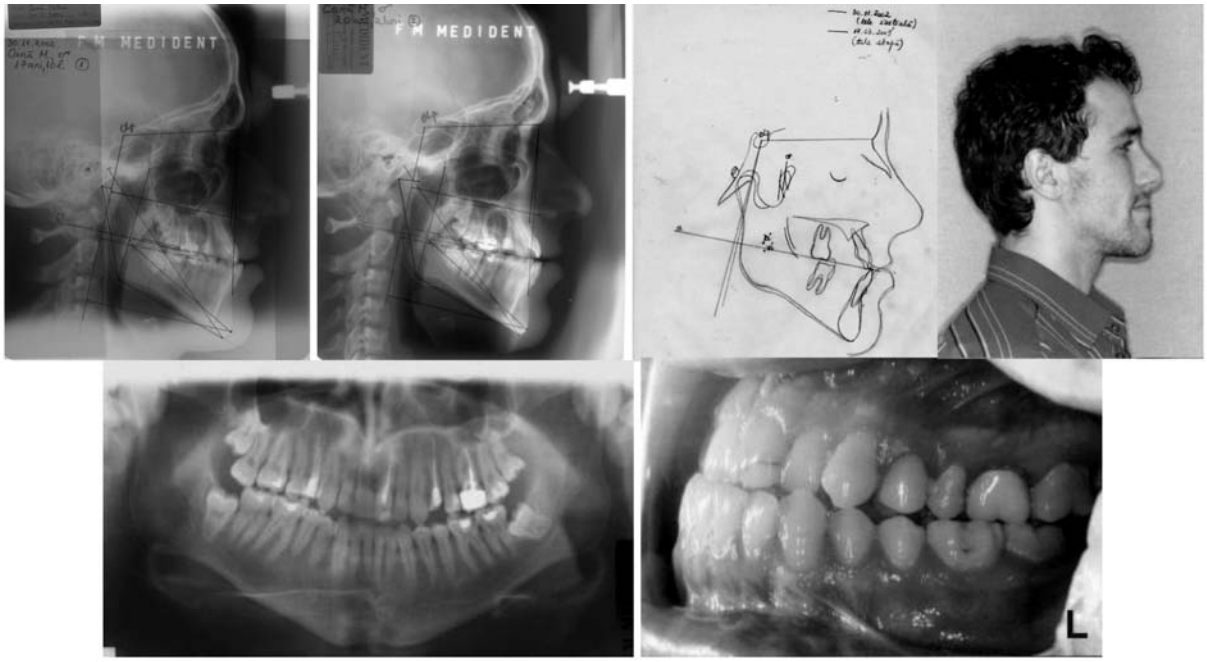

Figure 5. Patient C. M., male, lateral skull radiographs at the ages of 17 years and 10 months, 20 years and 2 months; tracings superimposition, profile aspect, orthopantomogram, intraoral aspect

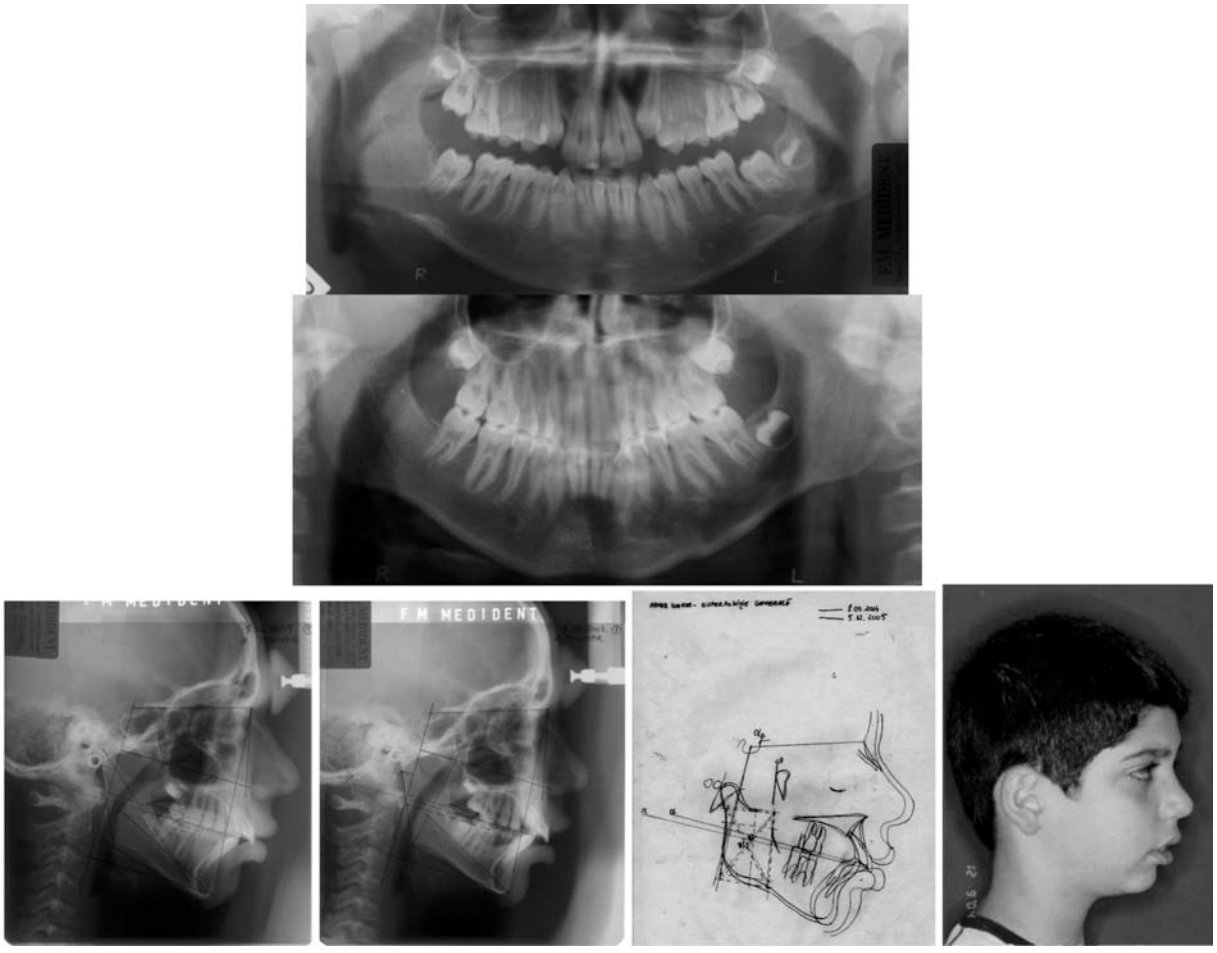

Figure 6. Patient A. T., male, orthopantomograms and lateral skull radiographs at the ages of 11 years and 12 years and 3 months; tracings superimposition, profile aspect

Another mandibular prognathism case, but superimposed on a hypodivergent growth pattern and tooth size close to macrodontia, presented an acute lack of space for the third molar eruption, even after an orthopaedic treatment stage. All 4 third molars had clear extraction indication, due to the space deficit (Fig. 5).

A typical Angle class II case, with mandibular micrognathia and a hyperdivergent growth pattern, offered excessive space to the third molars, a significant space being preserved even for the tooth 48, missed through anodontia (Fig. 6).

On the contrary, in the case of the another patient, also Angle class II with mandibular micrognathia, there was a total lack of space for third molars, visible at the age of 9 years and 11 months; another incriminating factor was generalised macrodontia (Fig. 7).

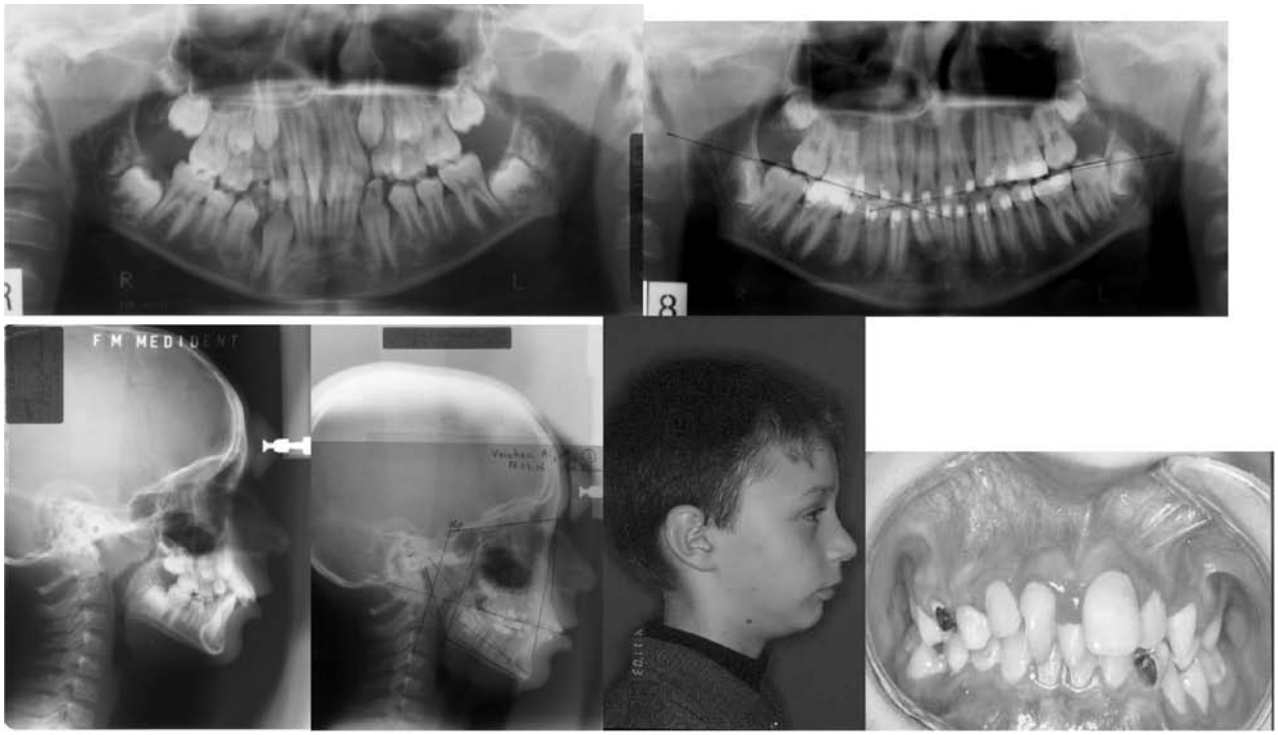

Figure 7. Patient V. A., male, orthopantomograms and lateral skull radiographs at the ages of 9 years and 11 months, 13 years and 8 months, initial profile and intraoral aspects

Discussion

The space necessary for the third molar's eruption and alignment depends on several interconnected factors:

- Mandible base dimension;
- Alveolar bone perimeter and its relation with the skeletal base dimension;
- In skeletal class III anomalies compensated at the dental level, the lower alveolar perimeter might be significantly reduced compared to the mandible skeletal base, due to anomaly compensation through lingual tipping, generating space deficit;
- Lower arch dental perimeter;
- Facial growth rotation pattern: hyper-divergent (high angle) or hypo-divergent.

Mandible macrognathia allows third molar alignment, but might not compensate the lack of space due to generalized macrodontia, with potential retro-molar crowding. Mandible micrognathia considerably reduces the eruption potential of the third molar through the lack of space it generates. The hyper-divergent growth pattern improves the lower third molar space in both micrognathia and macrognathia cases; the hypo-divergent growth pattern might be a reduction factor for the lower third molar space.

Generalized microdontia or macrodontia has a major influence on the evolution on the available space for the third molar. Generalized macrodontia can determine a lack of space even in cases with excessive mandible growth (mandible macrognathia). These remarks are in accordance with the studies of Hellman[3], Bjork et al[1,2], Richardson[5,6], Silling[9], Olive at al[10], who describe

the importance of the skeletal relations (prognathism/ retrognathism) in the aetiology of the lower third molar inclusion.

Clinical analysis and the additional examination show that the evolution of space necessary for the eruption of the third molar differs not only between the two samples (chosen based on their facial patterns and the types of the dento-maxillary anomalies) but also within each sample of patients.

The real question that arises is: which is more adequate orthodontic behaviour: (1) to extract the third molar in all conditions in which its eruption and development are, or could be, abnormal and unfavourable; (2) to create space by usual orthodontic treatment; or (3) to assist normal eruption and development of this molar? Probably the most efficient way to proceed is to be positioned in between the 2 extremes, keeping the third molar through an orthodontic procedure only when this is also imposed by other dental abnormalities. If the pathological condition involves only the third molar, it would be better to extract it.

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Humoral Immune Response with Oral Reticular Lichen Planus

SUMMARY

Still unknown, etiopathogenetic mechanisms of oral lichen planus need new evidence based on sensitive explorative steps, which be the evidence of immune response implication in the disease. For this reason we followed immunological status in 21 patients from different gender and age, with oral lichen planus, reticular form. We determined immunoglobulin A, G, and M, as well as component C3 and C4, and CIK in sera of all patients, in the phase of remission and exacerbation. All obtained results were compared with control group, and between themselves in 2 phases of the disease.

We found out low values in sera for immunoglobulin fractions in the phase of exacerbation, which we suppose to be a result of theirs incorporating in circular immune complexes (CIK). This is confirmed with increased CIK values with the examined group. Increased values of C3 with the examined group, and lowered values of C4 components play a role in activation of complement system's classical and alternative way. In this cascade reaction, one part of C3 incorporates in CIK and one part stays free in circulation, so it is increased in serum. C4 components assist in this reaction which late effect is activation of humoral immune response. In the remission phase, we found increased values of serum immunoglobulin fraction, CIK and C3, against low values of C4, confirming aberrant humoral immune response.

Keywords: Oral Lichen Planus; Reticular Form; Immunoglobulins; Humoral Immune Response

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Introduction

Owing to long poly-aspectual researches of oral lichen planus, contemporary medicine emphasises the fact that this type of disease is relatively common among people in population. Numerous figures and information, present in literature[4,6,10,17], are input to this information. Today, contemporary science and medicine disposes clinical, histological and histochemical research, using light and electronic microscopy, immune-fluorescent testing and many other procedures, but aetiology of oral lichen planus is still relatively unknown, and the theory of multi-causality comes from it. Mostly mentioned aetiological causers are: inheritance, endocrine disregulation, psychical factors, autoimmunisation, immunological mechanism etc.

While examining patients with clinically set and histologically verified diagnosis of lichen planus, Cerni et al[2] realized quantitative determination of immunoglobulin A, G and M (IgA, IgG and IgM) fractions present in complement C3 and C4, and circular immune complexes (CIK) in the phase of exacerbation of the disease. The study showed that all the examined parameters of the experimental group did not show digression from normal values at all. Ability of lymphocytes transformation in serum was also tested in the same group, but it was without statistic significance, so that the authors could not confirm the theory of IgA and IgM serum deficit in patients with lichen planus.

In the study of Sklavounou et al[12], levels of IgA, IgG and IgM were determined in serum, components C3 and C4 of the complement, and values of CIK in reticular and erosive-ulcerative form of lichen planus. Results showed significant reductions in serum levels of IgA and significant increase of levels of IgG. Values of IgM and

component C3 from the complement were in the normal framework. Similar results were achieved in the process of determination of levels of components C3 and C4 of the complement. The authors did not find significant difference between 2 clinical forms. It was assumed that the increased values of IgA, IgG and IgM in the reticular form result from the destroyed protein synthesis, and from unexplained mechanisms in metabolic processes, present in this type of the disease, while normal values of complement fractions, in both groups, suggest unimportance of the complement in the pathogenesis of the disease. The authors consider that humoral immune response does not predominate in patients with oral lichen planus. General conclusion from this study is that nor humoral nor cellular mechanism, as primary, can be included or excluded from pathogenesis of the oral lichen planus.

Conclusions of Jacyk and Greenwod[7] are different from findings of Sklavounou et al[12]. They confirmed the fact for normal values of IgA and IgG in all clinical forms of oral lichen planus, approaching closer to Stankler's hypothesis in 70's.

Whether humoral immunity is etiopatogenetically important for the disease, or is caused from certain pathological changes in the disease itself, is the question that Walsh et al[16] tried to answer. Monitoring patients with oral manifestation of lichen planus and examining serum values of IgA, IgG and IgM, and complement C3, they came to conclusion that IgG was significantly increased, in contrary to IgA, which was significantly decreased. Values of IgM and complement C3 were identical in the examined and in the control group. Results imply the conclusion that patients with lichen planus possibly have generalized immunological distemper in type of deteriorated humoral immunological response.

Taking into account controversial findings, we formed the aim of this study to monitor immunological status of patients with oral manifestation of lichen planus, reticular type.

Material and Method

21 patients from different sex and age, with diagnosis of oral lichen planus, were monitored at the Clinic for oral and periodontal diseases, with no difference on topographical distribution of changes. The examination did not involve patients with skin manifestation, and those that apart from skin manifestation, had oral manifestation. Diagnosis was set on the base of:

Completely taken history and

Objective clinical finding.

In all the patients, in both occasions (phases of exacerbation and remission), IgA, IgG and IgM, components C3 and C4, and CIK in serum were determined. For that purpose, blood was taken from vein, and analysis was carried out at the Institute of Transfusion.

Serum IgA, IgG and IgM were determined by micro-ELISA technique. Components C3 and C4 of the complement in serum were determined pursuant to the same method for immunoglobulins, with difference that specific antibodies for these components were set in partigen discs. CIK in serum were performed pursuant to method of poly-ethylene glycol (PEG).

The obtained results were compared with the control group and between themselves, in the both the examined stages. Control group was formed by 25 healthy people, clinically examined and confirmed that they did not have any kind of inter-current disease, nor lichen planus.

Results for all these examined parameters in the both stages of the disease, in the examined and the control group, were statically processed by Student's t-test for significance of differences.

Results

Results are presented in tables 1-4 and graphical drawings (Figs. 1 and 2).

Values of serum immunoglobulin levels of patients with reticular form of oral lichen planus, in the phase of exacerbation, are shown in table 1. Contrary to values at control group, values of all immunoglobulin fractions in the experimental group were highly significantly decreased ($p<0.001$).

