BALKAN JOURNAL OF DENTAL MEDICINE

Successor of the **BALKAN JOURNAL OF STOMATOLOGY**

Official publication of the **BALKAN STOMATOLOGICAL SOCIETY**

Volume 19 **No 1** **March 2015**

ISSN 2335-0245

BALKAN JOURNAL OF DENTAL MEDICINE

ISSN 2335-0245

Publisher: BALKAN STOMATOLOGICAL SOCIETY, Belgrade Serbia

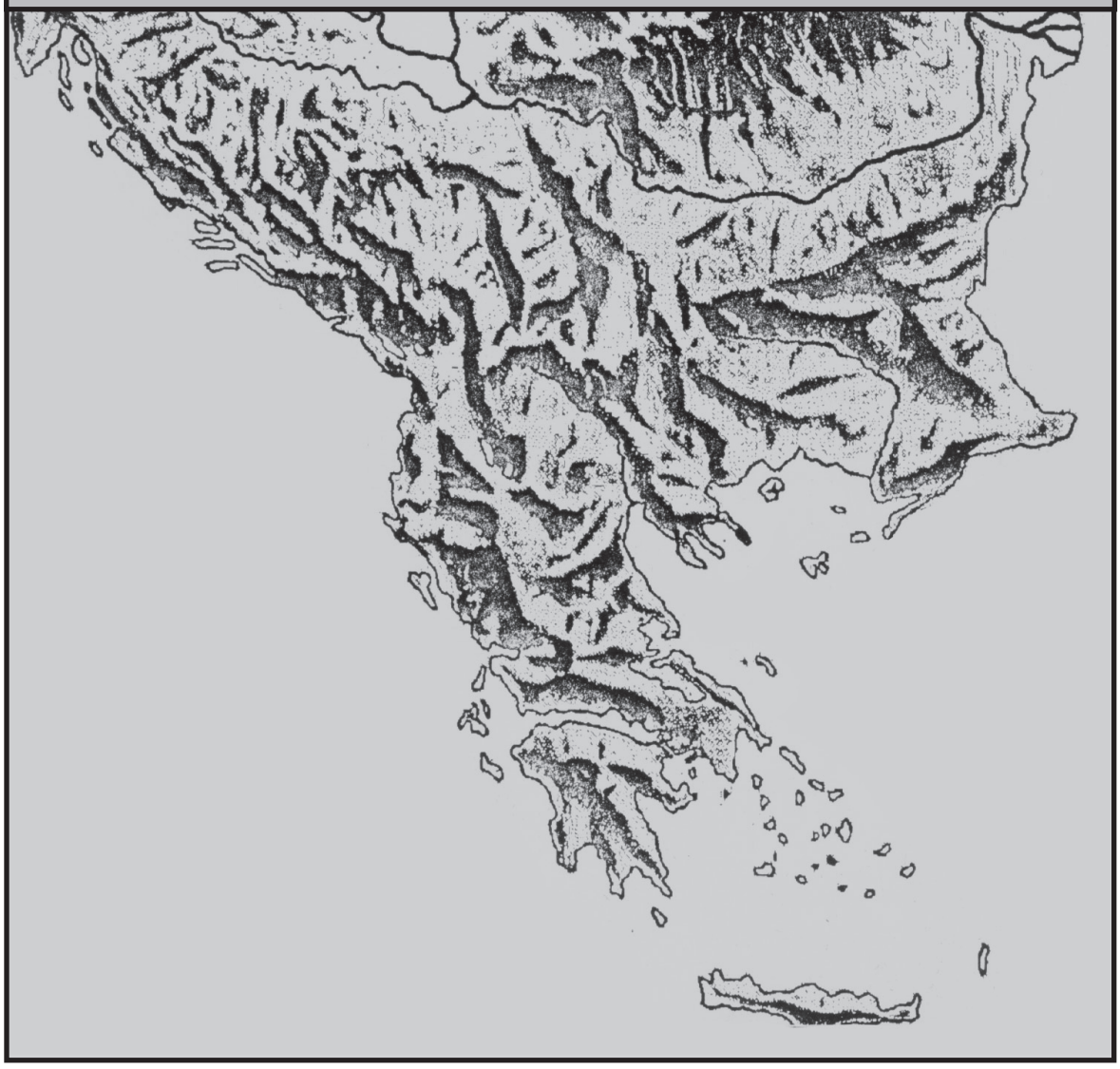
The whole issue is available on-line at the web address of the BaSS (www.e-bass.org)

BALKAN JOURNAL OF DENTAL MEDICINE

Successor of the **BALKAN JOURNAL OF STOMATOLOGY**

Official publication of the **BALKAN STOMATOLOGICAL SOCIETY**

Volume 19 **No 1** **March 2015**



ISSN 2335-0245

BALKAN JOURNAL OF DENTAL MEDICINE

ISSN 2335-0245

VOLUME 19 **NUMBER 1** **MARCH 2015** **PAGES 1-56**

Contents

BALKAN JOURNAL OF DENTAL MEDICINE

BaSS

ISSN 2335-0245

Indication and Technical Application of Stripping

SUMMARY

Interproximal enamel reduction is a technique used for creating space in cases of mild or moderate crowding. There are several indications and contraindications determined by the tooth shape and the percentage of the caries index. The instruments used for the technique are diamond-coated metal strips, diamond discs and burs, each with advantages and disadvantages. Another way of interdental enamel reduction is using chemical products such as orthophosphoric acid. There are opposite impacts on the enamel surface and the periodontal tissues, based on the literature review.

Keywords: stripping, indications, methods

A.A. Georgiadis[1], P. Darmanin[1], N. Topouzelis[2], I. Ioannidou-Marathiotou[2]

Aristotle University of Thessaloniki
School of Dentistry
[1]Student
[2]Department of Orthodontic
Thessaloniki, Greece

INFORMATIVE PAPER (IP)
Balk J Dent Med, 2015; 19:3-7

Introduction

Dental crowding is one of the commonest problems in dental practice and, particularly, in orthodontics. The main ways of resolving this problem are to increase the length of the dental arches and to reduce tooth mass, which is achieved through tooth extractions or by using the method of interproximal enamel reduction. Interproximal enamel reduction (stripping) is not a painful way to reduce enamel thickness at the contact points of the teeth, usually in order to create the space necessary to settle moderately crowded teeth or to change teeth morphology. The main role of the enamel is to protect teeth from abrasion and chemical or thermal stimuli. Stripping, as a way of resolving crowding problems in orthodontics, should be used when appropriate conditions, indications and criteria exist, and after informing the patient about potential consequences.

In 1944, Ballard first described enamel reduction to eliminate dental abnormalities responsible for crowding in the dental arch in the future[29], while Hudson, in 1956, presented a study in interproximal enamel reduction, involving lower anterior teeth[15].

In 1985, Sheridan described the technique of enamel reduction using a high speed air-rotor (Air-Rotor Stripping) and, since then, several studies have dealt with stripping, the space that may be gained using this technique and its potential biological consequences affecting the teeth and the periodontal tissues[21].

Indications

Favourable stripping factors are a triangular shape of teeth and a low caries risk index. A triangular shape of teeth allows gaining more space with less enamel reduction. The technique is applied in cases of mild or moderate crowding of both anterior and posterior teeth. As for the amount of crowding that may be tackled, options vary. It is claimed that up to 6.4mm or up to 9.8mm may be gained with molar and premolar stripping, removing almost 50% of the enamel of mandibular premolars and first and second mandibular molars[22,23,26].

Another view supports that the suitable size of crowding that can be resolved through interdental enamel reduction is 5-6mm[6]. It is important to consider: a) that enamel thickness is not necessarily related to tooth size and diminishes from the contact point to the cementoenamel conjunction; b) that enamel thickness of upper first premolars seems to be distal rather than

proximal (1.29mm distally on both sides, 1.08mm proximally on the right side and 1.19 mm proximally on the left side)[8]; c) that enamel thickness on molars is also increased distally rather than proximally and significantly increased when compared with that on premolars; d) that there is no difference in enamel thickness in relation to the gender of the patient[26]; e) that canine distal surface is preferred for air-rotor stripping (ARS), especially in cases of moderate crowding, due to greater quantity of enamel at this site[10].

In addition to crowding, the ARS technique can also be applied in cases of mild middle line deviation by a dental problem, cases of increased overjet or overbite or for aesthetic reasons, especially when the phenomenon of 'black triangles' appears in anterior areas (Fig. 1).

Figure 1. Stripping application in "black-triangle" phenomenon appearance with the use of a diamond-disc: A) Initial/primary situation; B) Stripping with diamond disc. The ¾ of the disc are covered with protective equipment (disc-guard); C) Enamel reduction in the surfaces of the teeth; D) Elimination of the "black-triangle" phenomenon after stripping and orthodontic treatment

Cases involving Bolton discrepancies are an indication for implementing the ARS technique, particularly when the discrepancy exceeds 1.5mm. A negative unilateral canine relationship may be caused by excessive second mandibular bicuspid width, where stripping can offer a solution[17]. Moreover, stripping may be applied when a second deciduous molar remains longer than necessary and the space for adaptive manoeuvring should be exploited, e.g. when second premolars are congenitally missing[19]. It is useful to consider that occlusal relations may be corrected or negatively affected after stripping and, therefore, it is necessary to evaluate beforehand the (occlusal) relationship of canine and molar cusps. Furthermore, the lateral occlusion of dental arches is usually determined by interproximal width of maxillary lateral incisors and maxillary incisors. According to C. F. Gugino, 70% of orthodontic cases are in need of stripping[17].

Contraindications

Nevertheless, stripping should be avoided in patients with a high caries risk index[23] and in cases of dysplasia or malformation when teeth have parallel adjacent surfaces - as stripping might cause root contact with consequent damage to the periodontium since alveolar and mid-alveolar bone resorption resistance is proportional to the interproximal width of the tooth crown[17] - and, of course, in cases when clinical and laboratory examination indicate that enamel reduction offers an insufficient solution to the problem.

As always, cases indicated for stripping should be treated in accordance with protocols appropriate for the technique used.

Stripping Techniques

Stripping techniques include the use of diamond discs, diamond-coated metal strips and burs. Firstly, thin metal strips or single coated diamond discs, which are both accurate and safe, are used to separate the teeth contact point. Additionally, separation may be effected by small separation rubber rings or brass wire, placed at adjacent dental surfaces. Diamond discs for interdental enamel reduction are available in several sizes and various surface diamond grains. Disc coating may be single or double.

Conventional low speed handles do not provide sufficient torque to the discs. Handles, instead, must operate at a speed of 4000-20000 rpm to generate the necessary torque, so that enamel reduction may be more effective[12].

A disadvantage of this method is that it entails a risk of injury to the cheek or the tongue; this is limited by using protective equipment that covers ¾ of the disc and leaves the disc cutting area uncovered (Fig. 1B).

Unfortunately, such equipment reduces working field visibility and, therefore, special care is required so as to avoid injuring the gingiva, to control the amount of enamel reduction and to avoid destroying the anatomical contour of the teeth. Wedging the disc between the teeth should also be avoided, because it may have negative consequences for the patient (e.g. tongue injury). Stripping with diamond coated discs should be completed by polishing the surface with a Sof-Lex disk; this results in a smooth enamel surface and removes enamel surface furrows that favour plaque concentration, as indicated in a recent study comparing different stripping methods in deciduous and permanent teeth[1]. It has been observed that enamel roughness, following the use of diamond disks and polishing with Sof-Lex discs, is so mild that it is comparable with the enamel surface of teeth that have not undergone any stripping (with intact enamel surfaces). Another advantage of this method is its short duration, since it can be limited to 2.2 minutes per session[32].

Diamond-coated metal strips provide another method for interproximal enamel reduction. They are indicated in cases of mild crowding, when a small amount of enamel needs to be removed. Metal strip coating may be single or double and of course, medium or fine grain size. The strips can be used manually with the assistance of special handles or be motorized with a special low speed handpiece (Fig. 2A). First of all, metal strips are placed between teeth contact points. Then the strips are forced to perform buccal-to-lingual movements until a sufficient amount of enamel has been removed.

Diamond-coated metal strips include the more advanced intensive ortho-strips. Specifically, these are strips used with low speed handles and they reduce enamel through anterior-posterior movements. Depending on their function, type and grain size, such strips may be selected from a wide range available[9]. The thinner ones are capable of reducing enamel from 0.140 to 0.160mm and may be used to separate the contact point of teeth or to polish at the final stage of the procedure. The medium ones, that can reduce enamel from 0.270 to 0.330mm, are used both for enamel reduction and for contouring the tooth after the procedure. Finally, coarse grain strips can cut enamel from 0.370 to 0.560mm and are used for the main enamel reduction in cases of crowding and for the removal of dental materials, such as composite resin or amalgam. A very important advantage of metal strips is that they can access areas regardless of the slope and shape of the teeth, which is an advantage discs do not offer. Appropriate care and attention are necessary on the part of the dentist during the procedure, so as to avoid any injury to the gingiva and periodontal tissues. Moreover, special attention should be paid to the fact that the area gained after interdental enamel reduction may be misleading because teeth move away from each other due to the movements and tools used during the procedure. After removing the strip, the teeth move back to their normal position in the periodontium (rebound phenomenon) and that is when the actual space gained through interdental enamel reduction becomes apparent[23] (Fig. 2B).

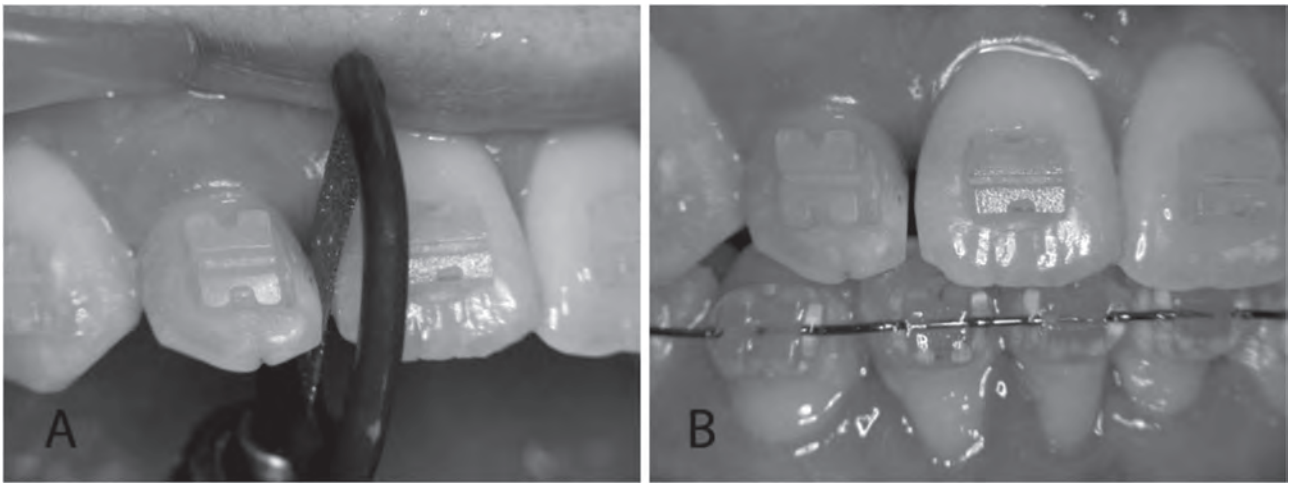


Figure 2. A) Stripping with a motorized metal strip; B) The real space gained (after stripping and rebound-phenomenon)

Burs placed on electric handpieces or air rotors provide another method for stripping. Sometimes there is a problem of controlling and regulating the speed of the handpiece when an air rotor is used and this entails the risk of damaging the tooth or soft tissues. An electric handpiece, on the other hand, can achieve the same speed as an air rotor while being capable of controlling and regulating speed at levels as low as 100rpm[12]. Burs used in stripping are appropriately designed so as to leave thin and smooth interproximal enamel surfaces. Burs should be safe-tipped, as this provides the advantage of creating a ledge on the enamel surface because their tips have no cutting edge.

Unlike strips and discs, for which a 50% enamel reduction is recommended, the ARS method is much more conservative and offers only a 1/3 decrease in the contact area. This corresponds to 0.5 mm in the widest area and 1 mm at the contact point[3]. More specifically, the stages of the technique using burs described by Sheridan are the following: First, a safe-tipped crosscut fissure carbide 699L bur is used for initial enamel reduction between teeth. Then, a 100 micron medium-grit, tapered diamond bur is used for smoothening and contouring teeth, followed by a 30 micron fine-grit, tapered diamond bur for extra smooth surface. Finally, a 15 micron extra-fine grit, tapered diamond bur is used to complete polishing.

In order to control the amount of enamel reduction at contact points, it is recommended to use a 0.40" steel indicator in the form of an open spring placed under the contact point between teeth. Enamel reduction is performed up to the point that allows the indicator to be removed. If more enamel reduction is required, stripping continues after the indicator has been removed. In this method, a water spray is necessary to avoid overheating; this also flushes the area, cleans the contact point and extends bur life. The highest amount of enamel reduction recommended at each contact point is 1mm. Enamel is thinner in mandibular incisors and maxillary lateral incisors; in these cases, the amount of enamel that can be removed is limited to 0.5mm at each contact point[9,24]. However, in a previous study, Hudson limits the enamel that can be removed to 0.2mm at each contact point for central incisors, 0.25mm for lateral incisors and 0.3mm for canines, thus gaining a space of 3mm[15]. Furthermore, there are views that up to 4mm crowding among anterior teeth may be resolved by reducing 0.3mm of enamel at each adjacent surface of each incisor (central and lateral) and 0.4mm at each canine surface[27].

Another way of achieving enamel reduction is through the use of chemical products. The particular chemical process followed is using 37% orthophosphoric acid on adjacent surfaces resulting in etching and further facilitating mechanical reduction. This method leaves a smoother enamel surface and increases potential enamel remineralisation in the area[16].

The structure and morphology of teeth that have undergone the procedure should be as close as possible to that of normal teeth so that proper contact points may be achieved following tooth alignment. An additional aim is to create a larger contact area and to avoid a conical tooth shape on the gingiva side, because this is a factor that favours plaque accumulation[23].

Effects of the Technique

In the past, stripping was suspected as a risk factor for future dental caries of teeth that had undergone this procedure, because, after the end of stripping, several furrows remain on the enamel surface, a fact that favours plaque accumulation[16,18,20]; besides, enamel that has been stripped is more vulnerable to demineralisation[7]. Therefore, finer diamond tools should be used for enamel reduction, since they leave shallower furrows than coarse grain, and the enamel surface should be polished after stripping[19,32].

A disadvantage of the technique is that pulp temperature may increase during stripping. Particular attention should be paid to the fact that during stripping with carbide burs, pulp temperature rises above the critical 5.5ºC. This requires taking precautions to cool the teeth during the procedure, especially when the dentist uses a high speed handpiece[4]. It has been proved that the use of an 8-blade tungsten carbide bur for enamel reduction, in combination with a Sof-Lex disc for polishing, provides the advantage of leaving an enamel surface that is smoother than one that had not been stripped at all[1].

Moreover, it has been shown that enamel surfaces that have undergone the procedure of enamel reduction using stripping in accordance with appropriate method protocols did not become more vulnerable to decay than teeth that had not undergone stripping, since new caries occurred in 2.5% of the teeth stripped as compared to 2.4% of intact enamel surfaces in a total of 43 patients[30]. This fact is supplemented by another research study indicating that stripping leads to enamel demineralisation followed by remineralisation over a period of 9 months[10].

However, the dentist should take into consideration caries and caries risk index of each patient. A local fluoride compound should be applied on surfaces that have been subjected to the procedure[28,30]. Other authors suggest application of sealants after interproximal enamel reduction[25]. Furthermore, it has not been proven that conditions like tooth hypersensitivity, reaction to hot or cold stimuli, periapical lesions or bone loss during orthodontic tooth alignment are a result of stripping, and no negative effects on periodontal health have been

found[31]. Due to the correction of the crowding, tooth surfaces in interproximal areas can be cleaned better, thus reducing plaque accumulation and, therefore, periodontal disease. It has also been proved that there is no difference in periodontal tissues after stripping, while, in some cases, stripping improves periodontal health, even in teeth that have been orthodontically aligned[2,5,11,31]. Particular attention should be paid to the fact that, unlike the extraction method, neither canine-to-canine distances nor the perimeter of dental arches change but remain almost the same after stripping in class I patients[13]. In addition, stripping does not alter a patient's profile (something that might be caused by tooth extraction), while it improves tooth size and shape, thus helping eliminate the phenomenon of 'black triangles'.

Moreover air-rotor stripping (ARS) helps create flat surfaces at mandibular incisor contact areas, reduces incisor labial tipping, and, therefore, reduces potential future relapse[19]. Additionally, ARS helps perfect tooth alignment as opposed to tooth extraction[13]. A final point to be noted is that the time of the overall orthodontic treatment period is reduced, as compared to extraction method[14].

References

1. *Arman A, Cehreli SB, Ozel E, Arhun N, Çetins Ahin A, Soyman M.* Qualitative and quantitative evaluation of enamel after various stripping methods. *Am J Orthod Dentofacial Orthop*, 2006; 130:131.e7-131.e14.
2. *Artun J, Kockich VG, Osterberg SK.* Long-term effect of root proximity on periodontal health after orthodontic treatment. *Am J Orthod Dentofacial Orthop*, 1987; 91:125-130.
3. *Ballard R, Sheridan JJ.* Air-rotor stripping with the Essix anterior anchor. *J Clin Orthod*, 1996; 30:371-373.
4. *Baysal A, Uysal T, Usumez S.* Temperature Rise in the Pulp Chamber during Different Stripping Procedures. *Angle Orthod*, 2007; 77:478-482.
5. *Betteridge MA.* A method of treatment for incisor crowding. *Br J Orthod*, 1979; 6:43-48.
6. *Betteridge MA.* The effects of interdental stripping on the labial segments evaluated one year out of retention. *Br J Orthod*, 1981; 8:193-197.
7. *Bonetti AG, Zanarini M, Serena IP, Marchionni S, Checchi L. In vitro* evaluation of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) effect on stripped enamel surfaces. A SEM investigation. *J Dent*, 2009; 37:228-232.
8. *Carvalho AM, Vellini-Ferreira F, Scavone-Junior H, Ferreira RI.* Mesiodistal width and proximal enamel thickness of maxillary first bicuspids. *Braz Oral Res*, 2010; 24:58-63.
9. *Chudasama D, Sheridan JJ.* Guidelines for Contemporary Air-Rotor Stripping. *J Clin Orthod*, 2007; 41:315-320.
10. *El-Mangoury, Moussa, Mostafa, Girgis.* In vivo remineralization after air-rotor stripping. *J Clin Orthod*, 1991; 25:75-78.
11. *Fillion D.* Apport de la sculpture amilaire interproximale a l'ortodontie de l'adulte. *Rev Orthop Dento Faciale*, 1993; 27:189-214.
12. Florman M, Lobiondo PE, Partovi M. Creating Space with Interproximal Reduction. A Peer-Reviewed Publication.
13. *Germec-Cakan D, Taner TU, Akan S.* Arch-width and perimeter changes in patients with borderline Class I malocclusion treated with extractions or without extractions with air-rotor stripping. *Am J Orthod Dentofacial Orthop*, 2010; 137:734.e1-734.e7.
14. *Germeç D, Taner TU.* Effects of extraction and nonextraction therapy with air-rotor stripping on facial esthetics in postadolescent borderline patients. *Am J Orthod Dentofacial Orthop*, 2008; 133:539-549.
15. *Hudson AL.* A study of the effects of mesiodistal reduction of mandibular anterior teeth. *Am J Orthod*, 1956; 42:615-624.
16. *Joseph VP, Rossouw PE, Basson NJ.* Orthodontic microabrasive reproximation. *Am J Orthod Dentofacial Orthop*, 1992; 102:351-359.
17. *Langlade M.* Diagnostic orthodontique. Paris: *Maloine S.A*, 1981; pp 574-575.
18. *Piacentini C, Sfodrini G.* A scanning electron microscopy comparison of enamel polishing methods after air rotor stripping. *Am J Orthod Dentofacial Orthop*, 1996; 109:57-63.
19. *Proffit W, Fields HW.* Contemporary Orthodontics. St. Louis: *Mosby*, 2000.
20. *Radlanski RJ, Jiiger A, Schwestka R, Bertzbach F.* Plaque accumulations caused by interdental stripping. *Am J Orthod Dentofacial Orthop*, 1988; 94:416-420.
21. *Sheridan JJ.* Air-rotor stripping. *J Clin Orthod*, 1985; 19:43-59.
22. *Sheridan JJ.* Air-rotor stripping update. *J Clin Orthod*, 1987; 21:781-788.
23. *Sheridan JJ.* The Updated Air-Rotor Stripping (ARS) Manual. (in review). New Orleans: Raintree Essix LLC, 2005.
24. *Sheridan JJ, Hastings J.* Air-rotor stripping and lower incisor extraction treatment. *J Clin Orthod*, 1992; 26:18-22.
25. *Sheridan JJ, Ledoux PM.* Air-rotor stripping and proximal sealants - a SEM evaluation. *J Clin Orthod*, 1989; 23:790-794.
26. *Stroud JL, English J, Buschang PH.* Enamel thickness of the posterior dentition: Its implications for nonextraction treatment. *Angle Orthod*, 1998; 68:141-146.
27. *Tuverson DL.* Anterior interocclusal relation. *Am J Orthod*, 1980; 78:361-370.
28. *Twesme DA, Firestone AR, Heaven TJ, Feagin FF, Jacobson A.* Air-rotor stripping and enamel demineralization in vitro. *Am J Orthod Dentofacial Orthop*, 1994; 105:142-152.
29. *Vickers DI.* Proximal stripping and enamel surface roughness. Masters Thesis. Univ. of Sydney: Department of preventive dentistry, 1982; pp 3-4.
30. *Zachrisson BU, Minster L, Øgaard B, Birkhed D.* Dental health assessed after interproximal enamel reduction: Caries risk in posterior teeth. *J Orthod Dentofacial Orthop*, 2011; 139:90-98.
31. *Zachrisson BU, Nyøygaard L, Mobarak K.* Dental health assessed more than 10 years after interproximal enamel reduction of mandibular anterior teeth. *J Orthod Dentofacial Orthop*, 2007; 131:162-169.
32. *Zhong M, Jost-Brinkmann PG, Zellmann M, Zellmann S, Radlanski RJ.* Clinical Evaluation of a New Technique for Interdental Enamel Reduction. *J Orofac Orthop*, 2000; 61:432-439.

Correspondence and request for offprints to:

Nikolaos Topouzelis
Aristotle University of Thessaloniki
Dental School, Department of Orthodontics
GR-54124 Thessaloniki, Greece
E-mail: ntopouz@dent.auth.gr

BALKAN JOURNAL OF DENTAL MEDICINE

BaSS

ISSN 2335-0245

Relationship among Lower Arch Length, Arch Width and Arch Perimeter in Crowding and Non-Crowding Groups

SUMMARY

Crowding is one of the causes of class I malocclusion. The purpose of the present study was to examine the relationship between arch length, arch width and arch perimeter in crowded and non-crowded arches, as well as to made comparison of the right and left sides between them and to find out the contributing factor in lower arch crowding. The study groups consisted of 60 subjects aged 16 to 21 years. First group consisted of 30 pairs of dental study models with class I normal occlusion. The second group consisted of 30 pairs of study models with class I crowding. Measurements of arch length and width were made as defined by Lavelle and Foster, using Korkhaus callipers. Arch perimeter was measured by Lundstrom method's using manual calliper with sharp points. Differences between these measurements were made by Mann-Whitney U test (Z/U).

According to our study, the arch length and arch perimeter were not associated factors in contribution to lower arch crowding. In association of contributed factors on the lower arch crowding, we could mention the width of the arch, because the differences between the two groups was significantly different.

Key words: Arch Length; Arch Width; Arch Perimeter; Crowding

Mimoza Selmani, Julijana Gjorgova

University "St. Kiril and Metodij"
Faculty of Stomatology
Department of Orthodontics
Skopje, FYR Macedonia

ORIGINAL PAPER (OP)
Balk J Dent Med, 2015; 19:8-12

Introduction

Crowding of teeth is considered as the most common type of malocclusion[7]. The relationship between arch dimensions and crowding has become subject of interest to many investigators which has lead to many conflicting and contradictory views. Arch dimension is explained by arch width, arch length and arch perimeter. An investigation performed by Howe et al[13] compared crowded and non-crowded groups using study models. They indicated that arch dimension made a greater contribution to dental crowding than tooth size. Other investigators found the same correlation between arch dimensions and dental crowding[8,10,21].

