

BALKAN JOURNAL OF STOMATOLOGY

Official publication of the **BALKAN STOMATOLOGICAL SOCIETY**

Volume 17

No 1

March 2013



ISSN 1107 - 1141

Editor-in-Chief

Ljubomir TODOROVIĆ, DDS, MSc, PhD
 Faculty of Dentistry
 University of Belgrade
 Dr Subotića 8
 11000 Belgrade
 Serbia

Editorial board**ALBANIA**

Ruzhdie QAFMOLLA - Editor
 Emil KUVARATI
 Besnik GAVAZI

Address:
 Dental University Clinic
 Tirana, Albania

ROMANIA

Alexandru-Andrei ILIESCU - Editor
 Victor NAMIGEAN
 Cinel MALITA

Address:
 Faculty of Dentistry
 Calea Plevnei 19, sect. 1
 70754 Bucuresti, Romania

BOSNIA AND HERZEGOVINA

Maida GANIBEGOVIĆ - Editor
 Naida HADŽIABDIĆ
 Mihael STANOJEVIĆ

Address:
 Faculty of Dentistry
 Bolnička 4a
 71000 Sarajevo, BIH

SERBIA

Dejan MARKOVIĆ - Editor
 Slavoljub ŽIVKOVIĆ
 Zoran STAJČIĆ

Address:
 Faculty of Dentistry
 Dr Subotića 8
 11000 Beograd, Serbia

BULGARIA

Nikolai POPOV - Editor
 Nikola ATANASSOV
 Nikolai SHARKOV

Address:
 Faculty of Dentistry
 G. Sofriiski str. 1
 1431 Sofia, Bulgaria

TURKEY

Ender KAZAZOGLU - Editor
 Pinar KURSOGLU
 Arzu CIVELEK

Address:
 Yeditepe University
 Faculty of Dentistry
 Bagdat Cad. No 238
 Göztepe 81006
 Istanbul, Turkey

FYROM

Julijana GJORGOVA - Editor
 Ana STAVREVSKA
 Ljuben GUGUČEVSKI

Address:
 Faculty of Dentistry
 Vodnjanska 17, Skopje
 Republika Makedonija

GREECE

Anastasios MARKOPOULOS - Editor
 Haralambos PETRIDIS
 Lambros ZOULOUMIS

Address:
 Aristotle University
 Dental School
 Thessaloniki, Greece

CYPRUS

George PANTELAS - Editor
 Huseyn BIÇAK
 Aikaterine KOSTEA

Address:
 Gen. Hospital Nicosia
 No 10 Pallados St.
 Nicosia, Cyprus

International Editorial (Advisory) Board

Christoph HÄMMERLE - Switzerland	George SANDOR - Canada
Barrie KENNEY - USA	Ario SANTINI - Great Britain
Predrag Charles LEKIC - Canada	Riita SUURONEN - Finland
Kyösti OIKARINEN - Finland	Michael WEINLAENDER - Austria

BALKAN STOMATOLOGICAL SOCIETY**Council:**

President: Prof. H. Bostanci
 Past President: Prof. P. Koidis
 President Elect: Prof. N. Sharkov
 Vice President: Prof. D. Stamenković
 Secretary General: Prof. A.L. Pissiotis
 Treasurer: Prof. S. Dalampiras
 Editor-in-Chief: Prof. Lj.Todorović

Members:

R. Qafmolla
 P. Kongo
 M. Ganibegović
 S. Kostadinović
 A. Filchev
 D. Stancheva Ziburkova
 M. Carčev
 A. Minovska
 T. Lambrianidis
 S. Dalambiras

A. Adžić
 M. Djuričković
 N. Forna
 A. Bucur
 M. Carević
 M. Barjaktarević
 E. Kazazoglu
 M. Akkaya
 G. Pantelas
 S. Solyali

BALKAN JOURNAL OF STOMATOLOGY

Official publication of the **BALKAN STOMATOLOGICAL SOCIETY**

Volume 17

No 1

March 2013



ISSN 1107 - 1141

Contents

RP	C. Dimova M. Pandilova I. Kovacevska B. Evrosimovska Z. Georgiev	Evidence-Based Dentistry - Between Science and Clinical Practice	5
LR	V. Tzifa A. Arhakis	Sealant Retention in Pits and Fissures: Preparation and Application Techniques. A Literature Review	9
OP	B. Vanlıoğlu Y.K. Özkan M. Uçankale E. Cansız O. Kayabaşı	Effect of Post Material, Cement and Amount of Coronal Destruction on Stress Distribution: 3D FEA Study	18
OP	Z. Belazelkoska M. Popovska L. Zendeli-Bedzeti	Preventive and Etiological Aspect of Oral Candidiasis	26
OP	Y. Benderli K. Gökçe	Effects of Various Rates of Polyacrylic Acid and Fluoride on <i>Streptococcus Mutans</i> and <i>Actinomyces Viscosus</i>	31
OP	A. Belazelkovska M. Popovska G. Spasovski V. Radojkova-Nikolovska A.Minovska Z. Belazelkoska K. Mitic	Oral Clinical Findings in Patients with Chronic Renal Failure	37
OP	M. Koray M. Özgül D. Oflluğlu A. Saruhanoğlu H. İşsever H. Tanyeri	The Effect of a New Topical Agent on Recurrent Aphthous Stomatitis	44

OP	S. Carceva Salja	Profile Convexity Changes after Maxillary Protraction Therapy in Patients with Class III Malocclusion	48
CR	K. Tolidis P.G. Liokatis C. Boutsiouki D. Michailidou	Avulsed Tooth Revascularization after 13-Hour Extra-Oral Time	54

Evidence-Based Dentistry - Between Science and Clinical Practice

SUMMARY

It is generally accepted that the more experience a physician or a dentist possess - better the quality of health care delivery. However, recent studies had shown that there is, in fact, an inverse relationship between the number of years of practice and the quality of care provided. Evidence-Based Dentistry (EBD) is a process that restructures the way in which we think about clinical problems. It is an approach to clinical problem solving that has evolved from a self-directed and problem-based approach to learning rather than the more traditional didactic form. The American Dental Association's definition is by far the most comprehensive, as it captures the core elements of EBD and it is namely patient-centred definition - the EBD is an approach to oral health care that requires judicious integration of systematic assessments of clinically relevant scientific evidence, relating to the patient's oral and medical condition and history, with the dentist's clinical expertise and the patient's treatment needs and preferences.