Table 1. Serum levels (g/l) of IgA, IgG and IgM of the patients with reticular type of oral lichen planus in the exacerbation phase and the control group

	Control group n = 25			Experimental group -exacerbation n = 21		
	IgA	IgG	IgM	IgA	IgG	IgM
$\overline{X}$	2.70	12.75	1.55	2.10	9.49	0.85
SD	0.63	5.27	0. 43	0.38	0.79	0.08
Se	0.04	0.35	0.02	0.08	0.17	0.01
t				4.27	2.82	7.41
p				<0.001	<0.001	<0.001

Table 2. Serum levels (g/l) of IgA, IgG and IgM of the patients with reticular type of oral lichen planus in the remission phase and the control group

	Control group n = 25			Experimental group - remission n = 21		
	IgA	IgG	IgM	IgA	IgG	IgM
$\overline{X}$	2.70	12.75	1.55	4.20	15.55	2.50
SD	0.63	5.27	0. 43	0.13	5.05	0.68
Se	0.04	0.35	0.02	0.02	1.10	0.14
t				10.84	2.32	0.07
p				<0.001	<0.05	<0.001

Table 3. Serum levels of complement components C3, C4 and CIK of the patients with reticular type of oral lichen planus in the exacerbation phase and the control group

	Control group n = 25			Experimental group - exacerbation n = 21		
	C3	C4	CIK	C3	C4	CIK
$\overline{X}$	0.65	0.33	0.05	0.81	0.20	0. 10
SD	0.14	0.10	0.02	0.10	0.07	0.5
Se	0.02	0.02	0.001	0.02	0.01	0.01
t				2.25	4.90	3.62
P				<0.05	0.001	<0.001

Table 4. Serum levels of complement components C3, C4 and CIK of the patients with reticular type of oral lichen planus in remission phase and the control group

	Control group n = 25			Experimental group-remission n = 21		
	C3	C4	CIK	C3	C4	CIK
$\overline{X}$	0.65	0.33	0.05	0.70	0.30	0.09
SD	0.14	0.10	0.02	0.10	0.09	0.04
Se	0.02	0.02	0.001	0.02	0.01	0.008
t				1.33	1.03	10.54
P				0.2	0.2	<0.001

Table 2 is review of serum values of immunoglobulins in patients with the reticular form of lichen planus in the phase of remission compared to the same values of the control group. Levels of IgA and IgM in the experimental group were again highly increased compared to serum values of the control group ($p<0.001$). On the other hand, pertaining to the IgG values, there has been identified statistically less significance ($p<0.05$).

Figure 1 is a graphic display of all the examined immunoglobulin fractions of the experimental group, at both stages of the disease (the stages of exacerbation and remission), as well as of the control group.

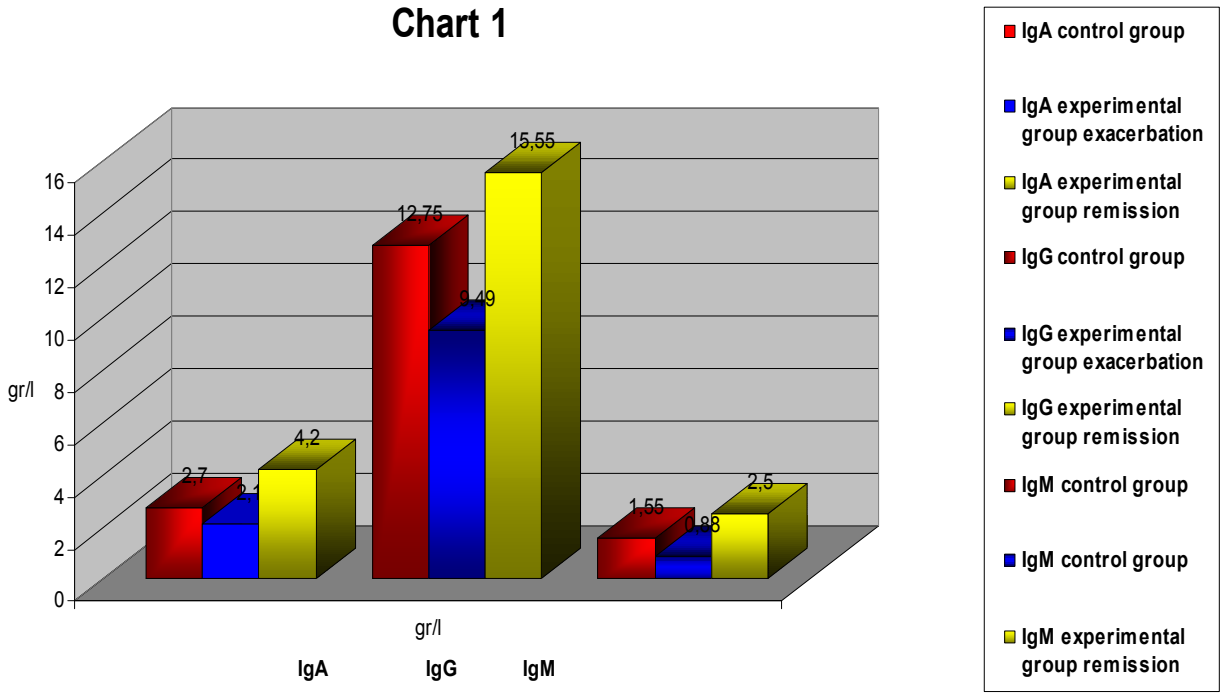

Figure 1. Serum levels of IgA, IgG and IgM in patients with reticular type of oral lichen planus at stages of remission and exacerbation

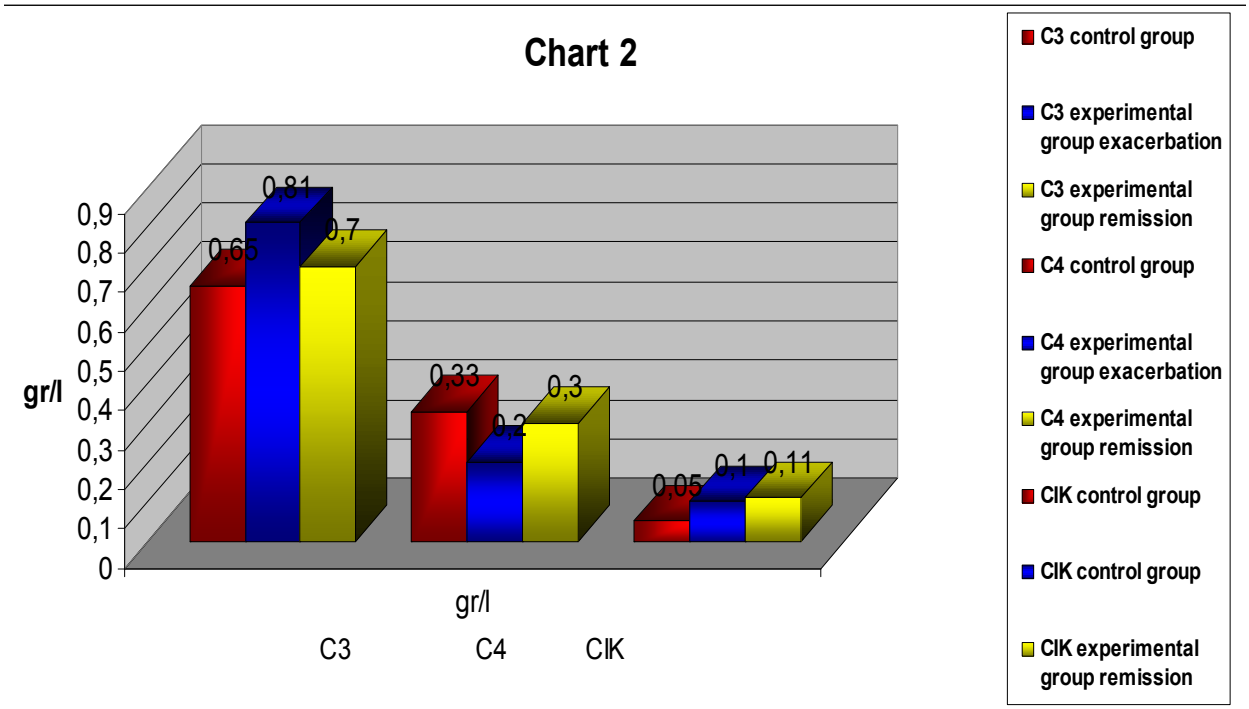

Figure 2. Serum levels of complement components C3, C4 and CIK in patients with reticular type of oral lichen planus at stages of remission and exacerbation

Table 3 demonstrates the serum levels of CIK concentration, and C3 and C4 components of the complement, in both the control and the experimental group at the stage of exacerbation. A highly significant difference in values has been identified for CIK and C4 serum components of the complement ($p<0.001$), while it was less different, although still statistically significant ($p<0.05$) for the C3 component of the complement.

The serum levels of CIK concentration, as well as the C3 and C4 components of the complement of the control and the experimental group at the stage of remission have been demonstrated in table 4. The table displays almost doubly increased CIK values of the experimental group in comparison with the control group ($p<0.001$), while values of the C3 and C4 components were not significant ($p<0.2$).

The CIK values, and C3 and C4 components of the experimental group in both stages of the disease and the control group have been displayed in figure 2.

Discussion

The ambiguous and complex mechanisms of the oral lichen planus currently impose the need for investigation of predominance of the immune components in etiopathogenesis of the disease. All former studies concerning participation of humoral immunoglobulin and serum complement resulted in discrepancies and contradiction. Therefore, there is no adequate information to prove whether oral lichen planus is caused by changes in the humoral or cellular immune system, or neither of these 2 systems is involved in clinical manifestation of the disease.

Sklavounou et al[12] have pointed out the significant reduction in the IgA serum levels, as well as the significant increase in the IgG level, whereas the IgM and the C3 component of the complement have been within the normal range. Based on a relatively small sample, Stankler[13] has concluded that the serum levels of both IgA and IgM were greatly reduced. Jacyk and Greenwood[7], attempting to verify Stankler's[13] results, have noticed a significant decrease in IgM levels only. On the other hand, Cerni et al[2], having examined 30 patients with oral lichen planus, informed us of normal values for A, G and M immunoglobulin, as well as C3 complement. In the preliminary announcement of Griffith et al[5], the fact for non-existence of any quantitative deflections of IgA and IgG serums has been emphasised.

Sun et al[15] have demonstrated a significant increase in the IgG level of the reticular and erosive forms of the disease; however, they did not believe that humoral immunodeficiency is the cause of the oral lichen planus. Another impressive element of that examination was the increased serum IgM level. Relating to this finding, they consider that this is a response of the body to a possible secondary oral infection during the presence of

mucosal erosion. Sklavounou et al[12] have an explanation of numerous contradictory elements in many studies, believing that this divergence has resulted from the different criteria of patient selection.

Our findings demonstrate the fact that the exacerbation stage of the disease is followed by a significant decrease in all immunoglobulin factions in patients with reticular type compared to the control group ($p<0.001$). These results are partially in accordance with the findings of Stanker[13], Sklavounou et al[12], Jacyk and Greenwood[7], but not with findings of Griffith et al[5] and Sun et al[15]. On the other hand, in regard with levels of the IgA and IgM at the stage of remission, we noticed a highly significant increase in comparison with the control group, apart from the IgG. Our findings regarding the CIK values are identical with the findings of Sallay and Dori[9], Banoczy[1], Chaterijee and Guha[3], but different from findings of Weksler[16].

We consider that low levels of serum IgA, IgG and IgM and, simultaneously, the increase of CIK component are due to their embedding into CIK. CIK, which is associated with IgG and IgM, activates the complement in a traditional manner, whereas CIK containing IgA is not identifiable from the complementary faction and activates the complement in an alternative manner. The large presence of CIK at this stage of the disease indicates active humoral immune response by activation of the complementary system, as a major mediator in the antigen-antibody reactions. Its primary function consists of instigation and enforcement of numerous humoral and cellular effector systems. According to Stites et al[14], the complement is one of the basic humoral effector mechanisms, which is particularly important and incredibly responsible for certain tissue disorders and destructive modifications. They assume that the deficiency and hyper-production of immune complexes can result in emergence and progression of the disease.

In the study of Sklavounou et al[12], the C3 component levels were not within the normal range. Griffith et al[5] also did not identify any quantitative abnormalities of the C3 complement component. The authors state that this component is deposited in the skin lesions of lichen planus. However, these findings do not suggest a pathological process with certainty, because this component, according to them, can also be biologically synthesized in the skin. Sun et al[15] noticed significant reduction of C4 component within the erosive and reticular form, whereas the C3 component was in within the normal values in both clinical forms.

The results we got concerning the C3 component of the complement showed slightly increased levels in the phase of exacerbation ($p<0.05$). In the phase of remission, the levels of the C3 component were decreased in comparison to the phase of exacerbation, but without significant difference, for all clinical forms ($p<0.2$).

The increased values of the component C3 of the complement in this phase of the disease are explained with the significant role of the complementary system in the oral lichen planus. Regarding that, we think that special accent should be put on the alternative way of activation, which starts with activation of component C3 of the complement. During the acute phase of the disease, i.e. in the phase of exacerbation, the increased production of the C3 component is most probably due to the tendency of the body to overcome the existing state of illness, which is mainly based on activation of the alternative way. Initial condition for its activation is the presence of C3b component, which is constantly produced in physiological circulation[14]. One part of the newly-made C3a components and C3b is built in the circulation, and another part stays free in it, with repercussion in the decrease of this component of the serum. The results in the phase of emission indicate the fact that IgA, IgG and IgM are also increased, in contrast to the lightly decreased values of the C3 component of the complement and CIK in comparison to the exacerbation phase.

Considering the fact that the oral lichen planus is a disease present until the end of one's life, with occasional worse situations or spontaneous or therapeutic improvements, in shorter or longer remissions, which last for different periods, the opinion of many oral pathologists about the need of specific antigen which would stimulate or help the immune response of the body is highly justified. The phase of remission is a reticular state of the patient, when subjective symptoms are absent and clinically we have objective improvement. The stabilization of the immunological status in this phase of the disease reflects the increase of all immunoglobulin fractions, found in our group of examinees.

It is most probable that the factor (antigen), which stimulates the immunological system, is present somewhere in the body, but provocation of its activation is necessary for the phase of exacerbation. When antigen penetrates again, the immunoglobulins present in the circulation merge with the antibodies and form CIK. With the implantation of the C3 component, the high values of CIK and decreased values of C3 component in the phase of remission could be explained, as opposed with the phase of exacerbation.

The results of the concentration of the C4 component of the complement, obtained from our study, showed the decreased levels in both phases of the disease (remission and exacerbation) in comparison to the control group. These results could be explained by changes of the humoral immunity, with the complementary system included together with the mediator enzyme function of the C4 component of the complement in forming CIK. We consider these finding are in accordance with functions of this component. This enzyme is mediator in reaction of the complement, so considering the high values of CIK in both phases of the disease, the decrease of its circulation level is logical and expected.

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In Vitro Evaluation of *Candida Albicans* Adherence to Silicone-Based Soft Lining Materials

SUMMARY

Colonization of soft lining materials with microorganisms, especially Candida albicans, is a common clinical problem. Silicone-based soft lining materials have been found to be particularly susceptible to Candidal adhesion. This study investigated the Candida albicans adhesion to 6 silicone-based soft lining materials (Molloplast-B, Mollosil, Ufigel P, Ufigel C, Soft Liner and Luci-sof). For each soft lining material, 7 specimens (10mm×10mm×3mm) were prepared. Sterile specimens were contaminated with Candida albicans. Adherent cells were fixed in methanol and stained with crystal violet and calculated by light microscopy. Scheffe F-test and ANOVA were used to analyze the data (p=0.001).

The results of this study showed that adherence of C. albicans occurred for all silicone-based soft lining materials. Significant differences were found in the Candidal adhesion among soft liners. Silicone-based soft lining materials tested have been found to exhibit particular Candidal adhesion.

Keywords: Soft Lining Materials; Candida Albicans

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Introduction

Denture stomatitis is an erythematous pathogenic condition of the denture-bearing mucosa that is mainly caused by microbial factors, especially *Candida albicans*[1-3]. *Candida albicans* is recovered more often and in high numbers from the fitting surface of the denture than the palate, indicating that the dentures act as a reservoir of infection and that yeast adhesion to the denture surface is a normal prerequisite for colonization of the palate[4-8]. Plaque formation is believed to be initiated by microbial adhesion to the surfaces of teeth or dental materials; subsequent microbial colonization may occur either by division of these adherent organisms or by cohesion of floating cells to adherent ones[9]. It has been reported that the higher the surface free energy of the substrata, the higher the amount of adhesion of microorganisms[10,11].

Permanent soft denture liners have been a valuable asset for dentists and, because of their visco-elastic properties, they act as shock absorbers and reduce and distribute the stresses on the denture-bearing tissues. Their use for patient comfort and the treatment of the atrophic ridge, bone undercuts, bruxism, xerostomia, and dentures opposing natural teeth has been known to be clinically beneficial[12-16]. However, these materials have some physical and microbial disadvantages. One of the most serious problems has been colonization and infection of the material surface by *C. albicans*, resulting in denture stomatitis[17-28.] Colonization may reduce the intraoral life of the soft lined denture, but little is known about the degree of adhesion in relation to soft lining materials.

Soft lining materials can be categorised into 5 main types: natural rubbers, vinyl copolymers, acrylic-based soft lining materials, silicone-based soft lining materials and fluoropolimers. Acrylic-based and silicone-based soft lining materials are more popular in clinical use. Both type are available in auto-polymerizing and heat-polymerizing forms, differing in the percentage of plasticizers, cross-linking agents, catalysts and fillers. In general, silicone-based soft lining materials are considered to be more successful clinically because of colour stability[28,29], compatibility with denture cleansers[18,30], low water absorption and hardness changes[15,27,28,31,32]. However, Candidal adhesion of silicone-based soft lining materials is contradictory. While some researches have shown that silicon-based soft lining materials did not support Candidal adhesion[20,25,26,33], others claimed that silicone-based soft lining materials were found to be more prone to microbial

adhesion because of their rough surface texture[18,34]. The aim of this study was to evaluate the *Candida albicans* adhesion to silicon-based soft lining materials.

Material and Methods

Preparation of Specimens

Table 1 lists the soft lining materials used in this study. The tested liners were fabricated according to manufacturers' recommendations. 7 specimens were prepared for each soft liner. Squares of soft liner materials with a 10 mm length, 10 mm width and 3 mm thick, were prepared in a stainless-steel mould and polymerized. They were saturated with a sterile water for 24 hours at room temperature[19,32,33].

Table 1. Type and manufacturer of silicone-based soft denture materials

Material	Type	Manufacturer
Ufigel C (UC)	Autopolymerized	VOCO, Cuxhaven, Germany
Mollosil (ML)	Autopolymerized	DETAX GmbH & Co. Ettingen, Germany
Softliner(SL)	Autopolymerized	PROMEDICA Neumünster/Germany
Ufigel P (UP)	Autopolymerized	VOCO, Cuxhaven, Germany
Molloplast B (MB)	Heat- polymerized	DETAX GmbH & Co. Ettingen, Germany
Luci-sof (LS)	Heat- polymerized	DENTSPLY International Inc. York, USA

Preparation of Candida

A reference *C. albicans* (ATCC 2091, Istanbul University, School of Medicine, Kükens) was used to investigate adhesion of the soft liners. *Candida* strains were incubated in Sabouraud's broth supplemented with sucrose 500 mmol/L overnight at 37°C. This medium was used because previous studies have shown increased Candidal adherence to acrylic resin after culture in Sabouraud's broth supplemented with sucrose[22]. Candidal growth was harvested after 48 hours by centrifugation (3000 g. 15 minutes, 10ºC). The Candidal cells were washed in phosphate-buffered saline solution (PBS), 0.15 mol/L, pH 7.2[26,27]. This procedure was repeated 2 times.

Adherence Assay

The principle of the experiment was to contaminate sterile specimens of the tested soft lining materials with *Candida albicans* and to determine the count of viable adherent cells[37].

At the commencement of the experiment, soft lining material specimens were autoclaved (15 minutes/121ºC/ PSI) to ensure that the specimens were sterile. Sterile specimens were deposited in 20 ml of yeast suspension in sterile universal bottles. The materials were incubated for 1 hour at room temperature[22,38,39]. After contamination, the suspensions were discarded and the specimens washed twice with PBS with gentle rocking to remove non-adherent cells. Excess PBS solution was drained from specimens. After materials were dried, adherent cells were fixed in methanol, stained with crystal violet and examined by light microscopy. Adherent cells in 30 fields of view (0.25 mm^2 per field) were enumerated and the results were expressed as yeast cells/mm^2 of the material[22,40]. Scheffe F-test and ANOVA were used to analyze the data (p=0.001).