Bishara et al[2-5] in the longitudinal study of the changes in dental arches and dentition between early childhood to adulthood, 25-45 years, and 6 weeks to 45 years, observed the increased late incisor crowding in both arches, which was more pronounced in the mandibular anterior segment. Similar findings have been observed in untreated subjects by Lundstrom[15], Sinclair[23] and Little[14]. Hamid and Rahbar[12] found significant relationship between arch dimensions and crowding rather than to tooth size in a Pakistani sample. Carter and McNamara[8] also reported significant reduction in the upper and lower arch lengths with time. Nimkarn et al[18], while studying records of 20 males and 20 females at the University of Pittsburgh by using their study casts, found that only males demonstrated significant correlation between inter-molar arch width discrepancy and crowding.

Crowding may occur due to different reasons, for example growth, decrease in dental arch length, maturation, aging of dentition, mesial drift, soft tissue pressures and tooth morphology[24]. Sanin and Savara[22] evaluated 150 children and reported that children without crowding in the

permanent dentition had larger anterior and posterior widths of the mandibular dental arch. Nordeval et al[20] compared 27 adults with ideal occlusion with slight mandibular crowding and reported no differences in inter-canine width between the groups. The objective of present study was to examine the relationship between arch length, arch width and arch perimeter in crowded and non-crowded arches, to compare right and left side of the lower arch on crowded and non-crowded groups and to define possible contributing factor in lower arch crowding.

Subjects and Methods

The study groups consisted of 60 dental study models of the subjects aged 16 to 21 years. First group consisted of 30 pairs of dental study models with class I (normal) occlusion, selected from students the Dental Faculty Pristine, and they had a complete set of permanent teeth in both jaws (including erupted third molars) with normal occlusion, which had not undergone orthodontic treatment. The second group consisted of 30 pairs of study models with class I crowding, selected from patients seen at the Department of Orthodontics, Dental Faculty Clinical Centre in Pristine.

The inclusion criteria were:

1. complete lower dental arch
2. Angle Class I molar relationship
3. no artificial dental crowns and no anomalies of crown morphology
4. no orthodontics treatment in maxillary and mandibular arch.

All the measurements were made by a single investigator to eliminate inter-examiner variability, and were assessed at least twice.

The following lower arch dimensions were measured on the study models (Fig. 1):

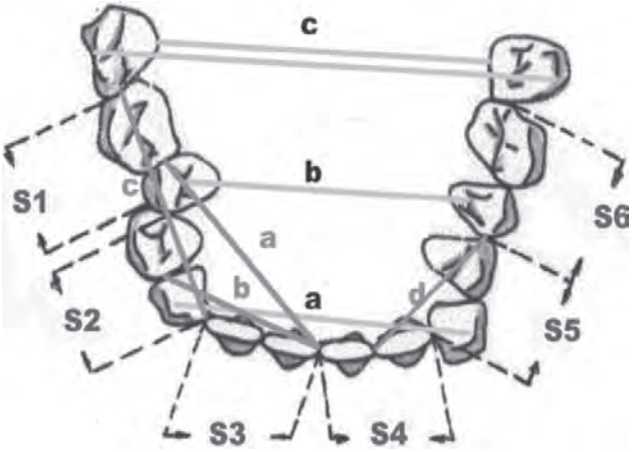


Figure 1. Measurements of lower arch dimensions on study models (Niedzielska)20

1. **Length** - Measurements of arch length were made as defined by Lavelle and Foster, using Korkhaus callipers. These measurements were made in segments (anterior and posterior for right and left sides) and represented

a - distance between the mesial edge point of the medial incisor, and the middle point of the mesial surface of the lower first molar;

b - distance between the mesial edge point of the medial incisor and the middle point of the canine distal surface;

c - distance between the middle point of the mesial surface of the canine and the middle point of the distal surface of the lower first molar;

d - distance between the mesial edge point of the lateral incisor and the middle point of the distal surface of the first premolar.

2. **Width** - Measurements of arch width were also made as defined by Lavelle and Foster, using Korkhaus callipers.

a - distance between the canine cusps (inter-canine width);

b - distance between the second premolars measured in the middle of the intercuspal fissure (inter-premolar width);

c - distance between the buccal surfaces of the lower second molars + distance between the lingual second molar surfaces, divided by two (inter-molar width).

3. **Arch perimeter** was measured by Lundstrom method on the right and left side, using manual calliper with sharp points.

S1 arch segment: first molar and second premolar of the right dental quadrant.

S2 arch segment: first premolar and the canine of the right quadrant;

S3 arch segment: lateral and medial right incisors;

S4 arch segment: medial and lateral left incisors;

S5 arch segments: canine and first premolar in the left quadrant;

S6 arch segment: second premolar and first molar in the left quadrant.

Statistical Analysis

Differences between these measurements of the lower arch/"crowded" and "non-crowding" (right and left side) groups were made by Mann-Whitney U test (Z/U). Significance was determined by $p<0.05$.

Results

Results related to differences in the measurements of the lower arch length between crowded and non-crowded groups, right and left sides, are shown in table 1. Values of arch length (**a-d**) on the right and left side were greater in non-crowded group; however, differences were significant for a on the right side and for b on the both sides.

Table 1. Differences of the measurements of lower arch length between crowding and non-crowding groups, right and left side

Parameter	Rank Sum crowding	Rank Sum non-crowding	U	Z	p	Valid N crowding	Valid N non-crowding
a - right	736.50	1093.50	271.50	-2.64	0.008*	30	30
b - right	689.50	1140.50	224.50	-3.33	0.000*	30	30
c - right	897.50	932.50	432.50	-0.26	0.80	30	30
d - right	884.00	946.00	419.00	-0.46	0.65	30	30
a - left	812.00	1018.00	347.00	-1.52	0.13	30	30
b - left	658.50	1171.50	193.50	-3.79	0.000*	30	30
c - left	981.00	849.00	384.00	0.98	0.33	30	30
d - left	858.50	971.50	393.50	-0.84	0.40	30	30

Differences of analyzed parameters for lower arch width between crowded and non-crowded groups are shown in table 2. As it can be seen, a significant difference was found for all 3 measured widths - inter-canine (**a**), inter-premolar (**b**) and inter-molar (**c**).

Differences of arch perimeters on the right and left sides (S1-S6) between crowded and non-crowded group are shown in table 3. Values of these segments were greater in crowded group in all relationships, but differences for segments S1, S2, S3, and S4 were not statistically significant. Segment S5 of the crowded group was significantly greater compared to non-crowded group, and segment S6 in the lower arch of the crowded group was significantly greater compared to the non-crowded group.

Table 2. Differences of the measurements of the lower arch width between crowded and non-crowded groups in the mandible

Parameter	Rank Sum crowded	Rank Sum non-crowded	U	Z	p	Valid N crowded	Valid N non-crowded
a	546.00	1284.00	81.00	-5.46	0.000*	30	30
b	660.50	1169.50	195.50	-3.76	0.000*	30	30
c	581.00	1249.00	116.00	-4.94	0.000*	30	30

Table 3. Differences of the measurements of the lower arch perimeter between crowding and non-crowding groups (S1-S6)

Parameter	Rank Sum crowding	Rank Sum non-crowding	U	Z	p	Valid N crowding	Valid N non-crowding
S1	1038.00	792.00	327.00	1.82	0.07	30	30
S2	1026.50	803.50	338.50	1.65	0.10	30	30
S3	957.00	873.00	408.00	0.62	0.53	30	30
S4	1028.50	801.50	336.50	1.68	0.09	30	30
S5	1044.00	786.00	321.00	2.01	0.04*	30	30
S6	1133.50	699.50	234.50	3.19	0.001*	30	30

Table 4. Differences between measurements of the lower arch length and arch perimeter in the crowding group on the right and left side

Parameters	Rank Sum right side	Rank Sum left side	U	Z	p	Valid N right side	Valid N left side
a	823.00	1007.00	358.00	-1.36	0.17	30	30
b	940.00	890.00	425.00	0.37	0.71	30	30
c	847.00	983.00	382.00	-1.01	0.31	30	30
d	850.50	979.50	385.50	-0.95	0.34	30	30
S1/ S6	793.00	1037.00	328.00	-1.80	0.07	30	30
S2/ S5	886.00	944.00	421.00	-0.43	0.67	30	30
S3/ S4	836.00	994.00	371.00	-1.17	0.24	30	30

Differences between measurements of the lower arch length on the right and left side are presented in table 4. There were not significant differences between right and left side when the arch length and arch perimeter were measured in the crowding group.

Differences between measurements of the lower arch length and arch perimeter in non-crowding group on the right and left side are shown in table 5. Also, in non-crowding group, we could not find significant differences between these measurements between right and left side.

Table 5. Differences between measurements of the lower arch length and arch perimeter in the non-crowding group on the right and left side

Parameters	Rank Sum right side	Rank Sum left side	U	Z	p	Valid N right side	Valid N left side
a	912.00	918.00	447.00	-0.04	0.96	30	30
b	921.50	908.50	443.50	0.10	0.92	30	30
c	918.50	911.50	446.50	0.05	0.96	30	30
d	838.00	992.00	373.00	-1.14	0.25	30	30
S1/ S6	908.00	922.00	443.00	-0.10	0.92	30	30
S2/ S5	907.50	922.00	442.50	-0.11	0.91	30	30
S3/ S4	915.00	915.00	450.00	0.00	1.00	30	30

Discussion

Crowding is one of the causes of class I malocclusion. Arch form and arch dimensions are 2 important factors in case assessment, diagnosis and treatment planning. Forsberg[9] found that the arch dimensions were more important factor for crowding than the tooth dimensions. Mills[17] in a study of 230 males between 17 and 21 years of age, found a significant association between crowding of teeth and arch width. Furthermore, he stated that little variation existed between crown diameters of persons with and without mal-alignment. In investigations performed by Howe et al[13] comparisons were made between crowded and non-crowded groups using study models. They indicated that arch dimension made a greater contribution to dental crowding than tooth size. McKeown[16] in a study of 65 dental casts collected from subjects between 18 and 25 years of age, found that arch width and crowding were strongly correlated and that a narrow arch predisposed to crowding of teeth. This is an agreement with our study because in that age lower arch width in crowded group was narrow compared with non-crowded group, the difference being significant. It seems, therefore, that narrow arch predisposes to crowding of teeth.

Since the lower arch represents the basic foundation on which the occlusion would be built on[14], announcement about the form of the lower dental arch is of at most importance for orthodontists, even more than the maxillary one. Therefore in our study, all the parameters were measured and analyzed in the lower arch, especially when it comes to the importance of orthodontics treatment.

Some investigators[1,6,11] suggest that other morphological characteristics, such as tooth shape and arch dimensions, play an important role in space discrepancies and these parameters have great implications in orthodontic diagnosis and treatment planning. In our study, we aimed to determine differences in non-crowded and crowded lower arches in terms of arch dimensions to better understand the morphological relationships of these variables with dental crowding.

Previous studies[13] verified that arches with crowding were shorter than those without crowding, which was confirmed in our study where we observed that the arches of the crowded group were shorter in relation with non-crowded arches, but not significantly in all of the analyzed parameters of the arch length. According to Hamid and Rahbar[12], arch length was found greater in non-crowded arches compared to crowded arches, and the differences were statistically significant ($p<0.05$). This is compatible with our findings, because in the non-crowding group the lower arch length was significantly greater than in the crowding group only for some of the analyzed parameters.

Furthermore, we have the arch perimeter greater in crowded group in relation to non-crowded group, but differences between the groups were not significant, except for segments S4 and S5 of the lower arch; therefore, according to our study, the arch length and arch perimeter of the lower arch are not dominant factors that contribute to lower arch crowding. It seems that the width of the lower arch contributes to crowding more as the differences between the 2 groups were significant.

In all the undertaken measurements, when we compared the right and left side of the arch length and arch perimeter of the crowding and non-crowding groups, we did not find significant differences between them.

The findings of our study may be important for orthodontic treatment planning of lower arch crowding correction, as it may have several possibly helpful points to overcome difficulties in orthodontics treatment.

Conclusions

Based on the results of our study, the following conclusions might be drawn:

A significantly greater difference was found between the crowded and non-crowded groups for inter-canine, inter-premolar, and inter-molar widths of the lower arch;

Arch length was greater in non-crowded arches as compared to crowded, but differences were not significant for all of the analyzed parameters;

Arch perimeter was greater in crowded group in relation to non-crowded group, but differences were not significant;

Non significant association was found between crowded and non-crowded arches when the right and left sides were compared.

References

1. *Agenter MK, Harris EF, Blair RN*. Influence of tooth crown size on malocclusion. *Am J Orthod DentofacOrthop*, 2009; 136:795-804.
2. *Bishara SE, Jakobsen JR, Treder JE, Stasi MJ*. Changes in the maxillary and mandibular tooth size arch length relationship from early adolescence to early adulthood. A longitudinal study. *Am J Orthod Dentofac Orthop*, 1989; 95:46-59.
3. *Bishara SE, Treder J, Damon P, Olsen M*. Changes in the dental arches and dentition between 25 and 45 years of age. *Angle Orthod*, 1996; 66:417-422.
4. *Bishara SE, Jacobsen JR, Treder J, Nowak A*. Arch width changes from 6 weeks to 45 years of age. *Am J Orthod Dentofac Orthop*, 1997; 111:401-409.
5. *Bishara SE, Treder JE, Jacobsen JR*. Facial and dental changes in adulthood. *Am J Orthod Dentofac Orthop*, 1994; 106:175-186.
6. *Bernabé E, del Castillo CE, Flores-Mir C*. Intra-arch occlusal indicators of crowding in the permanent dentition. *Am J Orthod Dentofac Orthop*, 2005; 128:220-225.
7. *Bushcang PH, Shulman JD*. Incisor crowding in untreated persons 15-50 years of age. *Angle Orthod*, 2003; 73:502-508.
8. *Carter GA, McNamara JA*. Longitudinal dental arch changes in adults. *Am J Orthod Dentofac Orthop*, 1998; 114:88-99.
9. *Forsberg CM*. Tooth size and spacing in relation to eruption or impaction of third molars. *Am J Orthod Dentofac Orthop*, 1988; 94:57-61.
10. *Gilmore CA, Little RM*. Mandibular incisor dimensions and crowding. *Am J Orthod*, 1984; 86:493-502.
11. *Gianelly AA*. Arch width after extraction and nonextraction treatment. *Am J Orthod Dentofac Orthop*, 2003; 123:25.
12. *Hamid MW, Rahbar MI*. Dental crowding and its relationship to tooth size and arch dimensions. *Pak Oral Dent J*, 2005; 25:47-52.
13. *Howe RP, McNamara JA, Oconnor K*. An examination of dental crowding and its relationship to tooth size and arch dimension. *Am J Orthod*, 1983; 83:363-373.
14. *Little RM, Wallen TR, Riedel RA*. Stability and relapse of mandibular anterior alignment. First premolar extraction cases treated by traditional edgewise orthodontics. *Am J Orthod Dentofac Orthop*, 1981; 80:349-365.
15. *Lundström A*. Changes in crowding and spacing of the teeth with age. *Dent Pract*, 1968; 19:218-224.
16. *Mckeown M*. The diagnosis of incipient arch crowding in children. *NZ Dent J*, 1981; 77:93-96.
17. *Mills LF*. Arch Width, Arch Length and Tooth Size in Young Adult Males. *Angle Orthod*, 1964; 34:124-129.
18. *Nimkarn Y, Miles PO, O'Reil MT*. The validity of maxillary expansion indices. *Angle Orthod*, 1995; 5:321-326
19. *Niedzielska I*. Third molar influence on dental arch crowding. *Eur J Orthod*, 2005; 27:518-523.
20. *Nordeval K, Wisth PJ, Boe OE*. Mandibular anterior crowding in relation to tooth size and craniofacial morphology. *Scand J Dent Res*, 1975; 83:267-273.
21. *Randzic D*. Dental crowding and its relationship to mesiodistal crown diameters and arch dimension. *Am J Orthod Dentofac Orthop*, 1988; 94:50-56.
22. *Sanin C, Savara BS*. Factors that affect the alignment of the mandibular incisors. *Am J Orthod*, 1973; 64:248-257.
23. *Sinclair PM, Little RM*. Maturation of untreated normal occlusions. *Am J Orthod*, 1983; 83:114-123.
24. *Shah AA, Eleock C, Brook AH*. Incisor crown shape and crowding. *Am J Orthod Dentofac Orthop*, 2003; 123:562-567.

Correspondence and request for offprints to:

Prof. Julijana Gjorgova
"Bulevard Oktomvriska revolucija" 432/21
Skopje, Republic of Macedonia
E-mail: jgjorgova@yahoo.com

BALKAN JOURNAL OF DENTAL MEDICINE

BaSS

ISSN 2335-0245

Differences in Pharyngeal Characteristics According to Angle Class of Malocclusion

SUMMARY

Objectives*: To investigate potential differences in the pharynx, the soft palate, the pharyngeal tonsil, and the tongue between patients with different Angle Classes of malocclusion.*

Study Design*: Pre-treatment lateral cephalograms of 116 normal breathing individuals aged between 9 and 12 years were analyzed. 20 linear and 4 angular measurements, as well as 5 variables concerning the surface area of the pharynx and the soft palate were evaluated.*

Results*: The angle formed by the palatal plane and the base of the skull had lower values in Class II groups. The soft palate height was smaller in Class II, div. 1 group. The angle between the soft and hard palates was smaller in Class III, followed by Class I, Class II, div. 2, and Class II, div. 1, with increasing values. The distance of the tongue from the palatal plane was larger in Class I and Class III groups. The surface area of the oropharynx was larger in Class III than in Class II groups. The total surface area of the pharynx had higher values in Class III than in Class II/1.*

Conclusion*: Subjects with Class II malocclusion may be more prone to develop respiratory related disorders, such as obstructive sleep apnea, followed by Class I and Class III subjects.*

Keywords: Malocclusion; Pharynx; Airway

A. Diamantidou[1], N. Topouzelis[2], S. Sidiropoulou-Hadjigianni[2], N. Gkantidis[3]

[1]Private Practice, Thessaloniki, Greece
[2]Aristotle University of Thessaloniki
School of Dentistry, Department of Orthodontics
Thessaloniki, Greece
[3]University of Bern
Department of Orthodontics and
Dentofacial Orthopedics
Bern, Switzerland

ORIGINAL PAPER (OP)
Balk J Dent Med, 2015; 19:13-20

Introduction

In recent years, the increased interest for Obstructive Sleep Apnoea (OSA) has turned the attention of numerous researchers to assessing pharyngeal dimensions. Various techniques have been implemented to image the pharynx, including optic fibres, ultrasonography, and cineradiography[1,2], while the most commonly used methods are CT/CBCT scanning and MRI[3,4]. CT offers the advantage of detailed 3D evaluation of hard-tissue pharyngeal structures, but it is an expensive, high radiation dose method. Also, through MRI, the volume, size, and shape of airways can be measured without ionizing radiation, but this is an even more expensive method, difficult to be performed[5].

On the other hand, lateral cephalometric radiography has been extensively used in craniofacial research, although its use to study anatomical structures such as the pharynx, the soft palate, and the tongue is limited. It is a routinely used radiographic technique in orthodontics, with low cost and low radiation dose. Thus, it can well point to the study of craniofacial features, which have been associated with OSA[2]. A recent study that evaluated MRI and cephalometric images confirmed that the lateral cephalogram is a valid method for measuring dimensions of the nasopharyngeal and retropalatal region[6].

Research based on cephalometry identified high correlation of a specific skeletal morphology, such as a small mandible in a retrognathic position, with OSA[7-9]. Further evidence supports that anteroposterior jaw relationships are associated with airway morphology, which directly affects the OSA[4,10,11]. However, such studies have focused on skeletal criteria for defining their study groups. This is an appropriate methodology for research hypothesis evaluation, but the use of this

information in everyday clinical practice needs special educational background by the dentist. On the other hand, the Angle Classes of malocclusion is a widespread and easy to use diagnostic tool, even for not specially trained clinicians. If a specific relation between the Angle Classes of malocclusion, which is an easy and convenient diagnostic element, and anatomical parameters that affect the airway is evident, this could be used to indicate increased predisposition to airway related disorders, such as the OSA syndrome.

Therefore, the purpose of this study was to evaluate the size and position of the pharynx, the soft palate, the pharyngeal tonsil, and the tongue through lateral cephalometric radiographs and investigate potential differences in these parameters in different Angle Classes of malocclusion in preadolescent individuals with normal breathing.

Subjects and Methods

The study sample consisted of lateral cephalometric radiographs selected from the archive of the Orthodontic Department, Aristotle University of Thessaloniki, based on the following criteria: a) Caucasian origin; b) age between 9 and 12 years; c) free medical history; d) no previous orthodontic treatment; e) no breathing problems detected by clinical examination or mentioned by the patient/parent; f) no surgical excision of the tonsils and/or adenoids; g) no clefts, syndromes or severe craniofacial malformations; h) high quality pre-treatment lateral cephalometric X-rays; and i) malocclusion pattern that could be assigned to 1 of the 4 groups described below:

- Class I: bilateral Class I molar and canine relationship with normal overbite and overjet;
- Class II, division 1: bilateral Class II molar and canine relationship with overjet > 4mm
- Class II, division 2: bilateral Class II molar and canine relationship with overbite > 4mm and overjet < 4mm
- Class III: bilateral Class III molar and canine relationship with overjet ≤ 0mm.

The archive from 2000 to 2004 was searched in a consecutive manner to identify the first 30 subjects that fulfilled the inclusion criteria. In order to obtain sex-balanced groups each gender was allowed to be predominant at most by 1 subject in each group. Regarding the Class III group, only 26 subjects fulfilled the inclusion criteria and were used for the study. A detailed description of the study sample is presented in table 1.

Table 1. Description of the sample used in the present study

Malocclusion group	Gender	Age (Mean ± SD
Class I	14 M, 16F	10.7 ± 0.9
Class II, division 1	14M, 16F	10.5 ± 0.6
Class II, division 2	15M, 15F	10.7 ± 0.7
Class III	13M, 13F	10.3 ± 0.9
Total	56M, 60F	10.6 ± 0.8
P-value	ns[1]	ns[2]

[1]Chi-spuare, [2]One-way ANOVA

All radiographs were obtained from the same radiographic unit that had a magnification of 7%, which was not corrected. Patients were standing with the Frankfurt Horizontal plane parallel to the floor and were asked to have teeth in maximum intercuspation with slight contact, lips in relaxed position, and not to swallow.

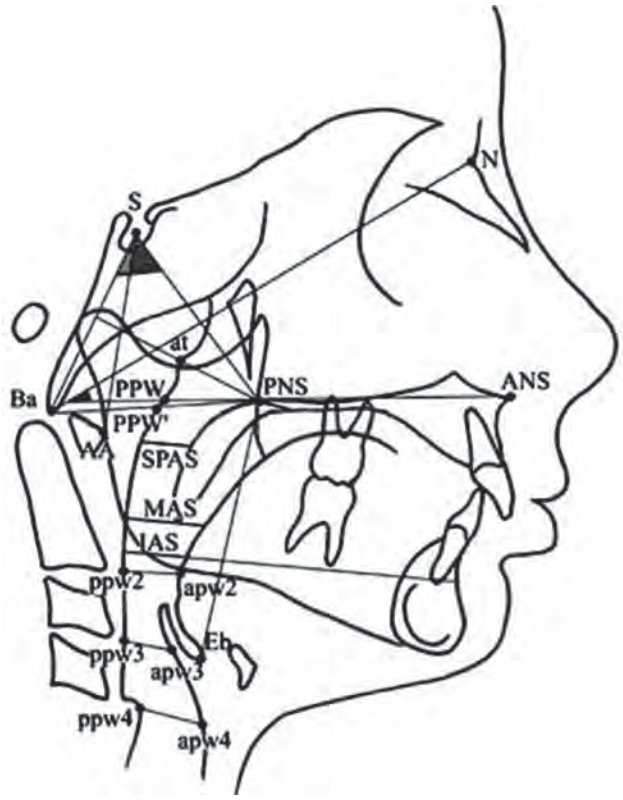


Figure 1. Parameters used to evaluate pharyngeal relationships: PNS-PPW, PNS-at, PNS-PPW', apw2-ppw2, apw3-ppw3, apw4-ppw4, SPAS, MAS, IAS, PNS-Eb, S-AA, NL-BaN, AA-Ŝ-PNS, Ba-Ŝ-PNS.

The X-rays were scanned and 41 points (21 to hard tissues, 19 to soft tissues) were digitized on screen by one investigator using the Viewbox 3 Software, which was appropriately modified for the needs of this study. 20 linear and 4 angular measurements that concerned the soft tissues of the pharynx (Fig. 1), the soft palate (Fig. 2), the pharyngeal tonsil (Fig. 3), and the tongue position (Fig. 4) were obtained. Moreover, 5 variables concerning the surface area of the pharynx and the soft palate[12] (Fig. 5) were calculated using Adobe Photoshop 6.0 software. A detailed description of the variables used in the present study along with the relevant references is provided in Appendix 1.

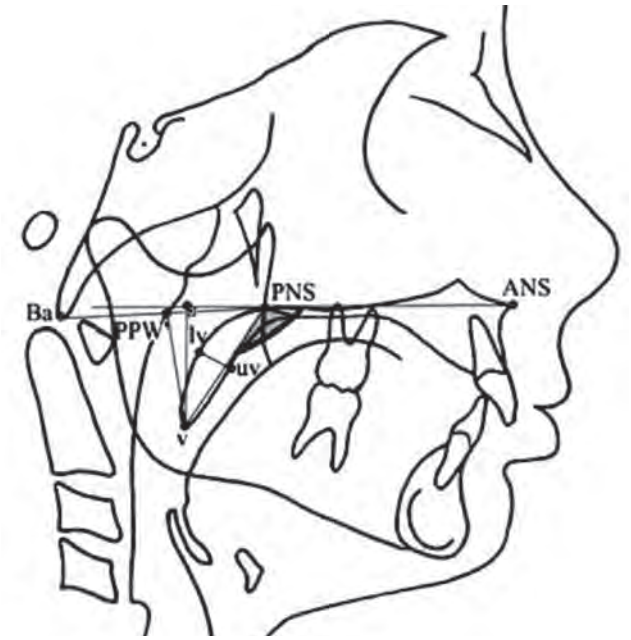


Figure 2. Parameters used to determine soft palate relationships: PNS-v, lv-uv, v- PPW´, v⊥NL, NL-v.

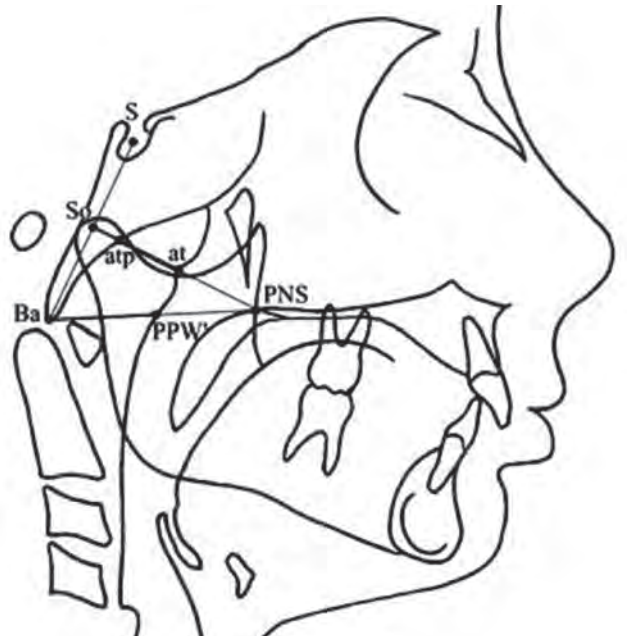


Figure 3. Parameters used to evaluate pharyngeal tonsil relationships: at-atp, PPW'- Ba, at-So.

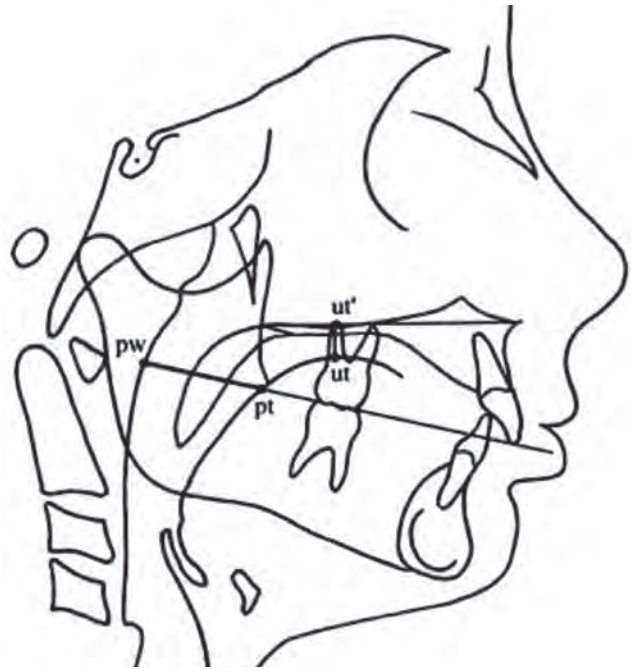


Figure 4. Parameters used to evaluate tongue position: pt-pw, ut-ut'.