This paper outlines this role, together with the advantages and problems of introducing an evidence-based approach to dentistry.

Keywords: Dentistry; Dental Education; Evidence-Based Dentistry

**Cena Dimova¹, Maja Pandilova²,
Ivona Kovacevska¹, Biljana Evrosimovska²,
Zlatko Georgiev²**

¹“Goce Delcev” University
Faculty of Medical Sciences, Stip, FYROM
²“Ss. Cyril and Methodius” University
Faculty of Dentistry, Skopje, FYROM

REVIEW PAPER (RP)

Balk J Stom, 2013; 17:5-8

Introduction

Information, innovations and changes are base points and world-wide interest in making dental health services more effective and containing dental health care costs without compromising quality of dental care in the face of technological advances, demographic changes and increasing public expectation⁹. As an integrative scientific field of medicine, dentistry and dental health services are very closely connected with contemporary research and the update of dental practice. However, scientific dental literature and lectures directed at the modern dental practitioner have created some dilemmas and problems. In resolving a clinical decision, evidence rather than empiricism should dictate treatment⁴.

It is generally accepted that the more experience a physician or a dentist possess, better the quality of health care delivery. However, recent studies had shown that there is, in fact, an inverse relationship between

the number of years of practice and the quality of care provided²⁰.

Evidence-based dentistry (EBD) presents guidelines to determine the validity of study results and whether they can be applied to clinical practice^{2,3}. According to Sackett²⁷, EBD is defined as “integrating individual clinical expertise with the best available external clinical evidence from systematic research”. The aim of the EBD is to encourage ordinary dental practitioners in primary dental care to look for and make sense of the evidence available in order to apply it to everyday clinical problems. However, making clinical decisions based on evidence does pose several problems for the dental practitioner^{8,34}.

The aim of this review was to get a solution how to determine what a cutting edge technique is and what is useless when contradictory information exists, as well as to point out the advantages and problems of introducing an evidence-based approach to dentistry.

Evidence-Based Medicine - the Fields and Practice

Evidence-based medicine (EBM) requires integration of the best research evidence with our clinical expertise and our patient's unique values and circumstances²⁹.

Its' philosophical origins extend back to mid-19th century Paris and earlier, is the conscientious, explicit and judicious use of current best evidence in making decisions about the care of individual patients. The practice of the EBM means integrating individual clinical expertise with the best available external clinical evidence from systematic research. In the same time, the practice of EBM is a process of life-long, self-directed learning, in which care for our own patients creates the need for clinically important information about diagnosis, prognosis, therapy, and other clinical and health care issues²⁵.

EBM systematically searches a wide range of international medical journals applying strict criteria for the validity of research^{12,24,32}. Experts critically appraise the validity of the most clinically relevant articles and summarize them, including comments on their clinical applicability. EBM also publishes articles relevant to the study and practice of EBM^{11,31}.

The definition and description of EBM offered by Sackett et al^{26,27} is an appropriate context for better understanding evidence-based dental practice¹⁵.

EBM is sometimes called evidence-based health care, to broaden its application to allied health care professionals¹². Because EBM is used in allied fields, including dentistry, nursing and psychology, *evidence-based practice* (EBP) is a more encompassing term, for example Evidence-Based Practice in xxx (EBPx) as well as Evidence-Based Health Care, Evidence-Based Nursing, Evidence Based Library and Information Practice, Research Based Evidence^{14,16}. Evidence-Based Public Health is the process of systematically finding, appraising and using contemporaneous clinical and community research findings as the basis for decisions in public health^{11,17}.

Evidence-Based Dentistry - Definition and Elements

EBD is the concept of using current scientific evidence to guide decision-making in dentistry. Actually, EBD is a process that restructures the way in which we think about clinical problems. It is an approach to clinical problem solving that has evolved from a self-directed and problem based approach to learning rather than the more traditional didactic form²³. The first introduction with EBD in the scientific field of dental medicine was referred

by Gordon Guyatt and the Evidence-Based Medicine Working Group at McMaster University in Ontario, Canada in the 1990s. A new paradigm for medical education designed to incorporate current research into education and practice was developed to help practitioners to provide the best care for their patients⁷.

The American Dental Association's definition^{1,29} is by far the most comprehensive, as it captures the core elements of EBD and it is namely patient-centred definition. They define it as "an approach to oral health care that requires:

- The judicious integration of systematic assessments of clinically relevant scientific evidence, relating to the patient's oral and medical condition and history,
- The dentist's clinical expertise, and
- The patient's treatment needs and preferences."

Business-centred definition, which David ME adapted from Health Affairs (2005) referred by Sackett²⁷, emphasized that "Evidence based dentistry is a set of principles and methods intended to insure to the greatest extent possible, clinical decisions, guidelines and other types of policies that are based on and consistent with good evidence of effectiveness and benefit".

EBD supplies guidelines to help the clinician make an intelligent decision. In and of itself, EBD does not give definitive answers. It does not exchange the tyranny of the expert for the tyranny of the literature²². As Sackett's definition³⁰ states, EBD relies first on clinical expertise. Shaw³⁰ pointed out in his recent leader on the Cochrane Collaboration that even when there is good evidence for a particular intervention or therapy, it is often many years before it comes into general use.

Evidence-Based Dentistry - Necessity in the Update Dentistry

Graduates from dental schools are up to date with the best practice in current dentistry at the time they graduate. Some of this knowledge gradually becomes out of date as new information and technology appear. It is important for dentists to be able to keep up to date with developments in diagnosis, prevention and treatment of oral disease, as well as newly discovered causes of diseases, especially in regards to patient safety³³. The problems of introducing evidence based dentistry are amount of evidence, quality of evidence, and practice based on authority rather than evidence^{5,6}.

Amount of evidence: Currently over 2 million biomedical articles are published annually in some 20,000 journals. There are about 500 journals related to dentistry. Clearly not all of these articles are relevant to all areas of dental practice, nor can one hope to read any more than a minority of them¹⁸.