Results

Figure 1 shows the mean adherence and standard deviation values of *Candida albicans* to the silicone-based soft lining materials. For all the tested materials, the adhesion was observed for *Candida albicans*. The adherence of *C. albicans* was the highest with Ufigel P lining material, and the lowest with Ufigel C lining material .

One-way ANOVA results are shown in table 2. Statistically significant differences were found among soft liners by means of Candidal adhesion (p=0.001). The results of comparisons between materials tested are shown in table 3.

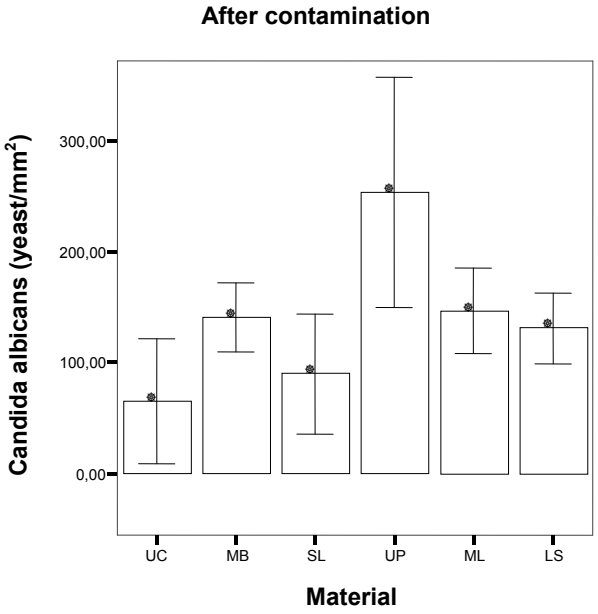

Figure 1. The mean adherence and standard deviation values of Candida albicans to the silicone-based soft lining materials

Table 2. One-way ANOVA results

Source	df	Sum of square	Mean square	F-test
Between groups	5	147274,690	29454,938	8.656
Within groups	36	122495,714	3402,659	
Error	41	269770,405		

p=0.001

Table 3. The results of comparisons between materials

Material(n= 7)	Mean (Standard deviation) (yeast/mm^2)
UC	65.14 (56.24)
MB	141.29 (31.49)
SL	89.86 (54.03)
UP	253.29 (103.95)
ML	146.29 (38.63)
LS	131.43 (32.33)

* Vertical rods shows statistical significance

Discussion

The adherence of a microorganism to a surface is classically considered to be a 2-stage process. The initial interactions between the 2 surfaces are non-specific and reversible, although the secondary phase is caused by specific intermolecular interactions. Many approaches have been used to explain the initial adherence of microorganisms to surfaces, including the thermodynamic approach to adhesion, which describes the adhesion of microorganisms to surfaces in terms of the surface free energies of the surfaces and the microorganisms. In addition, the hydrophobicity of the microorganisms has been theorised as a reason for high adherence and also for electrostatic interactions between surfaces. The second phase of the adhesion process involves specific adhesin-receptor interactions. The microorganism carries adhesins that bind stereochemically to complementary receptors on the surface. This stage is necessary for the tight binding of the microorganisms to the surface, which permits colonisation. In addition to tightly binding the microorganisms to the surface, the irreversible interactions are also responsible for the site-specific colonisation of the oral microorganisms, which provides a selective advantage for microorganisms that possess the relevant adhesins. Adhesins have been postulated to be associated with the microorganism's surface appendages that, by virtue of their small radius, are unable to overcome the energetic barrier of the primary force [22].

Other factors associated with the adherence of yeast to surfaces include surface roughness, presence of salivary proteins, presence of other adherent microorganisms, strain variability, concentration, viability of yeast cells, and culture conditions[22].

Because plaque formation is believed to be initiated by microbial adhesion to the surfaces of teeth or dental materials, subsequent microbial colonization may occur either by division of these adherent organisms or by cohesion of floating cells to adherent ones[9].

In this study, a simple *in vitro* model was used to compare the adherence of *C. albicans* with silicone-based soft lining materials. It was aimed to provide a reproducible assay for comparison of materials in which further variables could be examined in the future studies. For this reason, the material surfaces were reproduced in a highly polished stainless-steel mould in order to eliminate the variability of surface roughness. The concentration, viability, and culture conditions were kept constant. Adhesion was initially carried out on surfaces with no saliva coating to produce a reproducible assay before the introduction of variables. Crystal violet dye was used in this study, because crystal violet stains the adherent cells only. For all the tested materials, the adhesion was observed for *Candida albicans*. The highest *C. albicans* adherence was observed for Ufigel P, whereas the lowest for Ufigel C. Although both materials have similar chemical composition, results were very surprising. These differences may be their consistency and mixing procedure. In this study, surface roughness, and concentration, viability, and culture conditions of the assay were kept constant, except surface free energy and chemical properties of the materials tested. Conflicting reports have been published regarding the role of the materials' surface free energy on the degree of microorganism adhesion. It has been reported that the higher the surface free energy of the substrata, the higher the amount of adhesion of microorganisms[39,40]. This unclear situation highlights the importance of the surface properties of the lining materials and surface tensions of the suspending denture cleansing medium, both not measured. Therefore, there is a need for further investigations.

Conclusion

The ability of Candida albicans to adhere to 6 silicone-based soft liners was examined. The following conclusions may be made:

All the tested soft liners showed some degree of Candidal adherence;

The adherence of C. albicans was the highest with Ufigel P, and the lowest with Ufigel C;

There were statistically significant differences among soft liners for Candidal adhesion.

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In Vitro Evaluation of the Ability of Ray-Pex 5 to Determine the Working Length in Teeth with Simulated Apical Root Resorption

SUMMARY

The aim of this study was to evaluate in vitro the accuracy of Ray-Pex 5 to determine the working length in teeth with simulated apical root resorption. 40 recently extracted human single rooted teeth with mature apices were used for this study. To simulate apical resorption, we used a #3 carbide round bur (#330 S.S. White) to create an abnormal defect at the root apex of each tooth. 2 operators compared the electronic versus direct visual measurements, and the accuracy of the electronic apex locator was evaluated within ± 0.5, ± 1, and ± 1.5 mm, respectively. Ray Pex 5 was accurate 81.25%, 97.5%, and 100.0% by direct visual measurements within 0.5 mm, 1 mm, and 1.5 mm, respectively. There was no statistically significant difference between the 2 operators ($p<0.05$).

Keywords: Root Resorption, apical; Working Length; Ray-Pex 5

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Introduction

The exact determination of the tooth root canal working length is one of the most important steps in endodontic treatment, and makes the difference between success and failure. The working length corresponds to the distance between the coronal reference point and the apical constriction. Ideal root canal treatment should terminate at the apical constriction. Kuttler[1] showed that the average apical constriction is 0.524 - 0.659 mm coronal to the apical foramen; the determination of this area has traditionally been made by tactile-feedback and radiography.

Traumatic injury and chronic inflammation of the pulp or periodontal ligament, or both, results in apical root resorption, which will make the determination of the working length extremely difficult due to the fact that the apical constriction will be pathologically altered[2]. Root resorption is either a physiologic or pathologic process which results in the loss of cementum and dentin[3]. All pathological root resorption of dental origin are inflammatory in nature, and the most common stimulating factor is pulpal infection[4]. The surrounding periapical bone contains osteoclasts, and following injury to the cementum or dentin, infected dentinal tubules may stimulate the inflammatory process with osteoclastic activity, consequently initiating apical root resorption[5].

Most root canals associated with apical root resorption no longer have an apical constriction. Obtaining closure of the apex in these cases is synonymous with the apical closure in the blunderbuss canal. Besides the difficulty in instrumenting to close this type of root canals, there is also a great difficulty in determining the working length[6]. In these cases, combination of digital-tactile sense and radiography has important limitations.

Nowadays, many electronic apex locators (EAL) have been introduced to the market. Using a ground clip on the patient's lip and a file-probe inside the canal, the EAL determines the location of the actual anatomic apical foramen. These devices can be very technique-sensitive, leading to inconsistent results and frustration for the device user. However, when used on a consistent basis, they are a quick and accurate way to determine working lengths[7,8].

Several research papers have showed that EAL cannot determine accurately the working length in teeth with open apices, and only a few papers report the use of an EAL in teeth with apical resorption. Nguyen et al[9] used the Root ZX and were able to identify the location of the

apical constriction even when this anatomic landmark was eliminated.

Ray-Pex 5 (VDW GmbH, Germany) is a fourth generation device and records impedance measurement based on the advanced multi-frequency system and uses the latest digital technology. According to the manufacturers, the combination of using only 1 frequency at a time and basing measurements on the root mean square values of the signals increases the measurement accuracy and the reliability of the device. The accuracy of the measurements reportedly is not affected by vital pulp tissue, NaOCl, EDTA, saline solution, blood, or the various clinically encountered exudates.

The purpose of this study was to evaluate the accuracy of Ray-Pex5 in determining canal length in teeth with simulated apical root resorption.

Material and Methods

40 human single rooted teeth with mature apices, recently extracted due to periodontal disease or orthodontic reasons, retained in thymol solution 1%, were used for this study. The access cavity preparation was accomplished with a diamond round bur, the contents of the pulp chamber were removed, the root canal was irrigated with 5.25% NaOCl, and dried with cotton pellets. The incisal edges were prepared with a sand paper disk in order to keep a standard reference point for the measurements. The cleaning and shaping of root canals was carried out according to the step down technique (M.A.F = 25). Intermittent irrigation was performed with 5.25% NaOCl solution. To simulate apical resorption, we used a #3 carbide round bur (#330 S.S. White) to create an abnormal defect at the root apex of each tooth (Fig. 1).

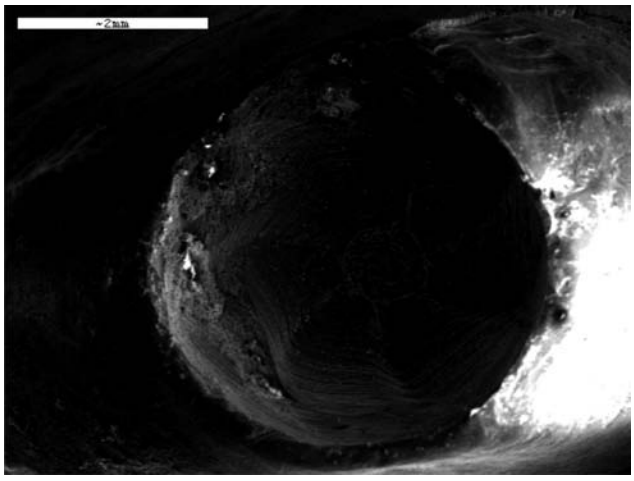

Figure 1. Simulated apical root resorption in tooth, with a #3 carbide-round bur (SEM, x 30)

In a wax sheet of 2 mm thickness, 40 holes were made and the specimens were inserted into them up to their neck, and stabilized with sticky wax. A gel of 0.9% NaCL solution and methyl-cellulose was prepared to simulate the periapical tissues, proposed by De Moor et al[10], and was placed in a plastic box of 20 x 6 x 3 cm. The wax sheet was placed at the top of the plastic box so that the roots of the teeth were embedded into the gel.

The electronic apex locator that was tested in the present study was Ray-Pex 5. Based on the canal size, a K-file was attached to the file holder and was inserted into the root canal until the signal on the display flashed "APEX." The file was then retracted until the EAL digital display showed 0.5 mm, which generally confirms that the instrument is at the apical constriction. The rubber stop on the inserted file was set to the prepared incisal edge, the standard reference point for the measurements. The files were then removed from the canals and their lengths were measured with a digital caliper (Mitutoyo Corp. Tokyo, Japan) to the nearest 0.5 mm. After the measurements with the apex locator, direct visual ones were performed. The distance from each incisal edge till the point where the tip of the file reached the outer surface of the root, was measured. This direct visual measurement was reduced by 0.5 mm and recorded. 2 operators compared the electronic *versus* the direct visual measurements, and the accuracy of the EAL was evaluated within ± 0.5, ± 1, and ± 1.5 mm, respectively.

Results

The results can be seen on table 1. According to the results obtained by the 2 operators, Ray-Pex 5 measurements were accurate 81.25%, 97.5%, and 100.0% by direct visual measurements within 0.5 mm, 1 mm, and 1.5 mm, respectively. There was no statistically significant difference between the two operators ($p<0.05$).

Table 1. Accuracy of Ray-Pex 5 obtained from 40 teeth

Distance to the apical foramen	Operator A		Operator B		Total	
	Number of teeth	%	Number of teeth	%	Number of teeth	%
± 0,5	33	82.5	32	80	65	81.25
± 1	39	97.5	39	97.5	78	97.5
± 1,5	40	100	40	100	80	100

Discussion

Although different papers point out that EAL don't give correct results in teeth with open apices, only a few

papers report the use of an EAL in teeth with apical root resorption[11,12]. When apical constriction is altered as a result of apical root resorption, it is very difficult to determine the working length by radiographic methods alone.

In this study we followed the methodology of Goldberg et al[2] who tested the measurement accuracy of Root ZX in teeth with simulated apical root resorption *in vitro*, and found that Root ZX was accurate 62.7%, 94% and 100% by direct visual measurements within ±0.5mm, ± 1mm, ±1.5 mm respectively. In this study, electronic readings obtained with Ray-Pex 5 showed an accuracy of 81.25%, 97.5%, and 100.0% by direct visual measurements within 0.5 mm, 1 mm, and 1.5 mm, respectively. Shabahang et al[13] suggested that an error tolerance of 1 mm is clinically acceptable. Our results are in agreement with those of Goldberg et al[2], who evaluated the accuracy of Root ZX in 50 extracted teeth with simulated apical root resorption.

Only in 1 case in our study the distance to the apical foramen exceed 1.5 mm, which is in disagreement with the results of Dunlap et al[14] who found 2 measurements in the necrotic group that were >1.5 mm beyond the apical constriction using the Root ZX, and both came from the same patient whose 2 teeth had periapical radiolucencies, and it is conceivable that these periapical lesions, with their lack of periodontal ligament and periapical bone, might have caused abnormally long readings. It is also possible that apical root resorption may have occurred, thus resulting in the destruction of the apical constriction

In teeth with incompletely formed roots, the diameter of the apex is wider[15]. Huang[16] observed that when the width of the apex is greater than 0.5 mm, electronic measurement have been found to deviate significantly from the actual length. In a clinical study, Suehde and Tulim[17] showed that the length was correctly determined in only 7 out of 11 cases of teeth with incompletely formed root. Also, Berman and Fleischman[7] reported 5 such cases where the length measured with an EAL was shorter than the actual length. However, investigating mature teeth with apical root resorption *in vivo* by EAL, the root canal typically has a decreasing taper toward the defect, making it possible to place a file into the canal and have its apical most terminal aspect contact dentin and achieve an accurate electronic measurement.

The results of this study showed that the Ray-Pex 5 might be useful in determining the working length in a variety of clinical conditions, including extensive apical root resorption.

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Microscopic Evaluation of Different Sealant Materials

SUMMARY

Objectives: *The aim of this study was to compare in vitro penetration and adaptation of light curing ormocer based fissure sealant and non-filled low viscosity sealant material.*

Materials and Methods: *Extracted human molars (n = 108) were randomly assigned to 2 groups. Admira Seal and Teethmate F-1 were used as sealant materials in the study. Each tooth was sectioned using an Isomet cutter into 2 portions bucco-lingually, producing mesial and distal tooth halve. The penetration, adaptation and voids of 2 materials were evaluated according to fissure shape and depth at the stereo-light microscope, and from each group 10 samples were examined under scanning electron microscope (SEM).*

Results: *Under light microscope and SEM, ormocer based fissure sealant (Admira Seal) and non-filled low viscosity sealant material (Teethmate F-1) did not show significantly different statistical results when penetration and adaptation were compared (p>0.05).*

Keywords: Sealant; Retention; Penetration; Adaptation

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Introduction

Dental caries is a disease that has undergone dramatic changes in its prevalence addition - it is becoming primarily a disease affecting the pits and fissures, especially in the permanent dentition[1]. The anatomy of the fissure can favour plaque stagnation, particularly during eruption of the tooth[2,3]. This happens because the tooth is below the occlusal plane and its surface tends to be missed by the toothbrush.

Pit and fissure sealants were specifically designed for purpose of preventing caries, and have been demonstrated to be effective[4]. An unfilled or lightly filled resin is used to penetrate the fissures and prevent plaque accumulation on the occlusal surface[5]. Active lesions covered by the resin do not progress further, and the possible development of new lesions at other sites in the fissure is prevented[6]. There is ample evidence that caries does not progress as long as the fissure remains sealed[7-9]. Even radiographically evident caries has been shown not to progress over a 10-year period[10] provided it is sealed off from the oral environment with a composite restoration. Thus, sealing appears very effective in conserving sound tooth structure[5,11].

Early sealants were based on methylmethacrylate or cyanoacrylate cements. Most contemporary compositions are unfilled (or only lightly filled) and based on difunctional monomers, such as those used for the matrix of composites. The principal monomer may be diluted with lower molecular weight species (e.g., triethylene glycoldimetacrylate, TEGDMA) to reduce the viscosity[1]. Ormocer based on sealant materials have been investigated. Ormocer has filler material consist of a special glass ceramic and highly disperse silica, incorporated into this cross-linked inorganic and organic matrix network.

Self-curing materials have to be applied when they are fluid enough to penetrate the pit or fissure and so that they begin to cure before running away from the site. This combination of characteristics sometimes causes problems in obtaining adequate penetration. If occlusal surfaces are appropriately oriented during the procedure to control flow, then light-curing materials are actually simpler to use. They can be applied and allowed to flow for a convenient time before exposure to a visible light source for curing.

Penetration is a function of both capillary action and viscosity. If the site is well cleaned, etched, rinsed, and dried, then acrylic monomers, such as BIS-GMA, tend to wet the surface reasonably well. Even if the opening in the pit or fissure is small, if there is good wetting, then capillary action will tend to draw the material into the orifice. The viscosity must be low enough to allow penetration of the material into the defect site. Complete penetration of sealant is not absolutely critical. It is possible to occlude only the neck region of a fissure and produce clinically acceptable results[1,12]. The penetration of a sealant depends on the configuration of the pit or fissure, the presence of deposits and debris within the pit or fissure, and the properties of the sealant itself. Anatomy of pits and fissures may be helpful in understanding the effects of sealants in the prevention of dental caries. The shape and depth of pits and fissures vary considerably, even within one tooth. Adhesion of sealants to etched enamel has been improved last decade[13-15]. The **aim** of this study was to compare the penetration and adaptation of Admira Seal (Ormocer based fissure sealant) and Teethmate F-1 (unfilled, low-viscosity sealant material) *in vitro*. Teethmate F-1 features a specific co-polymer-system, which does not change its main polymer structure, while releasing fluoride constantly. Besides it performs not only a micro-retentive but a chemical adhesion to the enamel structure by monomer MDP and thus enables strong adhesion and long durability of the fissure sealing.