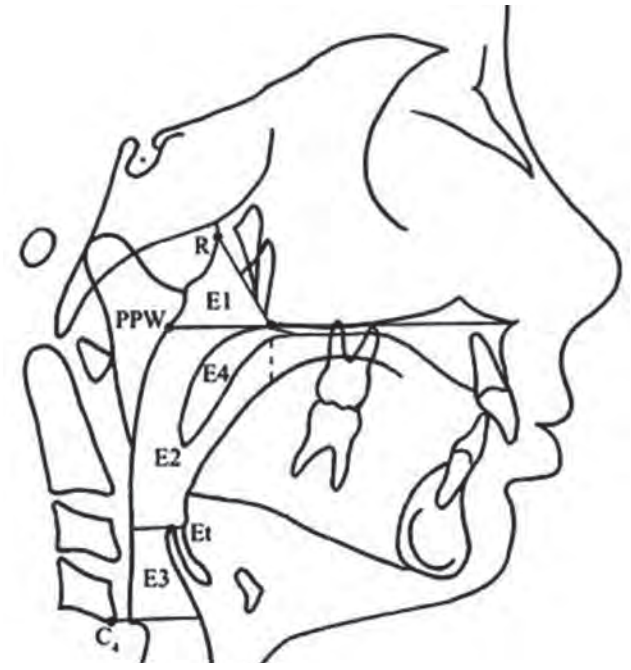


Figure 5. Parameters used to evaluate the surface area of the pharynx and the soft palate: E1, E2, E3, E4, E5.

APPENDIX 1.

Detailed description of the cephalometric variables used in the study along with the relevant references.

Description of the parameters used to evaluate pharyngeal relationships (Figure 1).

PNS-PPW: The width of the rhinopharynx, measured as the distance between the posterior nasal spine and the posterior pharyngeal wall.[1]

PNS-at: The distance between the posterior nasal spine and point *at* on the pharyngeal tonsil.[2,3]

PNS-PPW': the distance between the posterior nasal spine and point PPW' on the posterior pharyngeal wall.[2,3]

apw2-ppw2: the distance between points apw2 and ppw2, which corresponds to the pharyngeal width at the lowest axis border.[4]

apw3-ppw3: the distance between points apw3 and ppw3, which corresponds to the pharyngeal width at the lowest edge of the 3rd cervical vertebra.[1]

apw4-ppw4: the distance between points apw4 and ppw4, which corresponds to the pharyngeal width at the lowest edge of the 4th cervical vertebra.[4]

SPAS (upper airway space): the distance from the posterior wall of the soft palate to the posterior pharyngeal wall at the level of the mid soft palate, along a line parallel to the Go-B plane.[5]

MAS (middle airway space): the distance from the base of the tongue to the posterior pharyngeal wall at the level of the uvula along a line parallel to the Go-B plane.[5]

IAS (lower airway space): the distance from the base of the tongue to the posterior pharyngeal wall, along Go-B plane.[5]

PNS-Eb: the vertical dimension of the upper airway, measured as the distance between points PNS and Eb.[5]

S-AA (posterior craniocervical height): the distance between points S and AA.[6]

NL-BaN: the angle formed by the palatal plane and the base of the skull.[7]

AA-Ŝ-PNS: anterior craniocervical depth.[6]

Ba-Ŝ-PNS: posterior craniocervical depth.[6]

Description of the parameters used to evaluate soft palate relationships (Figure 2).

PNS-v: the length of the soft palate, measured as the distance between the posterior nasal spine and the uvula.[8]

lv-uv: the thickness of the soft palate, measured as the distance between points lv and uv.[8]

v-PPW´: the height of the soft palate, measured as the distance between the uvula and point PPW´ on the posterior pharyngeal wall.[9]

v⊥NL: the height of the soft palate, measured as the projection of point v on the palatal plane.[9]

NL-v: the angle formed between the soft and hard palates.[9]

Description of the parameters used to evaluate pharyngeal tonsil relationships (Figure 3).

at-atp: the width of the pharyngeal tonsil between points at and atp.[1]

PPW'-Ba: the width of the pharyngeal tonsil between PPW' and Ba.[1]

at-So: the width of the pharyngeal tonsil between at and So.[10]

Description of the parameters used to evaluate tongue position (Figure 4).

pt-pw: the distance of the tongue from the posterior pharyngeal wall, measured between points pt and pw.[11]

ut-ut': the distance of the tongue from the palate, measured between the highest point on the tongue dorsal surface (ut) and point ut'.[11]

Description of the parameters used to evaluate the surface area of the pharynx and the soft palate (Figure 5).

E1: the rhinopharyngeal surface area, which is encompassed by points R, PNS and PPW.[5]

E2: the oropharyngeal surface area, which is encompassed by the lowest edge of the rhinopharynx, the posterior surface of the soft palate, the posterior undersurface of the tongue, a line parallel to the palatal plane that goes through point Et, and the posterior pharyngeal wall.[5]

E3: the hypopharyngeal surface area, which is encompassed by the lowest edge of the oropharynx, the posterior surface of the uvula, a line parallel to the palatal plane that goes through point C4, and the posterior pharyngeal wall.5

E4: the surface area of the soft palate, which is encompassed by the perimeter of the soft palate, starting and ending at point PNS having gone through point v.[5]

E5: the total pharyngeal surface area (E1+E2+E3).

References for Appendix 1

1. *Athanasiou AE, Papadopoulos MA, Mazaheri M, Lagoudakis M.* Cephalometric evaluation of pharynx, soft palate, adenoid tissue, tongue, and hyoid bone following the use of a mandibular repositioning appliance in obstructive sleep apnea patients. *Int J Adult Orthodont Orthognath Surg* 9: 273-283, 1994.
2. *Linder-Aronson S, Hernikson CO.* Radiocephalometric analysis of anteroposterior nasopharyngeal dimensions in 6- to 12-year-old mouth breathers compared with nose breathers. *ORL J Otorhinolaryngol Relat Spec* 35: 19-29, 1973.
3. *Lowe AA, Santamaria JD, Fleetham JA, Price C.* Facial morphology and obstructive sleep apnea. *Am J Orthod Dentofacial Orthop* 90: 484-491, 1986.
4. *Athanasiou AE, Toutountzakis N, Mavreas D, Wenzel A.* Alterations on hyoid bone position and pharyngeal depth and their relationship after surgical correction of mandibular prognathism. *Am J Orthod Dentofacial Orthop* 100:259-265, 1991.
5. *Pae EK, Lowe AA, Sasaki K, Price C, Tsuchiya M, Fleetham JA.* A cephalometric and electromyographic study of upper airway structure in the upright and supine position. *Am J Orthod Dentofacial Orthop* 106: 52-59, 1994.
6. *Handelman CS, Osborne G.* Growth of the nasopharynx and adenoid development from one to eighteen years. *Angle Orthod* 46: 243-259, 1976.
7. *Ricketts RM, Roth RH, Chaconas SJ, Schulhof RJ, Engel GA.* Orthodontic diagnosis and planning. USA, Denver: Rocky Mountain Data Systems; 1982.
8. *Mazaheri M. Athanasiou AE. Long RE Jr.* Comparison of velopharyngeal growth patterns between cleft lip and/or palate patients requiring or not requiring pharyngeal flap surgery. *Cleft Palate Craniofac J* 31: 452-460, 1994.
9. *Akcam MO, Toygar TU, Wada T.* Longitudinal investigation of soft palate and nasopharyngeal airway relations in different rotation types. *Angle Orthod* 72: 521-526; 2002.
10. *Linder-Aronson S, Leighton BC.* A longitudinal study of the development of the posterior nasopharyngeal wall between 3 and 16 years of age. *Eur J Orthod* 5: 47-58, 1983.
11. *Ingervall B, Schmoker R.* Effect of surgical reduction of the tongue on oral stereognosis, oral motor ability, and the rest position of the tongue and mandible. *Am J Orthod Dentofacial Orthop* 97: 58-65, 1990.

Descriptive and conclusive statistics were used for the study. The normality of data and homogeneity of variances were tested and confirmed by the Kolmogorov- Smirnov test and Levene's test, respectively. One-way ANOVA analysis was used to check if there was a statistically significant difference among the mean values of the 4 groups. Further *post hoc* analysis with pair-wise comparisons was performed with Waller-Duncan test. The level of significance was set at $p < 0.05$.

To estimate the method error, 35 randomly selected X-rays were re-digitized by the same investigator, after a time period of 3 weeks. Dahlberg's formula was used to calculate consistency between the 2 measurements.

For most variables the method error was under 10% of biological variability. 2 variables, the rhinopharyngeal surface area (E1) and the rhinopharyngeal width at the lowest edge of the 4th cervical vertebra (apw4-ppw4) were highly inconsistent and were not included in the results.

Results

Descriptive statistics of each variable for the 4 groups, as well as group comparisons are presented in table 2. Significant group differences were identified for the following 6 variables: NL-BaN angle, vlNL distance, NL-v angle, ut-ut' distance, E2 surface area, E5 surface area. Further statistical analysis for detecting between-group differences resulted in the following findings:

Table 2. Descriptive statistics and group comparisons for each variable tested (mean ± SC)

	PNS-PPW	PNS-at	PNS-PPW'	apw2-ppw2	apw3-ppw3	apw4- ppw4
Class I	20,0 ± 5,8	13,5 ± 2,8	17,7 ± 4,8	10,6 ± 3,8	12,7 ± 5,4	16,2 ± 4,6
Class II/1	22,2 ± 6,1	14,9 ± 4,2	19,7 ± 5,9	9,4 ± 4,1	12,1 ± 5,5	14,1 ± 4,8
Class II/2	23,1 ± 4,3	18,6 ± 15,4	21,1 ± 4,2	8,9 ± 3,0	12,7 ± 4,7	14,2 ± 2,5
Class III	20,6 ± 5,1	14,7 ± 3,0	20,0 ± 5,1	10,6 ± 4,2	14,1 ± 5,4	13,5 ± 2,7
p	ns	ns	ns	ns	ns	ns

	PNS-Eb	S-AA	NL-BaN	AA-Ŝ-PNS	Ba-S-PNS	PNS-v
Class I	61,9 ± 6,0	49,3 ± 4,9	27,6 ± 2,7^{a}	42,4 ± 4,9	59,3 ± 4,7	32,3 ± 2,6
Class II/1	60,6 ± 6,2	50,6 ± 4,1	25,3 ± 3,0^{b}	42,2 ± 5,1	59,4 ± 5,3	32,4 ± 4,3
Class II/2	59,8 ± 4,2	49,1 ± 4,6	26,6 ± 2,5^{a}'	42,2 ± 4,0	59,8 ± 4,4	32,9 ± 3,6
Class III	60,8 ± 4,9	49,3 ± 4,9	28,1 ± 2,5^{a,b}'	41,1 ± 5,0	58,7 ± 5,3	32,4 ± 3,1
p	ns	ns	0.001*	ns	ns	ns

	NL-v	at-atp	PPW'-Ba	at-So	pt-pw	ut-ut'
Class I	131,1 ± 6,4^{a}	18,0 ± 3,2	27,7 ± 4,8	26,5 ± 3,0	22,1 ± 4,1	9,6 ± 3,5^{a}
Class II/1	136,2 ± 8,1^{b}	17,6 ± 3,2	26,4 ± 6,1	25,5 ± 3,4	20,4 ± 4,0	7,8 ± 3,0^{b}
Class II/2	132,7 ± 8,3^{a}	19,5 ± 16,0	25,4 ± 5,7	25,7 ± 5,4	20,9 ± 3,9	6,7 ± 2,4^{b}
Class III	126,6 ± 4,9^{c}	17,5 ± 3,0	25,3 ± 4,5	25,3 ± 3,0	22,1 ± 4,2	9,6 ± 3,0^{a}
p	0.000*	ns	ns	ns	ns	0.000*

	E4	E5
Class I	2,1 ± 0,3	11,3 ± 1,9
Class II/1	2,0 ± 0,6	10,5 ± 2,4^{a}
Class II/2	2,0 ± 0,4	10,8 ± 1,7
Class III	2,1 ± 0,5	12,0 ± 2,4^{b}
p	ns	0.048*

For each variable tested values with different letters as superscripts differ significantly ($a \neq b \neq c$ and $a' \neq b'$) (Waller-Duncan test)
ns - not significant
* - p<0

The angle formed by the palatal plane (NL) and the base of the skull plane (BaN) presented significantly lower values in Class II/1 than in Classes I and III and it also showed statistically significantly lower values in Class II/2 than in Class III cases;

The soft palate height, measured as the distance of the uvula from the palatal plane (v⊥NL) presented significantly lower values in Class II/1 group than in the other groups;

The angle between the soft and hard palates (NL-v) presented significantly lower values in Class III cases, followed by Class I and Class II/2 with higher values, and, finally, Class II/1 which presented even higher values;

The distance of the tongue from the palatal plane (ut-ut') presented significantly higher values in the Class I and Class III than in Class II/1 and II/2 groups. The surface area of the oropharynx (E2) presented marginally statistically significant higher values in the Class III than in Class II/1 and II/2 groups;

The total surface area of the pharynx (E5) presented significantly higher values in the Class III than in the Class II/1 patients group.

Discussion

In the present study we examined lateral cephalograms of untreated young individuals with normal breathing and concluded that the anatomy of the pharynx, the soft palate, and the tongue differs at specific features among the various Angle Classes of malocclusion. Mild overbite and overjet criteria were also applied for inclusion of a subject in the study to account for misleading molar relationships attributed to molar rotations, mesialization due to space loss, or similar reasons. No cephalometric inclusion criteria were applied in sample selection because this could insert bias on the results. Our aim was to assess the measured variables in groups defined by a simple and easy to use criterion, such as the Angle Class of malocclusion and not by more specific criteria that can be used only by specialists. The sample was representative of a regular orthodontic population, since it was selected in a consecutive manner (restricted just regarding gender) from the archives of an orthodontic clinic. Groups were also similar regarding distribution of chronological age, which is strongly correlated to skeletal age[13].

Longitudinal studies regarding growth of the pharynx concluded that changes in the complex pharyngeal soft tissues are accelerated between 6 and 9 years of age, while significantly less change occurs between 9 and 12 years of age[14]. Thus, by limiting the sample within this period (range: 9 to 12 years, mean: 10.6 ± 0.8 years), we minimized growth effect on the results. This is 1 reason for selecting subjects of this age. Another reason is that this is the age where people usually seek orthodontic treatment. Thus, our sample represented the major part of the untreated orthodontic population, where proper diagnosis and information to the patient and his/her parent should be provided. The balanced sex distribution allowed as not forming sub-groups depending on patients' gender and thus reducing the power of the study. In any case, previous studies did not show statistically significant differences between genders at this age[14,15].

Although most parameters evaluated did not differ significantly among the 4 groups, there were certain important findings. Of the angular measurements estimating the relationships of the pharynx, the angle formed by the palatal plane and the base of the skull (NL-BaN) was significantly smaller in Class II/1 and Class II/2 compared to Class III subjects. This parameter estimates the width of underlying skeletal elements of the nasopharynx and is considered important for detecting patients with nasal obstruction[16]. The lowest values reported for this measurement, in Classes II/1 and II/2, were probably caused by the more horizontally oriented posterior midline cranial base of the skull in Class II as compared to Class III group[17].

As for the tongue position, a statistically significant difference was found in the distance of the dorsal tongue surface from the palatal plane (ut-ut') in the 4 groups. Specifically, in Classes I and III the dorsal tongue surface (ut) lay at a lower position, while in Class II/1 and Class II/2 it lays at a shorter distance from the palatal plane (ut'). Moreover, the pt-pw measurement, which estimates the anteroposterior position of the tongue base, tended to present higher values in Classes I and III and lower values in Classes II/1 and II/2; however, this difference was not statistically significant. A likely explanation for these findings is that, in Class II, the tongue is restricted within a smaller space and pushed upward and backward, while in Class I and Class III the tongue spreads forward and downward.

Findings concerning the inclination of the soft palate are of particular interest. In Class III subjects, the angle between soft and hard palates (NL-v) was significantly smaller, while Class II/1 patients presented the highest values. Furthermore, in Class II/1, the distance of the uvula from the palatal plane (v⊥NL) was significantly shorter than in the other groups. On the other hand, the length of the soft palate (PNS-v) remained stable in the 4 groups. These may be caused by the position the tongue assumes in different Angle Classes, which may affect the corresponding activity of the palatoglossal muscles. For example, in Class III, the most anterior position of the tongue may activate the palatoglossal muscles more intensely and this can result in the soft palate being pulled further forward and downward than in the other groups. The measurements of the pharyngeal surface area presented statistically significant differences both in regards to

the oropharyngeal part and the total pharyngeal area. Specifically, in Class III, higher values were found for the oropharyngeal surface area compared to Classes II/1 and II/2, while the total pharyngeal surface area was significantly different only between Class III and Class II/1. These findings are in agreement with research studies based on skeletal criteria. It has been found that the smaller the ANB angle, the larger the oropharyngeal surface area[18]. In another study, no statistically significant differences were found for the surface area of the rhinopharynx between Class I and Class II/1[19]. In a study of normodivergent facial patterns, there was a significant tendency towards reduced dimensions of the upper airway in the lower palatopharyngeal and hypopharyngeal parts, in the following order: Class III, Class I and Class II[20]. The oropharyngeal height (PNS-Eb) has been correlated with the OSA syndrome[21].

The present study showed no statistically significant difference in this measurement in the 4 groups or any tendency for variation. The same is true for the posterior cranio-cervical height (S-AA). Besides, sagittal measurements of the oropharynx and the tongue position (SPAS, MAS, IAS, apw2-ppw2) showed a tendency to present higher values in Classes I and III and lower values in Classes II/1 and II/2 cases, however without any statistical significance. This fact, combined with the lower tongue position in Class III explains why the oropharyngeal surface area in the specific group was found to be larger when compared with Classes I/1 and II/2. This is in agreement with previous findings suggesting that the longer the mandible, the more increased the sagittal dimension at the lowest part of the oropharynx[22,23].

Future studies could investigate in greater detail possible correlations between pharyngeal relationships, occlusal relationships and particular craniofacial features, such as the vertical facial types, and their association with OSA. The 3D evaluation of nasopharyngeal and oropharyngeal structures and potential relationships with respiratory function or OSA symptoms could offer a better insight in the understanding of these issues. Moreover, a study similar to ours conducted in an untreated adult sample would also be of particular interest, although this sample is difficult to collect.

In conclusion, the present study revealed a certain relationship between Angle malocclusion classes and pharyngeal structures. This could indicate that Class II subjects may be more prone to develop respiratory related disorders, such as the OSA syndrome, followed by Class I and Class III subjects. Thus, clinicians should be more aware for this in certain Angle Classes of malocclusion. This is a simple, definite and well known diagnostic classification that can be easily performed during clinical examination, not only by specialists, but also by general dentists.

References

1. *Fujiki T, Takano-Yamamoto T, Noguchi H, Yamashiro T, Guan G, Tanimoto K*. A cineradiographic study of deglutitive tongue movement and nasopharyngeal closure in patients with anterior open bite. *Angle Orthod*, 2000; 70:284-289.
2. *Togeiro SM, Chaves CM Jr, Palombini L, Tufik S, Hora F, Nery LE*. Evaluation of the upper airway in obstructive sleep apnoea. *Indian J Med Res*, 2010; 131:230-235.
3. *Branstetter BF 4th, Weissman JL*. Normal anatomy of the neck with CT and MR imaging correlation. *Radiol Clin North Am*, 2000; 38:925-940.
4. *Grauer D, Cevidanes LS, Styner MA, Ackerman JL, Proffit WR*. Pharyngeal airway volume and shape from cone-beam computed tomography: relationship to facial morphology. *Am J Orthod Dentofacial Orthop*, 2009; 136:805-814.
5. *Farsaris N, Athanasiou AE, Goumas P*. Obstructive sleep apnea syndrome: Contemporary concepts regarding etiology, clinical characteristics, diagnosis and treatment management. *Hell Orthod Rev*, 2003; 6:2-48.
6. *Pirilä-Parkkinen K, Löppönen H, Nieminen P, Tolonen U, Pääkkö E, Pirttiniemi P*. Validity of upper airway assessment in children: a clinical, cephalometric, and MRI study. *Angle Orthod*, 2011; 81:433-439.
7. *Lowe AA, Ono T, Ferguson KA, Pae EK, Ryan CF, Fleetham JA*. Cephalometric comparisons of craniofacial and upper airway structure by skeletal subtype and gender in patients with obstructive sleep apnea. *Am J Orthod Dentofacial Orthop*, 1996; 110:653-664.
8. *Tangugsorn V, Skatvedt O, Krogstad O, Lyderg T*. Obstructive sleep apnea: a cephalometric study. Part I. Cervico-craniofacial skeletal morphology. *Eur J Orthod*, 1995; 17:45-56.
9. *Johal A, Conaghan C*. Maxillary morphology in obstructive sleep apnea: a cephalometric and model study. *Angle Orthod*, 2004; 74:648-656.
10. *Alves PV, Zhao L, O'Gara M, Patel PK, Bolognese AM*. Three-dimensional cephalometric study of upper airway space in skeletal class II and III healthy patients. *J Craniofac Surg*, 2008; 19:1497-1507.
11. *Kim YJ, Hong JS, Hwang YI, Park YH*. Three-dimensional analysis of pharyngeal airway in preadolescent children with different anteroposterior skeletal patterns. *Am J Orthod Dentofacial Orthop*, 2010; 137:306.e1-11.
12. *Smith RN, Brook AH, Elcock C*. The quantification of dental plaque using an image analysis system: reliability and validation. *J Clin Periodontol*, 2001; 28:1158-1162.
13. *Chatzigianni A, Halazonetis DJ*. Geometric morphometric evaluation of cervical vertebrae shape and its relationship to skeletal maturation. *Am J Orthod Dentofacial Orthop*, 2009; 136:481.e1-9.
14. *Taylor M, Hans MG, Strohl KP, Nelson S, Broadbent BH*. Soft tissue growth of the oropharynx. *Angle Orthod*, 1996; 66:393-400.
15. *Linder-Aronson S, Leighton BC*. A longitudinal study of the development of the posterior nasopharyngeal wall between 3 and 16 years of age. *Eur J Orthod*, 1983; 5:47-58.
16. *Langlade M*. Diagnostic orthodontique. Paris; Maloine S.A Editeur. 1981; pp145-205.

17. *Gkantidis N, Halazonetis DJ*. Morphological integration between the cranial base and the face in children and adults. *J Anat*, 2011; 218:426-438.
18. *Ceylan I, Oktay H*. A study on the pharyngeal size in different skeletal patterns. *Am J Orthod Dentofacial Orthop*, 1995; 108:69-75.
19. *Sosa FA, Graber TM, Muller TP*. Postpharyngeal lymphoid tissue in Angle Class I and Class II malocclusions. *Am J Orthod*, 1982; 81:299-309.
20. *Zhong Z, Tang Z, Gao X, Zeng XL*. A comparison study of upper airway among different skeletal craniofacial patterns in nonsnoring Chinese children. *Angle Orthod*, 2010; 80:267-274.
21. *Segal Y, Malhotra A, Pillar G*. Upper airway length may be associated with the severity of obstructive sleep apnea syndrome. *Sleep Breath*, 2008; 12:311-316.
22. *Trenouth MJ, Timms DJ*. Relationship of the functional oropharynx to craniofacial morphology. *Angle Orthod*, 1999; 69:419-423.
23. *Kikuchi Y*. Three-dimensional relationship between pharyngeal airway and maxillo-facial morphology. *Bull Tokyo Dent Coll*, 2008; 49:65-75.

Correspondence and request for offprints to:

Nikolaos Topouzelis, Professor
Aristotle University of Thessaloniki
Dental School, Department of Orthodontics
GR-54124 Thessaloniki, Greece
E mail: ntopouz@dent.auth.gr

BALKAN JOURNAL OF DENTAL MEDICINE

BaSS

ISSN 2335-0245

DMFT Index among Institutionalized Elderly

SUMMARY

Introduction. *Poor oral health among elderly is most common dental problem nowadays, especially among the institutionalized persons.*

Aim. *To detect DMFT index among the institutionalized elderly.*

Material and Method. *Oral examination was made to make adequate evaluation. A total number of 70 subjects were evaluated. DMFT index has been detected only with dental mirror and probe, without using additional instruments and methods.*

Results. *Average value of DMFT index in our survey was 24.84* ± 4.56 *(with* Confidence interval from 23.77 to 25.89). *M-component was dominant - 21.56* ± 7.79 *(*with Confidence interval from 15.74 to 23.38). D-component indicated by carious teeth and persistent roots had value *2.60* ± 3.54 *(with* Confidence interval from 1.77 to 3.42). Mean value of teeth with definitive fillings (F-component of DMFT index) was *0.34* ± 1.42 *(*with Confidence interval from 0.33 to 1.01).

Conclusion. DMFT index among the institutionalized elderly had one of the biggest values in the literature. M-component was dominant and indicator of the absence of many teeth. Therefore it is of great importance to prepare adequate protocol for oral health care among the institutionalized elderly.

Keywords: Gerodontology; Institutionalized Elderly; DMFT Index

M. Petrovski[1], K. Ivanovski[2], A. Minovska[1]

[1]University "Gotse Delchev"
Faculty of Medical Sciences, studies of dental medicin, Shtip, FYROM
[2]University "St. Kiril and Methodius"
Faculty of Dentistry
Department of Oral Pathology and Periodontology
Skopje, FYROM

ORIGINAL PAPER (OP)
Balk J Dent Med, 2015; 19:21-25

Introduction

Oral health is an integral part of the overall health and includes the health of the oral cavity or the health of teeth themselves, periodontal tissues, oral mucosa, the salivary glands and surrounding structures[13]. Poor oral health among the elderly is most common dental problem nowadays, especially among the institutionalized elderly. Edentulism may indicate an increased risk for numerous problems concerning general health.

The impaired general health of these people have an impact on their oral health, i.e. on the dental status, the presence of caries, periodontal health, oral hygiene, toothless, limited oral functions, problems associated with wearing dentures, total or partial presence of malignant and benign conditions and tumors, xerostomia, and other oral conditions that can cause pain or discomfort in the orofacial region. Also, oral health in turn, is conditioned by many factors such as dental caries and its complications, un-extracted untreated dental roots, different diseases of the oral mucosa and numerous oral infections, precancerous conditions and benign and malignant tumors, pain in the temporo-mandibular joint, and partial or a total toothless as well. Loss of teeth can affect chewing efficiency, the choice of food and, of course, the nutritional status in the elderly[17]. The oral health of the institutionalized elderly can be influenced by numerous other factors such as: multi-morbidity, depending on the maintenance of oral hygiene, limited skills and movements and use of numerous medications.

Krasta et al[15] noted that in recent decades among retirees in developed countries, the number of remaining teeth is increased, while the number of untreated teeth affected by caries in developed countries over the last 20 years has declined.

Interaction of highly prevalent xerostomia and inability to maintain satisfactory oral hygiene among institutionalized elderly leads to increased occurrence of dental plaque. Because of its presence the elderly have increased risk for caries and periodontitis. Therefore, special knowledge and manual dexterity are needed to allow proper oral health care among the elderly.

Recurrent caries around inadequate or old fillings and cervical caries or cavities on the root of the teeth are the most common types of caries in adults[29,30]. Root caries is a specific type of caries, which is characterized by the presence of hard-tissue lesion of one or more teeth in the area of the tooth root (the part of the tooth that is protected and covered with cement). Due to the process of apical migration of epithelial attachment and gingival recession, the primary place of occurrence of dental hard-tissue demineralization is the cervical region and the subsequent decomposition of cementum. In the cementum, demineralization affects quite large area and rapidly evolves, due to its histological and mineralogical structure. The process occurs in 2 ways: by direct decomposition of the dental cementum of the lesion and peeling between dentin and cement due to bacterial penetration[18].

According Alian et al[2], high prevalence of coronary dental caries and root caries can be seen in the old population worldwide and advanced dental caries and periodontal disease are considered to be the most important reason for extraction of teeth. Factors that are taken as reasons for the increased risk of caries in the elderly population include attacking factors ("attack factors"), such as dental plaque, the presence of specific microorganisms and nutritional factors and defensive factors ("defense factors"), such as: protective role of saliva and fluoride use.

Unsatisfactory oral health and hygiene among the institutionalized elderly are verified by Rihset al[25], demonstrated by the high percentage of lost teeth, dental caries and a high incidence of total edentulism.