Quality of evidence: Much of the ever increasing volume of evidence is produced to enhance career prospects rather than to increase knowledge. This can compromise quality¹⁸. Dental treatment decisions have been largely based on observations of historical response of a disease or condition to an intuitive treatment. Methods of treatment were based upon a good understanding of underlying disease and physiology (G.V. Black's "extension for prevention"; Caries control via plaque removal). Observations of outcomes, however, have rarely been validated or tested scientifically to see if they were valid¹³.

There is an overwhelming amount of evidence that comes from research and policy-making organisations, but there is no one organisation that synthesises and assesses all this evidence. Advances in dentistry are usually first reported in dental journals, and in order to keep up with new research, healthcare professionals need to feel confident that they can read and evaluate dental papers. Keeping abreast of new developments through reading current literature can seem onerous and hard to combine with a heavy clinical workload. Fortunately, having an understanding of how to interpret research results, and some practice in reading the literature in a structured way, can turn the dental literature into a useful and comprehensible practice tool²⁰.

There is world-wide interest in making health services more effective and containing health care costs without compromising quality of care in the face of technological advances, demographic change and increasing public expectation¹⁹.

Quality assurance and performance evaluation have become central issues in dental medicine. Sometimes dental care is suboptimal in many different dental conditions and clinical settings. Few existing studies have had the specific goal of evaluating the effects of experience on the quality of dental care. However, length of time in clinical practice has been included as part of a set of physician characteristics that might explain variations in quality or that may be confounders of the association between quality and other factors³³. However, comparatively few decisions in the health services are made as a result of good evidence.

The Impact of Evidence in Dental Practice

While the EBD can provide dental clinicians with clear answers to questions about specific treatments, this information is intended to be used in conjunction with clinicians' expertise and specific patient factors. EBD is not intended to be "cookbook" dentistry, but it is envisioned as a disciplined process where the best objective information of the risks and benefits is weighted with clinical experience and patient preferences³⁵.

EBD does not mean that clinicians need not study basic and dental material sciences. In fact, the opposite is true. To evaluate the research presented, clinicians need a

solid background on which to base their evaluations and decisions. However, most dentists in teaching institutions or clinical practice have not been prepared to deal with the conscious and conscientious integration of best evidence into clinical practice, and hence may be less than effective in adopting evidence-based practice³⁵.

The Impact of Evidence on Dental Community Research

EDB is relatively a new paradigm in dentistry and thus may not be a well-known concept to every dental graduate. It opens a new era in dental research. This movement can bring together traditional basic science researchers with clinical researchers, clinicians, and educators. Modern day dentistry presents great challenges to the practicing dentist to deliver care of the highest standard to patients. At times, there are situations where the clinician is confronted with doubts regarding diagnosis and treatment planning in spite of the knowledge and experience gained over time^{23,25}.

The current barriers that exist between the dental research community and the practicing community can diminish as evidence-based teams start to work on finding, appraising, summarizing, and analyzing evidence to answer clinically relevant questions. The gaps they identify in the evidence will constitute arguments for designing and implementing both prospective and retrospective studies to answer critical clinical questions³³. Moreover, EBD represents a potential strengthening of the complex process of science transfer, of translating research into practice. Dental research has a vested interest in this movement, because research results are the raw input to the process²⁰.

Conclusion

The challenges for dental research are to establish an international dialogue and collaboration to strengthen the evidence and to improve the processes through which clinicians integrate it into their treatment decisions. There are clear paths to meet these challenges, which require cooperation of dental research, dental education, and international funding agencies.

References

1. American Dental Association Council on Scientific Affairs. Professionally applied topical fluoride. Evidence-based clinical recommendations. *J Am Dent Assoc*, 2006; 137(8):1151-1159.
2. Bader J, Shugars D. The evidence supporting alternative management strategies for early occlusal caries and suspected occlusal dental caries. *J Evid Base Dent Pract*, 2006; 6:91-100.