Materials and Methods

2 different sealant materials, Admira Seal (Voco, Cuxhaven, Germany), a light curing ormocer based sealant, and Teethmate F-1 (Kuraray, Japan), non-filled low viscosity sealant were used. Non-carious 108 extracted human third molars were used, and surfaces were cleaned with polishing brush and water without any pumice, and rinsed well at least 20 seconds. Then teeth were mounted on a pink wax arc, and sealants were applied according to manufacturer's recommendations. 35% orthophosphoric acid gel was applied 15s (Vococid, Voco, Cuxhaven, Germany) for Admira Seal group (n= 54). For Teethmate F-1 group (n=54), 40% orthophospforic acid (K-etchant gel, Kuraray Dental, Okayama, Japan) was applied 15s. Sealant materials were applied with the tips onto the surfaces for 15s and polymerized by light cured (Chromalux 75, Mega-Physics Dental, Germany) for 40s. The restorations were checked for the marginal adaptation and surface texture with an explorer.

After the application of sealant materials, teeth were stored in 100% humidity during a week before microscopic evaluation. Each tooth was sectioned into 2 portions bucco-lingually, producing mesial and distal tooth halve, using an Isomet cutter (Isomet 1000 Precision Saw, Buehler, Lake Bluff, IL, USA). Tubulicid Plus (Dental Therapeutics AB, Nacka, Sweden) was applied 30s for each section in order to remove the smear layer. Samples evaluated in a stereo-light microscope (Leica 7.5 Mz, Microsystems Ltd. Business Unit SM, Heerbrugg, Switzerland) at x30 magnification for each group. 10 samples were selected randomly and evaluated under scanning electron microscope (JSM-5910LV, Tokyo, Japan).

The criteria for penetration:

1 = only top of the fissure sealed;
2 = 1/2 of the fissure sealed;
3 = 2/3 of the fissure sealed;
4 = the whole fissure sealed.

The criteria for adaptation:

1 = complete disconnection from enamel surface
2 = disconnection at some interfaces
3 = tight connection to enamel

The relation of penetration and adaptation with the depth and type (U, V, Y1, Y2) of fissures were compared under stereo-light microscope. The micro morphological types of the fissure system were classified as follows: 1) U-type; 2) V-type; 3) Y1-type; 4) Y2-type[16]. Fissure shapes were examined independently under light microscope by 2 experienced dentists (Kappa= 0.81). Voids that occurred in sealant were observed under stereo-light microscope as well.

The results were statistically analyzed with Mann-Whitney U test and the significant p value was 0.05.

Results

After section of the teeth, the results of distribution of Admira Seal and Teethmate F-1 according to fissure depth and type were imaged readily at stereo-light microscope (Tab. 1). As a first data there were no significant differences between 2 groups according to fissure depth and shape ($p > 0.005$).

There were 40 teeth (74.07%) in the Admira Seal group and 33 (61.1%) in the Teethmate F-1 group with penetration through the whole fissure (Tab. 2). In figure 1 (a and b), incomplete penetration of the whole fissure in Admira Seal group was shown. Debris, caries or air hole might be the reason of this incomplete penetration. 7 Y1 type, 6 U type, 23 V type and 2 Y2 type fissures were penetrated totally in the Admira Seal group. Five Y1 type, 19 U type and 7 V type fissures were penetrated totally in the Teethmate F-1 group (Tab. 3). In figure 2, incomplete penetration and poor adaptation of Teethmate F-1 was seen at light microscope (x30). According to penetration, there was no statistically significant differences between the groups ($p = 0.103$). Group Admira Seal had 38 (70.37%)

and group Teethmate F-1 had 40 (74.07%) teeth with high adaptation of sealant materials to enamel (Tab. 2).

In figure 3, high adaptation of Admira Seal to enamel walls was shown under SEM (x1000). 6 Y1 type, 5 U type, 24 V type and 3 Y2 type fissures were determined with the best adaptation to enamel in the Admira Seal group. 12 Y1 type, 18 U type, 9 V type and 1 Y2 type fissures were determined with the best adaptation to enamel in the Teethmate F-1 group (Tab. 4). Evaluating adaptation, no significant differences were found between the groups ($p>0.591$).

Voids were mostly observed at V type fissures in the Admira Seal group, while they were observed at U type fissures in the Teethmate F-1 group. Concerning depths of the fissures, the most common ones with voids were shallow in the Admira Seal, and medium in the Teethmate F-1 (Tab. 5). The voids seen in the Admira Seal are presented in figure 4.

Table 1. Relation of Admira Seal and Teethmate F-1 according to fissure depth and type

	Fissure Depth			Fissure type			
	Shallow	Medium	Deep	Y1	U	V	Y2
Admira Seal n=54	17 31.48 %	21 38.8 %	16 29.62 %	11 20.37 %	17 31.48 %	18 33.3 %	8 14.81 %
Teethmate F-1 n=54	14 25.92 %	27 50 %	13 24.07 %	16 29.62 %	22 40.74 %	10 18.51 %	6 11.11 %

Table 2. Relation between materials in terms of penetration and adaptation (%)

	PENETRATION				ADAPTATION		
	(1) Sealed only top of the fissure	(2) Sealed 1/2 of the fissure	(3) Sealed 2/3 of the fissure	(4) Sealed the whole fissure	(1) Complete disconnection to enamel	(2) Disconnection at some interfaces	(3) Tight connection to enamel
Admira Seal (n= 54)	0 0%	4 7.4%	10 18.5%	40 74.07%	2 3.7%	14 25.9%	38 70.3%
Teethmate F-1 (n= 54)	1 1.9%	9 16.7%	11 20.4%	33 61.1%	0 0%	14 25.9%	40 74.1%

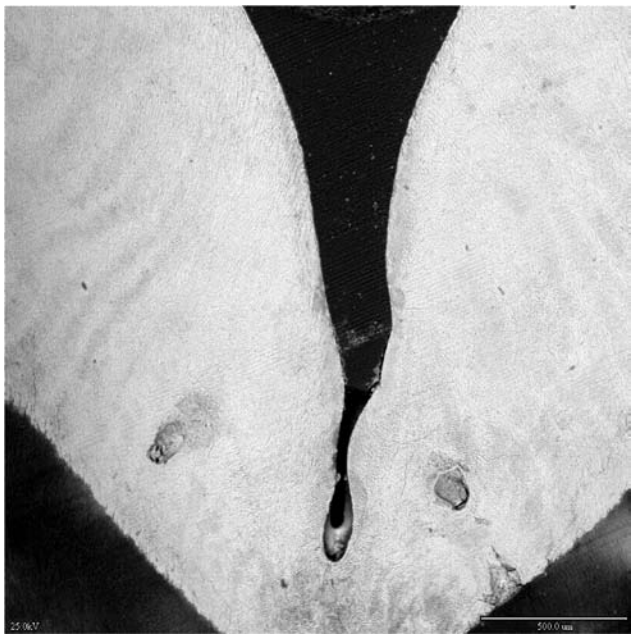

Figure 1a. Not totally penetrated Teethmate F-1 sealant material, seen under SEM (x50)

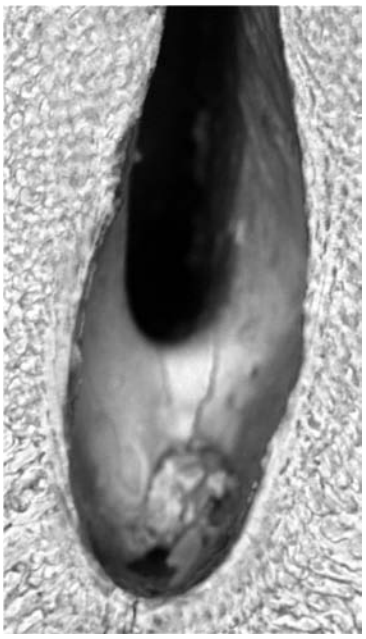

Figure 1b. The bottom of the fissure at figure 1a, seen under SEM (x350)

Table 3. Relation between penetration and fissure depth - fissure type (number)

		Fissure Depth			Fissure Type			
		Shallow	Medium	Deep	Y1	U	V	Y2
Sealed only top Of fissure	Admira Seal	0	0	0	0	0	0	0
	Teethmate F-1	0	0	1	0	0	0	1
Sealed ½ fissure	Admira Seal	0	0	4	2	0	0	2
	Teethmate F-1	2	2	5	6	0	0	3
Sealed 2/3 fissure	Admira Seal	0	2	8	3	2	0	5
	Teethmate F-1	0	6	5	5	3	0	3
Sealed whole fissure	Admira Seal	17	17	6	7	15	16	2
	Teethmate F-1	9	20	4	5	17	10	1

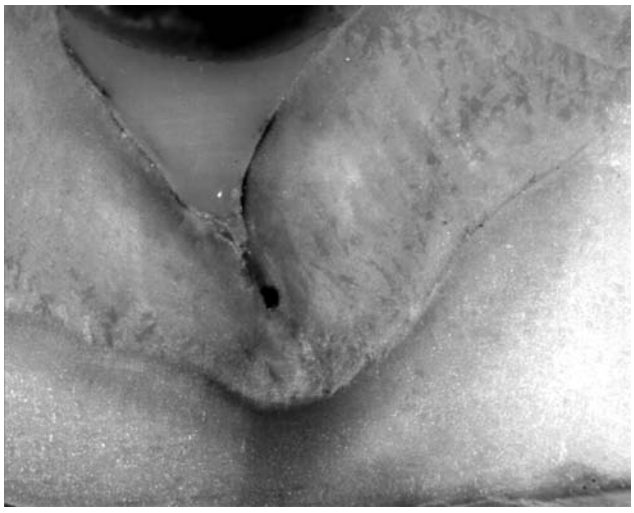

Figure 2. Not totally penetrated and poor adapted Teethmate F-1 sealant material, seen at light microscope (x30)

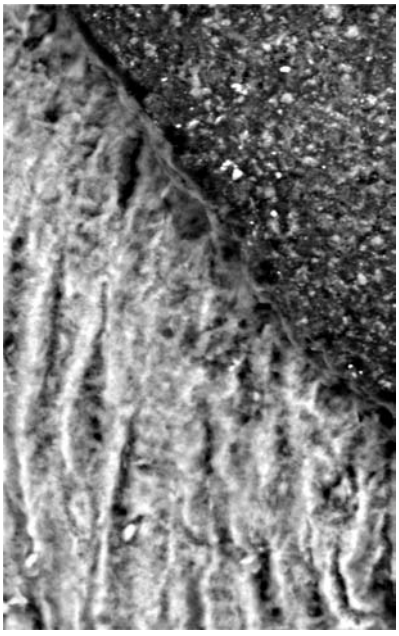

Figure 3. High adaptation of Admira Seal to enamel walls was shown under SEM (x1000)

Table 4. Relation between adaptation and fissure depth- fissure shape (number)

		Fissure Depth			Fissure Type			
		Shallow	Medium	Deep	Y1	U	V	Y2
Complete disconnection to enamel	Admira Seal	0	0	2	0	0	0	2
	Teethmate F-1	0	0	0	0	0	0	0
disconnection at some interfaces	Admira Seal	2	6	6	5	4	2	3
	Teethmate F-1	1	8	5	4	4	1	5
Tight connection to enamel	Admira Seal	17	15	6	6	14	15	3
	Teethmate F-1	16	19	5	12	18	9	1

Table 5. Accuracy of voids according the sealant materials (number)

		MATERIALS	WITOUT VOID	WITH VOID			
				DEEP 1/3	MEDIUM 1/3	SURFACE 1/3	TOTAL
FISSURE TYPE	Y1	Admira Seal	9	1	0	1	11
		Teethmate F-1	11	3	2	0	16
	U	Admira Seal	10	1	2	2	8
		Teethmate F-1	14	1	6	1	22
	V	Admira Seal	13	1	0	4	25
		Teethmate F-1	9	0	0	1	10
	Y2	Admira Seal	6	0	1	1	8
		Teethmate F-1	5	0	1	0	6
	TOTAL	Admira Seal	38	3	5	8	54
		Teethmate F-1	39	4	9	2	54
FISSURE DEPTH	SHALLOW	Admira Seal	8	0	0	9	17
		Teethmate F-1	7	0	0	7	14
	MEDIUM	Admira Seal	13	1	4	3	21
		Teethmate F-1	17	3	6	1	27
	DEEP	Admira Seal	14	1	1	0	16
		Teethmate F-1	11	1	1	0	13
	TOTAL	Admira Seal	35	2	5	12	54
		Teethmate F-1	35	4	7	8	54

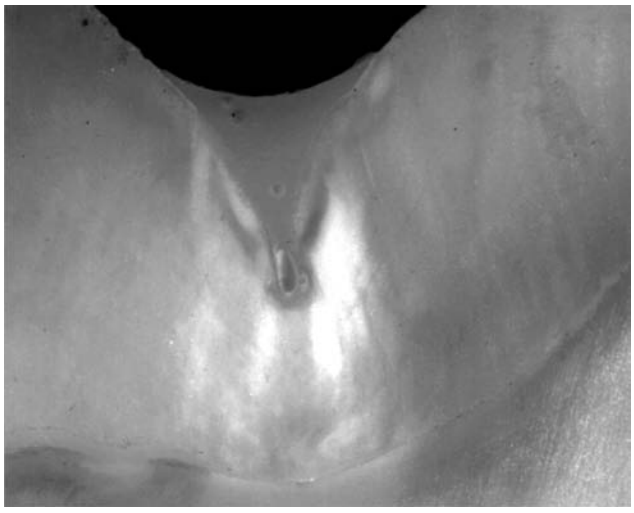

Figure 4. The voids were seen in Admira Seal

Discussion

In the present study, both sealant groups penetrated deeper into medium fissures than into shallow and deep fissures, but in the study of Percinoto et al[17], penetration was deeper into shallow than deep ones. The reason for this difference might be due to voids that occurred in some shallow fissures more than the other fissure depths.

Sealants adapted well to the vertical enamel walls at the orifice of deep fissures, but generally failed to penetrate into the deeper aspects[18]; also in the present study, it was found that deep fissures were not penetrated in deep aspects.

The significant impacts on penetration ability were the fissures type, material and mode of application[19]. In this study, Teethmate F-1 showed best total penetration at U type fissures, while Admira Seal showed the best total

penetration at V and U type fissures (Tab. 3). Concerning the material, 74.07% Admira Seal and 61.l% Teethmate F-1 sealant materials penetrated the whole fissure (Tab. 2). Although Admira Seal had 54% volume filler content and the Teethmate F-1 was unfilled, there were no significant differences when penetration and adaptation was compared. But Stavridakis et al[20] showed that the low viscosity and unfilled sealant material (Teethmate F-1) exhibited better marginal adaptation than its high viscosity counterpart.

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Influence of Age, Sex and Socioeconomic Factors on Dental Health*

SUMMARY

The purpose of this study was to investigate the relationship between the parameters of sex, age, family size, economic status, family and individual incomes, educational level and professional status, and the dental health. 424 patients were visually (mirror and explorer) and radiographically examined. DMFT, DMFS, FT, FS, MS, MT, DT and DS scores were used to collect data. Also, age, family size, economic status, family and individual incomes, educational level and professional status informations were recorded for these patients. Each of these parameters were compared to all DMF scores. One-way ANOVA test was used for the statistical analysis.

FT has been found significantly higher for women. It has been observed that, with regards to age, DMFT (7.27 in 13-20-year-olds and 18.72 in >51-year-olds) and DMFS (14.20 in 13-20-year-olds and 83.96 in >51-year-olds), FT, FS, MS and MT (major components) were increasing whereas DT and DS were decreasing. It has also been observed that as the size of the family increased, DT and DS increased, but FT decreased. As the income per person increased, DT and DS decreased, whereas FT increased. Moreover, as the level of education increased, MS and MT, DMFT and DMFS, decreased. Finally, in students compared to the employed and unemployed, the level of FT, FS, DMFT and DMFS were lower, whereas DT and DS were higher.

Age, family size, economic status, family and individual incomes, educational level and professional status may play significant roles in DMFT, DMFS, FT, FS, MS, MT, DT and DS indices.

Keywords: Caries; Age; Sex; Socioecenemic Factors

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Introduction

The development of dental caries is a complex interaction of etiologic factors, many modifying risks and protective factors[6]. Caries can be prevented not only by sub-doing the bacterial plaque, which associate with caries, through brushing and using antibacterial agents, but also by reducing the cariogenic challenge by changing the conditions that promote the establishment, multiplication and acid production of cariogenic bacteria, and by interfering with the dynamics of tooth demineralization[26]. The conditions that affect oral bacteria are related to social and economic factors because these factors directly and indirectly influence diet[26]. Indeed, caries can be considered as a "social disease"[15]. An understanding of socioeconomic factors that are associated with higher caries level during the economic development process may help in planning caries prevention programs in regions of the world experiencing rapid social and economic change[14]. There are few representative national surveys on caries prevalence in adults[6]. In the past, the DMF score was generally considered to lose its validity by adulthood, amongst other things due to the apparent fact that many missing teeth from then on were lost as a result of periodontal disease. Therefore, studies on the natural history of dental caries - i.e. the pattern of development of caries with age beyond adolescence - have been rather scarce, but recently several studies from different parts of the world show that caries susceptibility in adults is not restricted to particular age groups[27].

* This study was presented as a poster at the IADR/CED/NOF/ID in Istanbul, 25-28 August, 2004.

There has been a lack of interesting research on gender-based differences in health, and a lack of knowledge concerning women's health issues outside the field of obstetrics and gynaecology. For many years the primary acknowledged difference between men's and women's oral health was pregnancy gingivitis. As has been seen in medicine, dentistry must re-examine the attitude toward the misconception that women's oral health differs from men's only with respect to the reproductive process. There are, in fact, many areas in which women's oral health that differs from that of men, including oral hygiene behaviour, aesthetics, eating, temporomandibular disorders (TMD) and the hormonal influence on periodontal health[13].

The purpose of this study was to evaluate the relation between age, sex and socioeconomic condition on one side and dental status on the other.

Material and Method

This study was conducted between 2002-2004 on 424 patients referred to the Department of Conservative Dentistry, Faculty of Dentistry, Istanbul University. A questionnaire was used during the personal interview to collect the social and behavioural data. Questionnaires asked **about** patients' age, sex, family size (how many people live in the family), income per person, educational level and personal status. The questionnaires responses were recorded before examination of the patients. Then examination of the permanent dentition was conducted by 2 trained examiners. Post-stratification adjustments were computed on the basis of age (13-20, 21-40, 41-50, ≥51), sex (male/female), family size (1-2, 3-5, ≥6 person), annual income per person (<2000 $, 2000-4000 $, >4000 $), educational level (primary school, high school, university), and personal status (unemployed, employed, student).

Patients were examined both visually using mirror and explorer and radiographically. The clinical information was recorded on a dental chart that was specially designed to record restorations, carious lesions and missing teeth. At the start of the study examiners were given written and verbal instructions on how to complete the charting. On approximal surfaces, the examiner must be certain that the explorer has entered the lesion. Where any doubt existed, the surface was recorded as sound. DT, DS, FT, FS, MT, MS, DMFT and DMFS indices were used for evaluation to date which were obtained from patient's examination.

The correlation between obtained DT, DS, FT, FS, MT, MS, DMFT and DMFS values and patient's age, sex, income per person, educational level and personal status were investigated statistically using one way ANOVA test.

Results

The data evaluated according to sex showed a FT value of 4.19 for females and 3.09 for males. The difference between these 2 values was statistically significant ($p<0.05$).