According to Wyatt et al[35], about 80% of institutionalized elderly had more than 1 carious lesion, approximately half of them had coronary caries and about 70% had root caries. On average, according to this survey come 3.8 caries teeth of a patient despite the higher number of extracted teeth. The residents of long-time care facilities had presented significantly more carious lesions localized in dental crowns.

In a study published by Gati and Vieira[9], performed among individuals older than 75 years (mean age 85 years), they found that in almost all (or 97%) of subjects caries was presented, while ⅔ of individuals who participated in a survey had root caries, out of which 20% were untreated. According to this research, coronary active root caries was more common in men and in people who consume tobacco in the form of cigarettes or cigars.

Elderly patients' carriers of partial dentures are facing with another significant dental problem: caries in the area of the tooth neck because the retentional parts of the partial dentures are in contact with them. Their presence disables adequate level of oral hygiene presented with accumulation of dental plaque on the retentional parts of the partial dentures[10].

According Samnienget al[27] in patients with hyposalivation there is a higher number of teeth, caries prevalence and greater expression of periodontitis than in people with normal salivation.

Considering the poor oral health among institutionalized elderly, this research aimed to determine DMFT index among the institutionalized elderly.

Material and Method

The survey included a total of 70 subjects who were older than 65 years. In this institution for long-term institutionalized, most of the persons were functionally-dependent individuals, dominated with chronic diseases. The research was conducted in the period from April to July 2013, in the department "Mother Teresa", which is within the PHI Gerontology Institute "13th November" in Skopje.

When performing this research, all persons being in the terminal stage of the disease were excluded, subjects with dementia and all individuals who have cognitive disorders, patients with naso-gastric tube and patients placed on artificial ventilation were also excluded. All individuals who do not cooperate because of different behavioural disorders, aggression, or do not allow them to perform clinical examination were not included in the studied group as well. From the survey were also excluded people who do not understand the Macedonian language.

In order to make an objective assessment of the situation on the oral health among institutionalized elderly, an oral examination was done. This examination included determination of DMFT-index (originally: D - "Decayed", carious; M - "Missing", extracted; F - "Filling", teeth with definitive filling). The existence of dental caries during the study was revealed only by a dental mirror and probe without using additional instruments and methods.

The data obtained from the clinical examination were statistically processed using special software for statistical processing of data - Statistica 7.1.

Results

Determining the DMFT index is intended to define the morbidity and mortality of the remaining teeth

among the institutionalized individuals older than 65 years. Besides the general DMFT index, values for all its components were determined too. The mean value found for DMFT index was 24.84 ± 4.56 (Fig. 1).

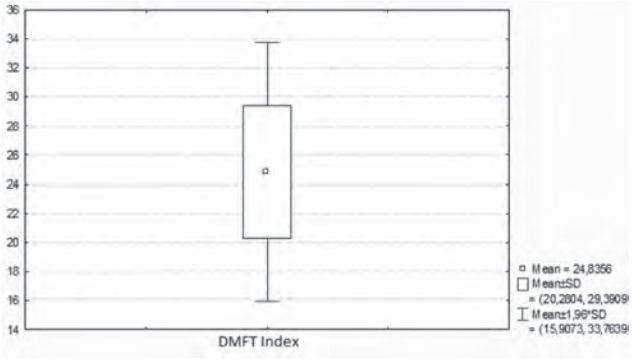


Figure 1. Average value of the DMFT index among examined institutionalized elderly

As it was expected for this population, the number of missing (extracted) teeth (MT) - as an indicator of the huge loss of teeth - was the biggest contributor responsible for the high values of the DMFT index. In particular, higher average number of extracted teeth was found among subjects older than 75 years (*versus* subjects aged 65-74 years). It has to be stressed the fact that elderly subjects where there was no caries observed were predominantly people who have total toothless.

The average value of untreated caries teeth (D-component of DMFT-index) among the institutionalized elderly in this study was 2.60 ± 3.54 per person. (Fig. 2). Average value of teeth with definite filling (F-component of DMFT-index) was 0.34 ± 1.42 per person studied. (Fig. 3).

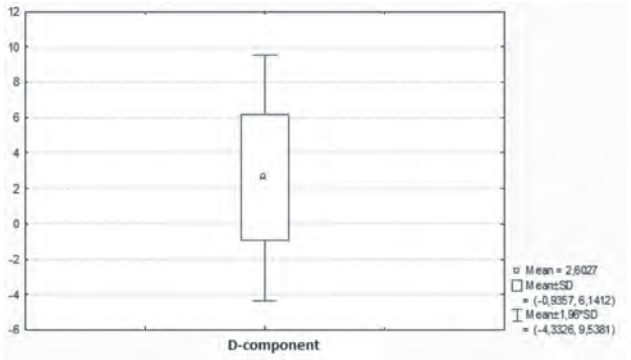


Figure 2. Average value for D-component of the DMFT index among examined institutionalized elderly

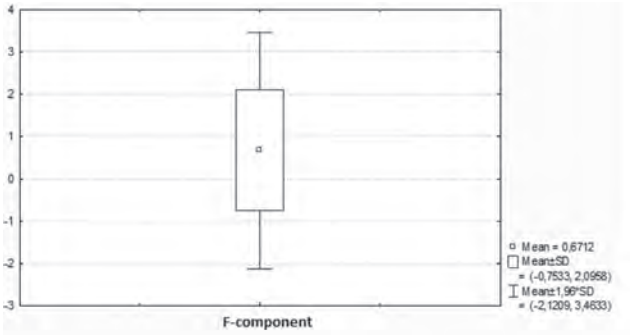


Figure 3. Average value for F-component of the DMFT index among examined institutionalized elderly

The mean value of lost teeth (M-component of DMFT-index) was 21.56 ± 7.79 per person. (Fig. 4).

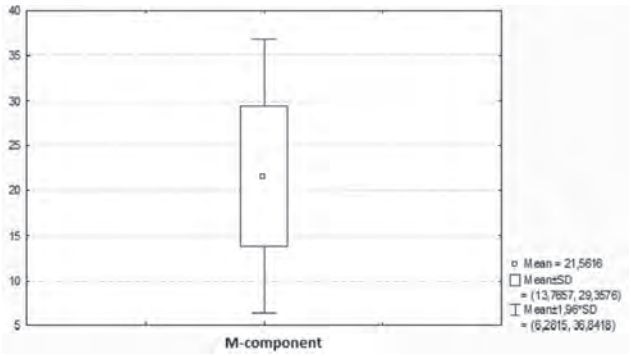


Figure 4. Average value for M-component of the DMFT index among examined institutionalized elderly

The mean value the number that refers to teeth that possess definitive restoration was quite low (20.5%) among subjects.

The mean value for the number of extracted teeth was 11.64 ± 4.21 for each jaw. The number of teeth lost among subjects was higher in the upper than in the lower jaw. It was found that the highest number of missing teeth was in trans-canine region.

Discussion

DMFT-index is used in order to statistically determine the number of carious, extracted and filled teeth per person and the average value of this index in the tested population. DMFT index is chosen because it is commonly used index for assessment of dental status in population. Primary, DMFT index was developed for

use in childhood and later is adapted for use in the elderly. Some authors believe that DMFT index has low validity for assessment of caries in adults, primarily because it is not able to reveal the true impact of caries on the oral health. This index refers only to the presence of the disease, its consequences and the need for treatment, and it can present high values especially in populations such as ours, where the presence of caries and extracted teeth is really high.

This index does not take into consideration the reasons for tooth loss - whether the tooth is lost due to caries or periodontal disease, risk for caries occurrence and dental assessment of the need for treatment. As can be noticed, the presence of dental caries among respondents varied greatly.

As it is expected in this population, the number of lost (extracted) teeth (MT) - as an indicator of the huge loss of teeth - was the biggest contributor responsible for the high values of the DMFT index.

The available data indicate a wide distribution of caries and the consequences of its presence around the world, having enormous social significance especially among the institutionalized elderly. D-component of the DMFT index itself comprises: carious teeth, teeth with definitely filling on one area while the other with caries, teeth with definite fillings that have recurrent or secondary caries, teeth with temporary fillings or teeth that have only remaining root.

M-component (number of missing teeth) is the dominant component of the DMFT index, not only in this study but also in numerous other studies concerning the institutionalized elderly. In particular, higher values of extracted teeth were found among respondents older than 75 years (versus respondents aged 65-74 years).

F-component (teeth with definite fillings) has the lowest value in the DMFT index. The low value of F-component may due to the neglect of the respondents for their oral health, irregular check-ups, as well as because the definitive fillings did not last, or their durability was reduced due to other factors.

The value of the DMFT index obtained in this research was 28.84 ± 4.71 (Fig. 1), which is quite high but often seen in the literature. Our results for DMFT index correlate with the data obtained by Simunkovićet al[29], Piuvezam and de Lima[23], Unluer et al[31] as well as data published by Vrbic et al[34]. Lower index values for DMFT index concerning the institutionalized elderly are presented by Ruiz-Medina et al[26], Philip et al [21], Zusman et al[36], Ahluwalia et al[1], Samson et al[28], Comfort et al[7], Lo et al[16], Bourgeois et al[4], Vrbic et al[33]. Higher values for DMFT index are quite rare in the literature and observed in Brazil by Rihs et al[25], Gaiao et al[12], Van Wyk et al[32] , and Petelin et al[20].

The determined averaged value for D-component of the DMFT in our study (Fig. 2) coincides with the data presented by Lo et al[16], Zusman et al[36], Corneo et al[7] and Carter et al[5], unlike King and Kapadia[14], Esmeriz et al[11], Rihs et al[25] and Ambjøsrnsen[3] , who showed a lower representation of carious lesions of the remaining teeth. Greater representation of carious teeth i.e. D-component of the DMFT index in institutionalized elderly noticed Raoet al[24] and Piuvezam and de Lima[22].

M-component of the DMFT index is the dominant component in most studies and researches, not only among the institutionalized elderly, but among the elderly as a whole population[19]. Results published by King and Kapadia[14] and Esmeriz et al[11] match with the same from this research - 25.41 ± 8.03 (Fig. 4). Lo et al[16], Zusman et al[36], Cornejo et al[7], Ambjøsrnsen[3] and Chlamers et al [6] in their studies showed lower mean values for M-component of the DMFT index. Unlike them, Rihs et al[25] and Piuvezam and de Lima[22] showed higher values for extracted teeth.

Values concerning F-component as a factor indicating the subjective views of subjects to improve their own oral health in most of the published results are around 1. Similar results to those of our research 0.69 ± 1.46 (Fig. 3) published Lo et al[16] and King and Kapadia[14]. Lower representation of teeth with definite fillings among institutionalized elderly published Cornejoet al[7], unlike Rihs et al[25], Zusman et al[36], Esmeriz et al[11] and Ambjøsrnsen[3], who indicated higher representation of this component.

Conclusion

Based on the data and analysis of the results, it may be concluded that there is a presence of large number of extracted teeth and caries among institutionalized elderly, and low representation of teeth with definitive filling. There was a high value of DMFT index, with predominance of M-component.

References

1. *Ahluwalia KP, Cheng B, Josephs PK, Lalla E, Lamster IB.* Oral disease experience of older adults seeking oral health services. *Gerodontology*, 2010; 27(2):96-103.

Alian AY, McNally ME, Fure S, Birkhed D. Assessment of Caries Risk in Elderly Patients Using the Cariogram Model. *J Can Dent Assoc*, 2006; 72(5):459-463.

2. *Ambjøsrnsen E.* Decayed, missing, and filled teeth among elderly people in a Norwegian municipality. *Acta Odontol Scand*, 1986; 44(2):123-130.
3. *Bourgeois D, Berger P, Hescot P, Leclercq MH, Doury J.* Oral health status in 65-74 years old adults in France, 1995. *Rev Epidemiol Sante Publique*, 1999; 47(1):55-59.

4. *Carter G, Lee M, McKelvey V, Sourial A, Halliwell R, Livingston M*. Oral health status and oral treatment needs of dependent elderly people in Christchurch. *NZMJ*, 2004; 117(1194:1-10.
5. *Chalmers J, Hodge C, Fuss J, Spencer A, Carter K*. The Prevalence and Experience of Oral Diseases in Adelaide Nursing Home Residents. *Australian Dental Journal*, 2002; 47:123-130.
6. *Comfort AO, King T, Moveni M, Tuisuva J*. Dental health of Fiji institutionalized elderly (2003). *Pac Health Dialog*, 2004; 11(1):38-43.
7. *Cornejo M, Pérez G, Costa-de Lima K, Casals-Peidro E, Borrell C*. Oral Health-Related Quality of Life in institutionalized elderly in Barcelona (Spain). *Med Oral Patol Oral Cir Bucal*, 2013; 18(2):e285-292.
8. *Daniel Gati, Alexandre Vieira*. Elderly at Greater Risk for Root Caries: A Look at the Multifactorial Risks with Emphasis on Genetics Susceptibility. *Int J Dent*, 2011.
9. *Ergün Kunt G, Kökçü D, Ceylan G, Yilmaz N, Umut Güler A*. Pulpal blood flow changes in abutment teeth of removable partial dentures. *Bosn J Basic Med Sci*, 2009; 9(4):296-300.
10. *Esmeriz CEC, Meneghim MC, Ambrosano GMB*. Self-perception of oral health in non-institutionalised elderly of Piracicaba city, Brazil. *Gerodontology*, 2012; 29:e281-e289.
11. *Gaiao LR, de Almeida MEL, FilhoGB, Leggat P, Heukelbach J*. Poor Dental Status and Oral Hygiene Practices in Institutionalized Older People in Northeast Brazil. 2009; doi:10.1155/2009/846081
12. *Glažar Irena*. Procjena oralnog zdravlja štićenika ustanova za njegu starijih osoba, (Oral helath evaluation in the institutionalized elderly). PhD Thesis, Medical Faculty of Rijeka, Croatia. 2010.
13. *King T, Kapadia D*. Oral health and treatment needs of institutionalised elderly and disadvantaged population of Figi (1997). *Pacific Health Dialog*, 2003; 10(1):35-40.
14. *Krasta I, Vidzis A, Brinkmane A, Cema I*. Evaluation of Oral Therapeuthical and Surgical Treatment Needs among Retirement Age Population in Different Countries. *Acta Chirurgica Latviensis*, 2011; 11:125-129.
15. *Lo EC, Luo Y, Dyson JE*. Oral health status of institutionalised elderly in Hong Kong. *Community Dent Health*, 2004; 21(3):224-226.
16. *Mason J, Peare MS, Walls AWG, Parker L, Steele JG*. How do factors at different stages of the life course contribute to Oral-health-related quality of life in the middle age for men and women. *J Oral Health*, 2006; 12(2):125-131.
17. *Matovska Lj*. Cariology. Sigmapres: Skopje, 2011; p 72. (in Macedonian)
18. *Milstein L, Rudolph MJ*. Oral health status in an institutionalised elderly Jewish population. *SADJ*, 2000; 55(6):302-306.
19. *Petelin M, Cotič J, Perkič K, Pavlič A*. Oral health of the elderly living in residential homes in Slovenia. *Gerodontology*, 2012; 29:e447–e457.
20. *Philip P, Rogers C, Kruger E, Tennant M*. Caries experience of institutionalized elderly and its association with dementia and functional status. *Int J Dent Hyg*, 2012; 10(2):122-127.
21. *Piuvezam G, de Lima KC*. Factors associated with missing teeth in the Brazilian elderly institutionalised population. *Gerodontology*, 2013; 30(2):141-149.
22. *Piuvezam G, de Lima KC*. Self-perceived oral health status in institutionalized elderly in Brazil. *Arch Gerontol Geriatr*, 2012; 55(1):5-11.
23. *Rao A, Sequeira P, Peter S, Rajeev A*. Oral health status of the institutionalized elderly in Mangalore, India. *Indian J Dent Res*, 1999; 10(2):55-61.
24. *Rihs LB, da Silva DD, da Luz Rosário de Sousa M*. Dental caries in an elderly population in Brazil. *J Appl Oral Sci*, 2009; 17(1).
25. *Ruiz-Medina P, Bravo M, Gil-Montoya JA, Montero J*. Discrimination of functional capacity for oral hygiene in elderly Spanish people by the Barthel General Index. *Community Dent Oral Epidemiol*, 2005; 33(5):363-369.
26. Samnieng P, *Ueno M, Shinada K, Zaitsu T, Wright FA, Kawaguchi Y*. Association of hyposalivation with oral function, nutrition and oral health in community-dwelling elderly. *Thai Community Dent Health*, 2012; 29(1):117-123.
27. *Samson H, Strand GV, Haugejorden O*. Change in oral health status among the institutionalized Norwegian elderly over a period of 16 years. *Acta Odontol Scand*, 2008; 66(6):368-373.
28. Simunković SK, *Boras VV, Pandurić J, Zilić IA*. Oral health among institutionalised elderly in Zagreb, Croatia. *Gerodontology*, 2005; 22(4):238-241.
29. *Steele JG, Sheiham A, Marcenes W, Fay N, Walls AW*. Clinical and behavioural risk indicators for root caries in older people. *Gerodontology*, 2001; 18(2):95-101.
30. *Unluer S, Gokalp S, Doğan BG*. Oral health status of the elderly in a residential home in Turkey. *Gerodontology*, 2007; 24:22-29.
31. *vanWyk GW, Farman AG, Staz J*. Tooth survival in institutionalized elderly Cape Coloreds from the Cape Peninsula of South Africa. *Community Dent Oral Epidemiol*, 1977; 5:185-189.
32. *Vrbic V, Homan D, Zavrsnik B*. Oral health in Slovenia, Yugoslavia. *Community Dent Oral Epidemiol*, 1991; 19(2):72-73.
33. *Vrbic V, Vulović M, Rajić Z, Topić B, Tatić E, Malić M, Milić D, Aurer-Kozelj J, Neceva L, Redzepagić S, et al*. Oral health in SFR Yugoslavia in 1986. *Community Dent Oral Epidemiol*, 1988; 16(5):286-288.
34. *Wyatt CCL*. Long-term Care Hospitals. Part II: Dental Caries Status. *J Can Dent Assoc*, 2002; 68(6).
35. *Zusman SP, Ponizovsky AM, Dekel D, Masarwa AE, Ramon T, Natapov L, et al*. An assessment of the dental health of chronic institutionalized patients with psychiatric disease in Israel. *Spec Care Dentist*, 2010; 30(1):18-22.

Correspondence and request for offprints to:

Mihajlo Petrovski
St. “Ivan Turgjenjev” 10 a
1000 Skopje, FYR Macedonia
E-mail: m_5rovski@yahoo.com

BALKAN JOURNAL OF DENTAL MEDICINE

BaSS

ISSN 2335-0245

Assessment of Knowledge and Attitudes to Preserve Oral Health among Older People Aged 60+ in FYROM

M. Šapurić[1], F. Tozja[2]

[1]PZU "ProdentIdadija", Skopje
[2]Institute of Public Health, Faculty of Medicine, Skopje, FYROM

SUMMARY

Introduction. *In the last decade, the impact of oral health on overall health status in the elderly has attracted considerable attention. Elderly people are often not aware that oral diseases and tooth loss can be prevented. Oral health behaviour involves acquisition of individual habits for preservation and maintenance of oral health, such as regular brushing and brushing teeth, using fluoride-rich toothpastes and dental floss, reducing sugar in the diet and habit of regular visits to the dentist. The aim of this research was to assess knowledge and attitude toward maintenance and preservation of oral health in people over 60 years in FYROM.*

Methods. *This is a cross-sectional study of the population, older than 60 years in FYROM, who use dental services and treatment in public health institution, at the Faculty of Dentistry in Skopje (Clinic of Mouth and Periodontal Diseases) and in the Health Centre in Skopje. As an instrument of this research, a questionnaire which included issues related to oral health behaviour was used for the targeted population. 193 patients answered the questions in this questionnaire.*

Results. *The age of respondents was significantly associated with the frequency of brushing their teeth and interdental spaces, as well as the habits of frequent dental care visits to the dentist. The gender of the patients had a highly significant effect on the use of fluoride toothpastes and the time that had passed since their last visit to the dentist ($p<0.01$). Education level in elderly patients significantly affected the knowledge of the individual oral health care.*

Conclusion*. Respondents with higher education brushed their teeth and interdental spaces more frequently and had regular dental check-ups. Furthermore, they almost always used fluoride toothpaste when brushing their teeth. The increased concern for their own health correlated to a higher level of knowledge, awareness and attitudes about oral health care, and follow recommendations by a dentist. In FYROM, oral health behaviour of patients above 60 years is far behind the level of care that is achieved in higher developed countries.*

Keywords: Oral Health Care; Oral Health Behaviour; Population, old

ORIGINAL PAPER (OP)
Balk J Dent Med, 2015; 19:26-32

Introduction

In the past decade, the impact of oral health on overall health in older people attracted a lot of attention, globally. The health authorities around the world are especially faced to the problem of the increased burden of oral diseases in elderly patients[1,2]. The percentage of elderly people in developed countries today is higher than in developing countries. By 2050, an increase in the population over the age of 65 is expected, including those of aged 80 years or above. Currently this age group accounts for less than 2% of the total world population,

but the number of old people is expected to increase from the current 90 million (in 2005) to 400 million in 2050[3]. Similarly, the age structure of population in FYROM has evidently faced significant changes - in the period from 1999 to 2009, the proportion of young people (0-14 years) decreased from 22.8% to 17.7%. Proportionally, the elderly population (65 and over) increased from 9.8% to 11.6% - in 2012, this percentage was 11.9%[4].

In highly developed countries, the economic conditions permit funding oral-health systems and most of these countries even give priority to classical treatment of diseases rather than prevention[5-7]. However, classical treatments of oral diseases are very expensive for the elderly, even in highly-industrialized countries. Therefore, prevention of oral diseases is more important and it depends on the oral health habits of elderly patients[8,9]. One of the main reasons that patients from lower socio-economic environments do not visit a dentist is the insufficient awareness for dental diseases. Older people are often unaware that oral disease and tooth loss may be treated and prevented[10]. It is very interesting that only a few countries have national data about oral health habits among elderly patients. Where the information is available, there are large differences between countries across the region in terms of the frequency and types of oral health habits among older persons[11]. Oral-health behaviour involves the acquisition of individual habits of preservation and maintenance of oral health, such as regular teeth brushing, regular use of fluoride-rich toothpastes, interdental cleaning, reduced intake of sugar, and regular dental check-ups.

Oral-hygiene habits are acquired early in childhood and if so, it is hard to change them in later life[12]. Annual visits to dental offices have been suggested as an acceptable indicator of appropriate use of dental care. The dentists are facing the challenge to preserve as many natural teeth among older people; today, more and more elderly people, especially in countries that are highly-developed, give priority to dental treatment, which will allow preservation of their natural teeth and a nice look of the whole dentition[13,14].

Oral health among older population in FYROM is alarming and below international standards. Investigations that have estimated the impact of oral health and quality of life in people older than 65 years in this country have shown that oral health in this population is not on a satisfactory level, but given the overall score - 43, which was obtained in this study in which the OHIP-14 questionnaire was used, it proved to be relatively well (given that the total score of the present questionnaire was 52, indicative of poor oral health)[15]. Therefore, it is necessary to fully achieve goals of the Health Strategy of the Country[16]. The **aim** of this study was to assess the knowledge elderly people (over 60 years of age) in FYROM, and their ability to maintain and preserve the oral health.

Materials and Methods

This is a transversal study (cross-sectional study) which includes the knowledge and skills to maintain oral health in patients aged 60 years and older, who receive dental care in public health institutions - "Health Centre" and "Dental Clinical Centre" in Skopje, Clinic of Mouth and Periodontal Diseases. The study applied a same methodology as that used in a similar survey in Lithuania in 2009[12] in order to make a comparison of the findings for knowledge and attitudes toward preserving the oral health of older patients between FYROM and a state which is a member of the European Union. Testing was done by a questionnaire, which included issues related to oral health behaviour of old patients, their individual attention to oral health and obtaining dental services, as well as the sources of information related to the preservation and maintenance of oral health[12,17,18].

The study population were patients older than 60 years who were treated in Public Health Facilities: "Health Centre" in Skopje and "Dental Clinical Centre", also in Skopje. The total number of patients who completed the questionnaire was 193 although the sample included 200 patients. The issues related to personal information about dental care and maintenance of oral health, frequency of tooth brushing, interdental cleaning, consumption of sugar and the use of fluoride in various forms were analysed. The questions were rated as: (1) "Yes, recently" - 2 points; (2) "Yes, before/earlier/some time ago" - 1 point; and (3) "Never" - 0 points. Account of points should theoretically range from 0-34 points.

All of the obtained data was put into a specially developed database. The study used $\chi 2$ test for assessing differences in the frequency and the coefficient of correlation. Statistical significance was determined at $p<0.05$ (statistically significant results).

Results

From all of the respondents (193), there were more women than men (Tab. 1). In terms of age, 54.92% were patients aged 60-69 years (106) and 45.08% (87) were patients aged 70 years and over. In relation to the frequency of tooth brushing, 33.7% of respondents used to wash their teeth once a day, and 34.9% of them were at the age of 60-69 years, while 32.2% of respondents were older than 70 years. More frequent tooth brushing, twice a day or more, was observed in 122 participants (63.2%), of whom31.6% were males and 35.1% females (Tab. 1). A noticeable high percentage (6.9%) of the participants older than 70 years said that they never brush their teeth, while 25.4% of respondents stated that they brush their teeth just after the meal. Regarding the frequency of cleaning interdental space,

the majority of respondents stated that they don't have this habit, which especially applied to the participants older than 70 years. Regarding the use of paste enriched with fluoride, 27.9% of respondents said they always use toothpaste with fluoride and 34.7% answered that they almost always used. Among the respondents with higher education, only 28.9% of them used fluoride toothpastes. Using a sugar in coffee or tea proved to be a common habit (Tab. 1). Regular visits to the dentist were recorded in 64.2% of the respondents, younger participants being more regular in that sense. Also, more of them were females. 68.1% of respondents with higher education said that they have a habit of going to the dentist for regular check-ups (Tab. 1).

Table 1. Maintaining of oral hygiene and health of elder people in FYROM

Parameter	Frequency	Age		Gender	
		60-69 years 106 (54.92%)	**70+ years** 87 (45.08%)	**Men** 79 (40.93%)	**Women** 114 (56.07%)
Tooth brushing	Once a day	37 (34.9%)	28 (32.2%)	25 (31.6%)	40 (35.1%)
	Often	69 (65.1%)	53 (60.9%)	51 (64.6%)	71 (62.3%)
	Never	0	6 (6.9%)	3 (3.80%)	3 (2.6%)
		p=0.02*		p=0.82	
	In the morning	96 (90.6%)	73 (83.9%)	69 (87.3%)	100 (87.7%)
		p=0.16		p=0.94	
	In the evening	75 (70.7%)	58 (66.7%)	56 (70.9%)	77 (67.5%)
		p=0.54		p=0.62	
	After a meal	31 (29.3%)	18 (20.7%)	22 (27.8%)	27 (23.7%)
		p=0.17		p=0.51	
Interdental cleaning	Once a day	8 (4.1%)	1 (1.2%)	4 (5.1%)	4 (3.5%)
	More often	6 (3.1%)	0	0	6 (5.3%)
	Weekly	9 (4.7%)	5 (5.7%)	3 (3.8%)	6 (5.3%)
	Rarely	51 (26.4%)	14 (16.1%)	21 (26.6%)	30 (26.3%)
	Never	119 (61.6%)	67 (77.1%)	51 (64.6%)	68 (59.6%)
		p=0.0003**		p=0.3	
The Use of Fluoride Toothpastes	Always	34 (32.1%)	20 (22.9%)	21 (26.6%)	33 (28.9%)
	Almost always	36 (33.9%)	31 (35.6%)	32 (40.5%)	35 (30.7%)
	Weekly	22 (20.7%)	17 (19.5%)	21 (26.6%)	18 (15.8%)
	Rarely	12 (11.3%)	12 (13.8%)	2 (2.5%)	22 (19.3%)
	Never	9 (4.7%)	7 (8.1%)	3 (3.8%)	6 (5.3%)
		p=0.23		p=0.005**	
Using of sugar in coffee/tee	No use	50 (47.2%)	33 (37.9%)	32 (40.5%)	51 (44.7%)
	1-2 cubes/teaspoons	45 (42.5%)	46 (52.9%)	40 (50.6%)	51 (44.7%)
	2-3 cubes/teaspoons	11 (10.4%)	6 (6.9%)	5 (6.3%)	12 (10.5%)
	4 or more cubes/teaspoons	0	2 (2.3%)	2 (2.5%)	0
		p=0.16		p=0.22	
Period since the last dental visit	Less than 1 year	72 (67.9%)	52 (59.8%)	60 (75.9%)	64 (56.1%)
	1-2 years	19 (17.9%)	20 (22.9%)	5 (6.3%)	34 (29.8%)
	2-4 years	11 (10.4%)	6 (6.9%)	8 (10.1%)	9 (7.9%)
	Over 4 years	4 (3.8%)	9 (10.3%)	6 (7.6%)	7 (6.1%)
		p=0.18		p=0.001**	
Regular visits to the dentist	Yes	36 (33.9%)	16 (18.4%)	23 (29.1%)	29 (25.4%)
	No	70 (66.1%)	71 (81.6%)	56 (70.9%)	85 (74.6%)
		p=0.015*		p=0.57	

Chi-square test: * - $p<0.05$ ** - $p<0.01$

Concerning the level of education among the participants, the majority of respondents had secondary education - 46.6%. The level of education correlated to frequency of tooth brushing - if the level of education was higher, the frequency of tooth brushing was higher. The largest percentage of respondents without basic education (23.1%) reported that they never brush their teeth (Tab. 2).