3. Bader J, Ismail A. Survey of systematic reviews in Dentistry. *J Am Dent Assoc*, 2004; 135:464-473.
4. Choudry NK, Fletcher RH, Soumerai SB. Systematic review: The relationship between clinical experience and quality of healthcare. *Ann Inter Med*, 2005; 142(260):73-94.
5. Durgesh NB. Evidence based education in Dentistry: Can it be implemented in India? *Int J Oral Maxillofac Pathol*, 2011; 2(2):44-45.
6. Haron IM, Sabti MY, Omar R. Awareness, knowledge and practice of evidence-based dentistry amongst dentists in Kuwait. *Eur J Dent Educ*, 2012; 16(1):e47-e52.
7. Hendricson WD, Rugh JD, Hatch JP, Stark DL, Deahl T, Wallmann ER. Validation of an instrument to assess evidence-based practice knowledge, attitudes, access and confidence in the dental environment. *J Dent Educ*, 2011; 75(2):131-144.
8. Hyanes B, Haynes GA. What does it take to put an ugly face through the heart of a beautiful hypothesis? (Editorial). *Ann Inter Med*, 2009; 150:JC3-2-3.
9. Iqbal A, Glenney AM. General dental practitioners' knowledge of and attitude towards evidence-based practice. *Br Dent J*, 2002; 192:587-591.
10. Jencks SF, Cuerton T, Burwen DR, Fleming B, Houck PM, Kussmaul AE, et al. Quality of medical care delivered to Medicare beneficiaries: A profile at state and national levels. *JAMA*, 2000; 284:1670-1676.
11. Jenicek M, Stachenko S. Evidence-based public health, community medicine, preventive care. *Med Sci Monit*, 2003; 9(2):1-7.
12. Jha AK, Perlin JB, Kizer KW, Dudley RA. Effect of the transformation of the Veterans Affairs Health Care System on the quality of care. *N Engl J Med*, 2003; 348:2218-2227.
13. Kay EJ, Blinkhorn AS. A qualitative investigation of factors governing dentists' treatment philosophies. *Br Dent J*, 1996; 180:171-176.
14. Lockwood S. 'Evidence of me' in evidence based medicine? *Br Med J*, 2004; 329:1033-1035.
15. Merijohn GK, Bader JD, Frantsve-Hawley J, Aravamudhan K. Clinical Decision Support Chairside Tools for Evidence-Based Dental Practice. *J Evid Base Dent Pract*, 2008; 8:119-132.
16. McGlynn EA, Asch SM, Adams J, Keesey J, Hicks J, DeCristofaro A, et al. The quality of health care delivered to adults in the United States. *N Engl J Med*, 2003; 348:2635-2645.
17. Mulrow CD. The medical review article: state of the science. *Ann Inter Med*, 1987; 106:485-488.
18. Newman MG. Clinical decision support complements evidence based decision making in dental practice. *J Evid Base Dent Pract*, 2007; 7:1-5.
19. Osheroff JA, Teich JM, Middleton BF, Steen EB, Wright A, Detmer DE. A roadmap for national action on clinical decision support. June 13, 2006.
20. Padiyar N, Agarwal S, Tandon P. Evidence based dentistry: Why and How? *J. Int Oral Health*, 2011; 3(1):1-6.
21. Prabhu. S, Joseph J, Saravanan. S. Knowledge, Attitude and Perceived Barriers towards practice of Evidence Based Dentistry among Indian postgraduate dental students. *J Dent Med Sci*, 2012 Sep-Oct; 1(2):46-51.
22. Rajshekar AS, Sunder KK, Lakshminarayan N. Knowledge and Attitude towards Evidence based Dentistry among Postgraduate students of two Dental Colleges in Davangere City. *Journal of the Indian Association Public Health Dentistry*, 2011; 18 (Suppl II):761-765.
23. Richards D, Lawrence A. Evidence based dentistry. *Br Dent J*, 1995; 179(7):270-273.
24. Risahmawati M, Sei E, Tomoko N, Shunzo K. Japanese Resident Physicians' attitude, knowledge and perceived barriers on the practice of Evidence based Medicine: a survey. *BMC Research Notes*, 2011; 4:374.
25. Rosenberg W, Donald A. Evidence-based medicine: an approach to clinical problem-solving. *Br Med J*, 1995; 310:1122-1126.
26. Sackett DL. Evidence-based medicine. *Seminars in Perinatology*, 1997; 21(1):3-5.
27. Sackett D, Richardson WS, Rosenberg W, et al. Evidence based Medicine: How to Practice and Teach EBM. New York: Churchill Livingstone, 1997.
28. Sackett DL, Haynes RB. On the need for evidence-based medicine. *Evidence based Medicine*, 1995; 1:5-6.
29. Schuster MA, McGlynn EA, Brook RH. How good is the quality of health care in the United States? *Milbank Q*, 1998; 76:517-563.
30. Shaw WC. The Cochrane Collaboration: Oral Health Group. *Br Dent J*, 1994; 177:272.
31. Straus SE, Richardson WS, Glasziou P, Haynes RB. Evidence-based medicine: how to practice and teach EBM. 3rd ed. London: Churchill Livingstone, 2005.
32. Thompson JW, Ryan KW, Pinidiya SD, Bost JE. Quality of care for children in commercial and Medicaid managed care. *JAMA*, 2003; 290:1486-1493.
33. Vashisth S, Bansal M, Gupta N, Chand RN. Evidence Based Dentistry an evolving concept. *Journal of the Indian Association of Public Health Dentistry*, 2011; 18:226-228.
34. Winning T, Needleman I, Rohlin M. Evidence based care and the curriculum. *Eur J Dent Educ*, 2008; 12(sup1):48-63.
35. Zamros YM, Ha LJ, San PP, Ramli AS. Evidence-based practice among a group of Malaysian dental practitioners. *J Dent Educ*, 2008; 72(11):1333-1342.

Correspondence and request for offprints to:

Doc. dr Cena Dimova
 "Goce Delcev" University
 Faculty of Medical Sciences - Stip
 2000 Stip, FYR Macedonia
 cena.dimova@ugd.edu.mk

Sealant Retention in Pits and Fissures: Preparation and Application Techniques. A Literature Review

SUMMARY

The effectiveness of sealants as a preventive measure has been well established. However, the desirable successful outcome depends on retention and, therefore, on the factors contributing to this. The purpose of this literature review is to highlight the most significant factors connected with preparation and application of the sealant materials and any consensus that exists about their contribution to the longevity of the sealant. The preparation of the surface is a matter of great importance, the choice of the cleaning method before etching, and its relevance to retention, remains the subject of discussion. Techniques such as air abrasion, laser, and mechanical preparation have been proposed and applied with varying results. Application techniques are also a controversial area, especially when it comes to the use of self adhesive agents and special bonding agents. Other factors, however, have also been the subject of study in the literature. These include the state of eruption, the nature of isolation, type of the tooth, the profile of the operator and the kind of sealant. More long-term clinical trials are needed to examine the relationship between preparation and application process and retention in order to achieve the desired aim of improved clinical success rates.

Keywords: Pit Sealants; Fissure Sealants; Retention

V. Tzifa, A. Arhakis

Aristotle University of Thessaloniki
School of Dentistry
Thessaloniki, Greece

LITERATURE REVIEW (LR)
Balk J Stom, 2013; 17:9-17

Introduction

Pits and fissures, forming part of occlusal tooth surfaces, are prone to decay or need for restoration in 70% of cases over a period of 10 years after eruption¹. Therefore, the need to use sealants in view of their ability to inhibit caries prevention has been underlined since the 1970s and 1980s². A significant consideration mentioned in the literature is that pits and fissures benefit less from fluoride due to their depth below the enamel surface and their close connection with dentin, and, therefore, are subject to rapid progression of caries³. An important number of randomized clinical trials have confirmed their significance in caries prevention on occlusal surfaces⁴. Every child or young person should be a candidate for using sealants when there are susceptible pits and fissures. The rationale of pit and fissure sealants is related to the fact that occlusal fissures are connected to the dentin and lesion can therefore spread along the enamel-dentin interface in a

fast rate³. Consequently, the effectiveness of sealants is well established for persons at high caries risk⁵.