According to age, DMFT, DMFS, FT, FS and MS values increased significantly ($p<0.05$). On the other hand, DT and DS values decreased significantly (Tab. 1).

According to the number of family members, it was found that while DT and DS were significantly excessive for family that had 6 or more than 6 people, FT was statistically lower (Tab. 2).

DT and DS were found to be significantly more excessive in the group with low personal income, while FT was found statistically lower ($p<0.05$). Also, FT was significantly higher in the group that annual person income was higher than that in the lower income group (Tab. 3).

Table 1. Comparison between age groups and DT, DS, FT, FS, MT, MS, DMFT and DMFS indices, set at p< 0.05

Age group	DT	DS	FT	FS	MT	MS	DMFT	DMFS
13-20 n=93	5.07	7.21	1.32	2.64	0.83	4.15	7.26	14.20
21-40 n=192	3.68	5.30	3.63	10.56	2.33	11.65	9.67	27.50
41-50 n=79	2.16	3.36	5.01	20.22	6.65	33.25	13.83	57.41
≥51 n=60	2.01	2.95	5.75	26.38	10.81	54.05	18.72	83.96

MS, MT, DMFT and DMFS values were significantly higher in the group with elementary school educational level compared to university group (Tab. 4).

A comparison between the groups including employed and unemployed people in the study showed that MT, MS, FS, FT, DMFT and DMFS values were lower and DT and DS values higher in the group including students (Tab. 5).

Table 3. Comparison between annual income per person and DT, DS, FT, FS, MT, MS, DMFT and DMFS indices, set at p< 0.05

Annual income for per person	DT	DS	FT
<2000 $ n=277	3.84	5.61	3.08
2000-4000 $ n=99	2.64	3.78	4.64
>4000 $ n=48	2.95	4.14	5.29

Table 2. Comparison between family size and DT, DS, FT, FS, MT, MS, DMFT and DMFS indices, set at p< 0.05

The number of family members	DT	DS	FT
1-2 Person n=75	3.05	4.441	4.80
3-5 Person n=293	3.37	4.86	3.56
≥6 Person n=56	4.48	6.66	2.85

Table 4. Comparison between educational level and DT, DS, FT, FS, MT, MS, DMFT and DMFS indices, set at p< 0.05

Educational level	MT	MS	DMFT	DMFS
Primary School n=154	6.00	30.00	13.35	50.22
High School n=124	2.68	13.40	9.47	29.12
University n=146	3.04	15.20	10.38	33.29

Table 5. Comparison between professional status and DT, DS, FT, FS, MT, MS, DMFT and DMFS indices, set at p< 0.05

Professional status	DT	DS	FT	FS	MT	MS	DMFT	DMFS
Unemployed n=142	3.59	5.07	4.18	15.13	5.13	25.65	12.87	45.45
Employed n= 182	2.87	4.20	4.30	16.12	4.87	24.35	12.10	45.41
Student n=100	4.35	6.41	1.84	3.75	0.85	4.25	7.12	14.72

Discussion

In this study, the relationship between dental health and age, sex, family size, economical factors (household income, income per person), educational level and personal status was determined. Investigations throughout history revealed the relationship between social characteristic and dental diseases and especially that change of social status effected obviously dental diseases. Urban life, wars and industrialization are some of the reasons that effect caries prevalence[6]. There is a tight relationship between social factors and personal behavioural factors especially related to the health. Social factors influence behaviour directly related to dental caries, such as oral hygiene, dietary habits, and dental care habits[6]. To understand the disease process and how caries presents itself in different groups in society, one needs to know about the disease in various "populations" or communities, as opposed to just at the individual patient level, which normally concerns the clinician providing dental care[24]. With community intervention studies, intervention are tested with natural communities, usually aimed at assessing the effect of modification of socio-behavioural patterns, environmental factors, primary health care intervention etc. Although the advantage of such studies is that the intervention take place under conditions that more closely approximate real life, this is achieved by having less control over the influence of potential risk factor[27].

In this study, present caries, restorations, and missed teeth in 424 patients were examined manually by using mirror and explorer and also radiographically and the

results were recorded. Caries was recorded as present when a lesion in a pit or fissure or on a smooth tooth surface had a detectably softened floor, undermined enamel or a softened wall. A tooth with a provisional restoration was also included in this category. On approximal surfaces, the examiner had to be certain that the explorer has entered lesion. Where any doubt existed, the surface was recorded as sound. True number of caries teeth and surface is considerably underestimated in epidemiologic studies conducted according to WHO criteria[6]. Pitts[23] has pointed out that noncavitated enamel lesions (D1 and D2) are about 3 times more common than our lesion in dentin (D3 and D4), particularly those with cavitation into the dentin.

In contrast to national epidemiologic surveys according to WHO criteria, surveys in Sweden routinely record approximal caries on the basis of bite wing radiographs, and enamel lesions (D1, D2) as well as noncavitated and cavitated lesions in dentin are detected. Compared to other national surveys, epidemiologic data from Sweden, which include noncavitated approximal lesions in dentin, are therefore overestimated[6].

In the present study, DMF index was used to evaluate the obtained results. Decayed, missing or filled (DMF) scores are used to collect epidemiologic data about the prevalence of coronal caries in permanent teeth or surfaces. Once the caries status of individuals had been recorded, the next step was to assign some score that expressed their accumulated caries experience; for example, simply by counting the number of surfaces (or teeth) that are decayed (D), filled (F), and missing (M). The sum of these provided a score for the individual. If surfaces have been counted, the score was turned DMFS, or if the teeth have counted, the score was termed DMFT[6]. It is reported that single components of DMFT index were generally more sensitive than the composite measures of dental status tested. Single measures do not present the problem of conflicting components. For example, while irregular dental attendance pattern is positively associated with number of filled teeth, it is negatively associated with the number of missing teeth[22]. For this reason, in this study, DT, DS, FT, FS, MT and FS values were also calculated, as well as the DMFT and DMFS complex scores, in order to evaluate the contribution of each component of the DMF index to DMF values and to achieve a more detailed analysis.

In this study the relationship between sex and dental health was analyzed. It was found that mean number of filling teeth (FT) in females (4.19) was statistically higher than that of males (3.09). This data indicated that women are more careful about their dental health than men, showing stronger tendency to treat their teeth. In a study conducted on adults in Oviedo in Spain, while higher DMFT values were observed for women, number of fillings was significantly higher in women than in men[3]. Another study, which was performed on 2110 people between 35-44 years of age in Quebec, Canada has shown that in women FS and MS were higher than that of men. On the other hand DS was higher in men[10]. People who visit dentist regularly have more treated tooth surface than people who do not visit dentist regularly. In addition, it was observed that women visit dentists much more regularly than men[12]. Tanzanian health workers seemed to play a significant role in influencing women's risk appraisal varies information obtained from the media and women's own risk experience had less importance[4]. Unexpectedly, the Tanzanian women appeared to be more optimistic regarding oral health hazards than did their Norwegian counterparts. And also, Canadians seemed to be significantly more optimistic about negative life events than the Japanese[17]. The results of these studies and our study showed similarity - women have higher FT values than men. However we can not compare them directly. There are many reasons that affect differences among countries, such as education, income, life style etc. In our country, number of employed women is less than of employed men. Thus, women have more free time to watch TV and follow media and take care of themselves. Women are more interested in health related TV commercials and, as a result, they are more sensitive on solving problems concerning their own health. Besides the process of urbanization provided women with conscious and chance to get to know other cultures with the help of advanced communication. As a natural result of this, everyday women become more and more aware of their role and position in the society and thus become more concerned about their oral health.

According to this study; DMFT, DMFS, FT, FS, MT, MS values statistically increased parallel to age, while DT and DS scores decreased. In adolescence, the caries increment seemed to level out and this was taken as further varying caries activity existed[27]. It was known to be common in the recent past, in population around ages of 20 in some industrialized countries, to have virtually all surfaces at risk filled (this means surfaces at which could microbial plaque accumulate and retain undisturbed for longer periods time), and such as a further change DMF with age mainly reflected an extension of restorations and tooth extractions[27]. On the other hand, it has been reported that the generally held view of caries experience being reduced with age may not be a result of reduced caries activity, but due to the reduced number of remaining teeth[27]. In contrast to the finding of the present study, it has been reported that adults and the elderly were as much at risk of developing new lesions as are children, also shown in the USA[16]. It has also been reported that dental caries on a population basis is the predominant cause of tooth loss even up to the age 60 years[2,9,11,19-21]. Cohort effect may be an important factor, i.e. each age chart is assumed to have had its own lifestyle, socio-economic background, etc.; therefore, the rate of which caries lesions develop early in life as a result of particularly favourable or unfavourable life conditions will strongly

influence caries levels later on in life[27]. Such cohort effects are, of course, of tremendous importance when interpreting caries data from today's populations, where dramatic changes in caries experience occur even between age groups only separated by a few years[27].

In the present study it was observed that as person number in the family increased, FT and DS values increased significantly, while FT values decreased. This could be related to the decrease of income per person because of rising person number in the family and reducing health expenses. Support of this relation, in our study, was the finding that with the increase income per person, DT and DS values statistically decreased, and FT significantly increased when family got higher income. Also as annual income level increased, FT was getting higher. In summary, as income are higher people paid more attention to their oral health and they have their teeth treated. Similarly to data of this study, in a study of the effect of family structure on dental caries, families with high birth rate (more than 3 children) tended to have more dental caries[25]. In the study made in Quebec, it was determined that a member of the family with lower income had an average of 4 or more decayed tooth surfaces and also inclined to caries more than 4 times than a member of a family with higher income[10]. It has been reported that income inequality was strongly associated with lack social mistrust and higher age adjusted mortality rates from a range of conditions, including coronary heart disease, unintentional injury and infant mortality[18]. It was concluded that the growing gap between the rich and poor affects the social organisation of communities, and has profound implications for the public's health[18].

In this study, the association between education and dental health was analyzed. DMFT and DMFS decreased significantly when education level was higher. Members who got university education had significantly better tooth health than members who got elementary education, and they were more concerned about their oral health. The results of the present study were also similar to the study conducted on 2110 persons, showing relationship between educational level and different components of DMFS. It was found that members who has elementary and high school education level have more missed teeth (5.8 versus 9.8), decayed surfaces (1.3 versus 2.3), and less filled surfaces (30 versus 19.6) than members who has university education level[10].

The study conducted in Sweden has investigated the relation between educational level and dental status on persons 35, 50, 65 and 75 years old. 69% of 65 years old person, 72% of 75 years old person and 22% of 35 years old person had low educational level. The relation between elementary school, secondary school and high school educational levels and percentage of intact, decayed, missing and filled surfaces in 50 year olds was evaluated. It was found that subjects with higher levels of education had greater percentage of intact tooth surfaces and lower percentage of missing surfaces than do those with less education[6]. It has been reported that low educational level is a very significant risk indicator for tooth loss, dental caries, and periodontal diseases, not necessarily because highly educated people are more intelligent or wealthier. The difference in dental status is attributable to the fact that highly educated people know how to learn from written information, to seek information about health promotion, and to apply theoretical information, for self-care[6].

In the present study, it was found that MT, MS, FT, FS, DMFT and DMFS values were significantly lower, and DT and DS values significantly higher in the student's group compared to the employed and unemployed groups. MT, MS, FT and FS values were high in the employed and unemployed groups, which mean that these individuals had more missed teeth and more treated teeth. On the other hand, considering only high DS and DT values in the student group, in spite of low FT and FS values, it seems that students do not pay attention to their dental health.

Job insecurity, change or loss are the critical periods in human life, which may have particular importance in determining health status of individuals and levels of health inequalities within populations[7]. In this study, the youngest age group was 13-20, which consisted mostly of adolescent students. Adolescent oral health status may reflect maternal care during childhood, rather than their current oral health-related behaviour[1]. Previous studies pointed to the influence of mothers on children's oral health-related behaviours, such as toothcleaning[8] and dental attendance[5]. It seems likely that mother-related factor may have a stronger influence on adolescent current oral health status than their own self-care practices or psychological status. High caries prevalence in the student group reveals the parents' insufficient oral health education in the family.

In conclusion, age, family size, socioeconomic position, including household income and income per person, individual's position (student, employed or unemployed), play a role in frequency of DMFT, FT, FS, MS, MT, DT and DS. It is important to consider these social and economic factors in forming each country's politics to improve public's dental health programme.

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Correlation of Blood Fibrinolytic Activity and Clinical Outcome After Oral Surgery Interventions

SUMMARY

The primary aim was to investigate how the oral surgical interventions, as psychophysical and operative trauma, influence the response of the blood fibrinolytic system, and whether the possible changes in the level of the activators and inhibitors of the fibrinolytic system could correlate to clinical results. Fourthly healthy subjects with operative tooth extractions were included in the research; 35 subjects, blood donors, constituted a control group. The influence of the interventions over the parameters of the blood fibrinolytic system was examined prior to surgery and immediately after the extraction with Elisa test. The values of the blood pressure and pulse frequency prior to the surgery; after the applying of the local anaesthesia and immediately after the performed interventions were the only objective parameters for the evident physical reflections of the stress. At the control investigations, after 24, 48, hours and seven days, certain changes like oedema, haematoma, pain and dry socket have been presented.

Decreased values of t-Pa and PAI-1 after the oral surgery were found at a high statistically significant difference. The analysis of diastolic blood pressure and pulse frequency values showed statistic significance, showing psychological dimension of oral surgery. There was a positive correlation between most of the parameters from the control examinations and the parameters (t-Pa, PAI-1, pro-activators and inhibitors) of the fibrinolytic system in the examined group.

Oral surgical intervention, as a stress factor, had influence and affects the fibrinolytic process through the effect upon pro-activators and inhibitors of the fibrinolytic system, and severity of the clinical outcome after tooth extraction. Parameters of fibrinolytic system, t-PA and PAI-1, can be the most sensitive markers of reaction to oral surgical stress.

Keywords: Tooth Extraction; Oral Surgery; Fibrinolytic System; Stress

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Introduction

Many authors[4-8,27] set an aim to evaluate the influence of the surgery stress and the operative trauma over certain parameters of coagulation and blood fibrinolysis.

According to Grand[8], the acute physical stress as the major surgery is, the insulin induced hypoglycaemia and the physical exercises are connected to acute increase of concentration of the factor VIII in circulation, as well as increased blood fibrinolytic activity. The mechanisms that are included in the production of these answers are partly under hormone control and it is obvious that the changes are mediated by the neuro-hormones adrenalin and arginine-vasopressin.

Kehlet[13] emphasized that surgery trauma and modified effects of pain are the reason for start of possible complications, as infection and the haemorrhage are. Exactly, as a consequence of the activation of humoral substances (prostaglandin, kinin, leukotriene, interleukin-1, as well as the tumour necrotizing factor), the creation of mentioned complications is possible[2]. The interlukin-1, as a mediator of an inflammatory reaction, and the tumour necrotizing factor lead to pro-coagulation

changes in the one-body cells. Here are the synthesis and secretion of the thromboplastin, antigen activity of F VIII: factor von Willebrand, then activity of the inhibitor of the plasminogen-1 (PAI-1) activator with the decreased production and secretion of the tissue type plasminogen activator (t-PA) at same time.

In contemporary scientific books[1,2,17] there is an elaboration of accidentally discovered cases with prolonged bleeding after tooth extraction[18] in patients with rare deficits of some factors of coagulation[17,18,23] or inhibitors of fibrinolysis[11,12,14,15,20,25,26].

The basic aim of this research was to determine whether the oral surgical interventions as psycho-physic and operating trauma influence the answer of blood fibrinolytic system. The second aim was to determine the correlation of values of the level of fibrinolytic system activators and inhibitors, and clinical outcome after oral surgery.

Material and Methods

These researches covered 40 healthy patients of both sex, 25-35 years of age. According to the history data, clinical and radiographic examination, indications for operative extraction were set up (roots with different chronic lesions in the molars region). The surgical interventions were done for 35-40 min, and the operative trauma was similar in the examinees according to the operative protocol. The interventions in the examined group were realized during the morning hours at the Clinic for Oral Surgery, University Dental Clinical Centre "St Panteleimon" in Skopje. The control group consisted of 35 healthy examinees of both sex, blood donors, 25-30 years of age, who didn't have any dental intervention. All examinees agreed to be included in our research.

Venous blood samples were taken before and after surgery from the examined group and also, from the control group. The blood samples were instantly distributed to the Institute of Blood Transfusion, Department for Haemostasis and Thrombosis, at the Medical Faculty in Skopje. There was a selective determination of the plasminogen activator - tissue-type plasminogen activator (t-PA antigen - t-PA-Ag) - INNOTEST t-PA, and of the inhibitors of plasminogen activator-antigen (Plasminogen activator inhibitor-1-antigen; PAI-1-Ag), with INNOTEST PAI-1 (Biopol Trinity Company, Ireland). Bought tests are immuno-enzymatic analysis - MIKRO-ELISSA method, with double antibody or sandwich method where the antigen is inserted.

As objective parameters of the physical reflection of stress the values of blood pressure and pulse rate were recorded in 4 phases: before surgery, after the application of local anaesthesia, during surgery, and at the end of it. Presence of fear according to the subjective statements was recorded in all the examinees. The control investigations were done 24, 48 hours and 7 days after surgery, recording the presence of oedema, haematoma, pain, dry socket and prolonged bleeding.

For statistics elaboration and analysis of the obtained data, the statistics programme "Statistica" was used.

Results

The distribution of the values for t-PA and PAI-1 for the examined group, before and after surgery, is showed in figure 1. The value of t-PA after the surgical extractions (3.55 ng/ml) is lower comparing to the value before them (4.43 ng/ml), showing statistically significant difference ($t = 3.29$; $p < 0.01$). The value of PAI-1 after surgical extractions (56.3 ng/ml) is significantly lower comparing to the average value of PAI-1 before surgery (71 ng/ml). Their analysis showed statistically significant difference ($t = 2.59$; $p < 0.05$).

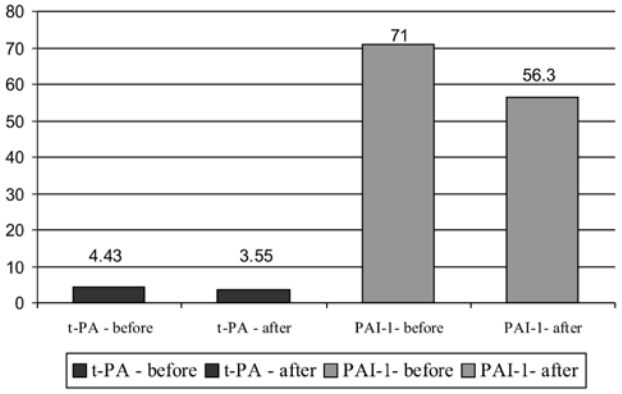

Figure 1. Values of t-PA and PAI-1 before and after oral surgery

The values of t-PA and PAI-1 before and after surgery in the examined group were significantly higher comparing to the same parameters at the control group, and the analysis of average values showed statistically significant difference in all of the examined relations (t-PA contr./t-PA before: $t=4.26$, $p<0.01$; t-PA contr./ t-PA after: $t=2.21$ $p<0.05$; PAI-1 contr./PAI-1 before: $t=7.89$, $p<0.01$; PAI-1 contr./PAI-1 after: $t=6.60$, $p<0.01$), which is shown in figure 2.