Table 2. Maintaining of oral hygiene and health of the respondents according to their level of education

		Level of education			
Parameter	**Frequency**	**No education** 13 (6.7%)	**Primary ed.** 43 (22.3%)	**Secondary ed.** 90 (46.6%)	**Higher ed.** 47 (24.3%)
Tooth brushing	Once a day	7 (53.8%)	26 (60.5%)	23 (25.6%)	9 (19.2%)
	More often	3 (23.1%)	17 (39.5%)	64 (71.1%)	38 (80.8%)
	Never	3 (23.1%)	0	3 (3.3%)	0
			p=0.000**		
	In the morning	7 (53.8%)	37 (86.1%)	85 (94.4%)	40 (85.1%)
			p=0.0005**		
	In the evening	6 (46.2%)	17 (39.5%)	65 (72.2%)	45 (95.7%)
			p=0.000**		
	After a meal	0	14 (32.6%)	21 (23.3%)	14 (29.8%)
			p=0.099		
Interdental cleaning	Once a day	0	0	2 (2.2%)	6 (12.8%)
	More often	0	0	2 (2.2%)	4 (8.5%)
	Weekly	0	0	5 (5.6%)	4 (8.5%)
	Rarely	0	0	34 (37.8%)	17 (36.2%)
	Never	13 (100%)	43 (100%)	47 (52.2%)	16 (34.1%)
			p=0.000**		
The Use of Fluoride Toothpastes	Always	1 (7.7%)	5 (11.6%)	28 (31.1%)	20 (42.5%)
	Almost always	1 (7.7%)	8 (18.6%)	43 (47.8%)	15 (31.9%)
	Weekly	0	14 (32.6%)	13 (14.4%)	12 (25.5%)
	Rarely	6 (46.1%)	15 (34.9%)	3 (3.3%)	0
	Never	5 (38.5%)	1 (2.3%)	3 (3.3%)	0
			p=0.000**		
Using of sugar in coffee/tee	No use	5 (38.5%)	13 (30.2%)	36 (40%)	29 (61.7%)
	1-2 cubes/teaspoons	8 (61.5%)	27 (62.8%)	44 (8.9%)	12 (25.5%)
	2-3 cubes/teaspoons	0	3(6.9%)	10(11.1%)	4 (8.5%)
	4 or more cubes/teaspoons	0	0	0	2 (4.3%)
				p=0.011*	
Period since the last dental visit	Less than 1 year	0	28 (65.1%)	64 (71.1%)	32 (68.1%)
	1-2 years	8 (61.5%)	9 (20.9%)	18 (20%)	4 (8.51%)
	2-4 years	0	4 (9.3%)	5 (5.6%)	8 (17.1%)
	Over 4 years	5 (38.5%)	2 (4.6%)	3 (3.3%)	3 (6.4%)
		p=0.000**			
Regular visits to the dentist	Yes	0	3 (6.9%)	32 (35.6%)	17 (36.2%)
	No	13 (100%)	40 (93.1%)	58 (64.4%)	20 (63.8%)
		p=0.0003*			

Chi-square test: * - $p<0.05$ ** - $p<0.01$

With regards to the number of natural teeth present in the mouth, 48.2% of the total number of respondents had 1-15 natural teeth; of those 54.92% were aged 60-69 years and 45.08% were over 70 years of age 21 or more natural teeth were found in 12.9% of the total number of respondents, of whom 16.7% were women (Tab. 3).

Table 3. Preserved teeth of the respondents related to age, gender and level of education

Parameter	Nr	Age		Gender	
		60-69 years 106 (54.92%)	**70+ years** 87 (45.08%)	**Men** 79 (40.93%)	**Women** 114 (56.07%)
Number of present teeth	0	18 (16.9%)	32 (36.8%)	19 (24.1%)	31 (27.2%)
	1-15	55 (51.9%)	38 (43.7%)	40 (50.6%)	53 (46.5%)
	16-20	20 (18.9%)	5 (5.8%)	14 (17.7%)	11 (9.6%)
	21+	13 (12.3%)	12 (13.8%)	6 (7.6%)	19 (16.7%)
		p=0.0025**		p=0.13	
Level of Education		**No education** 13 (6.7%)	**Primary ed.** 43 (22.3%)	**Secondary ed.** 90 (46.6%)	**Higher ed.** 47 (24.3%)
Number of present teeth	0	8(61.5%)	14(32.6%)	28(31.1%)	0
	1-15	5(38.5%)	23(53.5%)	43(47.8%)	22(46.8%)
	16-20	0	0	10(11.1%)	15(31.9%)
		p=0.00001**			

Chi-square test: * - $p<0.05$ ** - $p<0.01$

Discussion

In this research, the assessment of knowledge and attitudes concerning maintenance of oral health among elderly population, aged 60 years and over, in FYROM is presented. This was done on the basis of the answered the questionnaire. The age of respondents significantly corresponded to the frequency of tooth brushing and interdental cleaning, as well as to the habit of going to the dentist and doing regular check-ups. Respondents aged 70 years and older much more often than those aged 60-69 years said they had never cleaned their teeth ($p<0.05$). They also stated that they do not clean the interdental spaces very often ($p<0.01$), and do not go to regular dental visits ($p<0.05$). Gender of respondents in this age population has a significant effect on the use of fluoride toothpaste ($p <0.01$). Education level in patients over 60 years of age significantly affected their knowledge of the individual oral health care. The results show that respondents with higher levels of education brush their teeth more often (brush their teeth in the morning and evening), clean the interdental spaces, go to regular dental check-ups, and use fluoride toothpaste; they also said they do not use or use less sugar in their coffee or tea.

Brushing is the primary method for individual oral health care, which enables efficient level of dental plaque control, caries prevention and maintaining a healthy condition of the periodontal ligament[19,20]. Interdental tooth cleaning can be performed by dental floss or special interdental brushes for cleaning teeth, and is recommended once a day[21,22]. In industrialized countries, 40%-90% of seniors reported that they follow these recommendations[23,24]. The majority of respondents claimed that they use fluoride-enriched toothpaste[25,26]. The detrimental effect of using sucrose and refined sugar for oral health is well documented[27]. A general recommendation is to limit the intake of sugar, no more than 4 times daily, or to limit intake of simple sugars to less than 40 grams a day[28].

The presence of natural teeth is the main indicator for oral health status among older people[29,30]. The presence of 20 or more teeth among the elderly indicates the existence of a functional dentition without the need of prosthetic rehabilitation if the condition of teeth aesthetically pleases the patient[32].

In our research, 33.7% brushed their teeth once or twice a day (or several times per day), of whom 31.6% were men and 35.1% women. The results obtained in a similar study in Lithuania[12], showed that 37% of the

respondents brushed their teeth once a day and 30% of respondents were brushing the teeth twice a day (39% of them were women and 23% men).

As the participants' level of education increased, they brushed their teeth more often: 71.1% of the respondents with secondary education and 80.8% with higher education said that they brush their teeth twice a day and more. Similar results were obtained in a study in Lithuania[12], where 67% of respondents stated that they brushed their teeth twice per day and they were highly educated, compared to 11% who had only primary education and 26% with secondary education. The results from our study showed that 4.1% of all respondents were cleaning the interdental spaces once a day, in contrast to patients examined in the Lithuanian study, where the percentage was 19%. Regarding the use of fluoride toothpastes, 27.9% of all our respondents stated that they always used fluoride toothpaste, whereas 57% of respondents in Lithuanian research said that they used fluoride toothpaste and most of them were aged 60-69 years (67%) and with higher levels of education (87%).

The use of sugar in coffee or tea proved to be a custom among men and women: from the total number of respondents, 38.5 drank coffee or tea without sugar and 40% of that refers to men and 61.7% to women. In Lithuania[12], coffee or tea with 1 cube or teaspoon of sugar drank 26% of respondents and with 2 cubes or teaspoons of sugar drank 46% of respondents. Regular dental visits were recorded in 64.2% of respondents, mostly in younger of them (60-69 years), more often among women. 68.1% of respondents with higher education said that they had a habit of going to the dentist for dental control.

The number of teeth present in the mouth significantly associated with age and level of education among respondents, while it was not significantly associated with gender. From all of the respondents, 48.2% had 1-15 natural teeth, and 21 or more natural teeth was recorded in 12.9% of the total number of respondents. The results obtained in the Lithuanian study[12] showed that 40% of their respondents had 1-15 natural teeth and 25% had 21 or more teeth. Wearing removable dentures, very significantly depends on the level of education, considering the significantly lower number of respondents with no education, who wear removable dentures. From all of the subjects, who were included in our study, 59.6% were wearing removable dentures in both jaws, unlike the respondents in the study of Lithuania[12], where 32% of respondents said that wore removable dentures in both jaws.

A similar study, which aimed to investigate oral health behaviour, knowledge and ability of maintaining oral health among persons aged 35-44 years and 65-74 years, was made in China[33]. The results indicated that 32% of people in the age group of 35-45 years and 23% of the respondents aged 65-74 years, were washing their teeth twice a day, but only 55% of them used fluoride toothpaste. Annual dental visits were registered in 25% of the total number of respondents, while only 6% were on a check-control to the dentist in the last 2 years. Significant differences in the maintenance of oral health were found among respondents from urban areas and provinces. 43% of respondents, aged 35-44 years, consumed sweets every day. Knowledge about the causes of dental diseases and their prevention was low[33].

Oral health status in older people is reflected as a result of oral-health behaviour, oral diseases and their treatment during life. Preservation as many as possible natural teeth in elderly, will be a major challenge for dentists to preserve their teeth and oral health with a greater professional care[34-37].

Conclusion

By realizing the objectives set and based on the obtained findings, the following conclusions can be drawn:

1. Greater care for one's oral health is correlated with a higher level of knowledge and attitudes about oral health care and is in accordance with the recommendations for oral care and maintenance of oral health by the dentists;

2. Improved oral health behaviour and knowledge about oral health care is connected with the presence of a large number of natural teeth in the elderly;

3. Encouraging is the fact that a significant number of older people are brushing their teeth twice a day and also take a relatively good oral health care;

4. Oral health behaviour of elderly people, aged over 60 years, in FYROM is worse than in the industrialized countries, which is reflected in the small percentage of preserved natural teeth in the mouth. Based on the knowledge and attitudes of personal oral health care, we can modify the knowledge and provide a variety of dental treatments from different areas of dentistry, including advice on personal hygiene and maintenance of oral health.

References

1. *Petersen PE, Ueda H.* Oral Health in Ageing Societies. Integration of Oral Health and General Health. Report of the meeting at the WHO Centre for Health Development in Kobe, Japan, June 2005.
2. *Harford J.* Population ageing and dental care. *Community Dent and Oral Epidemiol*, 2009; 37(2):97-103.
3. United Nations, Department of Economic and Social Affairs, Population Division. World population ageing. 2007.

4. Statistical State Office. Statistical Review: Population and social statistics. Nov. 2011 accessed on this website http://www.stat.gov.mk
5. *Coleman P*. Improving oral health care for the frail elderly: A review of widespread problems and best practices. Geriatric Nursing, 2002; Vol. 23, No 4.
6. *Andersson K*. Oral health in old age. Perceptions among elderly persons and medical professionals. Division of Geriatric Dentistry, Institution of Odontology, KarolinskaInstitutet, Stoskholm, Sweden, 2006.
7. *Axelsson P, Albandar JM, Rams TE*. Prevention and control of periodontal diseases in developing and industrialized nations. *Periodontol 2000*, 2002; 29:235-246.
8. *Kelly M, Steele J, Nuttall N, Bradnock G, Morris J, Nunn J, Pine C, Pitts N, Treasure E, White D*. Adult Dental Health Survey. Oral Health in the United Kingdom 1998. Office of National Statistics. London: The Stationary Office, 2000; pp 13-400.
9. *Walker A, Cooper A* (Ed). Adult dental health survey. Oral health in United Kingdom, 1998. Office of National Statistics. London: The Stationery Office, 2000.
10. *Woods N, Whelton H, Kelleher V*. Factor Influencing the Need for Dental Care amongst the Elderly in the Republic of Ireland. Centre for Policy Studies, National University of Ireland, Cork, 2009, accessed on this website www.lenus.ie/hse/
11. *Petersen PE, Kwan S*. Evaluation of community-based oral health promotion and oral diseases prevention - WHO recommendations for improved evidence in public practice. *Community Dent Health*, 2004; 21:319-329.
12. *Vysniauskaite S*. Oral health behavior, conditions and care among dentate elderly patients in Lithuania: preventive aspects. 2009.
13. World Health Organization/Federation Dentaire Internationale. Global Goals for oral health by the year 2000. *Int Dent J*, 1982; 32:74-77.
14. *Petersen PE, Yamamoto T*. Improving the oral health of older people: the approach of the WHO Global Oral Health Programme. *Community Dent Oral Epidemiol*, 2005; 33:81-92.
15. *Sapuric M*. Public health aspects of oral health in elderly, over 65 years in the Republic of Macedonia - state and implications for strategic planning. Master's Thesis. March 2011.
16. Health Strategy of the Republic of Macedonia, accessed on the website http://www.moh-hsmp.gov.mk
17. *Todd JE, Lader D*. Adult Dental Health 1988. United Kingdom, 1990. London: Her Majesty's Stationary Office, 1991.
18. *Tseveenjav B, Suominen AL, Vehkalahti MM*. Oral health-related behaviours among dentate adults in Finland: findings from the Finnish Health 2000 Survey. *Europ J Oral Sciences*, 2012; 120(1):54-60.
19. ADA, 2007a. American Dental Association. Professionally applied topical fluoride: evidence-based clinical recommendations. *J Dent Educ*, 2007; pp 393-402.
20. ADA 2000. American Dental Association. Division of Communications for the Dental Patient. ADA 2000; 131:1095.
21. *Attin T, Hornecker E*. Tooth brushing and oral health: How frequently and when should tooth brushing be performed? *Oral Health Prev Dent*, 2005; 3:135-140.
22. *Berchier CE, Slot DE, Haps S, van der Wejden*. The efficacy of dental floss in addition to a toothbrush on plaque and parameters of gingival inflammation: a systematic review. *Int J Dent Hyg*, 2008; 6:265-279.
23. World Health Organization, Geneva. Oral Health Surveys - basic methods, 1st Edition, 1973.
24. World Health Organization, Geneva. Oral Health Surveys - basic methods, 2nd Edition, 1979.
25. World Health Organization, Geneva. Oral Health Surveys - basic methods, 3rd Edition, 1987.
26. World Health Organization, Geneva. Oral Health Surveys - basic methods, 4th Edition, 1999.
27. *Petersen PE, Aleksejuniene J, Christensen LB, Eriksen HM, Kalo I*. Oral health behaviour and attitudes of adults in Lithuania. *Acta Odontol Scand*, 2000; 58:243-248.
28. *Petersen PE*. Sociobehavioural risk factors in dental caries-international perspectives. *Community Dent Oral Epidemiol*, 2005; 33:274-279.
29. *Petersen PE*. The World Oral Health Report 2003: Continuous improvement of oral health in the 21st century - the approach of the WHO Global Oral Health Programme. http://www.who.int/oral_health/media/en/orh_report03_en.pdf,accessed May 2009.
30. *Petersen PE*. World Health Organization global policy for improvement of oral health. World Health Assembly 2007. *Int Dent J*, 2008; 58:115-121.
31. *Petersen PE*. Global Strategies for Promoting Oral Health - the approach of the World Health Organization, 2009.
32. Petersen PE, Bourgeois DM, Brathhall D, Ogawa H. Oral Health Information Systems towards measuring progress in oral health promotion and disease prevention. Bulletin of the World Health Organization, 83(9). September 2005.
33. *Sheiham A, Netuveli GS*. Periodontal disease in Europe. *Periodontol 2000*, 2002; 29:104-121.
34. *Ling Z, Petersen PE, Wang HY, Bain JY, Zhang BX*. Oral health knowledge, attitudes and behaviour of adults in China. *Int Dent J*, 2005; 55:231-241.
35. *Bourgeois DM, Llodra JC* (Ed). Health Surveillance in Europe. European Global Oral Health Indicators Development Project. Report Proceedings 2003. Paris: Quintessence International, 2004.
36. *Bourgeios DM*. Health Surveillance in Europe. European Global Oral Health Indicators Development Project. Final Report 2003-2005.
37. *Petersen PE, Kandelman D, Arpin S, Ogawa H*. Global oral health of older people. Call for public health action. *Community Dent Health*, 2010; 27(Suppl. 2):257-268.

Correspondence and request for offprints to:

Meri Šapurić
Teodosij Gologanov Str, bb
1000 Skopje, FYR Macedonia
E-mail: sapurik_meri@yahoo.com

BALKAN JOURNAL OF DENTAL MEDICINE

BaSS

ISSN 2335-0245

Dental Caries and Associated Socio-Demographic Factors in Adult People in Bulgaria

SUMMARY

***Objective**: The study aimed to establish the mean DMFT of adults over the age of 20 years in Bulgaria. We also aimed to determine any association between demographic factors, such as age, gender, general health status etc, and dental caries.*

***Material and Methods:** The study was conducted from 2006 to 2009. Data was collected from a randomly chosen representative sample of 1741 adults aged over 20 years, from 13 Bulgarian cities and villages. From these 1741 adults, 105 (6.03%) refused to complete the questionnaire. A total of 1636 (93.97%) were included in the survey, of which 766 (47%) lived in villages and small towns and 870 (53%) in the capital city Sofia and other cities. The average age of the sample was 39.6 years. 894 (54.6%) were male and 742 (45.4%) were female. Each participant completed a questionnaire about demographic and socio-demographic status. Afterwards, a clinical examination was carried out. Chi-square and one-way ANOVA were used to test for statistical significance of qualitative variables ($p<0.05$).*

***Results**: For the whole study sample, mean DMFT was 17.8 (SD 7.98). There was a statistically significant association between DMFT and age. Women had higher DMFT values (18.6 ± 7.66)) than men (15.2 ± 7.91). There was a link between DMFT and general health too. People with excellent general health had DMFT 11.5 (SD 6.39), whereas people with bad general health had DMFT 21.4 (SD 8.16).*

***Conclusion**: Mean DMFT score of adults in Bulgaria is 17.76 teeth. There is an association between some demographic factors and DMFT. Women, people with bad general health and older people have higher values of DMFT and need more care from dental health services.*

Keywords: DMFT; Dental Caries; Socio-Demographic Factors

Boyko Bonev[1], Nadya Avramova[1], Krassimira Yaneva[1], Dimitar Filchev[2]

Medical University, Faculty of Dental Medicine,
[1]Department of Dental Public Health
[2]Department of Prosthetic Dentistry
Sofia, Bulgaria

ORIGINAL PAPER (OP)
Balk J Dent Med, 2015; 19:33-37

Introduction

Dental health care delivery systems and oral health status have improved in more European countries during the recent decades. A number of studies report a reduction of dental caries, not only in children and adolescents[1,2]. Unfortunately, in many Eastern European countries, the prevalence of caries disease in these age groups is still high[3].

A study conducted by Hugoson at all[4] in Sweden for a period of 30 years (from 1973 to 2003) showed that the number of decayed teeth declined with 90% in 15 year olds and 79% in 30 year olds. Slade et al[5] regarding the oral health in adult population showed the reduction of DMFT from 14.9 to 12.6 for the period 1987/88-2004/06 in all age groups. Considering the structure of DMFT index, in1987/88 the mean number of decayed (D) teeth was 1.5, missing (M) teeth - 5.7 and filled (F) teeth - 7.8. In 2006 the index components were significantly reduced - decayed teeth were only 0.6, missing teeth 4.6 and filled teeth 7.4[5].

In 2003-2004 a survey was conducted in Hungary with respect to adult oral health. 4606 persons (2923 female and 1683 male) were included in the study. There were considerable variations in the value of DMFT index - it was 11.79 in the youngest age group and 21.90 in elderly people (65-74 year olds). In all age groups M-component of the index had the highest value. In comparison to the results of older studies in Hungary (Madlen et al[6]) it was found that the number of people with 20 and more teeth increased. For the period 1985-1991 in the age group of 35-44 year olds, the mean value of DMFT index decreased from 15.8 to 15.0. For the next period (1991-2000) this value increased to 15.7 and in 2004 it decreased to 15.4. The structure of DMFT index in this age group (in 1991) was as follows: DT 3, MT 10.2, FT 2.6; in 2004, the structure was: DT 2.2, MT 8.9, FT 4.3[6].

In a number of studies it is shown that values of DMFT index tend to vary significantly between and within different countries. Furthermore, these variations, especially with respect to the structure of the index, are determined by a wide range of socio-demographic factors (age, gender, education, frequency of dental visits, oral health behaviour, etc.)[5,7-12].The value of DMFT is higher in females. Rural residents and people with lower education are more likely to have missing teeth. Urban residents, higher education and better oral hygiene are associated with a lower chance of having decayed and missing teeth, but with a higher chance of having filled teeth[5,7,9-13].

The aim of this study was to establish the mean value of DMFT index in adults over the age of 20 years in Bulgaria. We also aimed to identify socio-demographic factors, such as age, gender and general health status, associated with decayed, missing and filled teeth. The following tasks were defined and executed for implementation of the aims above:

1. To determine the value of DMFT index in people over the age of 20 years in Bulgaria;
2. To determine the structure of DMFT index;
3. To identify some socio-demographic factors associated with DMFT index.

Materials and Methods

A cross-sectional epidemiological study was organized and done. Data for this study was collected between 2006 and 2009 from a randomly chosen representative sample of 1741 adults aged 20 years and over. The participants in the survey were residents of 13 Bulgarian cities and villages. Each participant completed a self-administrated questionnaire consisting of 13 questions. The items were about age, gender, level of education, occupational status, general health status, oral hygiene and other oral health behaviours. Afterwards, a clinical examination was carried out. All oral examinations were done by 1 calibrated examiner in natural light using a mirror and a dental probe, with the subject seated in an ordinary chair.

Of the target subjects in this cross-sectional survey (1741 adults) 105 (6.03%) refused to complete the questionnaire. A total of 1636 (93.97%) were included in the study. Of these, 766 (47%) lived in villages and small towns and 870 (53%) in the capital city Sofia and other urban centres. The average age of the sample was 39.6 years. 894 (54.6%) were males and 742 (45.4%) were females. The Ethical Committee of the Medical University-Sofia approved the study (number 299/15.05.2007). The research was carried out in compliance with the Helsinki Declaration. Verbal consent was obtained from each subject prior to data collection.

Chi- square and one-way ANOVA were used to test for statistical significance of qualitative variables ($p<0.05$).The data of the present study was processed and analyzed with statistical software R.

Results

In present survey the mean value of DMFT index was assessed for the whole study sample. It was 17.76 ± 7.98 (Fig. 1). The structure of DMFT index showed that everyone had 1.96 decayed teeth (DT), 7.41 filled teeth (FT) and 7.56 missing teeth (MT) on average.

Figure 1. Mean value and structure of DMFT-index

Considering the age structure of the study sample, it was found that DMFT index had the highest level in the adults aged 60 years and over (Tab. 1; Fig. 2). The level of the index was lower in the age group of 50-59 year olds, and it was the lowest in the youngest age group of 20-29 year olds. The values of the index were statistically significantly associated with age ($p<0.05$).

Table 1. Mean value ± SD of DMFT-index according to the age of the respondents

Age group / DMF-T Index	20-29	30-39	40-49	50-59	≥60	Total
Mean ±SD	10.33±5.30	14.17±6.59	19.11±6.93	21.22±7.17	24.21±7.11	17.76±7.98

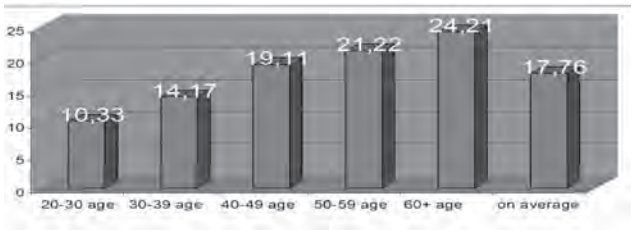


Figure 2. Mean value of DMFT-index according to the age structure of the study sample

There was a statistically significant association between the value of DMFT index and the gender of the dentate subjects in the study sample ($p<0.05$). Generally, females showed more decayed, missing and filled teeth than males (Fig. 3).

General health status was determined by means of self-assessment during the questionnaire survey. The respondents were asked to indicate 1 of possible assessments using a 5-point scale - poor, satisfactory, good, very good and excellent. Persons with excellent general health had the lowest value of DMFT index - 11.52. People with very good general health status showed higher value of the index - 12.27 and those with good health status – 17.53. Persons that indicated their health status as satisfactory had DMFT index 20.84. The level of DMFT index had the highest value in people with poor general health status - 21.44. There was a statistically significant association between the value of DMFT index and the general health status of the respondents (Tab. 2; Fig. 4).

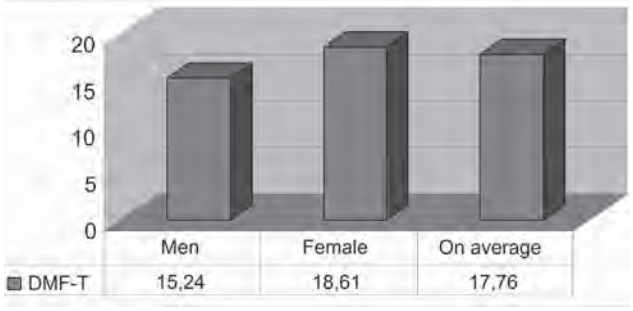


Figure 3. Mean value of DMFT-index according to the gender of the respondents

Figure 4. Mean value of DMFT-index according to respondents' self-assessment of general health status

Table 2. Mean value ± SD of DMFT-index according to the respondents' self-assessment of general health status

General health status / DMF-T Index	Excellent	Very good	Good	Satisfactory	Poor
Mean ±SD	11.52±6.39	12.72±6.87	17.53±7.65	20.84±7.39	21.44±8.16

It was found that DMFT index was not statistically significantly associated with the level of education of the persons included in the study (p>0.05). However, with increasing level of education, the mean number of decayed, missing and filled teeth decreased (Tab. 3; Fig. 5).

Table 3. Mean value ± SD of DMFT-index according to respondents' education

Education / DMF-T Index	Unknown education	Primary School	High school	College	University
Mean ± SD	25.50±9.19	23.63±7.53	16.79±7.85	19.14±8.66	14.93±7.26

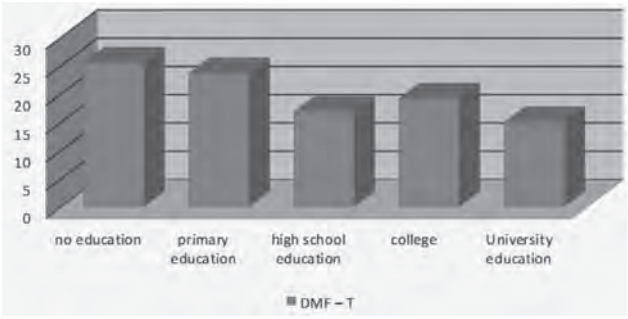


Figure 5. Mean value of DMFT-index according to respondents' level of education

Discussion

DMFT index is main indicator of the caries severity and its components represent different dimension of the disease process. For the last decades, the oral health status of the population in most of the developed European countries has been improved. This tendency is related to increasing needs of dental care, as a result of increased number of natural teeth. This statement is proven by a number of studies demonstrating reduction in the value of DMFT index[4,5]. The present findings show that there is a lack of similar tendency in dental health status of the adults in Bulgaria. For the whole study sample, it was found that the value of the index was very high (17.76). Furthermore, this value is higher than the level of the index in previous studies in Bulgaria[7,9].

According to the results of the current survey, everyone had on average 1.96 decayed teeth. The structure of the index was mainly constituted by filled and missing teeth. These results compared to the findings in other studies are very similar[6]. However, the mean value of 1.96 decayed teeth is still a high level of untreated teeth. These facts indicate main needs of dental care, which are related to treatment of dental caries and tooth extraction as a result of caries disease.