Retention still remains the main determinant factor for success, where this is defined in terms of long lasting protection, absence of open margins, and sound enamel of the occlusal surface. In other words, effectiveness and preventive ability is related to the complete retention of the material on the occlusal surfaces^{6,7}. Failure, on the other hand, is marked by an early loss, usually during the first year after application⁸, mainly due to inadequate adhesion⁹ or by gradual failure of the sealant when exposed to wear¹⁰. The statistics in the literature reveal a loss of 5-10% of sealant per year¹¹. With the endorsement of use of mainly resin-based materials for sealants, integration and retention has become the key for ensuring cariostatic effectiveness since such sealants present difficulties regarding the release of fluoride¹². In addition, good adaptation of the sealant can block micro-leakage and its detrimental impact on tooth integrity^{13,14}. Other research refers to retention in terms of "penetrability of the etched enamel, enough marginal

sealing and resistance to wear¹⁵. Taking into account that, micro-leakage does not necessarily mean loss of retention¹⁶.

Longevity, therefore, results from many factors contributing to better retention, such as fissure type, the kind of sealant, the profile of the operator, the nature of isolation, the type and place of the tooth, the enamel, eruption status and other factors^{2,17-21}. Notwithstanding the complexity of the issue, this review is an attempt to evaluate and report the most analyzed and important factors mentioned in the recent literature, focusing on preparation and application processes of sealant use.

Preparation Technique

Cleaning Methods

The importance of the method chosen for cleaning the surface is beyond question. Apart from conventional acid etching, other methods have been proposed and investigated. An early study suggested the lack of significant differences among the various selected cleaning methods in terms of the retention achieved²². However, since then, newer techniques have been introduced and the need to evaluate their effect on retention still remains paramount.

One of the most emphasized factors discussed in the literature concerns the mechanical preparation of the surface, also termed enameloplasty or fissurotomy. In the light of the evidence provided, enameloplasty is not recommended, as a routine choice, before etching the tooth surface^{22,23}. This is consistent with Hatibovic-Kofman et al²⁴, who found no significant differences between mechanically prepared surfaces and those that had been only etched. Other extensive reviews of the literature also concluded that there was no strong supporting evidence in favour of enameloplasty^{23,24}. This view is supported by Fiegal and Donly²⁶ who noted the lack of long-term clinical studies supporting enameloplasty, which could lead to its recommendation as a standard procedure. The European guidelines regarding pits and fissures suggest that excessive enlarging the fissures should be avoided since the literature does not relate high retention with the use of the bur²⁷.

However, on the same issue, evaluation of etch resistance in prismless walls of the enamel, it has been suggested that where pits are deeper, adequate etching might not be achieved, and thus, the prismless area of the walls should be removed²⁸. This is consistent with other authors who conclude that enameloplasty has a good effect on prismless enamel and enhances the bond to the surrounding enamel²⁹. In an *in vitro* study of a common sealant and preparation techniques, there were significantly better micro-leakage scores using enameloplasty, but significantly less advantage when it came to penetration³⁰. In addition, it has been suggested that bur preparation may remove accumulated

debris from the deeper places in fissures^{22,31} and aids the exposure and removal of decayed areas³². In the same double-blind study, it was also demonstrated that preparation resulted in better retention³². Similarly, it was demonstrated that better penetration can be achieved by combination of enameloplasty and acid etching for a resinous sealant material when compared with the use of a compomer applied with a bonding agent but without preparation; therefore, this should be preferred clinically³³. This was attributed to better flow properties of the material in widened fissures³³ and improved maintenance in cleaned cavities³⁴.

Another laboratory study also showed that mechanical preparation with a diamond-tapered bur led to better retention than with unprepared surfaces³¹. These results are consistent with another study using an electron microscope to evaluate the enlargement of fissures³². They suggested that the key to a strong bond is good penetration by the resin, which is facilitated by enameloplasty, and that enlargement enables more material to be applied, increasing resistance to loss. Their hypotheses were confirmed by microscope observations revealing superior adaptation levels and good retention patterns. This led them to propose the method for deep fissures.

It has also been proposed that mechanical fissure enlargement might be beneficial for maxillary molars²². In addition when sealed fissures subject to enameloplasties were tested for micro-leakage, there were significant differences²⁹. Specifically, teeth with enameloplasty performed better under stress, with the type of bur affecting the result slightly in favour of the round bur against the tapered one²⁹.

Attempts to broaden the armoury of techniques available for fissure preparation led to the creation of Carisolv system using sodium hypochlorite and 3 kinds of amino acids as chemical cleaning agents³⁴. This yielded smooth, normal surfaces, free of debris inside the cavity. These were somewhat rougher than brush-prepared surfaces, with no differences in micro-leakage between the 2 groups. However, possibility of achieving better retention with this type of chemical cleaning warrants further investigation.

A recent critical review of cleaning methods included only 2 clinical studies, finding no difference between cleaning with pumice and using water and a probe³⁶. The use of the probe is also supported clinically, but in terms of combining it with a bristle brush³⁷. Moreover, a literature search revealed that sealant retention was the same or higher after cleaning with a toothbrush in comparison with handpiece³⁶. Past practice recommended a prophylaxis cup or bristle and pumice. It was then thought that any paste used should be fluoride free to avoid jeopardizing the enamel bond²². However, more recent evidence seems to indicate that fluoride does not reduce retention ability^{26,36}. Nonetheless, recent guidelines do not recommend prophylaxis paste or pumice³⁸. Furthermore, an analysis has shown that handpiece-prophylaxis

reduced retention to an extent that surprised the authors³⁹. Added to this, a study comparing conventional pumice preparation with enameloplasty and air-abrasion found no difference in micro-leakage scores⁴⁰.

No evidence has been found for retention enhancement achieved by hydrogen peroxide (3%)²²; its clinical recommendation is due to its efficacy in removing debris over the conventional brush and pumice³⁷.