Average values of systole and diastole blood pressure at the examined group are shown on figure 3. The analysis of average values of systole pressure between the examined and the control group in all of the researching phases did not show statistical difference. The analysis of average values of the diastolic pressure between the examined and the control group in all of the researching

phases did not show statistical difference except in the relation before surgery/ after anaesthesia (t = 2.76, p<0.001).

The average values of the pulse rate at the examined group are shown in figure 4. The analysis of average values between the examined and the control group showed high statistical difference in relation control pulse rate/pulse rate before surgery (t = 4.33, p<0.001). In the rest of the researched relations, there was no significant difference (t = 0.33; 0.87; 0.95 and p<0.05).

Subjective statements for the presence of fear among the examinees with extractions are shown in table 1. Before the extraction, 13 of the examinees (32.5%) had no fear, while 27 (67.5%) felt fear; 22 examinees (55%) felt fear during the intervention.

After 24 and 48 hours from the extraction, no prolonged bleeding was seen among the examinees. The data for the appearance oedema, haematoma, pain, dry socket in the examined group during the control investigations after 24, 48 hours and 7 days are shown in table 2.

With the Spearman coefficient of correlation (Tab. 3), modest connection was noted between the values of t-PA and PAI-1 after oral surgery and parameters from the control examinations - a domination of values of the relation of PAI-1 after/oedema 24 hours; PAI-1 after/oedema 48 hours; PAI-1 after/dry socket 7 days; t-PA after/dry socket 7 days.

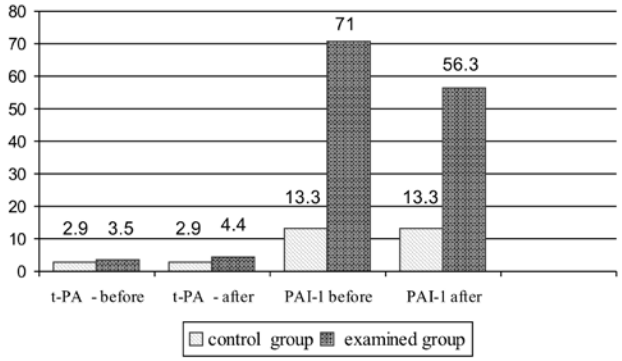

Figure 2. Values of t-PA and PAI-1 before and after oral surgery in the examined and control group

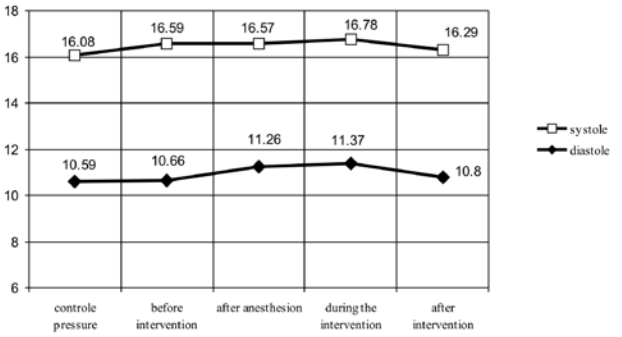

Figure 3. Values of systolic and diastolic blood pressure in the examined group

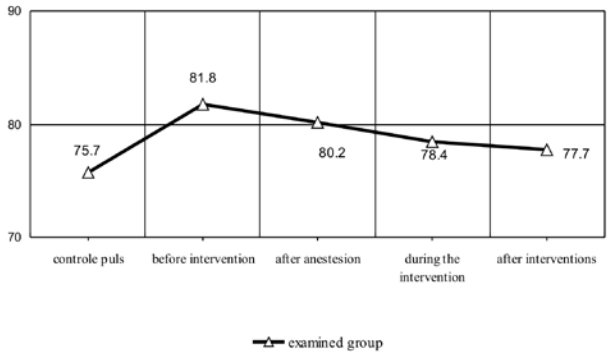

Figure 4. Values of the pulse rate in the examined group

Table 1. Presence of fear before and after surgery in the examined group

fear	n = 40			
	Before surgery	%	During surgery	%
absence	13	32.5	18	45
presence	27	67.5	22	55
a little	12	30	9	22.5
a few	7	17.5	9	22.5
a lot	8	20	1	2.5
increasing	0	0	3	7.5
differences	Chi Sqr.=1.74			

Table 2. Presence of oedema, haematoma, pain and dry socket after surgery in the examined group

(n = 40)	24 hours				48 hours				7 day			
	oedema		haematoma		oedema		haematoma		oedema		haematoma	
	N^0	%	N^0	%	N^0	%	N^0	%	N^0	%	N^0	%
absence	19	47.5	40	100	30	75	40	100	40	100	40	100
presence	21	52.5	0	0	10	25	0	0	0	0	0	0
	pain		dry socket		pain		dry socket		pain		dry socket	
	N^0	%	N^0	%	N^0	%	N^0	%	N^0	%	N^0	%
absence	17	42.5	40	100	22	55	30	75	40	100	35	87.5
presence	23	57.5	0	0	18	45	10	25	0	0	5	12.5

Table 3. Spearman coefficient of correlation between t-PA and PAI-1 after the interventions and the parameters of the control examinations in the examined group

parameters	Spearman R.	p
t-PA after surgery/oedema 24 h.	- 0.049	p = 0.76
t-PA after surgery/oedema 48 h.	0.043	p = 0.79
t-PA after surgery/oedema 7 days	/	/
t-PA after surgery/haematoma 24 h.	/	/
t-PA after surgery/haematoma 48 h.	/	/
t-PA after surgery/haematoma 7 days	/	/
t-PA after surgery/dry socket. 24 h.	/	/
t-PA after surgery/dry socket 48 h.	0.005	p = 0.97
t-PA after surgery/dry socket 7 days	0.101	p =0.53
PAI-1 after surgery/oedema 24 h.	- 0.357	p = 0.02
PAI-1 after surgery/oedema 48 h.	- 0.233	p = 0.14
PAI-1 after surgery/oedema 7days	/	/
PAI-1 after surgery/haematoma 24 h.	/	/
PAI-1 after surgery/haematoma 48 h.	/	/
PAI-1 after surgery/haematoma 7 days	/	/
PAI-1 after surgery/dry socket 24 h.	/	/
PAI-1 after surgery/dry socket 8 h.	- 0.32	p = 0.04
PAI-1 after surgery/dry socket 7 days	- 0.167	p = 0.30

Discussion

In our research there is an attempt to give an answer to the question whether minor oral surgery under local anaesthesia in patients with intact system of haemostasis can cause changes to some parameters of the fibrinolytic answer. Having in mind complexity of the haemostatic system, we meant to obtain a real clinical evaluation of the physiological activity of blood fibrinolytic system during surgical extractions.

Knowledge about the local haemostatic balance is significantly enlarged with the examination of patients with congenital and acquired defects of coagulation[11,14,15,20]. In that respect, knowledge of the specific type and strength of the prolonged bleeding is crucial for planning of a safe and suitable oral surgical treatment, with aim to minimize the risk of prolonged bleeding[3].

It is stated that the circulation at individuals that are not under stress, t-PA circulates as a complex with PAI-1[1]. It is indicative that normally, in the plasma, the bigger part or the whole t-PA is present in the complex with PAI-1. Stimulation like physical activity leads to the release of t-PA, which freely circulates and temporarily stops the PAI-1 effect. The stress, pain, physical exercises, adrenaline injecting or venous occlusion have been known as conditions that result in significant increase of the plasminogen activators' level[19,24,27]. Sprengers and Kluft[24] reported identical results.

The activation of plasminogen with t-PA is strengthened by the fibrin that creates matrix. In that mean, t-PA and the plasminogen are in suitable position including the conformation changes that make the activation suitable. During the resolution of the fibrin, the connection of the plasminogen and t-PA with the partly degraded fibrin is strengthened, enabling highly efficient fibrinolysis against the decreased concentrations of the involved components. At the same time, t-PA appears in complex with the C-inhibitor and α_2-MG[3,6,17].

Congenital plasminogen activator inhibitor-1 (PAI-1) deficiency is an extremely rare disorder characterized by a bleeding diathesis due to hyper-fibrinolysis as a result of the decreased PAI-1 activity[11,14,15,25,26]. Takahashy et al[25] and Tanimura et al[26] presented cases with partial quantitative deficit of the PAI-1 of the members of several families in Japan, that had prolonged bleeding episodes after trauma and tooth extraction. The characteristic disorders of the fibrinolysis with them were: shortened euglobulin lysis time, low level of PAI-1 activity with low levels of the PAI-1 antigens in the plasma and serum. For these patients 5% tranexemic acid as a solution for mouth washing was used in order to decrease bleeding during and after tooth extraction.

The results from our statistical analysis undoubtedly showed that during the surgical extraction in the examined group there were changes of the values of the researched parameters from fibrinolytic system, however without enormously difference; they were in the frames of physiological, referent limits.

Kaličanin and Lečić-Toševski[10] emphasize the Selye's concept: stress reaction is always the same i.e. stereotypical, no matter the kind of stress. Every phase of stress is accompanied by biological modifications and rather stereotypical and characteristic clinical manifestations caused by oscillations of the regulatory biological mechanisms.

However, comprehensive researches[16,19,21] showed that stress reaction cannot be described as stereotypical changes and processes. Most of the authors[16,19] agree that psychological, biological and social nature of humans are unique and undivided. So stress, in a best and most correct way, can be defined as a whole psycho-socio-biological answer of the organism towards the action of any agent that attacks its homeostasis.

Raikkonen et al[20] researched the effect of chronic stress, comparing to the t-PA and PAI-1 at 69 healthy men. The findings confirm the hypothesis that the chronic stress creates changes in the fibrinolytic system and suggest that the fatness, level of the insulin and triglycerides are in a very close correlation to the fibrinolytic parameters, in other words the increased synthesis of t-PA and PAI-1.

The measurements of blood pressure and pulse rate in this research did not show any bigger differences. Before surgery, after local anaesthesia, during and at the end of surgery, statistically significant differences were

not recorded; while the average values of the diastolic pressure and pulse rate showed statistically significant difference in certain analyzed relations.

Dimova[3] emphasized that stress is more present at the examinees with tooth extraction in the examinees with oral surgery. This is confirmed also subjectively with the personal statements of the examinees and objectively through the values of the diastolic pressure and pulse rate. These findings justify pre-surgical psychological treatment of the examinees before oral surgery, which helps in decreasing stress before and during surgery.

Our findings for the presence of oedema and haematoma in the period after the surgery show that there was a moderate correlation with the values of t-PA and PAI-1. In the period after the extraction, no haemorrhage was noticed, which was confirmed at the control examinations. This finding is normal as the examinees were with the intact haemostatic system.

Conclusions

The examinations from this study precisely determined fibrinolytic blood activity during oral surgery and emphasize following conclusions:

Operative extractions had influence on the fibrinolysis by releasing the pro-activators and inhibitors of the fibrinolytic system.

Immuno-enzymatic test of the fibrinolytic activity showed increased values of t-PA before surgery comparing to values of the control group, and some decrease after surgery; these values are in correlation with the increased values of PAI-1.

The values of the examined vital parameters, the blood pressure and pulse rate, as well as data for presence of stress before and during surgery, made a clear view for stress reaction of the examinees. The changes of the values of the diastolic blood pressure and the pulse rate confirm psychological dimension of surgery.

Correlations of the fibrinolytic system parameters with the control examinations parameters (oedema, haematoma, pain and dry socket) showed that there were correlation between the values of t-PA and PAI-1 after surgery, which implicate the clinical outcome of oral surgery. Parameters of the fibrinolytic system, t-Pa and PAI-1, can be used as the most sensitive markers of reaction to surgical stress.

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Surgically Created Orifice in the Buccal Mucosa for the Treatment of Parotid Duct Fistula

SUMMARY

The aim of this paper was to present the efficacy of surgically created orifice in the buccal mucosa in the treatment of parotid duct fistula. The treatment was performed in 7 patients with parotid duct fistula. The procedure comprised creation of an artificial orifice in the buccal mucosa by introducing a haemostat via an open extraoral wound and puncturing the mucosa. Through the newly created soft tissue channel, a soft rubber drain was inserted, leaving one end in the wound where it was fixed with 3/0 catgut suture; the other end was left to protrude into the oral cavity for the period of 10 days. The treatment was successful in all patients and can be recommended as a routine cost-effective method of parotid duct fistula management.

Keywords: Salivary Fistula; Stensen's Duct Injury

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CASES REPORT (CR)
Balk J Stom, 2009; 13:117-122

Introduction

The location of the Stensen's duct on the face may be visualized as the middle third of a line from the tragus of the ear to the upper lip. The duct is approximately 4-6 cm in length and 5 mm in diameter. The Stensen's duct then runs through the buccinator muscle into to oral cavity, followed by the buccal branch of the facial nerve. Trauma to the Stensen's duct frequently happens as a result of: traffic accidents, occupational trauma, accidental trauma, fights and war injuries[1,2].

Such kind of injuries always represents unpleasant condition for the patient, and treatment is difficult for the surgeon, associated with high percentage of failure. Different methods and procedures were developed in order to threat extraoral salivary fistula. Nicoladini first reported primary anastomosis of the parotid duct in 1896, and Morestin reported ligation of the proximal stump in 1917[4]. Basically the next procedures are in use at present: (1) conservative methods[5,7,8] using compressive dressings in combination with different medications, such as Bothulinus, Probanthine, Atropine and Glycopyrrolate; (2) microsurgical methods[3,11] referring to end to end anastomosis, venous grafts (in cases of missing parts of the Stensen's duct), intraoral rerouting of the proximal part of the duct, reconstruction of the missing parts of the Stensen's duct using mucosal of skin tubes[3]; (3) tympanic neurectomy[9]; (4) irradiation[6]; (5) Stensen's duct ligation[3]; (6) internalization of the fistula with a cutaneous cuff in cases of chronic fistulas[10].

The **aim** of this article was to describe a surgical technique of an artificial orifice creation in the buccal mucosa in order to treat a parotid duct damage and subsequent salivary fistula.

Case Report

The procedure was performed in 7 patients with massive lacerations of buccal region resulting in injury to the Stensen's duct and consequent extraoral salivary fistula during the period of 5 years. All patients were operated under local analgesia. Of those treated, 2 male patients, 26 and 32 years of age, are documented in this paper.

First patient sustained injury in a traffic accident, from pieces of a shattered wind shield. Unsuccessful repair was attempted at another department, resulting in

the extraoral fistula creation and skin wound breakdown (Figs. 1 and 2). Second patient was injured in a street fight by a sharp end of broken glass (Figs. 9-15). In both cases certain part of the duct was missing, so usual method considering end to end anastomosis was not possible.

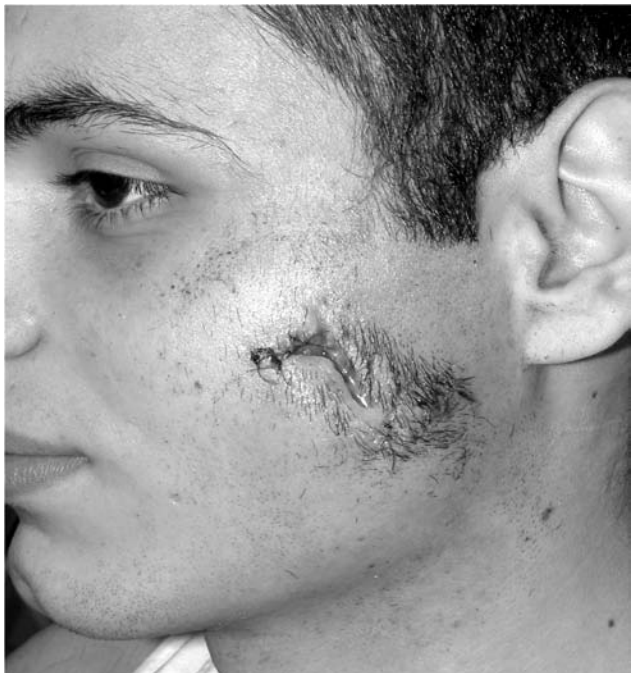

Figure 1.

Figure 2.

Patient on admission, with extraoral salivary fistula previously treated at another department

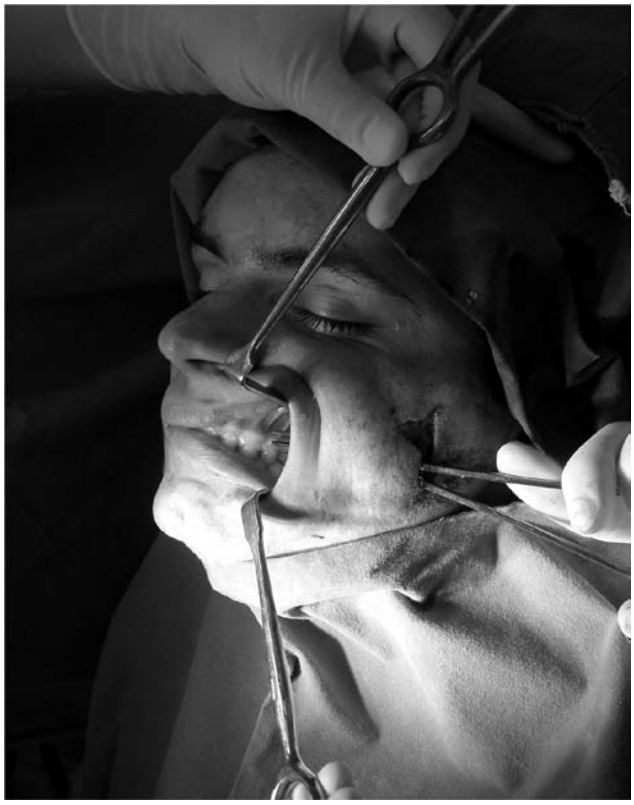

Figure 3.

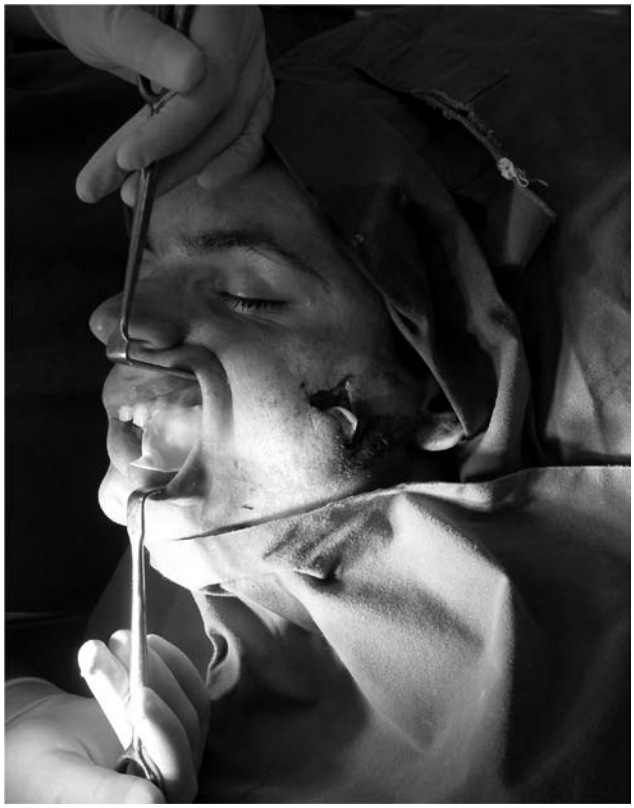

Figure 4.