The present findings demonstrate variations in dental health status among adults in the study sample. Differences in the dental health status were associated with a number of socio-demographic factors - age, gender, educational background and general health status. Generally, younger people had lower values of DMFT index than older ones. Females had higher values of DMFT index than males. Adults with poor general health had worse dental health status than these with excellent general health. Also, the results showed that level of education could be a prerequisite for good dental health. People with higher education had a lower chance of having decayed, missing and filled teeth than these with lower education. Although direct comparison of the present outcomes with previous studies is hindered by differences in the study designs, the results of the current study comply with previous suggestions that DMFT index is associated with different socio-demographic factors[7,9,10,12].

Conclusion

From the current study it can be concluded that:

1. The mean value of DMFT index in adults aged 20 years and over, living in Bulgaria, was high - 17.76;
2. The structure of DMFT index indicated that everyone had on average 2 decayed teeth;
3. DMFT index was statistically significantly associated with age, gender and general health status.

Acknowledgements: This study received support through Research Grant № 56/2007 from the Medical University Sofia, Bulgaria. The authors would like to thank all the participants for taking part in this survey.

References

1. *Marthaler TM, O'Mullane DM, Vrbic V.* The prevalence of dental caries in Europe 1990-1995. *Caries Res*, 1996; 30:237-255.
2. *Petersson HG, Bratthall D.* The caries decline: a review of reviews. *Eur J Oral Sci*, 1996; 104:436-443.

3. *Bjarnason S.* High caries levels: problems still to be tackled. *Acta Odontol Scand*, 1998; 56:176-178.
4. *Hugoson A, Koch G, Göthberg C, Helkimo AN, Lundin SA, Norderyd O, Sjödin B, Sondell K.* Oral health of individuals aged 3-80 years in Jönköping, Sweden during 30 years (1973-2003). II. Review of clinical and radiographic findings. *Swed Dent J*, 2005; 29(4):139-155.
5. *Slade GD, Spencer AJ, Roberts-Thomson KF.* Australia's dental generations. The National Survey of Adult Oral Health 2004-06; 2007:274.
6. *Madlen M, Hermann P, Jahn M, Fejerdy P.* Caries prevalence and tooth loss in Hungarian adult population: results of a national survey. BMC Public Health, 2008; 8:364.
7. *Zabov.* Dental caries prevalence among rural and urban population in Bulgaria. Zabolekarski pregled, 1926; №1 (in Bulgarian)
8. *Popov Y.* Needs of dental treatment in PRB (People's Republic of Bulgaria) population. PhD Thesis, Medical University Sofia, Bulgaria. 1973.
9. *Yaneva-Ribagina Kr.* Prevalence of dental diseases and need of dental care among Bulgarian population. PhD Thesis. Medical University Sofia, Bulgaria. 1998.
10. *Gökalp S, Guciz Doğan B, M. Tekçiçek, Berberoğu A, Ünlüer Ş.* National survey of oral health status of children and adults in Turkey. *Community Dental Health*, 2010; 27:12-17.
11. *Shah N, Sundaram RK.* Impact of socio-demographic variables, oral hygiene practices, oral habits and diet on dental caries experience of Indian elderly: a community-based study. *Gerodontology*, 2004; 21(1):43-50.
12. *Skudutyte R, Aleksejuniene J, Eriksen HM.* Dental caries in adults. *Acta Odontol Scand*, 2000; 58:143-147.
13. *Petersen PE, Kaka M.* Oral health status of children and adults in the Republic of Niger, Africa. *Int Dent J*, 1999; 49(3):159-164.

Correspondence and request for offprints to:

Boyko Bonev
Medical University, Sofia
Faculty of Dental Medicine
Department of Dental Public Health
1 G. Sofiiski Str, 1431 Sofia
Bulgaria
E-mail: boiko_bonev@abv.bg

BALKAN JOURNAL OF DENTAL MEDICINE

BaSS

ISSN 2335-0245

Influence of Irrigation with NaOCl and Chlorhexidine on Microleakage

SUMMARY

Background*: Irrigation during endodontic therapy is required in order to remove debris, tissue remnants, microbes and smear layer. Sodium hypochlorite (NaOCl) and Chlorhexidine (CHX) are the most commonly used irrigants. Although they are reported to have good antimicrobial effects, both have limitations. Hence, a combination of NaOCl and CHX has been proposed to compensate for these limitations. However, this association forms a dense, orange-brown precipitate that stains walls of the pulp chamber. The aim of this study was to clarify in vitro if this precipitate affects the microleakage of endodontic sealers.*

Material and Methods*: Extracted human teeth were used for this study. The teeth were cut at the height of the cervix and instrumented with NiTi rotary instruments. They were then divided into 2 experimental groups. In the first group (Group A) irrigations were performed with 2ml NaOCl 1%, 1ml EDTA 17% and 1ml CHX 0.2% and in the second (Group B) with 2ml NaOCl and 2ml CHX. Following this, they were obturated with gutta-percha and roth sealer. The microleakage was determined using a fluid filtration method. The measurements were repeated a month later. All analyses were performed using Fisher exact test.*

Results*: Microleakage of Group A was lower than microleakage of Group B but the difference was not statistically significant.*

Conclusion*: The precipitate that is formed by NaOCl and CHX did not affect microleakage of endodontic sealers.*

Keywords: Irrigation; Precipitate; Microleakage; Sodium hypochlorite; Chlorhexidine

Zoumpoulia Mylona, Christos Gogos, Nikolaos Economides

Aristotle University of Thessaloniki
School of Dentistry
Department of Endodontology
Thessaloniki, Greece

ORIGINAL PAPER (OP)
Balk J Dent Med, 2015; 19:38-42

Introduction

The primary aim of root canal treatment is to eliminate bacteria from the infected root canal and to prevent re-infection[1,2]. Although a mechanical action of instruments can reduce the number of bacteria, complete canal disinfection is difficult because of the complexity of the internal root canal anatomy[2,3]. In addition, mechanical instrumentation forms a smear layer on the canal surface[3]. As a result, irrigation is required to remove debris, tissue remnants, microbes and the smear layer[3]. A variety of irrigants have been used in an attempt to achieve these aims but no single preparation has been found to be completely predictable or effective[1].

Sodium hypochlorite (NaOCl) is the irrigant most commonly used during endodontic therapy in a concentration ranging from 0.1% to 5.25%. It is efficient in dissolving organic tissues as well as eliminating microorganisms[2-6]. Its germicidal ability is related to the formation of hypochlorous acid when in contact with organic debris[2]. However, in high concentrations, NaOCl is toxic and can cause inflammation in the periapical tissues, whereas in low concentrations it is ineffective against specific microorganisms[6,7]. Moreover, NaOCl is unable to completely remove the smear layer, it does not impart antimicrobial substantiveness, it corrodes surgical instruments and has a very unpleasant odor[2,6,7].

An alternative irrigant is chlorhexidine gluconate (CHX). CHX is a cationic bisbiguanide with a broad-

spectrum antimicrobial action that acts by absorbing onto microbial cell walls or by disrupting them, causing leakage of intracellular components[1,8]. It has an antibacterial efficacy comparable to that of NaOCl[9,10], while also being effective against certain NaOCl resistant bacterial strains[9,11]. In addition, CHX has the advantages of having both substantiveness[13] and low level of toxicity[12]; however, the inability of CHX to dissolve organic matter is a perceived drawback[14].

Both NaOCl and CHX have limitations despite their reported good antimicrobial effects[8]. Hence, a combination of NaOCl and CHX has been recommended to enhance their properties, on the basis that the antimicrobial effect of 2.5% NaOCl and 0.2% CHX used in combination is better than that of either solution alone[10]. However, this association forms a dense, orange-brown precipitate that stains the walls of the pulp chamber[2,7,15,16]. This insoluble precipitate is difficult to remove from the canal, occludes the dentinal tubules[3] thus preventing penetration of the intra-canal medicaments and compromises the seal of the obturated root canal[6]. Additionally, its presence imparts colour to the canal wall and causes tooth discoloration affecting aesthetic appearance[6,17]. Moreover, it has been reported that when mixed with NaOCl, CHX hydrolyzes and forms para-chloroaniline (PCA), a toxic and carcinogenic product[18].

In order to eliminate formation of the precipitate, the use of intermediate flushes of saline or distilled water in greater volumes to enhance dilution effect on NaOCl has been suggested, or to eliminate the formation of precipitate by flushing away the remaining NaOCl with absolute alcohol before using CHX as the final irrigant; However, in this case, the biocompatibility of alcohol with the periapical tissues remains a concern[2]. Irrigation with EDTA and/or drying with paper points in order to remove the NaOCl has also, been proposed[7,8,16,19]. Kuruvilla[10] managed to dissolve the precipitate with methanol. Alternatively, 0.1 mol/ml acetic acid also manages to dissolve the precipitate[10].

The aim of this study was to clarify *in vitro* if the precipitate caused by the NaOCl and CHX combination affects microleakage of endodontic sealers.

Material and Methods

40 single-rooted extracted human teeth were used for this study. Each tooth was placed in NaOCl for 2 hours for surface disinfection, and then stored in distilled water until use. 35 teeth of these were cut at the height of the cervix with a high speed bur leaving only the root. The working length was determined with a #10 K-File by introducing into the canal until the tip of the file was visible at the apical foramen and then subtracting 1mm. All the resulting 35 samples were subsequently prepared by rotary instrumentation with Protaper instruments using a F3 File as the finishing file. During instrumentation, irrigation was performed using NaOCl between each instrument. Then, these 35 teeth were randomly divided into 2 experimental groups of 15 teeth (A and B) and one positive control group of 5 teeth (C). The remaining 5 teeth from the initial 40 composed the negative control group (D).

The samples of group A were irrigated with 2ml NaOCl 2.5%, 1ml EDTA 17% and 1ml CHX 0.2 %. After each irrigation, root canals were dried with paper points. The teeth of group B were irrigated with 2ml NaOCl and 2ml CHX. Finally, the teeth of groups A and B were obturated using one point technique with gutta-percha Protaper points NoF3 and Roth sealer (Roth International LTD). Positive controls were left unfilled.

Thus the following groups were created:

Group A: This group comprised 15 teeth, which were prepared, irrigated with NaOCl, EDTA and CHX and obturated with gutta-percha

Group B: This group comprised 15 teeth, which were prepared, irrigated with NaOCl and CHX, and obturated with gutta-percha.

Group C: This group comprised 5 teeth which were prepared, irrigated with NaOCl but left unfilled.

Group D: This group comprised the first 5 teeth, which were not instrumented; these constituted the negative control group.

Microleakage was evaluated by using a modified fluid filtration study design previously reported by Pashley and Depew (1986). The specimens were attached to a plastic tube. Cyanoacrylate cement was applied circumferentially between root and plastic tube to fix the specimens tightly. A 20 HL glass micropipette was connected to the plastic tube on the outlet side of the specimen. Distilled water was used to fill all pipettes, syringes and plastic tubes. The whole set-up was then placed in a water bath (20°C) and, using a syringe, the air bubble was adjusted to a suitable position within the capillary. A pressure of 30 kPa (0.296 atm) was applied on the coronal side. A 5-minute pressurization preload of the system was completed before taking readings. The position of the bubble was recorded. The quality of the seal of each specimen was measured at 2, 4, 6 minutes. The amount of microleakage was recorded in the units of HL/cm H_2O per minute. The measurements were repeated a month later.

The data was evaluated using SPSS software (Statistical Package for the Social Sciences). Analysis of Fisher's exact test was used to perform the comparison. The significance level was set at 5%.

Results

The results are presented in table 1 (immediate microleakage) and table 2 (microleakage 30 days later).

The positive controls showed fluid movement throughout the length of the canals (Group C), while the root canals in the negative control group did not display any fluid leakage (Group D). The microleakage of Group A after 30 das was lower than microleakage of Group B but the difference was not statistically significant. A month later, despite an expected rise in microleakage in both groups, the results were the same: there was no statistical difference between the groups.

Table 1. Immediate microleakage of group A and B

time	2 min		4 min		6 min	
	Group A	Group B	Group A	Group B	Group A	Group B
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	CL*	0	CL*	0	CL*
4	0	0	0	0	0	0
5	CL*	CL*	CL*	CL*	CL*	CL*
6	0	CL*	0	CL*	0	CL*
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	0	CL*	0	CL*	0	CL*
10	0	CL*	0	CL*	0	CL*
11	0	0	0	0	0	0
12	CL*	0	CL*	0	CL*	0
13	CL*	CL*	CL*	CL*	CL*	CL*
14	0	CL*	0	CL*	0	CL*
15	0	0	0	0	0	0

* - CL: cross leakage

Table 2. Microleakage for group A and B after 30 days

Time	2 min		4min		6 min	
	Group A	Group B	Group A	Group B	Group A	Group B
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	CL*	0	CL*	0	CL*
4	0	0	0	0	0	0
5	CL*	CL*	CL*	CL*	CL*	CL*
6	0	CL*	0	CL*	0	
7	0	0	0	0	0	0
8	CL*	CL*	CL*	CL*	CL*	CL*
9	CL*	CL*	CL*	CL*	CL*	CL*
10	0	CL*	0		0	
11	0	0	0	0	0	0
12	CL*	0	CL*	0	CL*	0
13	CL*	CL*	CL*	CL*	CL*	CL*
14	0	CL*	0	CL*	0	CL*
15	0	CL*	0	CL*	0	CL*

* - CL: cross leakage

Discussion

It is well established that biomechanical cleaning and shaping of the root canal system using files and antibacterial irrigants reduces the bacteria load but no irrigant can completely eliminate all organic and inorganic matter and at the same time impart a substantive residual antibacterial property to the canal wall dentin. A combination of NaOCl and CHX has been proposed in order to enhance their antimicrobial properties and to improve the chemical cleaning of root canal[10]. Although such a combination of irrigants may enhance their antimicrobial properties chemical interactions among the irrigants have to be considered.

As already mentioned, the combination of NaOCl and CHX results in the formation of a precipitate. Basrani et al[7] determined that this formation is immediate and independent of the concentration of NaOCl and CHX. The lowest concentration of NaOCl to cause the formation of precipitate was 0.19%[7]. When the NaOCl came in contact with CHX precipitate was formed and a colour change occurred immediately, which did not change with time[7]. As concentration of NaOCl increased, the colour varied from peach to brown and the precipitate thickened[7].

The precipitate covers the dentin walls and affects the patency of the dentinal tubules[3,17]. Bui et al[3] found a statistically significant reduction in the number of patent dentinal tubules in the experimental groups when compared with the negative control group, but the obstruction of dentinal tubules was not found to be significant in the apical third. There were no significant differences among all the experimental and control groups. This might be due to the fact that the apical third is more difficult to irrigate[3]. However, Akisue et al[8] assert

that the combination of 1% NaOCl and 2% CHX solutions results in formation of a flocculate precipitate that acts as a chemical smear layer reducing the permeability of the dentine in the apical third.

Formation of the precipitate could be explained by the acid-base reaction occurring when NaOCl and CHX are mixed[7]. CHX, a di-cationic acid (pH 5.5-6.0) has the ability to donate protons. NaOCl is alkaline and can accept protons from the di-cationic CHX[7]. This proton exchange results in the formation of a neutral insoluble substance, referred to as the "precipitate".

In regard to actual composition of the precipitate, there is no general agreement and more research needs to be done to clarify this[17]. Basrani et al[7] used x-ray photoelectron spectroscopy (XPS) and time of flight secondary ion mass spectrometry (TOF-SIMS) to identify this precipitate. They found that the precipitate contains a significant amount of para-chloroaniline (PCA). It has been reported that, when placed in an aqueous solution, CHX slowly hydrolyzes and forms para-chloroaniline. This occurs through the substitution of the guanidine group in the CHX molecule. Basrani's findings indicated that when mixed with NaOCl, the CHX molecules become hydrolyzed into smaller fragments, each forming a by-product. It has been suggested that the first bonds to be broken in this reaction are between carbon and nitrogen because of the low bond dissociation energy existing between the 2 atoms. Molecules with low bond disassociation energies are more prone to breakdown. This disassociation results in formation of PCA, among other fragments. PCA can further degrade to 1-chloro-4-nitrobenzene[3]. Krishnamurthy et al[2] maintain that PCA is the main product of the interaction of NaOCl and CHX, with the molecular formula NaC6H4Cl as analyzed by mass spectrometry. The presence of PCA was confirmed in this study by the Beilstein test for the presence of chlorine and the HCl solubility test for the presence of aniline[2]. The presence of chlorine in the para position of the benzene ring was finally confirmed using the nuclear magnetic resonance imaging technique[2]. However, Thomas and Sem[15] claimed that the reaction mixture of NaOCl and CHX does not produce PCA in any measurable quantity, and further investigation is needed to determine the chemical composition of the brown precipitate[15]. In addition, Nowicki and Sem[21] using one dimensional and dual dimensional NMR found that the precipitate consists of at least 2 sub-products of CHX, smaller than CHX, neither of them being PCA.

PCA has industrial uses in pesticides and dye sand has been demonstrated to be carcinogenic in animals[22]. Its degradation product, 1-chloro-4-nitrobenzene, is also a carcinogen[3]. As an aromatic amine, the primary toxic effect is methemogloblin formation[18]. Short-term exposure of humans to PCA results in cyanosis, which is a manifestation of methemoglobin formation[18]. Toxicological studies in rats and mice have shown that the haemopoietic system is the major target for PCA[18,22]. Chabra et al[18] conducted a 90-day study and found that methaemoglobin formation and accompanying haemolytic anaemia, extra-medullary haematopoiesis, and splenomegaly were indicative of erythrocyte toxicity and regenerative anaemia. They reported PCA to be carcinogenic in rats due to increased sarcomas in the spleen[18]. In male mice, there was an increase in hepato-cellular carcinomas and haemangiosarcomas of the spleen[18]. The amount of precipitate left behind is unclear. Concern exists that this precipitate might be absorbed onto the root surface and can slowly leach into the periapical tissues[3].

In addition to these concerns, the presence of this precipitate on the root surface might affect the seal of an obturated root canal, especially with resin sealers in which a hybrid layer is required[3]. Vivacqua-Gomez et al[6] studied the coronal microleakage after root-canal treatment using different endodontic irrigants and found that the combination of NaOCl with CHX showed the worst results when compared to 1% NaOCl and 17% EDTA, 2% CHX gel, distilled water[6]. Our findings are not in accordance with this study as we found that the irrigation method did not significantly affect the microleakage.

However, the precipitate formed is also of clinical relevance because of staining, reduction in the action of antiseptic solutions and possible leaching of PCA into the periapical tissue. In order to eliminate all these drawbacks, while still taking advantage of the combined action of NaOCl and CHX, various strategies have been employed. Hence, the use of intermediate flushes of saline or distilled water in greater volumes to enhance the dilution effect on NaOCl or to prevent its formation by flushing away the remaining NaOCl with absolute alcohol before using CHX as the final irrigant has been suggested. However, in this case, biocompatibility of alcohol with the periapical tissues remains a concern[2]. Irrigation with EDTA and/or drying with paper points in order to remove the NaOCl have also been proposed[7,8,16,19]. Kuruvilla and Kamath[10] managed to dissolve the precipitate with Methanol. In addition, the use of 0.1mol/ml acetic acid, which also dissolves the precipitate, has been proposed[10].

Acknowledgments. The authors thank Pr. Anastassios V. Katos, from the Department of Applied Informatics of University of Macedonia, Thessaloniki, for statistical analysis. This study was co-financed by the project "Scholarships SSF" from the resources of the European programme "Education and Lifelong Learning" of the European Social Fund - NSRF 2007-2013.

References

1. *Athanassiadis B, Abbott PV, Walsh LJ*. The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics. *Aust Dent J*, 2007; 52(1):64-82.

2. *Krishnamurthy S, Shashikala S.* Evaluation and Prevention of the Precipitate Formed on Interaction between Sodium Hypochlorite and Chlorhexidine. *J Endod*, 2010; 36:1154-1157
3. *Bui TB, Baumgartner JC, Mitchell JC*. Evaluation of the interaction between Sodium Hypochlorite and Chlorhexidine Gluconate and its Effect on Root Dentin.
4. *Spångberg L, Pascon EA*. The importance of material preparation for the expression of cytotoxicity during in vitro evaluation of biomaterials. *J Endod*, 1988; 14:247-250.
5. *Delany GM, Patterson SS, Miller CH, Newton CW*. The effect of chlorhexidine gluconate irrigation on the canal flora of freshly extracted necrotic teeth. *Oral Surg*, 1982; 53:518-522.
6. *Vivacqua-Gomes N, Ferraz CC, Gomes BP, Zaia AA, Teixeira FB, Souza-Filho FJ*. Influence of irrigants on the coronal microleakage of laterally condensed gutta-percha root fillings. *Int Endod J*, 2002; 35:791-795.
7. *Basrani BR, Manek S, Sodhi RN, Fillery E, Manzur A*. Interaction between Sodium Hypochlorite and Chlorhexidine Gluconate. *J Endod*, 2007; 33:966-969.
8. *Akisue E, Tomita VS, Gavini G, Poli de Figueiro JA*. Effect of the Combination of Sodium Hypochlorite and Chlorhexidine on Dentinal Permeability and Scanning Electron Microscopy Precipitate Observation. *J Endod*, 2010; 36:847-850.
9. *White RR, Hays GL, Janer LR*. Residual antimicrobial activity after canal irrigation with chlorhexidine. *J Endod*, 1997; 23:229-231.
10. *Kuruvilla JR, Kamath MP*. Antimicrobial activity of 2.5% sodium hypochlorite and 0.2% chlorhexidine gluconate separately and combined, as endodontic irrigants. *J Endod*, 1998; 24:472-476.
11. *Basrani B, Santos JM, Tjaderhane L, et al*. Substantive antimicrobial activity in chlorhexidine-treated human root dentin. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 2002; 94:240-245.
12. *Oncag O, Hosgor M, Hilmioglu S, Zekioglu O, Eronat C, Burhanoglu D*. Comparison of antibacterial and toxic effects of various root canal irrigants. *Int Endod J*, 2003; 36:423-432.
13. *Rosenthal S, Spangberg L, Safavi K*. Chlorexidine substantivity in root canal dentin. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 2004; 98:488-492.
14. *Okino LA, Siquera EL, Santos M, Bombana AC, Fingueiredo JA*. Dissolution of pulp tissue by aqueous solution of chlorhexidine digluconate and chlorhexidine digluconate gel. *Int Endod J*, 2004; 37:38-41.
15. *Thomas JE, Sem DS*. An In Vitro Spectroscopic Analysis to Determine Whether Para-Chloroaniline is Produced from Mixing Sodium Hypochlorite and Chlorhexidine. *J Endod*, 2010; 36:315-317.
16. *Vianna ME, Gomes BP*. Efficacy of sodium hypochlorite combined with chlorhexidine against Enterococcus faecalis in vitro. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 2009; 107:585-589.
17. *Marchesan MA, Pasternak Junior B, Afonso MM, Sousa-Neto MD, Paschoalato C*. Chemical analysis of the flocculate formed by the association of sodium hypochlorite and chlorhexidine. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 2007; May103(5):e103-105.
18. *Chabra RS, Huff JE, Haseman JK, Elwell MR, Peters AC*. Carcinogenicity of parachloroalinine in rats and mice. *Food Chem Toxicol*, 1991; 29:119-124.
19. *Basrani BS, Manek S, Fillery E*. Using diazotization to characterize the effect of heat or sodium hypochlorite on 2.0% chlorhexidine. *J Endod*, 2009; 35:1296-1299.
20. *Cobankara FK, Adanr N, Belli S*. Evaluation of the influence of smear layer on the apical and coronal sealing ability of two sealers. *J Endod*, 2004; 30:406-409.
21. *Nowicki JB, Sem DS*. An in vitro spectroscopic analysis to determine the chemical composition of the precipitate formed by mixing sodium hypochlorite and chlorhexidine. *J Endod*, 2011; 37:983-988.
22. *Beard RR, Noe JT*. Aromatic nitro and amino compounds. In: Patty's Industrial Hygiene and Toxicology. 1981; pp 2413-2489.

Correspondence and request for offprints to:

Dr Zoumpoulia Mylona
Aristotle University of Thessaloniki
School of Dentistry, Department of Endodontology
Thessaloniki, Greece
E-mail: myliaptol@windowslive.com

BALKAN JOURNAL OF DENTAL MEDICINE

BaSS

ISSN 2335-0245

Computerized Reconstruction of Pulpal Blood Vessels Examined under Confocal Microscope

SUMMARY

The purpose of this study was the evaluation of 3 different histological methods for studying pulpal blood vessels in combination with 2 types of confocal microscope and computer assisted 3-dimensional reconstruction. 10 human, healthy, free of restorations or caries teeth that were extracted for orthodontic reasons were used. From these teeth, the pulp tissues of 5 were removed, fixed in formalin solution, dehydrated and embedded in paraffin. Serial cross sections 5μm thick were taken from 3 of the above mentioned pulpal tissues and stained with CD34 according to the immunohistochemical ABC technique, while the rest 2 were stained with CD34 and Cy5 by means of immunofluorescence after serial cross sectioning of 10μm. 5 of the 10 teeth were fixed, decalcified, serial cross sectioned (30μm thickness) and stained with eosin. The physical sections were examined under 2 types of confocal laser microscope. Serial images were taken for each section, alignment of the images was followed and finally 3-dimensional reconstructions of the pulpal vessels were achieved.

The combined use of immunofluorescence, confocal microscope and automatic segmentation proved to be a useful method for the detailed study of pulpal vasculature. The above method provides deep knowledge of the form and spatial relationship even of the smallest pulpal blood vessels with neighbouring structures like odontoblasts, which are essential for the fully understanding of their role and function within the dental pulp.

Key words: CD34; Cy5; Immunofluorescence; 3D reconstruction

Anna Digka[1], Kleoniki Lyroudia[2], Lucie Kubinova[3], Georgia Karayannopoulou[4], Ioannis Marras[5], Ioannis Pitas[5]

[1]Private Dentist, Thessaloniki, Greece
[2]Aristotle University of Thessaloniki
Department of Endodontology, Dental School
Thessaloniki, Greece
[3]Academy of Sciences of the Czech Republic
Institute of Physiology
Department of Biomathematics
Prague, Czech Republic
[4]Aristotle University of Thessaloniki
Medical School, Thessaloniki, Greece
[5]Aristotle University of Thessaloniki
Department of Informatics, Thessaloniki, Greece

ORIGINAL PAPER (OP)

Balk J Dent Med, 2015; 19:43-49

Introduction

Dental pulp is the only soft dental tissue, surrounded by dentin, and it consists of odontoblasts, fibroblasts, antigen-presenting cells, stem cells, connective tissue fibres, and a broad vascular and nervous plexus[1,2]. The inner layer of the entire vascular system consists of endothelial cells (ECs). CD34 is a cell surface protein that is expressed by hematopoietic cells (HCs) and ECs. Antibodies against CD34 are extensively used as a pan-endothelial marker in immunohistochemistry[3,4]. Cy5-conjugated anti-CD34 has been used in immunofluorescent confocal microscopy[5]. Eosin, on the other hand, is a fluorescent red dye resulting from the action of bromine on fluorescein. It can be used to stain cytoplasm, collagen and muscle fibres for examination under microscope.

A variety of methods have been used until today in order to study the structure of the vascular plexus of the dental pulp. One of the above mentioned methods was that of resin casts in order to follow the dental pulp vessels' distribution[6], while later on, 3-dimensional reconstruction by using serial cross sectioning and light or transmission electron microscopy were used[7,8] .

The purpose of this study was the evaluation of 3 different histological methods for studying pulpal blood vessels in combination with 2 types of confocal microscope and computer assisted 3-dimensional reconstruction.

Material and Methods

Tissue Specimens

The research was approved by the Ethical Committee of the Aristotle University of Thessaloniki, Greece. 10 upper premolars from young patients (range 16-18 years), extracted for orthodontic reasons, were used. The teeth were free of caries and without restorations.

5 teeth were fixed immediately after extraction in a 10% formalin solution buffered at a pH of 7.2 for 48 hours. A longitudinal groove was prepared along the external surface of each tooth thereafter and a mechanical fracture was achieved in order to have an exposure of the dental pulp tissue. The exposed pulp tissue was gently removed and was put once again into a fresh formalin solution of the same concentration for 48h. The specimens were finally embedded in paraffin blocks and serial cross sections 5μm thick were taken from each specimen that was used for immunohistochemistry, while 10μm thick serial cross sections were taken for the immunofluorescence procedure. Immunohistochemistry for CD-34 was applied on the sections of 3 specimens. Immunofluorescence for CD-34 and Cy-5 was applied on 2 remaining specimens.