Air abrasion for the preparation of teeth for sealants is a fairly recent innovation. It has been suggested that it improves adhesion by eliminating steps of application of acid and rinsing²² and constitutes a less invasive procedure¹³. It has also been proposed for removing prismless enamel, superficial caries, and enlarging and cleaning fissures to achieve better retention⁴¹. In an investigation concerning preparing deciduous molars with air abrasion, a stronger bond was achieved with the combined use of air abrasion and acid etch than with acid etch alone¹³. The superiority of this combination over acid etch alone is consistent with other studies⁴¹⁻⁴³, but air abrasion alone is thought to be inadequate^{41, 44}. A recent paper concerning evidence-based

recommendations about pits and fissure sealants concluded that there was no clear evidence about the air abrasion-acid combination²³. Another study failed to find significant differences between the air abrasion and the pumice prophylaxis, when chosen as different procedures for testing sealant materials for leakage and success⁴⁵. However, some researchers have found similar retention results between air-abrasion and the conventional acid etching^{43, 46, 47}.

Comparison of sono-abrasion, acid etching and enameloplasty showed no significant differences between the 3 methods⁴⁸.

The Er,Cr: YSGG laser system has also been suggested for surface preparation. In an *in vitro* study⁴², acid etching, and the laser combined with acid etching performed similarly. In experiments to evaluate the shear bond strength of Er: YAG laser in the presence of saliva, the laser did not show comparable results to conventional etching with phosphoric acid⁴⁹. On the same subject, other authors have found that applying a sealant to fissures after laser preparation resulted in greater micro-leakage than using mechanical preparation³⁰ (Table 1).

Table 1. Cleaning methods and retention rate

Study	Number of teeth	Cleaning/preparation method examined	Retention/microleakage of the sealants
Geiger et al (2000) ³¹	90	Enameloplasty vs no mechanical preparation	Better retention rates (only 6,25% loss) for enameloplasty
Selecman et al (2007) ⁴⁵	100	Pumice prophylaxis and air abrasion	No differences between them
Garcia-Godoy and de Araujo (1994) ³⁵	32	Enameloplasty vs pumice prophylaxis	Better retention for enameloplasty
Lygidakis et al (1994) ³²	320	Enameloplasty vs bristle brush with and without paste	Better retention for Enameloplasty
Burrow et al (2001) ²⁸	55	Enameloplasty vs no mechanical preparation	Better retention for enameloplasty
Hatovic-Kofman et al (2001) ²⁴	180	Enameloplasty vs air abrasion	Same retention
Yamada et al. (2008) ³⁴	50	Preparation with Carisolv	Greater surface roughness, perhaps better retention
Knobloch et al., (2005) ¹³	40	Air abrasion	Better retention only together with acid etching
Salama and Al-Hammad (2002) ³³	30	Enameloplasty vs no mechanical preparation	Better penetration for enameloplasty
Moslemi et al (2011) ⁴²	45	Er,Cr: YSGG laser vs air abrasion	Better retention with air abrasion together with acid etching
Zervou et al (2000) ²⁹	60	Enameloplasty vs no enameloplasty	Better microleakage scores for enameloplasty
Yazici et al (2006) ⁴¹	162	Air abrasion with acid etching vs acid etching alone	Better retention with air abrasion together with acid etching
Lepri et al (2008) ⁴⁹	24	Er: YAG laser	Not better retention
Kanellis et al., (1997) ⁴⁴	300	Air abrasion alone vs acid etching	Similar results for occlusal surfaces
Duangthip and Lussi (2003) ⁵⁸	90	Air abrasion together with acid etching vs pumice prophylaxis	Not better results versus traditional pumice prophylaxis
Blackwood JA et al (2002) ⁴⁰	60	Pumice prophylaxis vs enameloplasty, versus air abrasion	Same results between them (all followed by acid etching)
Ellis et al (1999) ⁴³	84	Air abrasion	Better retention scores together with acid etching
Lupi-Pegurier et al (2004) ⁴⁸	90	Sono-abrasion alone vs enameloplasty	Similar results

Isolation Type

Literature strongly supports that saliva contamination is detrimental to sealant retention^{21,50-52}. The moisture and the saliva encourage creation of an organic layer in contact with the etched surface and thereby jeopardize retention⁵³.

The best way of optimizing isolation is by using a rubber dam^{2,22,54}. In other words, even 1 second of contact with saliva can reduce bond strength by 50 to 100%⁵⁴. In a systematic review of the literature the authors concluded that with auto-polymerised resin based sealants, retention seems to be unaffected when cotton rolls are chosen². These conclusions are also supported by the European guidelines where, while the dam still remains the optimal means of isolation, the use of cotton rolls with the right amount of water and an evacuation tip, are thought to be an effective and easy way of achieving isolation²⁷. The Irish guidelines arrive at the same conclusion³⁸. Researching the same issue, a double blind study by Lygidakis et al³² also found no differences between the type of isolation chosen. This is also confirmed by Straffon et al⁵⁶ who, in a study about sealant efficacy, found that the retention rate was up to 95% when using cotton rolls and 94,3% for a rubber dam⁵⁶.

In a clinical study that tested the differences between the isolation obtained using cotton rolls and the Isolate system (IS - a plastic device placed in the cavity with an integral light source, tissue retraction and isolation ability covering half of the cavity), the latter showed reduced working time and, as a result, the authors hypothesized, better retention. Confirming this would require further and longer term examination⁵⁷. For others, better isolation can be obtained by correct patient positioning, the use of a mouth mirror when retracting tissues, and placing the evacuation tip over the roll to take off most of the moisture²². However, the idea of using extra drying agents after the etching was not proved to be beneficial^{58,59}. When there is inadequate isolation, glass ionomer sealants can be an adequate temporary solution^{4,53}.

Etching

All failures involving adhesion, are due to non-optimal processes concerning cleaning and preparing the micro cavity of the fissure. The vast amounts of data from investigations, however, leave much room for discussion. Firstly, retention is related to completely acid conditioned enamel^{13,28,59,60}. The tags created are the mechanism of the bond that constitutes retention. The etchant is provided as a gel or liquid and the acids used are mainly ortho-phosphoric acid, maleic acid and nitric and citric acids³. Examination of the literature indicates that both liquid and gel produce good results²². Assisting flow by the use of a brush is suggested for both^{22, 59}.