Haemostat used to puncture into the mouth and to place soft rubber drain in order to establish artificial salivary fistula

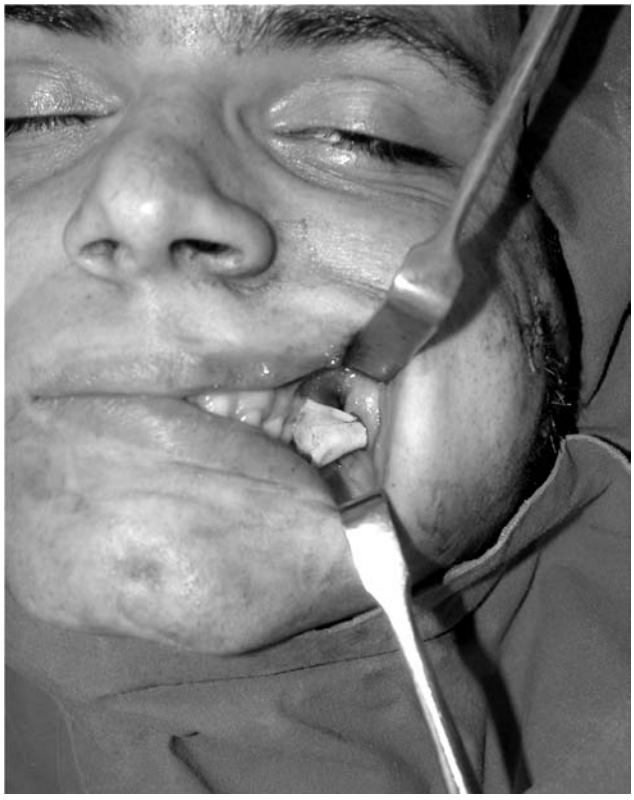

Figure 5. Rubber drain protruding into the mouth

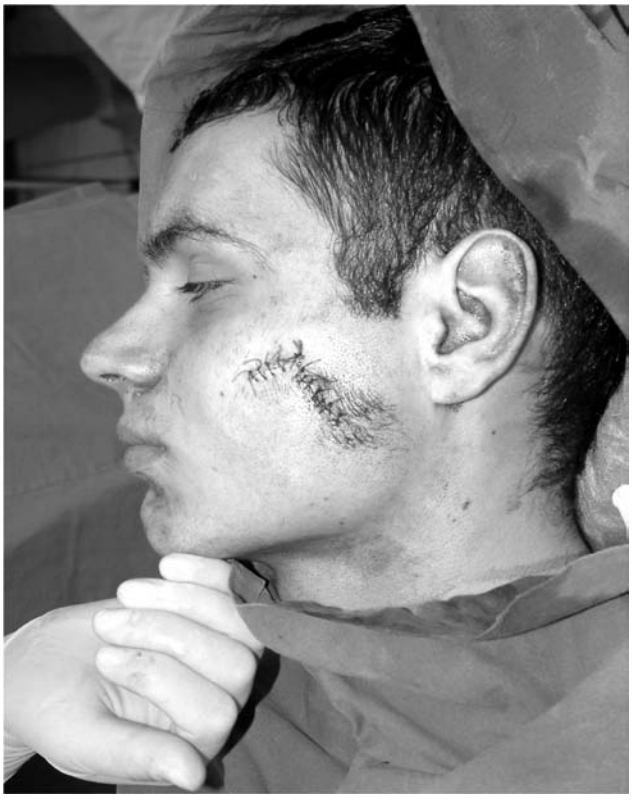

Figure 6. Final result

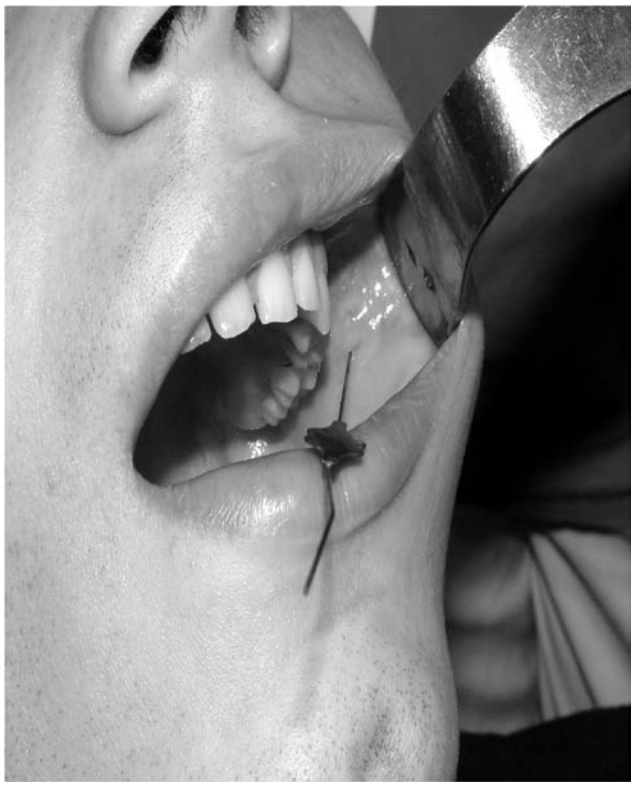

Figure 7. New orifice

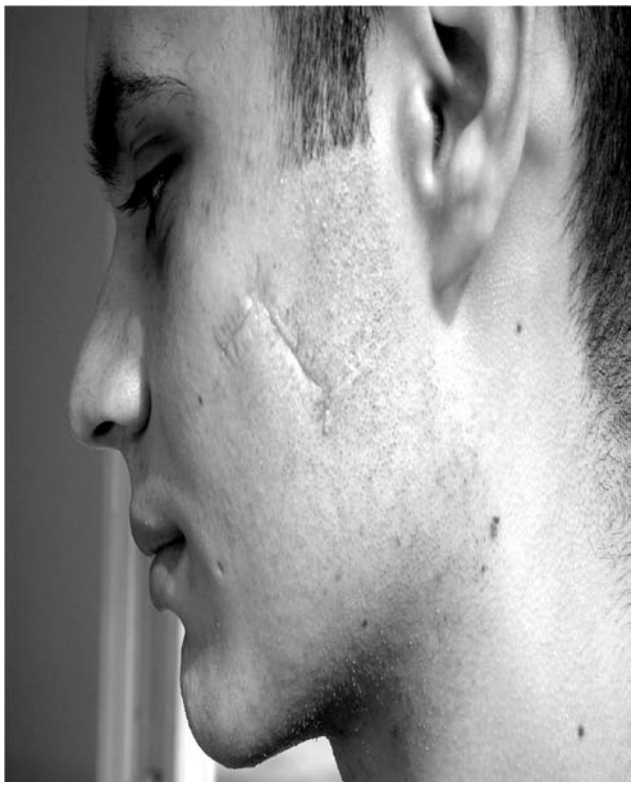

Figure 8. Final result, 6 week

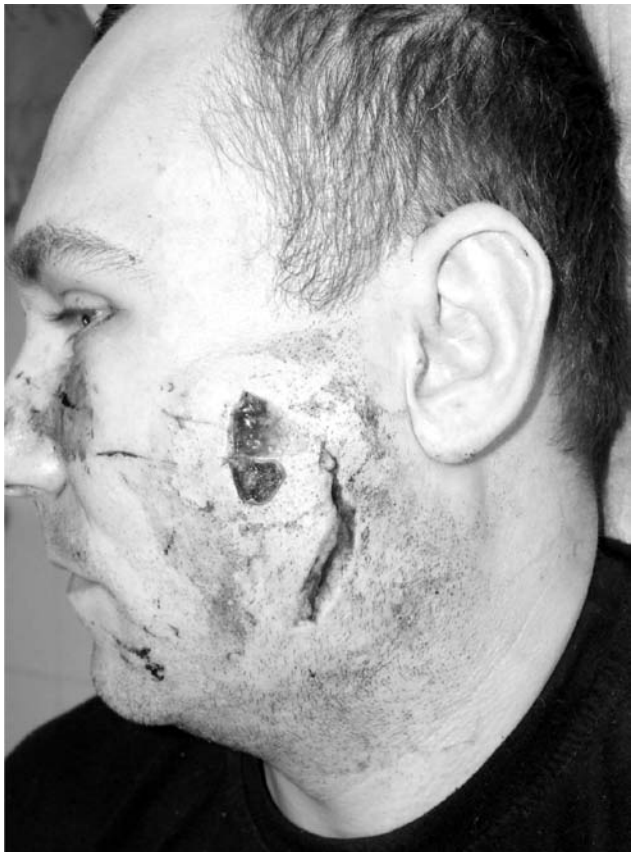

Figure 9. Patient on admission s postoperatively

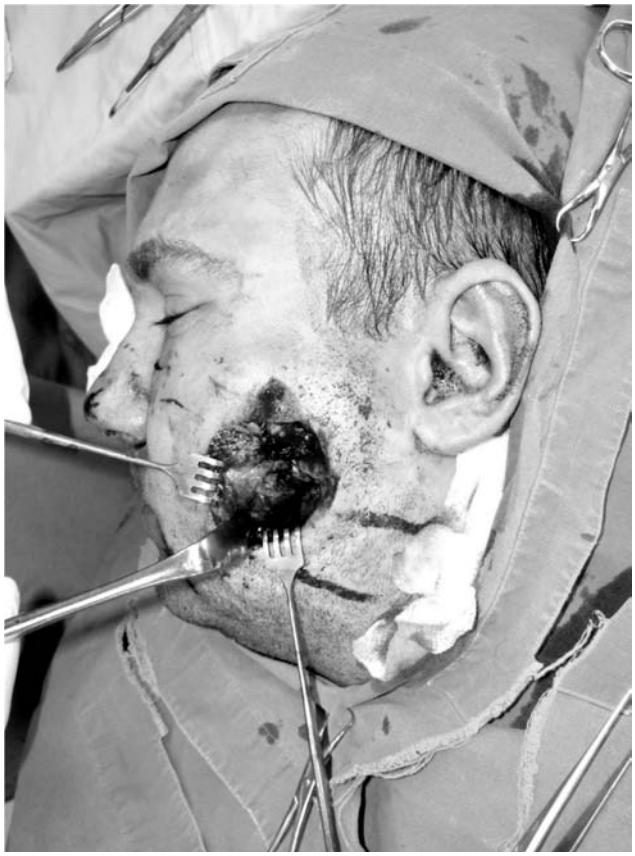

Figure 10. Inspection of the wound

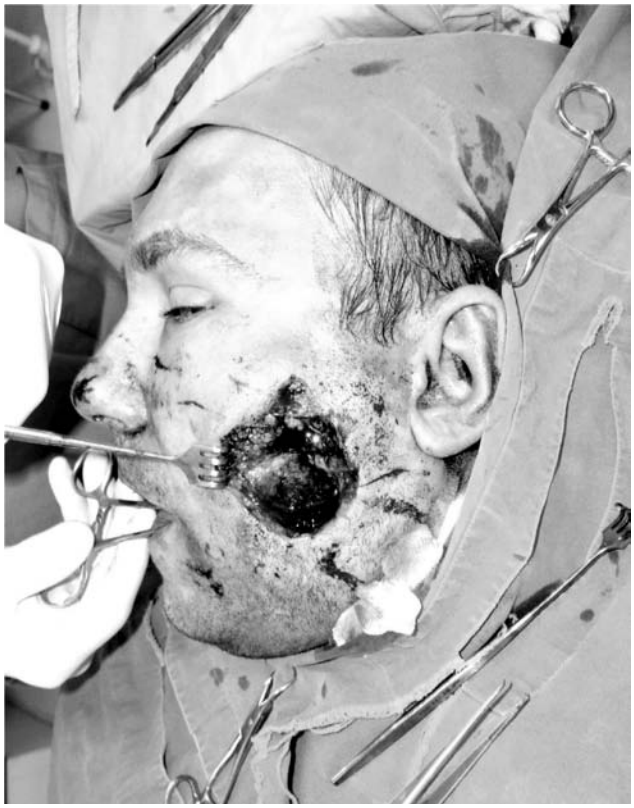

Figure 11. Creating pathway for intraoral salivary fistula

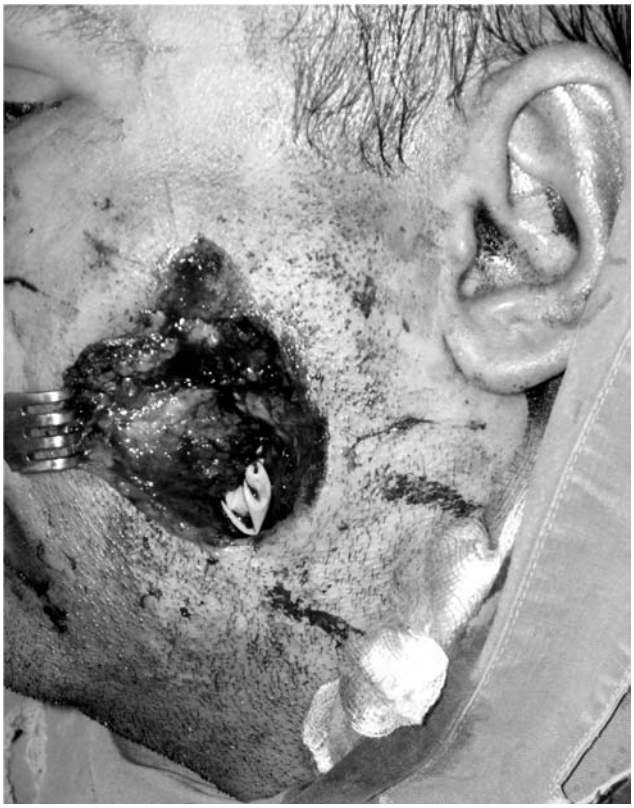

Figure 12. Soft rubber drain placed into the wound and protruding into the mouth

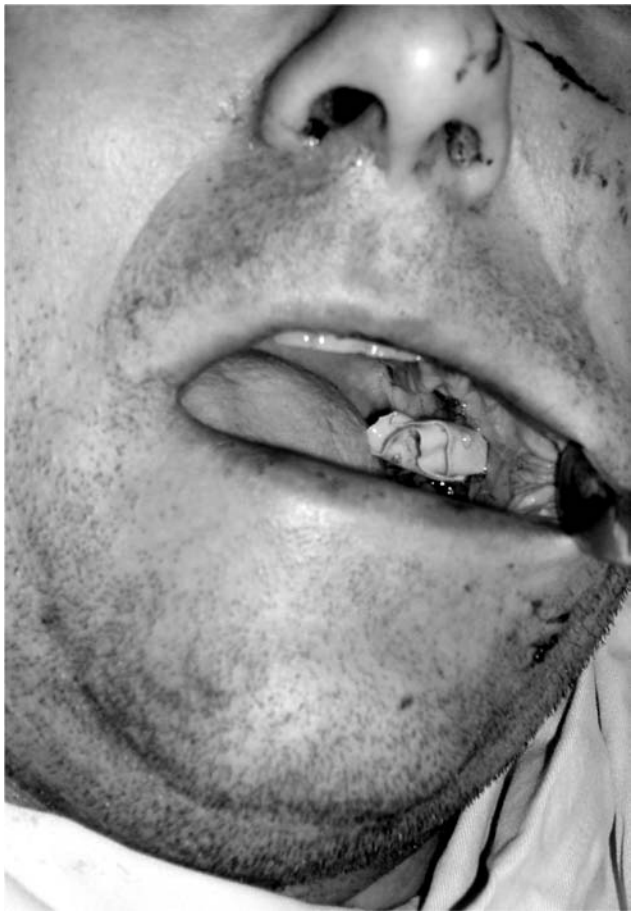

Figure 13. Soft rubber drain protruding into the mouth

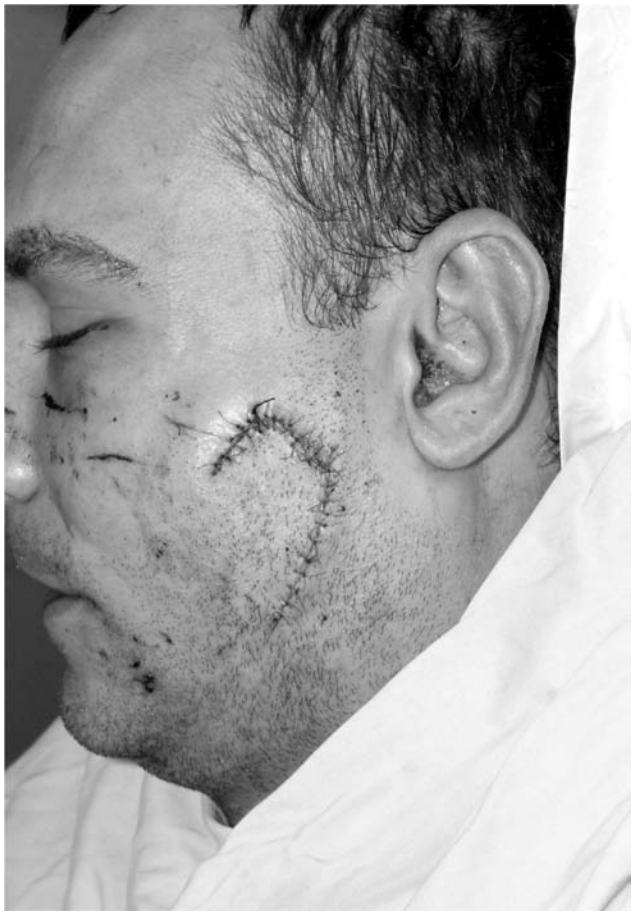

Figure 14. Final result

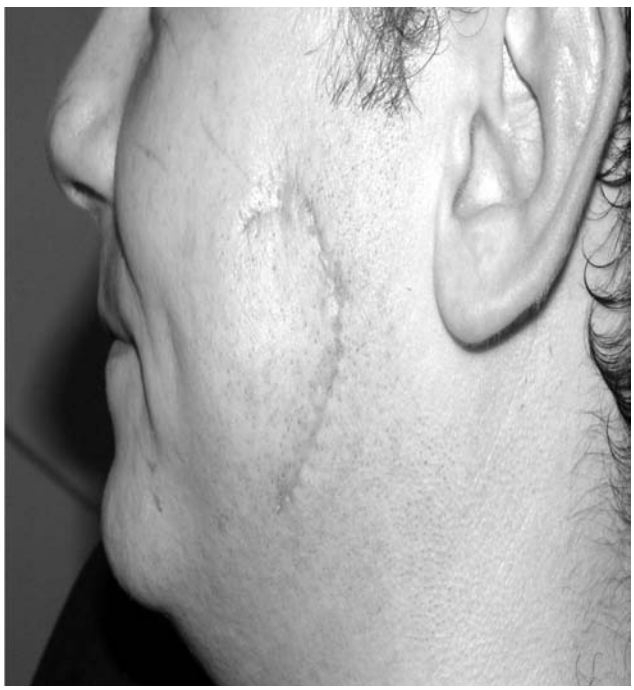

Figure 15. Patient 6 weeks postoperatively

Surgical Technique

A curved haemostat was introduced through the open wound into the mucosa that was punctured by exerting a pressure (Fig. 3) in order to place soft rubber drain into the wound cavity (Fig. 4). Through the newly created soft tissue channel, a soft rubber drain was inserted leaving one end in the wound where it was fixed with 3/0 catgut suture. The other end was left to protrude into the oral cavity for the period of 10 days. Subcutaneous tissue was sutured with interrupted 4-0 vicryl sutures and skin was closed with 5-0 ethhicrin interrupted sutures (Figs. 5 and 6). Skin sutures were left in place for 2 weeks in order to prevent possible wound breakdown (Figs.7 and 8).