5 teeth were decalcified after the fixation period. Serial cross sections 30μm thick were taken from the above specimens and eosin stained.

All serial cross sections were examined under confocal microscope.

Antibodies-Immunohistochemistry

The avidin:biotinylated enzyme complex (ABC) technique was used in the cases selected to be treated following the immunohistochemical method. The sections were deparaffinised, endogenous peroxydase was inhibited by using 1.6% hydrogen peroxide in methanol, washing with distilled water was done and heating in a microwave oven followed in a citrate buffer 10mM pH 6 for 21min.

Rinsing with Tris-buffered saline (TBS) (DAKO) was done thereafter and incubation with rabbit serum (DAKO) in TBS 1/5 for 20min was performed. After that, an incubation with mouse monoclonal primary antibody CD34 (Novocastra) in 1/20 dilution was performed overnight. The sections were rinsed with phosphate-buffered saline and incubation was followed with Biotinylated Rabbit Anti-Mouse immunoglobulins (DAKO) in 1/200 dilution for 30min. Washing with TBS and incubation with StrepABC complex HRP (DAKO) at 1/100 dilution for 30min followed. Colour was developed with chromogen 3, 3-diaminobenzidine (DAKO) for 5 min, while washing with TBS and nuclear counterstaining with Harris haematoxylin for 45 sec was achieved. Normal mouse serum replaced the primary antibody in negative control sections.

Antibodies-Immunofluorescence

The specimens selected for immunofluorescence, were serial cross sectioned (10μm thick). The sections were deparaffinised and treated with 2.73% hydrogen peroxide and 0.1% sodium azide diluted with distilled water. After trypsin digestion the sections were first incubated with CD34 monoclonal antibody diluted at 1/20 with TBS containing 55% foetal calf serums for 12h at 4ºC and then with Cy TM5-conjugated secondary antibody diluted at 1/100 with phosphate-buffered saline for 12h at 4ºC. The slides were mounted in Mowiol.

Decalcification and Eosin Staining

After fixation with a 10% formalin solution, the teeth were decalcified by using a 5% solution of trichloroacetic acid. Decalcification was controlled roentgengraphically. After decalcification teeth were transferred into 10% formalin with 0.5% of eosin for 3 days, dehydrated and embedded in glycol-methacrylate. Thereafter, 30μ thick serial sections were taken.

Laser Scanning Confocal Microscopy

a. Observation of the sections 5μm thick stained with CD34 was done by using Confocal Laser Scanning Microscope (CLSM) Bio-Rad MRC 600 (using excitation wavelength of 488nm), equipped with an oil immersion Nikon CFN Plan Fluor objective (40x, N. A. = 1.3). From each physical section, 11-14 images were taken at various overlapping locations of each optical section. 6 serial optical sections, 1μm apart, were taken from each paraffin section.
b. Observation of the sections 30μm thick, stained with eosin, was also done by using CLSM Bio-Rad MRC 600 equipped with an oil immersion Nikon CFN Plan Fluor objective (40x, N. A. = 1.3). From each physical section, 15-30 images were taken at various overlapping locations of each optical section. 6 serial optical sections, 5μm apart, were taken from each glycol-methacrylate section. .
c. The sections for immunofluorescence were observed in a Leica TCS SP2 AOBS CLSM using 20x oil immersion objective (HC PL APO CS, NA=0.70). For Cy-5 detection, excitation with helium-neon laser at a wavelength of 633 nm and emission at 649-732 nm was applied in one channel (red), while in the second channel (green), the autofluorescence images were acquired at the excitation wavelength of 514 nm and detection range 572-709 nm.

3-Dimensional Reconstruction and Visualization

2 CLSMs were used, namely Bio-Rad MRC 600 and Leica TCS SP2 AOBS. CD34 and eosin staining was used for imaging the cross-sections. As no single microscopy image could cover the entire section, a number of overlapping images were taken from each serial optical section. Consequently, digital image stitching was

performed using the GlueMRC software to obtain one image per physical section[9]. 3-dimensional reconstruction and visualization of the vascular plexus were achieved using either surface or volume rendering.

a. For surface rendering, vessel segmentation was achieved automatically using colour and texture information10,11, or using active contour approaches12. Semi-automatic segmentation has also been used in case that better segmentation accuracy was needed. The alignment of serial sections was performed automatically through image registration13 with manual fine tuning. Then the segmented serial sections were interpolated14 and the vessel surfaces were triangulated. Finally, 3D visualization of the obtained vessel surfaces was achieved by using surface rendering15.
b. For surface rendering fully automatic image registration16,17 and alignment algorithms were used to register the serially acquired optical slices with respect to a reference slice18,13. Such algorithms rely on a global energy function[19] with variables the rigid transformation parameters (2D translation and rotation) of a physical slice with regard to its neighbourhood. A volumetric rendering algorithm, using opacity, shading, depth and light effects, was then applied[20] for better visualization and morphological analysis of the 3D reconstructed data. Image warping techniques can be used to cover eventual spurious holes in the tissue[21].

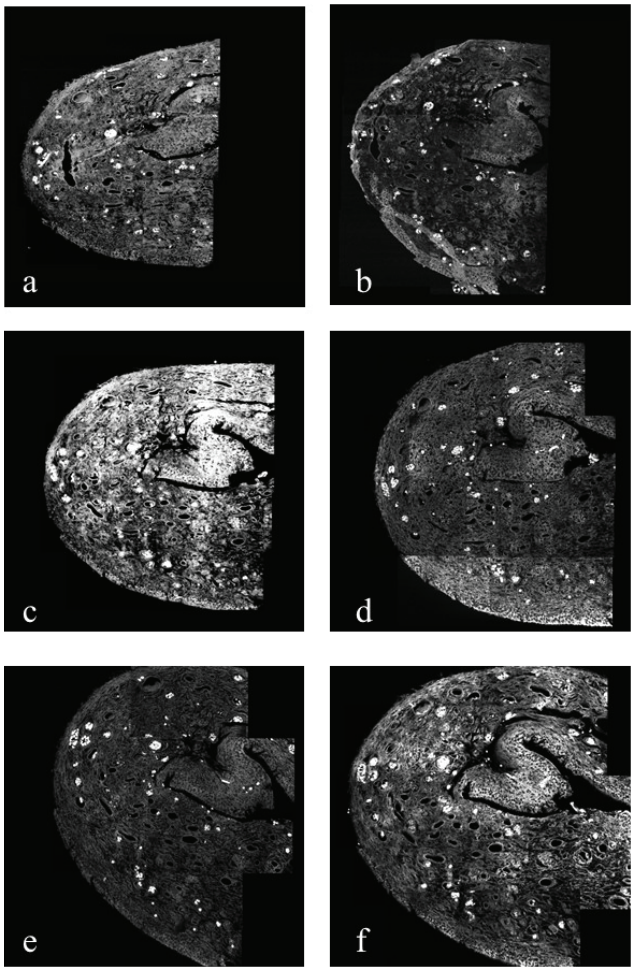


Figure 1. Serial paraffin cross sections (a-f), 5μm thick, stained for CD34 with immnunohistochemical ABC technique and studied by confocal scanning laser microscope BioRad MRC 600

Results

a. By using immunohistochemistry (CD34) and confocal microscope BioRad MRC 600, the structure of the dental pulp and its pulpal vessels was shown (Fig.1). 3-dimensional reconstruction of the above case is observed in figure 2. The course and spreading of the pulpal vessels, as well as the presence of vessels of different sizes are shown in the above 3-dimensional reconstruction.
b. By using eosin staining of decalcified teeth and BioRad MRC 600, the appearance of the pulpal vessels' plexus is clearly seen (Fig. 3). 3-dimensional reconstruction of the pulpal vessels' plexus is shown in figure 4. The distributions of the vessels within pulpal tissue as well as the presence of larger and smaller vessels and their spatial relationship inside dental pulp are shown in figure 4. Spreading of vessels' plexus covers the entire pulpal space, while in the wider root canal the vascular plexus appears to be denser and the presence of larger vessels is more intense than in the narrower root canal.

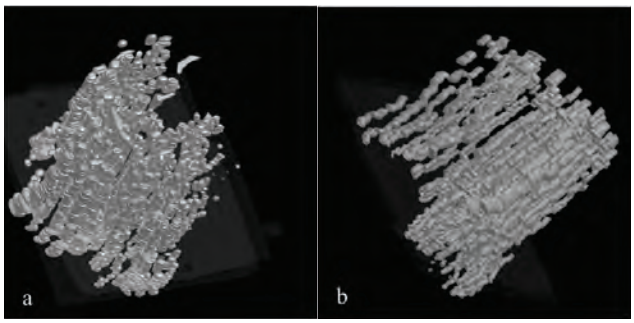


Figure 2. 3-dimensional volume reconstructions of 6 serial paraffin cross sections, 5μm thick, stained for CD34 with immnunohistochemical ABC technique and imaged using the confocal scanning laser microscope BioRad MRC 600. The course and spatial arrangement of the dental pulp vessels are shown

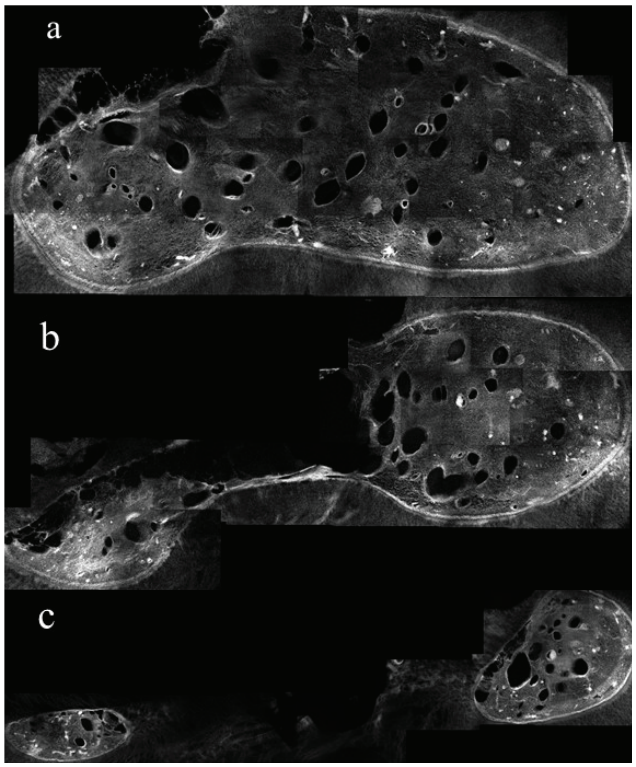


Figure 3. 3 histological sections of decalcified and glycol-methacrylate embedded specimen, 30μm thick, stained with eosin and studied by confocal scanning laser microscope BioRad MRC 600, from the coronal (a), middle (b) and apical (c) part of the pulp

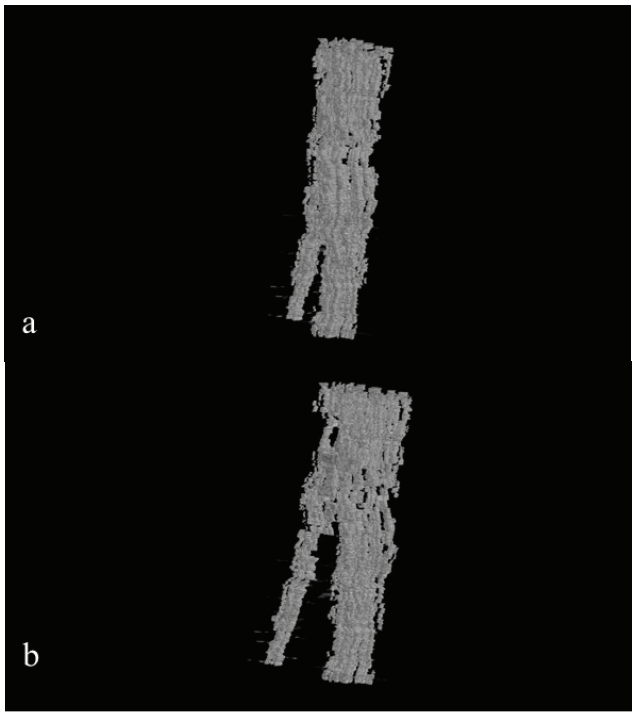


Figure 4. 3-dimensional volume reconstructions of serial cross sections of a decalcified and glycol-methacrylate embedded specimen, 30μm thick, stained with eosin and imaged using the confocal scanning laser microscope BioRad MRC 600. The course and spatial arrangement of the dental pulp vessels in both root canals are shown

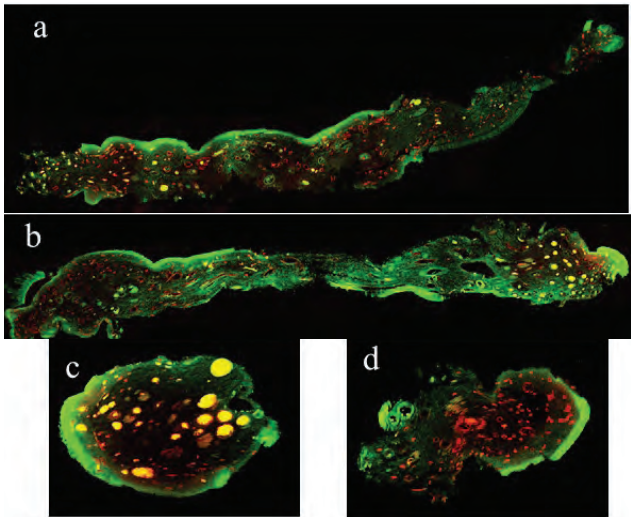


Figure 5. 3 histological paraffin sections, 10μm thick, stained for CD34-Cy5 by immunofluorescence and studied by Leica TCS SP2 AOBS confocal laser scanning microscope, from the coronal (a), middle (b) and apical (c and d) part of the pulp, x40. Dental pulp vessels' wall is shown intense red, the odontoblasts green and the erythrocytes intense yellow

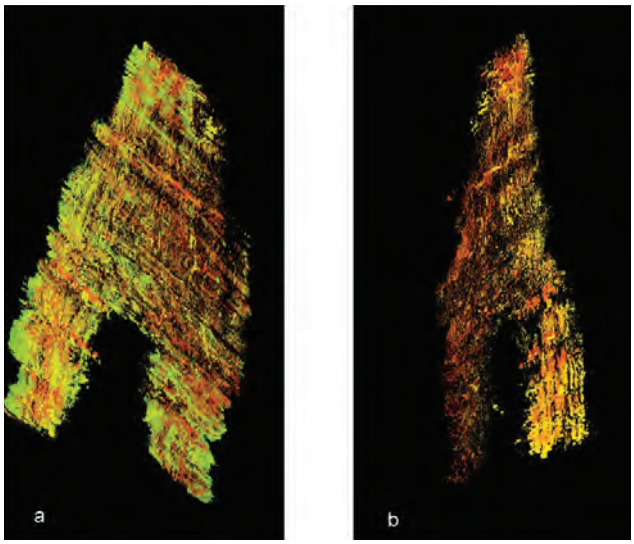


Figure 6. 3-dimensional volume reconstructions of 300 serial cross sections stained for CD34-Cy5 by immunofluorescence and imaged using the Leica TCS SP2 AOBS confocal laser scanning microscope. a: In this three-dimensional volume reconstruction, the course of the vessels in the dental pulp is shown (orange-red color) and their spatial arrangement with the odontoblasts (green colour); b: 3-dimensional volume reconstruction of the same specimen after the appliance of filters software for opacity. In this reconstruction only the course of the dental pulp vessels is shown

c. The most impressive and detailed image regarding the pulpal vessels' plexus is seen by using the immunofluorescence (CD34-Cy5) method (Fig. 5). A 3-dimensional reconstruction of the whole pulpal vessels' plexus is shown on figure 6. A number of 300 physical serial cross sections were taken from the above pulp tissue that was examined under Leica

TCS SP2 AOBS CLSM. In the above 3-dimensional reconstruction, the presence of the vessels appears extremely intense. Inside the root canals, the presence and apical-coronal course of larger vessels is obvious, while in the coronal part of the pulp the huge number of smaller vessels is rather impressive (Fig. 6b). Furthermore, not only the course, distribution of pulpal blood vessels and their spatial relationship inside the pulp are visualized (Fig. 6a), but their relationship with the other pulpal cells, like odontoblasts, are shown as well (vessels are shown red, the odontoblasts green and the erythrocytes inside the vessels yellow).

Discussion

In our previous study[5], combining immunohistochemical and immunofluorescence application for the detection of dental pulp vessels, immunofluorescence (CD34-Cy5) proved to be superior to immunohistochemical ABC technique. Recently a combination of immunohistochemistry for both pulpal blood vessels and nerves has been described (CD34 and S100) applied in ground tooth sections[22].

The corrosion cast method, used in the past[6], enables observation of several characteristic types of vasculature. However, it concomitantly has limitations in the study of biological functions, as no vascular cells' wall remains on the resin casts. The great advantage of our method in comparison with that of resin casts is that the dental pulp tissue retains its integrity and the connective tissue surrounding the dental pulp vessels remains intact. Furthermore, it is of great value that serial cross sections can be stored and examined under both conventional light and confocal microscope and the digital images can also be stored and re-examined in the near future. The reconstructions of vessels are based only on image processing of the pulpal sections and not on dissolving the surrounding connective tissue.

The method of serial cross sectioning and computerized 3D reconstruction has been used by other research groups[23- 29] for examination of different tissues. The above method has been employed as well, in order to study the distribution of pulpal blood vessels and nerves by using a limited number of serial dental pulp paraffin sections[7,8].

The quality and success of 3D reconstruction depends greatly on the number and thickness of the serial sections that are used, the histological method that is followed and the method of the reconstruction that is applied. Thus, in this study confocal microscope in combination with eosin staining, immunohistochemical application of CD34 and immunofluorescence CD34-Cy5 was used.

Confocal microscope is a powerful tool for visualizing fluorescent specimens. The principal advantage of confocal microscopy over conventional wide-field microscopy is that it reveals in more detail the 3-dimensional structure of the specimen under examination. Fluorescent specimens viewed with a conventional wide-field fluorescent microscope appear blurry and lack contrast because fluorophores throughout the entire depth of the specimen are illuminated, and fluorescence signals are collected not only from the plane of focus but also from the areas above and below. A confocal microscope selectively collects light from a thin (<1μm) optical section at the plane of focus in the specimen. Structures within the focal plane appear more sharply defined than with a conventional microscope because there is essentially no flare of light from out-of-focus areas. A 3-dimensional view of the specimen can be reconstructed from a series of optical sections at different depths. The capability for optical sectioning makes confocal microscopy well suited for studying the structure and function of cells using immunofluorescence reagents, organic dyes, fluorescent-fusion proteins and fluorescence in situ hybridization [30].

The use of confocal microscope proved to be more useful than the light microscope immunofluorescence because all the details of the pulpal vascular plexus in the space can be observed. Confocal microscope provides the capability to use thicker serial sections (in the cases of eosin staining 30μm), a fact that results to a less number of serial sections for examination and also enables us to examine a larger amount of histological sections and longer parts of dental pulp in less time.

In this study, due to confocal microscope, the decalcified and eosin stained specimens gave us the ability to use thicker (30μm) sections. This procedure provides better visualization of the pulpal vessels' course and distribution in the space although it appears the great disadvantage of the lack of specificity concerning vessels' detection, since eosin isn't an endothelial marker.

Immunohistochemistry by using CD34 on paraffin sections that were much thinner (5μm) provide the specific vessels' detection, since CD34 is considered to be a pan- endothelial marker. 3D reconstructions from the above sections also reveal the vessels' course and distribution within the entire volume of pulp tissue.

Both the above methods have the disadvantage that are very time-consuming, since in every section the contours of each vessel should be manually delineated for segmentation, procedure that restricts the number of dental pulp tissue specimens that can be examined and reconstructed.

Immunofluorescence for CD34-Cy5 specimens in combination with confocal microscopy and volumetric rendering provided the best 3D reconstruction result in our work. This method combines tissue specificity and rather thick paraffin sections (10μm) and requires no manual

segmentation of the vessels. Therefore, it is time efficient and also retains in the 3D reconstructions the histological structure of the surrounding tissues. Thus, in the above 3D reconstructions the vessels' spatial arrangement with the neighbouring structures can be shown.

Single sections cannot give information concerning the spatial arrangement of a structure or an arrangement of several structures and their mutual relationships. Knowledge of the form of an organ and the perception of its spatial arrangement with the neighbouring structures is essential for the fully understanding of its form and function. The volume reconstructions that we have achieved can be visualized in 3 dimensions, rotated in a proper perspective angle for a better convenience to obtain an accurate and fully perceptive 3D representation of the volume of interest.

The above possibility gives our method great value since it allows a fully comprehension of the course and spreading of detailed pulpal blood vessels' plexus in the space and at the same time enables us to study histological sections from which the volumes have been created.

Acknowledgments: The authors have to thank Dr. T. Jirasek, M. Jirkovska, I. Kostopoulos and N. Nikolaidis for their kind assistance during this work. This study has been partially supported by the following projects: (1) "Analysis of geometry of teeth and its constituents" (Funded by the General Greek Secretariat of Research Technology) (1999-2001) (Co-operation with the Czech Rebublic), and (2) "3-D Reconstruction of the human dental vessels' plexus by using immunohistochemistry and confocal microscope" (Funded by the General Greek Secretariat of Research Technology) (2003-2005) (Co-operation with the Czech Rebublic).

References

4. *Avery J, Chiego D*. Essentials of Oral Histology and Embryology. A Clinical Approach. Mosby Elsevier. 4th Edition. 2014.
5. *Luuko K, Kettunen P, Fristad I, Bergreen E*. Structure and Functions of the dentin-pulp complex in Cohen's Pathways of the pulp. Mosby Elsevier. 10th Edition. 2011.
6. *Vermeulen PB, Gasparini G, Fox SB, et al*. Second international consensus on the methodology and criteria of evaluation of angiogenesis quantification in solid human tumors. *Eur J Cancer*, 2002; 38:1564-1579.
7. *Hasan J, Byers R, Jayson GC*. Intra-tumoural microvessel density in human solid tumours. *Br J Cancer*, 2002; 86:1566-1577.
8. *Digka A, Lyroudia K, Jirasek T, Kasampalidis I, Karayannopoulou G, Kubinova L*. Visualisation of human dental pulp vasculature by immunohistochemical and immunofluorescent detection of CD34: A comparative study. *Aust Endod J*, 2006; 32(3):101-106.
9. *Takahashi K, Kishi Y, Kim S*. A scanning electron microscope study of the blood vessels of dog pulp using corrosion resin casts. Three-dimensional reconstruction of the pulpal vessels. *J Endod*, 1982; 8:131-135.
10. *Lyroudia K, Nikolaidis N, Pitas I, Zervas P, Palakidis K*. Computerized three-dimensional reconstruction: A method to study pulpal vessels and nerves. *J Endod*, 1993; 19:604-608.
11. *Lyroudia K, Nikolaidis N, Pitas I, Palakidis K*. Three computer methods to reconstruct pulpal blood vessels and nerves. *J Endod*, 1995; 21:501-504.
12. *Karen P, Jirkovská M, Tomori Z, Demjénová E, Janácek J, Kubínová L*. Three-dimensional computer reconstruction of large tissue volumes based on composing series of high-resolution confocal images by GlueMRC and LinkMRC software. *Microsc Res Tech*, 2003; 62(5):415-422.
13. *Chang YC, Lee DJ, Wang YG*. Color-texture segmentation of medical images based on local contrast information. Proc. IEEE Symposium Series on Computational Intelligence, Honolulu, Hawaii, USA, 2007.
14. *Krinidis M, Pitas I*. Color texture segmentation based on the modal energy of deformable surfaces. *IEEE Transactions on Image Processing*, 2009; 18(7):1613-1622.
15. *Jiang Y, Meng J, Babyn P*. X-ray image segmentation using active contour model with global constraints. Proc. IEEE Symposium Series on Computational Intelligence, Honolulu, Hawaii, USA, 2007.
16. Krinidis *S, Nikou C, Pitas I*. Reconstruction of serially acquired slices using physics-based modelling. *IEEE Transactions on Information Technology in Biomedicine*, 2003; 7(4):394-403.
17. *Bors A, Kechagias L, Pitas I*. Binary morphological shape-based interpolation applied to 3-D tooth reconstruction. *IEEE Transactions on Medical Imaging*, 2002; 21(2):100-108.
18. *Nikolaidis N, Pitas I*. 3D image processing algorithms. Wiley, 2000.
19. *Zitova B, Flusser J*. Image registration methods: A survey. *Image and Vision Computing*, 2003; 21:977-1000.
20. *Tait R, Schaefer G, Hopgood AA, Nakashima T*. High performance image registration using a distributed blackboard architecture. Proc. IEEE Symposium Series on Computational Intelligence, Honolulu, Hawaii, USA, 2007.
21. *Krinidis S, Nikou C, Pitas I*. Alignment of serially acquired slices using a global energy function. In Proceedings of the IEEE Engineering in Medicine and Biology Conference (EMBS '01), Istanbul, Turkey, 2001.
22. *Ben-Jemaa R, Schmitt F*. A solution for the registration of multiple 3D points sets using quaternions. Lecture Notes in Computer Science. In: Proceedings of the 5th European Conference on Computer Vision (ECCV '98), Freiburg, Germany. Vol. 2, pp 34-50, 1998.
23. *Sramek M*. 20 years of volume rendering. Spring Conference on Computer Graphics, Slovakia, 2006.
24. *Plath N, Knorr S, Goldmann L, Sikora T*. Adaptive image warping for hole prevention in 3D view synthesis. *IEEE Trans Image Process*, 2013; 22(9):3420-3432.
25. *Steiniger BS, Bubel S, Boeckler W, Lampp K, Seiler A, Jablonski B, Guthe M, Stachniss V*. Immunostaining of pulpal nerve fibre bundle/arteriole associations in ground serial sections of whole human teeth embedded in technovit 9100. *Cells Tissues Organs*, 2013; 198(1):57-65.

26. *Radlanski FR*. Morphogenesis of human tooth primordial: the importance of 3D computer-assisted reconstruction. *Int J Dev Biol*, 1995; 39:249-256.
27. *Peterková R, Peterka M, Vonesch JL, Ruch JV*. Contribution of 3-D computer-assisted reconstructions to the study of the initial steps of mouse odontogenesis. *Int J Dev Biol*, 1995; 39:239-247.
28. *Witter K, Lesot H, Peterka M, Vonesch JL, Misek I, Peterkova R*. Origin and developmental fate of vestigial tooth primordia in the upper diastema of the field vole (Microtus agrestis, Rodentia). *Arch Oral Biol*, 2011; 50(4):401-409.
29. *Arnold WH, Gaengler P, Kalkutschke L*. Three-dimensional reconstruction of approximal subsurface caries lesions in deciduous molars. *Clin Oral Invest*, 1998; 2(4):174-179.
30. *Arnold WH, Gaengler P, Saeuberlich E*. Distribution and volumetric assessment of initial approximal caries lesions in human premolars and permanent molars using computer-aided three-dimensional reconstruction. *Arch Oral Biol*, 2000; 45(12):1065-1071.
31. *Arnold WH, Meiselbach V*. 3-D reconstruction of a human fetus with combined holoprosencephaly and cyclopia. *Head Face Med*, 2009; 29:5-14.
32. *Sohn WJ, Gwon GJ, An CH, Moon C, Bae YC, Yamamoto H, Lee S, Kim JY*. Morphological evidences in circumvallate papilla and von Ebners' gland development in mice. *Anat Cell Biol*, 2011; 44(4):274-283.
33. *Smith LC*. Basic Confocal Microscopy. In: In situ Hybrization and Immunohistochemistry. Current Protocols in Molecular Biology. Published on line: January 2008 in Wiley Interscience.