On the subject of etching time, most authors believe that 15 or 20 seconds are as effective as etching for 60 seconds^{22,25, 61} although some found differences between

40 and 60 seconds, in favour of the latter⁶³. Furthermore, earlier results concerning optimal etching time for bond strength have shown no significantly different results from varied etching times⁶³. The authors found that for the thin gel, application for 60 seconds produced different results from 20 seconds. However, they questioned the clinical significance of this⁶³. Generally, they recommended application for 20 or 30 seconds.

Regarding etchant viscosity, the previous study did not find any advantage between the liquid, the thick and the thin forms of the etchant⁶³.

Rinsing and Drying

On the subject of the optimum time for rinsing the surface, there is a belief that less than 20 seconds is sufficient and that the vigour of washing and drying is more important for success than the time itself²². However, others propose that rinsing for 30 seconds with water and air should be followed by 15 seconds drying⁶⁴. Another reported opinion is that, when a gel etchant is used, one should rinse for almost 90 seconds, as opposed to only 60 seconds with the liquid form³. It should be noted that the presence of oil in the air-water syringe can compromise etching^{22,53}.

Application Technique

Another topic that is abundantly studied is the use of adhesive agents and their contribution to retention. Sufficient retention is obtained by thorough application⁵¹.

Bonding Agent underneath the Sealant

There are interesting data regarding the concept of achieving better retention by the use of a bonding agent before placing the sealant. The general belief is that the use of an agent provides extra protection and retention in terms of moist conditions, better flow and more flexible combination of the primer adhesive and resin²⁶. In an early 2-year clinical evaluation between 2 sealant products placed with and without bonding agent, 1 sealant showed better rates with the bonding agent but only in the earliest stage of evaluation⁶⁵. The other sealant had the same retention results irrespective of the bonding agent; thus the authors did not support a stand point that retention is enhanced by a bonding agent. The same conclusions have been reported by other authors⁸. Similarly, in a split-mouth study forming part of an oral school program, the authors were surprised to find that no differences were seen in retention, with or without a bonding agent⁶⁶. It should also be added that they used rubber dam for isolation⁶⁶.

However, a recent study demonstrated enhanced retention performance when using a bonding agent than with the acid technique alone⁶⁷. For similar protocols,

when micro-leakage was tested as a side-effect of marginal adaptation, the best results were obtained with the use of underlying bond agent⁶⁸. Therefore, the authors felt that application of sealants together with a bonding agent, as an additional stage, seemed to be “their golden standard”⁶⁸. In a 5-year clinical study by Feigal et al (1993), the single-bottle systems (fifth generation) enhanced retention in terms of reducing risk of loss by half, whereas previous generation agents showed no such success (no long term success)⁵⁵. More success was achieved on buccal and lingual surfaces due to their ability to absorb forces on these more flexible areas. On the same subject, on salivary contaminated enamel, the use of adhesive systems provided the best sealing^{51, 53}. Feigal et al⁵⁵ carried out a 2-year clinical study where sealants were successfully retained despite 10 seconds of contamination by using Scotchbond® as an underlying hydrophilic layer. They found equal retention with the conventional way of applying a sealant, thus suggesting a very good clinical solution for retention when there is insufficient moisture control.

Positive results were also produced by a study of children with MIH, where the use of a 2-step etch and rinse, single bottle adhesive (5th generation) enhanced sealant retention⁶⁹. Authors attributed this to the ability to achieve deep penetration in the etched surface and good tag formation⁶⁹.

Another interesting topic is bond layer thickness; failure has been attributed to very thin bond layers⁵⁹. Concerning the same issue Feigal et al⁵⁵ recommended a full 2, 3 second thinning of the layer with the air syringe in terms of evaporating the moisture and enhancing success. The acetone constituent of the adhesive can also enhance bond strength⁶⁹. When examining the use of bonding agents in the context of an oil-contaminated surface due to the air-water-syringe, they found that the use of an acetone-based bonding agent enhanced adhesion, whereas an ethanol-based bonding agent produced no such effect⁵³.

Self-Adhesive/Etching Agents

Regarding self adhesive agents, it was proposed that combination of avoiding the rinsing stage, and the ability to obtain better adhesion, made them as an interesting concept in terms of retention^{26,52,70}. Thus, if they can effectively bond the sealant to the enamel, they will simplify the process for patients inconvenienced by standard etching-rinsing procedures. Consequently, studies examining the bond strength of these self adhesives are very important, in coming to conclusions about their retention ability⁷¹. This is thought to be a very interesting topic for achieving more ergonomic application of sealants and, therefore, is analyzed as far as possible in the present paper.

An *in vitro* study, testing the adhesion level of a sealant with a self-etching system, showed similar

retention to sealants applied alone to uncontaminated enamel, and higher than sealants applied with the etch and rinse system; leading to the conclusion that single-bottle adhesives have the ability to penetrate deeply into micro-spaces⁵¹. In the same study, different curing procedures did not affect the result. Promising results for the future use of self etching agents were also obtained by a 2-year study of resin restorations, where clinical results showed longevity and good retention⁷¹.

More information about the capability of self adhesive agents is demonstrated in a study comparing phosphoric acid with the Adper Prompt L-Pop®⁷². It achieved similar results and higher retention when the agent was applied in 2 layers and separately cured; it also showed better performance than other adhesives. However, another 12-month school-based programme studied the same sealant and showed that the conventional procedure with phosphoric acid produced much better results than the self-etching primer adhesive⁷³. Moreover, a RCT study undertaken for the UK National Health community dental service, which tested Xeno 3® as a self etching agent against traditional acid etching with the use of Prime & Bond®, found that the former had lower retention rates and reinforced the superiority of the latter, which is in line with most of the relevant literature⁷⁴. Additionally, another *in vitro* study of the use of self-etching primer agents showed same results in comparison with conventional etch and rinse procedures, but performed better when a ceromer product was used as a sealant material, indicating that their use can be a good choice as alternatives to traditional etching with phosphoric acid⁷⁴. In another retrospective study⁷⁶ regarding the clinical rates of Dyract Seal, a sealant applied under no rinsing conditions with a rubber dam, the findings were lower with respect to reported results for conventional sealants, but the authors suggest it can be a good short-termed choice for deciduous teeth and when there is a pronounced gag reflex.