Patients were placed on the soft and liquid diet with additional i.v. fluid replacement. Ceftriaxon 2x1gr, metronidazole 3x500mg, were administered during 7 days. After that period, additional peroral 3x500mg amoxicillin and metronidazole 3x400mg were given next 7 days. Half an hour before every meal, 0.5mg atropine was administrated subcutaneously.

Results and Discussion

After the sutures were removed, operative wounds were healed, and without the presence of salivary fistula (the most frequent complication in standard procedures used in such kind of injury)[3], and 100% success was obtained in both presented cases.

Chronic fistulous condition may develop often undiagnosed[10]. All the methods above described are less efficient with the emphasis on the early diagnosis. Conservative methods combining compressive dressings, different medications in order to diminish parotid secretion (such as Atropine, Botulinum, Glucopyrrolate), and dietary regiments are frequently used. But these methods are time consuming, and associated with a low success rate[5,7,8]. Microsurgical procedures, first of all, require adequate training and equipment, and are associated with high success rate when parts of the duct are not missing[3,12]. Delayed microsurgical reconstructions using mucosal or skin tubes offer modest results[12]. In cases of the distal part of the duct destruction, intraoral rerouting of the proximal part gives good results[12]. Venous grafts are recently used in cases of missing parts of the duct[11]. Tympanic neurectomy is a method to reduce parotid secretion as secreto-motor fibres for the parotid are carried by the tympanic branch of the glossopharyngeal nerve, and reach the gland via the auriculotemporal nerve, but results are variable[9]. Stensen's duct ligation leads to the atrophy of the parotid gland, but carries the risk of chronic parotitis[12]. Irradiation carries the risk and the potential carcinogenic effect[6]. In chronic fistulous conditions, internalization of fistula with a cutaneous cuff represents innovative, simple and highly effective procedure[10].

It should be emphasized that early detection of the injury to the Stensen's duct is an imperative in the treatment of such type of injuries[10-12]. Surgically created orifice in the buccal mucosa for the treatment of parotid duct fistula is a simple method for Stensen's duct reconstruction compared to the other methods[9- 12].

Conclusion

Surgically created orifice in the buccal mucosa is a procedure proved to be effective; it is a simple new method in the treatment of parotid duct salivary fistula.

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Extensive Tooth Wear: A Case Report

SUMMARY

This article presents an extensive tooth wear in a 25 year old female patient with congenital adrenal hyperplasia. Detailed history, dietary investigation, oral examination, and gastric evaluation revealed that severe tooth wear was a result of gastroesophageal reflux, dietary habits and bad habit of chewing nails, and not of a systemic disease.

Keywords: Tooth Wear; Tooth Abrasion; Tooth Erosion; GERD

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CASE REPORT (CR)
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Introduction

Non-carious loss of dental hard tissue, generically termed as tooth wear, is caused by combine processes of erosion, abrasion and attrition. Clinically, dental erosion is used to describe the physical results of a pathologic, chronic, localised, painless loss of dental hard tissue chemically etched away from the tooth surface by acid, without bacterial involvement[5,13,19]. On the other hand, abrasion is used to describe the pathological wearing away of dental hard tissue through abnormal mechanical processes involving foreign objects or substances repeatedly introduced in the mouth and contacting the teeth, while attrition represents physiological wearing away of hard tissue as a result of tooth-to-tooth contact with no foreign substance intervening [5,8].

This report presents a 25 year old female patient with clinical manifestations of a severe tooth wear caused by several reasons.

A Case Report

A 25-year-old female patient referred to the Department of Operative Dentistry, Faculty of Medicine, University of East Sarajevo for conservative treatment due to a severe tooth wear. Her chief complaint was related to shortening of her maxillary frontal teeth that had started nine years ago. The past dental history revealed that patient as well as dentists hadn't noticed changes on other teeth.

Detailed discussion with patient about dietary habits revealed that she used to consume one piece of fresh fruits daily (apple, orange), and a glass of soft drinks - lemonade or cola beverages at least 3 times a week. She also used to drink 2 coups of herbal tea and coffee twice a day. Patient did not sip or hold beverages over a long period of time in the mouth. However, she admitted a habit to drink salad dressing from time to time. She denied smoking and drinking alcohol.

Concerning gastric disturbances, patient reported that, during past 5 years, she had been experiencing hart-burn, once or twice a month, usually after certain spicy meals, but she denied any complaints about sour mouth or any gastric pain early in the morning, and gave no history of vomiting or bulimia. She has not been exposed to acidic environments and had no history of bruxism or any history of jaw para-function, but she has had a bad habit of chewing nails since childhood.

Data about oral hygiene habits showed that patient regularly brushed her teeth twice a day and usually after meals, with a medium toothbrush and non-abrasive fluoride toothpaste, predominantly using horizontal motion. Brushing teeth lasted from 2 to 3 minutes. She did not use dental floss or mouthwash, but she chewed gum regularly.

The patient's medically history revealed that, when she was 20, she consulted medical practitioner for treatment of menstrual irregularity when congenital adrenal hyperplasia was diagnosed. Since than, the patient has been on corticosteroid therapy (dexamethasone, 1mg per day) and oral contraceptive drugs. Osteopenia was diagnosed 2 years ago when densitometry (DEXA) revealed T-score-2.1 (L1-L4 region). Calcium and vitamin D were also prescribed.

Clinical Examination

The patient's oral hygiene was good, with some pigmented plaque on mandibular molars. No calculus was present. The gingiva and oral mucosa were normal.

For clinical assessment of tooth wear we used Tooth Wear Index (TWI) developed by Smith and Knight[15] - with scores from 0 to 4, as well as index for erosive lesion proposed by Lussi[11], with grading of facial surfaces from 0 to 3, and oral and occlusal surfaces from 0 to 2. For presenting distribution and severity of tooth wear, using TWI, teeth were subdivided into 4 zones: anterior maxillary, posterior maxillary, anterior mandibular and posterior mandibular. The anterior teeth included central and lateral incisors and canines, whereas the posterior included the first and second premolars and first, second, and third molars.

The maxillary central incisors showed facial, incisal and palatal enamel and dentine loss, restored with composite resin (Figs. 1-3). All of patient's maxillary incisors and canines showed loss of enamel and dentin from their incisal surfaces to the palatal surfaces (Figs. 2 and 3). Oral surfaces of these maxillary teeth were smooth, shiny and hard, while buccal-oral teeth diameter was smaller. According to the index proposed by Lussi, only grade II severe lesions were found on palatal surfaces, while score 4 was found for the Smith & Knight index (Tab. 1). Shallow cervical lesions were present on facial aspects of anterior maxillary teeth (Lussi index-grade 1; TWI mean score 0.8). Moderate enamel loss was seen on oral and occlusal surfaces of first and second maxillary premolar and first and second maxillary molars (Figs. 2 and 3). Whole occlusal morphology disappeared on premolars, while rounding of the cusps and grooves occurred on molars. Grade II (Lussi index) was found for occlusal surfaces of these posterior teeth, whereas the highest score of TWI (2.0) was for oral and occlusal surfaces.

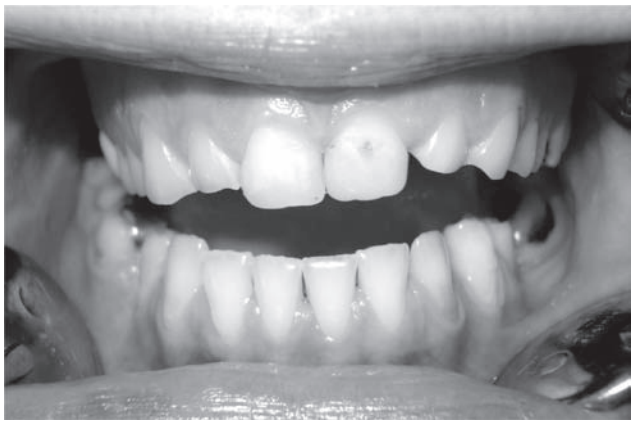

Figure 1. Tooth wear of facial and incisal surfaces of maxillary central incisors restored with composite resin; loss of incisal edges and facial tooth wear of maxillary second incisors and canines

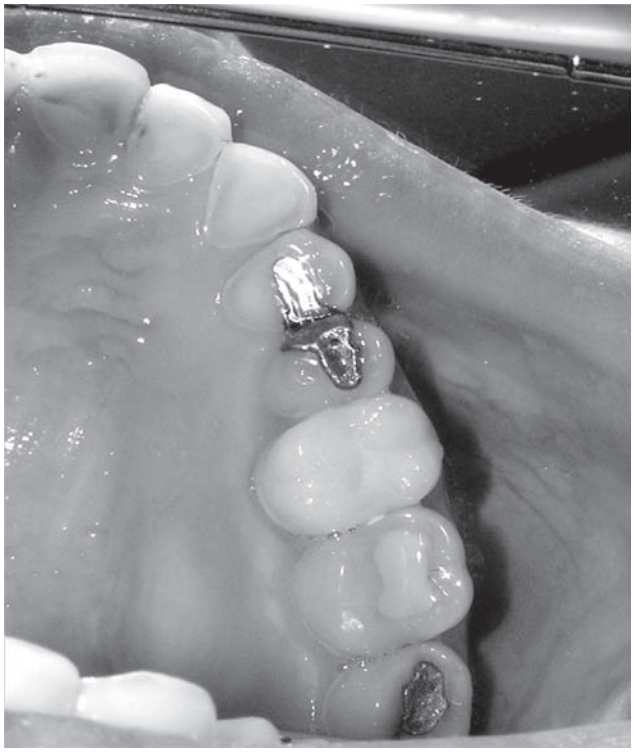

Figure 2. Loss of incisal/occlusal and palatal enamel from right maxillary incisors, canines, premolars and molars

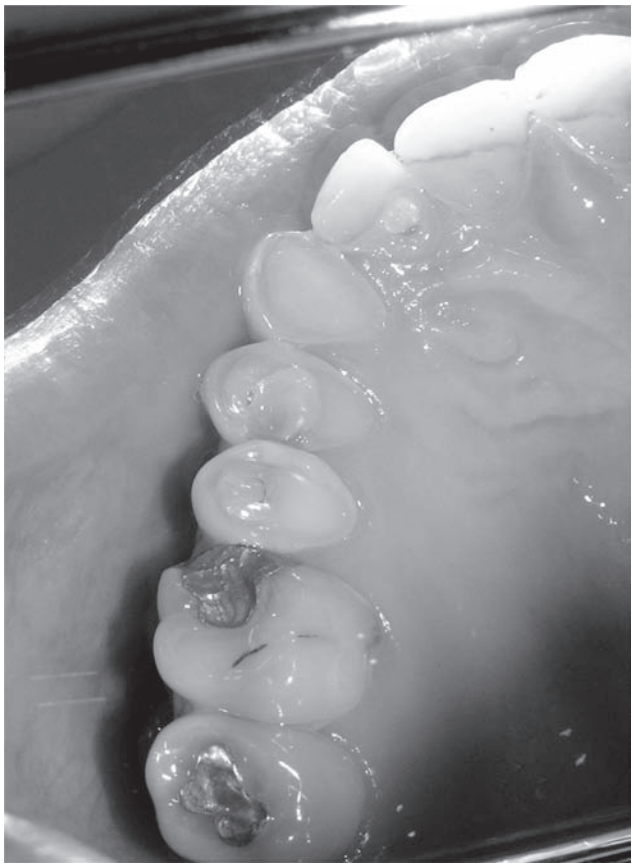

Figure 3. Loss of incisal/occlusal and palatal enamel from left maxillary incisors, canines premolars and molars

Table 1. Tooth wear index (TWI) score

Groups of teeth	cervical (mean ± SD)	buccal (mean ± SD)	lingual (mean ± SD)	***occlusal/incisal (mean ± SD)***
anterior maxillary	0.83 ± 0.75	1.00 ± 0.89	4.00 ± 0.00	1.67 ± 1.03
posterior maxillary	0.50 ± 0.53	0.00 ± 0.00	2.00± 1.69	2.00 ± 1.69
anterior mandibular	1.67 ± 1.21	0.50 ± 0.83	0.00 ± 0.00	0.33 ± 0.81
posterior mandibular	1.86 ± 1.77	1.00 ± 0.81	0.43 ± 0.53	1.14 ± 1.07

anterior teeth - incisors and canines,
posterior teeth - premolars and molars

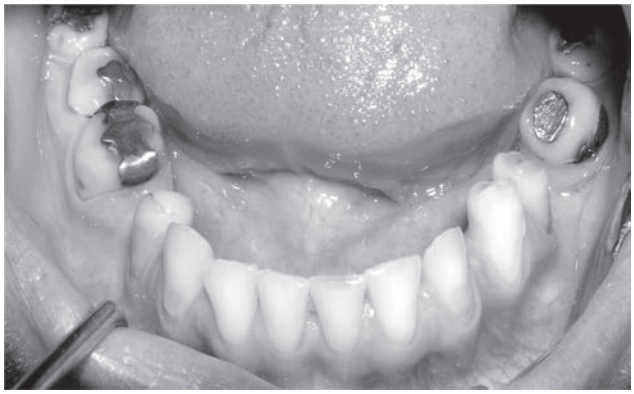

Figure 4. Loss of enamel from incisal edge of left central incisor, cervical third of facial surfaces of mandibular teeth, and occlusal surfaces of mandibular posterior teeth

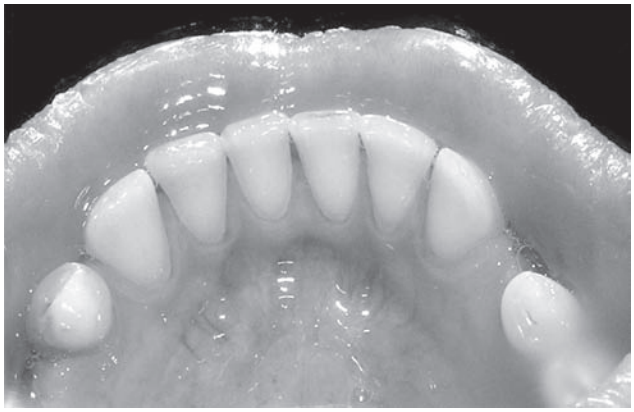

Figure 5. Initial loos of enamel in cervical third of lingual surfaces of mandibular anterior teeth (33, 41, 42, 43)

Dentin was exposed on incisal edge of mandibular central incisor (Fig. 4). More or less enamel loss in cervical third of facial surfaces was present in almost all mandibular teeth (Fig. 4). Lingual cervical tooth wear was present on mandibular anterior teeth and first premolars (Fig. 5). For cervical third, TWI score was 1.67 for mandibular anterior, and 1.86 for mandibular posterior teeth, and grade 2 was found for index proposed by Lussi. Dentin was exposed on occlusal surfaces on left second premolar and mandibular molars (Fig. 4). For these surfaces, TWI score was 1.14, while grade 2 was assessed for Lussi index.

During oral examination, it was also observed that amalgams restorations had raised margins. Carious lesions were observed on approximal surfaces of maxillary canine and incisors. For further observing and managing of tooth wear, the models of the patient's teeth were also made.

Clinical examination also included a determination of salivary flow rate. A non-stimulated and paraffin-chewing stimulated whole saliva were collected by the spitting method and salivary flow rate were 0.22 ml/min and 0.80 ml/min (low flow rate according to Ericsson and Hardwick) [6], respectively.

We referred the patient to a gastroenterologist for evaluation of gastroesophageal reflux disease (GERD). The report from the medical evaluation confirmed pathological reflux and the gastroenterologist prescribed the appropriate therapy to the patient.

Discussion

In the present case, according to patient description, tooth changes started as wear of the incisal edges and labial surfaces of frontal maxillary teeth 9 years ago. Concerning the fact that the patient's dietary habits included frequent consumption of acid salad dressing, it is possible that this acid was initial factor for wearing of her frontal teeth. It is well known that patients exposed to acids from an extrinsic source mostly exhibit a damage of the labial or incisal surfaces of the upper anterior teeth[10] with severity decreasing posteriorly, which was in accordance with our findings. Also, it can be supposed that acid from salad dressing caused superficial demineralization of dental hard tissue of other teeth, but these sub-clinical erosive lesions had been probably overlooked by dentists (facts from a case history). Having in mind the patient's long term bad habit of chewing nails, it could be supposed that abrasion of incisal edges

induced by this habit aggravated erosions induced by acid consumption. It is in accordance with findings that extrinsic acids, as well as intrinsic acids, decrease the wear resistance of dental hard tissue[4].

Clinical examination revealed that the most severe tooth wear was observed on palatal and incisal/occlusal surfaces of anterior and posterior maxillary teeth, while the most affected surfaces in the mandible were cervical thirds of facial and lingual surfaces of anterior and cervical thirds of buccal, and occlusal surfaces of posterior teeth. The distribution of the tooth wear, essentially incisal and palatal on maxillary anterior teeth and occlusal and buccal cervical on mandibular posterior teeth is the most common dental sign of the effects of gastric acids due to GERD or frequent vomiting[18]. Erosion of lingual aspect of mandibular anterior teeth, observed in our patient, is the rarest form of dental erosion, and it is found uniquely in chronic gastroesophageal reflux and in older bulimic patients[16]. Clinical finding of tooth wear pattern in our patient indicated that gastric acid, but not vomiting and bulimia, was most likely the main cause of wear since patient complained only about hart-burn, and medical diagnosis of GERD was established. The association of GERD with dental erosion has been established in a number of studies, and moreover, dental erosion may serve as a diagnostic sign of acid reflux[7,9,12]. Majority of people experience only mild reflux symptoms, which are commonly tolerated[2], or experience no GERD symptoms at all. Bartlett et al[2] introduced the term "silent reflux" to describe such patients, and suggested that in the absence of reflux symptoms, the oral manifestations of GERD may be the only clinical sign of pathological reflux. In one dental study, up to 25% of subjects presenting with dental erosion were observed to have pathological levels of reflux despite not having any symptoms of GERD[1]. Moreover, development of erosive lesion, especially lingual erosion of mandibular teeth, depends on the reduced salivary flow[16]. In connection with this is the fact that in our patient we estimated low flow rate for non-stimulated and stimulated whole saliva.

Loss of dental hard tissue observed in cervical third of buccal surfaces of almost all mandibular teeth was caused by action of acids, but probably aggravated by patient's oral hygiene habit. Namely, according to the dental history, patient frequently used toothpastes with horizontal tooth-brushing technique after meals. Several studies have shown that the loss of tooth substance after ingestion of erosive food stuffs is accelerated by tooth-brushing[4].

Having in mind that patient has a medical history of non-classic form of congenital adrenal hyperplasia (CAH), we analyzed possible association with the observed tooth wear. The disease is characterized by increased circulating levels of androgens. Androgen excess in adulthood may result in an increased bone mineral density and in a reduced fat mass percentage[17]. Since the data concerning the influence of CAH on teeth is very rare and related only to faster development of dentition[3,14], it seems that there is no relationship between CAH and tooth wear in our patient.

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