Correspondence and request for offprints to:

Anna Digka
36 Ermou Str.
54623, Thessaloniki
Greece
E-mail: annadiga@hotmail.com

BALKAN JOURNAL OF DENTAL MEDICINE

BaSS

ISSN 2335-0245

Excessive Tongue Amyloidosis as the Diagnostic Sign of Multiple Myeloma: a Case Report

SUMMARY

Background: *Deposition of amyloid in oral mucosa may be related to systemic disorders, including immune-related diseases and malignancies.*

Clinical Presentation: *We describe a case of 76-year-old patient with excessive, painless, multi-nodular tongue enlargement, and petechiae on the vermilion border and perioral skin that appeared 2 months ago. The biopsy detected subepithelial, Congo's Red positive amyloid depositions. Consequent laboratory investigation and bone marrow biopsy confirmed the diagnosis of multiple myeloma stage 2 (International Prognostic Index - IPI).*

Conclusion: *Multi-nodular excessive tongue enlargement could be of high significance as initial sign of undiagnosed, underlying systemic disease including severe malignancy like multiple myeloma.*

Keywords: Oral Amyloidosis; Macroglossia; Multiple Myeloma

Stylianos Dalampiras[1], Dimitrios Andreadis[2], Ioannis Kostopoulos[3], Florentia Stylianou[4], Ioannis Papadiochos, Athanasios Poulopoulos[2]

Aristotle University, Thessaloniki, Greece
[1]School of Dentistry
Department of Oral and Maxillofacial Surgery
[2]School of Dentistry
Department of Oral Medicine and Pathology
[3]School of Medicine
Department of Histopathology
[4]School of Dentistry, student
[5]"Evangelismos" General Hospital
School of Dentistry, Athens, Greece

CASE REPORT (CR)
Balk J Dent Med, 2015; 19:50-52

Introduction

The term amyloidosis represents extracellular deposits of amyloid, a group of unrelated proteins present as a homogenous eosinophilic material. The current classification of amyloidosis is based on the nature of precursor plasma proteins that form these fibril deposits. Amyloid depositions may occur in a single (localized) or in many organs (systematic) and amyloidosis is also divided into primary or secondary based on its relation with other concurrent systemic diseases, including malignancies[1,2].

Manifestations of amyloidosis in the head and neck area may be usually present in its secondary form, affecting orbit, sinuses, salivary glands, pharynx/larynx and oral cavity in 10-40% of the cases. Potential sites of oral lesions are buccal, palatal and gingival mucosa and tongue, manifesting rarely as excessive macroglossia[3]. Oral lesions can often represent an early manifestation, especially in cases of amyloid light chain depositions (AL amyloidosis)[4] and provide the first sign of multiple myeloma (MM) or other monoclonal gammopathies[5,6].

Microscopically, amyloid depositions are detected through specific staining with Congo Red, and the apple-green birefringence appearance under polarized light[7].

This report describes a case of an elder patient with excessive tongue enlargement (macroglossia) as the initial sign of AL amyloidosis related to underlying multiple myeloma.

Case Report

A 76-year-old patient was referred complaining for a 2-month painless, multi-nodular enlargement of the tongue (Fig. 1), leading to discomfort, dysphagia and speech impairment. Clinically, the nodules were solid in palpation, with normal or bluish colour, and normal covering mucosa. Also, petechiae on the vermilion border and the skin around both lips were observed. The patient's information for his medical history was unclear.

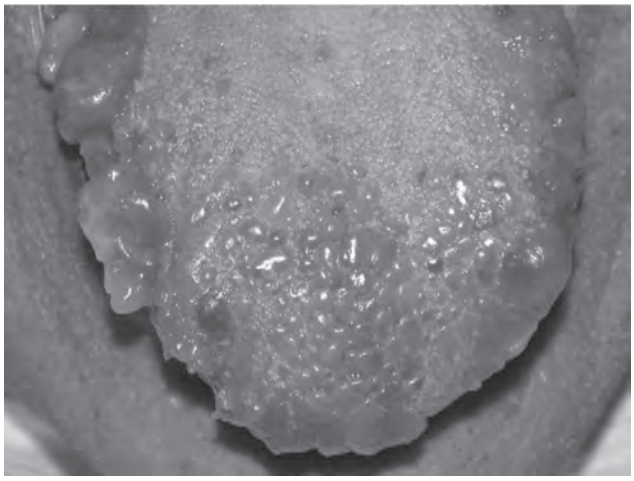


Figure 1. Excessive nodular enlargement of the tongue at the time of diagnosis

Under local anesthesia a partial biopsy was taken from a nodular mass of the lateral border of the tongue; histological examination revealed a subepithelial, multi-nodular amorphous, fibrillar accumulation (Fig. 2a), positively stained for Congo-Red, having a reddish colour under the light microscopy (Fig. 2b) and apple green birefringence under polarized light.

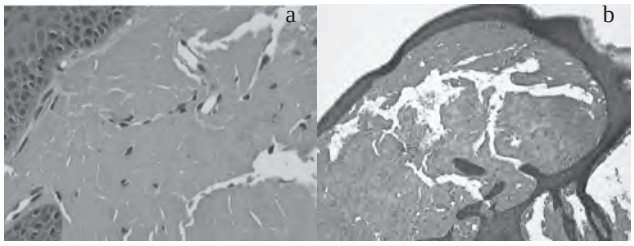


Figure 2. Histological finding. **a.** *Subepithelial, multinodular amorphous fibrillar accumulation (H&E x 20);* **b.** *Positive staining for Congo-Red. The multinodular amorphous fibrillar accumulation with a reddish colour (x10)*

The laboratory investigation revealed increased free light chain Kappa in serum (5360mg/l) and urine (2170mg/l), κ/λ ratio 324, anaemia (Hb= 9.7 g/dl), urine Bence- Jones protein and chronic renal failure (Creatinine 2 mg/dl). In addition, bone marrow biopsy showed plasmacytic infiltration (22%), but the imaging analysis failed to detect any clear bone lesion. According to the findings, the diagnosis of Multiple Myeloma stage 2 by the International Prognostic Index (IPI) was settled and combined treatment consisting of Bortezomid, Melphalan and Dexamethazone was administrated with good tolerance from the patient and efficacy for the disease based on laboratory tests. However, during the follow-up, the tongue enlargement excessively increased (Fig. 3), causing obstruction. Carpal tunnel syndrome, swelling of the lower extremities and a solid mass at the right shoulder (scapula) were observed, too. Also, the ultrasound of the heart showed wall hypertrophy with sigmoid inter-ventricular septum. Finally the patient died 5 years after the diagnosis and treatment.

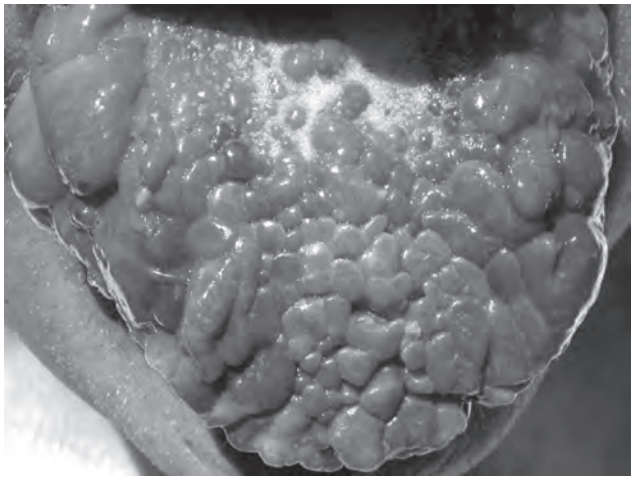


Figure 3. Tongue enlargement 55 months later

Discussion

Systemic amyloidosis can be associated with immunocytes' dyscrasia, such as multiple myeloma, monoclonal gammopathy, or macroglobulinemia, or presents as a complication of an underlying chronic inflammatory or tissue destructive process such as Crohn's disease, tuberculosis, rheumatoid arthritis, ankylosing spondylitis and chronic osteomyelitis[1]. Etiopathogenesis of amyloidosis is not clear. The proposed schema of the pathogenesis includes production of amyloid fibrils in the extracellular matrix, causing tissue damage or impairment. It is widely accepted that the major constituent of amyloid fibres in patients with multiple myeloma consists of light chain fragments termed Bence-Jones proteins, and the light-chain variable region of the immunoglobulin represents the main component of AL-amyloid deposits. It is thought that light chain proteins may be secreted by macrophages[8]. The class of light chain involved in amyloidosis in MM is most often λ versus κ in a ratio 3:1[9], which is in contrast to our case with κ chains.

Oral manifestations can often represent the initial sign, especially in the case of amyloid light chain depositions (AL amyloidosis), and provide a sign of multiple myeloma or other monoclonal gammopathies[5,6]. Interestingly, oral amyloidosis has been referred to be related with chronic periodontitis. Periodontitis can increase the levels of APRs and potentiate the development of amyloidosis by increasing the levels of

systemic inflammatory mediators. Additionally, deposition of amyloid in periodontal tissues causes accelerated periodontal destruction and bone loss[10].

Our patient suffered from oral amyloidosis associated with multiple myeloma and he died 60 months after diagnosis and treatment. 7 to 20 percent of patients with multiple myeloma manifest amyloidosis. When diagnosis of amyloidosis is established in patients with multiple myeloma it forecasts a poor prognosis due to renal impairment caused by the excretion of excessive light chains. The prognosis for patients with AL amyloidosis depends on the degree of the organ involvement. Also the median survival in λ-light chain disease is reported to be significantly shorter than κ-light chain diseases. Generally, the prognosis of a patient is poor in cases of late diagnosis and if left untreated, with a median survival of 1 to 2 years[11,12].

Conclusion

Although oral amyloidosis rarely involves oral mucosa or may be present as a localized lesion, its detection in oral mucosa could be of high importance as a clinical sign suspicious for amyloid depositions accompanying systemic disorders, ranging from immune-related diseases, inflections or severe malignancies (such as multiple myeloma). Noteworthy, in multiple myeloma cases amyloidosis is also related to prognosis and hence affects the therapeutic procedure of the disease.

References

1. *Petersson T, Konttinen YT*. Amyloidosis-Recent Developments. *Semin Arthritis Rheum*, 2010; 39(5):356-358.
2. *Robbins J*. Diseases of immunity. In: *Cortan RS, Kumar V, Collins T* (eds). Pathologic basis of disease. 6th ed. Philadelphia: Saunders Co, 1999; pp 188-259.
3. *Stoopler ET, Sollecito TP, Chen SY*. Amyloid deposition in the oral cavity: a retrospective study and review of the literature. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 2003; 95(6):674-680.
4. *Eisen D, Lynch DP*. Oral manifestations of systemic diseases. In: *Eisen D, Lynch DP* (eds). The mouth. Diagnosis and treatment. St Louis: Mosby, 1998; pp 212-236.
5. *Van der Waal RIF, van de Scheur MR, Huijgens PC, Starink TM, van der Waal I*. Amyloidosis of the tongue as a paraneoplastic marker of plasma cell dyscrasia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 2002; 94:444-447.
6. *Salisbury PL Jr, Jacoway JR*. Oral amyloidosis: A late complication of multiple myeloma. *Oral Surg*, 1983; 56:48-50.
7. *Angiero F, Seramondi R, Magistro S, Crippa R, Benedicenti S, Rizzardi C, Cattoretti G*. Amyloid Deposition in the Tongue: Clinical and Histopathological Profile. *Anticancer Res*, 2010; 30:3009-3014.
8. *Kyle RA, Greipp PR*. Amyloidosis (AL) clinical and laboratory features in 229 cases. *Mayo Clin Proc*, 1983; 58:665.
9. *Khan MF, Falk RH*. Amyloidosis. *Postgrad Med J*, 2001; 7:686-693.
10. *Cengiz MI, Wang HL, Yildiz L*. Oral involvement in a case of AA amyloidosis: a case report. *J Med Case Rep*, 2010; 4:200.
11. *Durie BG, Kyle RA, Belch A, Bensinger W, Blade J, Boccadoro M, Child JA, Comenzo R, Djulbegovic B, Fantl D, Gahrton G, Harousseau JL, Hungria V, Joshua D, Ludwig H, Mehta J, Morales AR, Morgan G, Nouel A, Oken M, Powles R, Roodman D, San Miguel J, Shimizu K, Singhal S, Sirohi B, Sonneveld P, Tricot G, Van Ness B*. Myeloma management guidelines. A consensus report from the scientific advisors of the International Myeloma Foundation. *Hematol J*, 2003; 4:279-298
12. *Kyle RA, Gertz MA*. Primary systemic amyloidosis: clinical and laboratory features in 474 cases. *Semin Hematol*, 1995; 32:45-59.

Correspondence and request for offprints to:

Dr Dimitrios Andreadis
Aristotle University of Thessaloniki
School of Dentistry
Department of Oral Medicine/Pathology
GR-54124, Greece
Email: dandrea@dent.auth.gr

BALKAN JOURNAL OF DENTAL MEDICINE

BaSS

ISSN 2335-0245

Occupational Maxillofacial Trauma: Report of a Rare Case

SUMMARY

Maxillofacial trauma, any physical trauma in the face, can involve soft tissues (lacerations, avulsions, bruises etc), bone injuries (fractures and dislocation), avulsed or fractured teeth (dental issues) and special regions (nerves, eyes, salivary glands etc). As the most exposed part of the human body, the face can be susceptible to injuries in work-related accidents. Occupational accident in the maxillofacial region rates 0.9-5% and, in some cases, can reach 9%. Based on their occupation, patients are classified as farm and forestry workers, construction workers, factory workers, craftsmen, service workers, and office workers.

This paper describes a rare case of a farm-related maxillofacial trauma in a 50-year-old woman and a brief review of the relevant literature.

Keywords: Trauma, occupational; Trauma, maxillofacial

Theodoros Dervisoglou[1], Foivos Dalabiras[2], Stilianos Dalabiras[3]

Aristotle University, Dental School
[1]Private dentist
[2]Dental student
[3]Department of Oral and Maxillofacial Surgery
Thessaloniki, Greece

CASE REPORT (CR)

Balk J Dental Med, 2015; 19:53-56

Introduction

Occupational maxillofacial trauma rates between 0.9-5%[1,6] and can rarely reach about 9%[2], varying due to socioeconomic, cultural, season, environmental and geographic factors, regional government, methods of transportation, recorded period and population density[1,3-5,7]. It seems to be rare, although studies have reported that work-related accidents, along with road traffic accidents (RTA's) and assaults-interpersonal violence represent the most common causes of maxillofacial injuries[4].

It is often complex and challenging involving the healing (treatment) of facial bone fractures, dentoalveolar trauma and soft tissue lesions. Particularly the treatment of patients suffering from occupational injuries is very important because it concerns a purely productive age and so, a quick and efficient recovery of these patients is required to return to their job[4,6]. Patients may present with facial injuries alone or with associated trauma to other systems[6]. The nature of the work is classified as:

1. agriculture and forestry;
2. construction;
3. manufacturing;
4. other services (craftsmen, office and service workers, transportation and warehousing etc)[2,7].

In this paper, a rare case of a farm-yard maxillofacial injury is presented. We also discuss the danger of agricultural jobs, some measures that can be taken, and some general but significant elements about maxillofacial injuries.

Case Report

A 50-year-old woman, farm worker, joined in urgent outpatient clinic of a provincial hospital after an injury of the lower lip and mandible, with tooth dislocation, fracture of the alveolar process in the same area and a condyle fracture of the opposite side. The patient reported that the trauma was caused in a field of sunflowers while she was working in the sunflowers seed harvest. Soft tissues of the lower lip were sutured in the emergencies of the local hospital.

Clinical and radiographic assessment revealed a fracture of the anterior right part of the mandibular alveolar process, along with the teeth #43 and #45 displaced lingually (Figs. 1 and 2). A second fracture was depicted in the panoramic radiography at the condyle neck of the opposite side of the mandible (Fig. 1). No sign of paraesthesia was detected.

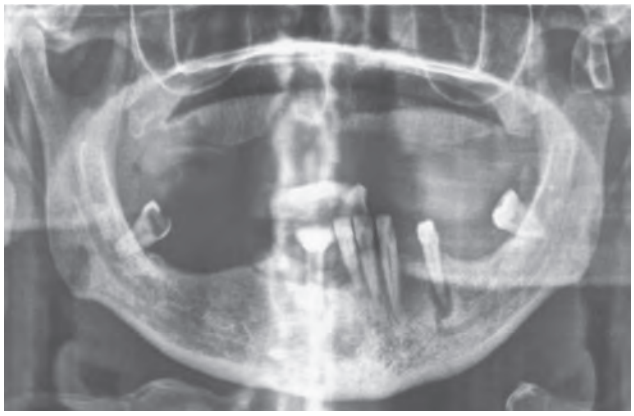


Figure 1. A panoramic radiography reveals a fracture of the alveolar process in the anterior aspect of the mandible and a fracture of the left condyle

Figure 3. The sunflower seed shell removing machine

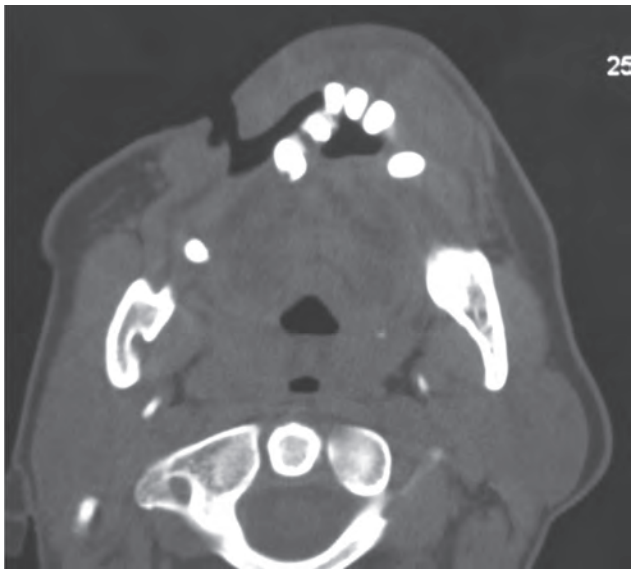


Figure 2. The lip laceration is well depicted in this CT-Scan image

Figure 4. Patients image 40 days after

After the initial deal, the patient was referred to the Department of Craniomaxillofacial Surgery (University clinic in the General Hospital G. Papanikolaou). Teeth #31, #32 and #33 were mobile. Under local anaesthesia, all luxated and displaced teeth, along with the fractured alveolar process, were stabilized with an external fixation, using an Essig-type splint. The patient stayed for monitoring, and after 3-4 days she was discharged. She showed significant improvement during post-operative control. In the case of patients reporting, the injury was done within seconds: the rotating machine in use (Fig. 3), caught her overcoat and with the power of rotation it pulled her throughout. Before her co-workers were able to do anything, her lip touched the rotary cylinder and caused the injury by its rotary force.

The patient was re-examined in the follow-up process, and after a period of 40 days she showed an uneventful healing of the wound in the skin of the lower lip (Fig. 4).

Discussion

Sunflower farming is significant and widespread in several parts of Greece. The plantation belongs mostly to small family growers, which have limited resources, and their harvest gets in a manner that does not ensure the safety of workers. Farming is well known for its hazards, ranking among the most dangerous occupations for fatal

and non-fatal injuries. The methods used, can be called primitive, for the worker is completely unprotected on this machine: the process of these plants takes place in a rotary machine, which does not provide security, due to the fact that the worker touches the plant with bare hands on a rotary cylinder in order to detach the seeds from the sunflower. For many families it is the only crop and only income source (selling oil or seeds). This case is characteristic of the total lack of security measures in a region of the Balkans, where the population is rural and the methods used for the harvest and processing of agricultural products are dangerous. Unfortunate is the fact that in such works, children are involved and this is very dangerous, since severe injuries of the facial skeleton and soft tissues may lead to disorders in children's physical and mental development, or even death[8,9]. The case presented here is not the only accident reported in the same family. A relative of our patient (a female cousin) was a victim of a similar accident while working in the same machinery some time ago. She had an upper limp amputated.

Unfortunately, especially in the maxillofacial area, there is not concrete data for such accidents. Sporadic reports are made and a lack of information exists for the outcome of such events. Fortunately those accidents are few in number. In the international literature, there are several reports and articles on occupational accidents, generally. Eggensperger et al[4] report a study in 42 patients suffering from occupational maxillofacial fractures (3 year survey, 2006). The mean age of the patients was 44.4 ages, male:female ratio=41:1. 69% of these injuries occurred in farm and forestry workers. The same authors agree that injuries generally are the main cause of reduced productivity, due to loss of days at work, even more than heart disease and cancer combined. Our patient had to stay out of work for at least 1 month.

The consequences of maxillofacial fractures remain of great significance, aesthetically and functionally. Rarely can it lead to death, with the exception of non stop bleeding and when inhalation of blood from oral bleeding is combined with a state of depressed consciousness - death may result from aspiration and asphyxia[3]. Another memorable risk factor is the fact that injury that takes place on farms and can cause serious infection (brain contamination) due to the fact that in the injury area, there is grass, mud, gravel and manure[10].

Possible mechanisms of injury in agricultural work are: hit by object or an animal, fall from height and of course during operating or assisting in operating farming equipment and wood processing. Work-related accidents are related to 3 causes: (1) human error, (2) machinery or apparatus failure, and (3) improper use of equipment due to a lack of training and/or instruction[11,12]. The majority of publication shows huge superiority of men towards women in occupational accidents and consequent injuries[1,7,11].

It is very important to know the differences between the population groups as well as the culture of it because it seems directly related to the causes of fractures and their severity. The only way to control the fact that these accidents occur is to understand the causes, habits leading to them, and thus be able to prevent them in any form. This requires the collection of data (sex, age, cause, circumstances of the accident, type and location), which must be recorded by a special unit recording and collecting maxillofacial trauma reports (cases, patients). This is possible to happen locally in large academic teaching modules that can be responsible for the treatment of such incidents in large cities-populations. Precautionary measures should be taken - even though they are not mandatory by law, as right information and training of workers across the work piece farm machinery etc, wear face and mouth guards, face shields or full-face protection helmets and spectacles, protective clothing and obviously not consume alcohol during work.

The list of machines available on the internet for the process of the sun flowers, did not appear anywhere the existence of such mechanism as the one that caused the accident. We suppose that this device was not fulfilling criteria of safety and probably was not officially patented in the EU.

References

5. *Özkaya Ö, Turgut G, Kayali MU, Ugurlu K, Kuran I, Bas L*. A retrospective study on the epidemiology and treatment of maxillofacial fractures. *Turkish Journal of Trauma & Emergency Surgery (UlusTravmaAcilCerrahiDerg)*, 2009; 15(3):262-266.
6. *Roccia F, Boffano P, Bianchi FA, Gerbino G*. Maxillofacial injuries due to work-related accidents in the North West of Italy. *Oral Maxillofac Surg*, 2013; 17:181-186.
7. *Kostakis G, Stathopoulos P, Dais P, Gkinis G, Igoumenakis D, Mezitis M, Rallis G*. An epidemiologic analysis of 1,142 maxillofacial fractures and concomitant injuries. *Oral Surg Oral Med Oral Pathol Oral Radiol*, 2012; 114(Suppl 5):S69-S73.
8. *Eggensperger MN, Danz J, Heinz Z, Iizuka T*. Occupational Maxillofacial Fractures: A 3-Year Survey in Central Switzerland. *J Oral Maxillofac Surg*, 2006; 64:270-276.
9. *Venetis G, Dalampiras S, Tilaveridis I, Kyrgidis A, Karathanos G, Zouloumis L*. Do socio-economic factors affect maxillofacial trauma? Proceeding of the EACMFS. Prague, 2014. E-poster
10. *Gassner R, Tuli T, Hachl O, Rudisch A, Ulmer H*. Cranio-maxillofacial trauma: a 10 year review of 9543 cases with 21067 injuries. *J Cranio-Maxillofac Surg*, 2003; 31:51-61.
11. *Yamamoto K, Matsusue Y, Murakami K, Horita S, Matsubara Y, Sugiura T, Kirita T*. Maxillofacial fractures due to work-related accidents. *J Cranio-Maxillofac Surg*, 2011; 39:182-186.

12. *Lewandowski B, Szymańska J*. Agriculture-related severe craniofacial injuries in rural children and adolescents. *Ann Agric Environ Med*, 2008, 15:59-62.
13. *Bancej C, Arbuckle T*. Injuries in Ontario farm children: a population based study. *Injury Prevention*, 2000; 6:135-140.
14. *Kumar A, Shankar R, Pandey PR, Keshri V, Singh K, Sharma V*. Five years' experience at a single centre of craniocerebral injury from winnowing fan blades. *Journal of Clinical Neuroscience*, 2010; 17:178-181.
15. *Hachl O, Tuli T, Schwabegger A, Gassner R*. Maxillofacial trauma due to work-related accidents. *Int J Oral Maxillofac Surg*, 2002; 31:90-93.
16. *Lee HK, Chou HJ*. Facial fractures in work-related injuries. *Asian Journal of Oral and Maxillofacial Surgery*, 2010; 22:138-142.

Correspondence and request for offprints to:

Theodoros Dervisoglou
K. Palaiologou 30, Alexandroupolis
68100 Greece
E mail: thdervis@gmail.com

BALKAN JOURNAL OF DENTAL MEDICINE

BaSS

ISSN 2335-0245

Instructions to authors

The BALKAN JOURNAL OF DENTAL MEDICINE provides contributors with an opportunity to publish review and original papers, preliminary (short) communications and case reports.

Review papers (RP) should present an analytic evaluation of certain problems in stomatology based on a critical approach to personal experience and to the published results of other authors.

Original papers (OP) should be related to the results of scientific, clinical and experimental research. They should investigate a certain stomatological problem using adequate scientific methods and comment the obtained results in accordance to the previously published observations of other authors.

Preliminary (short) communications (PC) should concern the preliminary results of current research.

Case reports (CR) should be related to uncommon and rare clinical cases, interesting from diagnostic and therapeutic viewpoints. Case reports may be related to innovations of surgical techniques as well.

Contributors from Balkan countries should send their manuscripts to domestic National Editorial Boards (addresses are cited on the second page of the Journal) for reviewing. Contributors from non-Balkan countries should send their manuscripts to the Editor-in-Chief (Prof. Ljubomir Todorovia, Faculty of Stomatology, Clinic of Oral Surgery, Dr Suboti}a 8, 11000 Belgrade, Serbia, fax: +381 11 685 361).

No fees are awarded for the submitted papers. Original copies of papers, as well as illustrations, will not be returned. Following acceptance of a manuscript for publication, the author will receive a page proof for checking. The proofs should be returned with the least possible delay, preferable bu e-mail (ljubatod@eunet.yu) or the regular mail.

Offprints can be obtained on the author's request, the cost being paid by the author.

Preparation of manuscripts

All manuscripts should be submitted in correct English, typed on one side of the standardized paper, in single spacing, with ample margins of not less than 2.5 cm, and the pages numbered.

Papers submitted for publication should be accompanied by a statement, signed by all authors, that they have not already been published, and are not under consideration by any other publication.

One copy of the manuscript with one set of figures and tables is required. Every article should also be submitted as a MS Word file on CD. The manuscript and the e-file must be identical, and the CD should contain no other file. The disk should be clearly labeled with the title of the article and the name(s) of the author(s).

The manuscripts should be set out as follows: title page, summary, text, acknowledgements if any, references, tables and captions of illustrations.

Title page. The title page should give the following information: 1) title of the paper, 2) initials, surname and the institution address of each author, 3) name, address, telephone and E-mail of the author responsible for correspondence and to whom requests for offprints should be sent and 4) sources of support in the form of grants if any.

Summary. This should consist of not more than 200 words summarizing the contents of the paper. It should include the title of the paper, but without the names of authors and institutions. Key word should be included, according to Index Medicus.

Text. The complete title should preceed the text (but without authors and institution names). Headings should be appropriate to the nature of the paper. Normally, only two categories of headings should be used: major ones should be typed in capital letters in the centre of the page and bolded; minor ones should be typed in lower case (with an initial capital letter) at the left hand margin and bolded.

All illustrations, labeled as figures (such as photographs, line drawings, charts or tracings) should be submitted as high-contrast prints, black and white, suitable for publications. They must be marked on the back with the title of the paper, numbered with arabic numerals in the same order as they are cited in the text, and the top edge indicated with an arrow. Photomicrographs should have the magnifications and details of staining techniques shown. Short explanatory captions of all illustrations should be typed on a separate sheet.

Tables should be typed on a separated sheet. Each table should have a short heading (title) above and any footnotes, suitably identified, below. Tables should be numbered consecutively with arabic numerals. Do not submit tables as photographs. Ensure that each table is cited in the text. Abbreviations are not desirable.

References. References in the text should use superscript numerals as they appear in the list of references, with or without the name(s) of the author(s). The list of references at the end of the paper should be typed on a separate sheet, arranged alphabetically and numbered, and should include all references cited in the text. For review papers, references can be arranged consecutively and numbered (by Arabic numerals) as they are cited. The accuracy of references is the responsibility of the author.

Titles of journals should be abbreviated as used by Index Medicus. The format for references should be: year-volume-first and last page. References to monographs should also include place and the name of the publisher, and the page(s) referred to.

Examples:

1. Brown JS, Browne RM. Factors influencing the patterns of invasion of the mandible by squamous cell carcinoma. Int J Oral Maxillofac Surg, 1995; 24:417-426.
2. Sternbach RA. Pain patients - traits and treatment. New York, London, Toronto, Sydney, San Francisko: Academic Press, 1974; pp 20-30.
3. Koulourides T, Feagin F, Pigman W. Experimental changes in enamel mineral density. In: Harris RS (ed). Art and Science of Dental Caries Research. New York: Academic Press, 1968, pp 355-378.