Additionally, the self adhesive (Adper Promt-L-Pop) has produced comparable results to conventional bonding systems in a study of 3 different procedures about bond values with respect to authors' reference for more confirmation data⁶⁷. However, the same self adhesive, when used with 2 different polymerization regimes, failed to produce good retention in recently erupted molars⁷⁷. In a similar study⁶⁷, the self adhesive achieved comparable results to the use of a bonding agent prior to the sealant, in contrast to other studies that authors refer to in their paper. When the same self etching agent was compared with acid etching, it failed to produce better results⁶². This agrees with another recent study of a new self etch sealant for which there were, at that time, no supporting data. It produced a lower level of bonding than conventional sealants. More research is needed in an *in vivo* environment to assess its likely clinical performance⁷¹.

The recent literature reflects consensus that traditional etch and rinse techniques still remain procedure of choice. In the evidence-based recommendations of Beauchamp et al²³, the self-etching bond agents are not recommended as replacements for traditional etching due to their low retention scores. This is consistent with another recent evidence-based article which did not find sufficient data to support their use and concluded they should not be recommended²⁵. Yazici et al⁷⁸ also reported, from their clinical testing, the superiority of the traditional etch-and-rinse method. Their histological explanation for the failure of the self adhesive was that

it could not provide as effective an etched pattern as the phosphoric acid when applied separately and rinsed. Similar explanations for the failure of self-adhesives by observing their histological pattern appears in another study concerning the differences in penetration achieved by the 2 methods in question⁷⁹.

When considering how the properties of self-etching systems could be improved, it is notable that their adhesion to mechanically prepared enamel is better than to non-prepared surfaces, with the exception of 1 total-etch adhesive, which showed strong bonding no matter the preparation procedure⁸⁰ (Table 2).

Table 2. Retention rate of self adhesives vs simple etch-rinsing process

Study	Number of teeth	Product(s)	Overall outcome of success of self adhesive
Celiberti and Lussi (2005) ⁶³	80	Xeno 3 [®]	No significant difference in retention rate
D. Gillet et al (2002) ⁷⁵	24	Prompt [®]	No significant difference in retention rate
Dos Santos KT et al (2008) ⁷⁹	36	Adper Prompt L-Pop [®]	No significant difference in retention rate
Venker DJ et al. (2004) ⁷³	208 students of a school-based sealant program	Prompt-L-POP [®]	No significant difference in retention rate
M.E Asselin et al (2008) ⁶⁸	63	Adper Prompt L-Pop [®]	No significant difference in retention rate
J. M. Gomes-Silva et al (2008) ⁵²	45 with saliva contamination	Clearfil S3 Bond [®]	No significant difference in retention rate
AR Yazici et al (2009) ⁷⁸	244	FuturaBond NR [®]	No significant difference in retention rate
L. Burbridge et al., (2006) ⁷⁴	162	Xeno 3 [®]	No significant difference in retention rate

Filling Level of the Fissure

There is some evidence about the effect of the filling level of the fissure. Geiger et al. demonstrated in an *in vitro* study, that overfilled fissures seem to suffer greater sealant loss than those that are border filled³¹. This can be explained by the existence of parts of the material exposed to higher levels of light and therefore undergoing greater shrinkage^{30,31,33}. The general consensus seems to be that all pits and fissures should be covered to obtain good retention and that the thickness of the material is of less importance⁸¹. The existence of bubbles within the sealant was not generally detrimental, except when they occurred in the margins. This leads to recommendations that, when polymerizing a sealant with load particles, it should be applied under vibration^{59,60}. There are also suggestions that better penetration occurs when material is left to flow for 20 seconds before curing than when it is left for a shorter time²² or left as long as possible¹⁵.

The use of an air polishing device helped investigators to achieve good retention in an early study⁵⁹. However, others found that the device does not allow material to flow deeply enough due to the trapped air³⁵.

In the end, the meticulous examination of sealant margins after placement with the use of a probe to reveal

indications of early retention failure remains of crucial importance^{22,82}.

Conclusion

The retention ability of a sealant is crucial because most failures are of an adhesive nature. Thus, when retention is jeopardized, the overall anti-cariogenic effectiveness of the sealants is in question, too¹².

When it comes to the factors as examined in the current study, some have been analyzed in greater depth than others, and for some there is a greater degree of consensus in the literature. The data given for the type and position of the tooth is not so clear, and sealant retention on primary molars is still a field that requires more investigation⁴. One of the factors most analysed regarding clinical impact is preparation of the surface before application of the sealant. In the literature, fissurotomy seems to enhance retention, but is not recommended as a routine preparation due to its invasive nature. More supporting data is needed about the use of air abrasion and lasers since that extant is unsatisfactory. It is interesting to

note that some references are to sealants as part of micro-therapeutic invasive technique, rather than as a solely preventive procedure⁴.

Numerous studies confirm the use of cotton rolls as free of risk to the isolation required. However, the most important factor for isolation and therefore ensuring retention and longevity is the state of eruption, where the literature suggests having as full an eruption status as possible²⁷. Regarding the idea of applying a bonding agent under the sealant, the facts are positive overall. However, concerning the recently discussed use of self adhesives because of their ergonomic characteristics, the results are still inconclusive and more clinical trials are needed⁷¹. More data is also required about sealant materials to connections to be made between level of viscosity and the type of material and sealant retention^{4,5}.

The main idea pervading the available literature is that the central issue is no longer the effectiveness of sealants, but how the factors discussed above contribute to the desired clinical outcomes of every day sealants practice.

References

1. Eklund SA, Ismail AI. Time of development of occlusal and proximal lesions: implications for fissure sealants. *J Public Health Dent*, 1986; 46:114-121.
2. Muller-Bolla M, Lupi-Pégurier L, Tardieu C, Velly AM, Antomarchi C. Retention of resin-based pit and fissure sealants: A systematic review. *Community Dent Oral Epidemiol*, 2006; 34:321-336.
3. Arhakis A, Damianaki S, Toumba KJ. Pit and Fissure Sealants: Types, Effectiveness, Retention, and Fluoride Release: A Literature Review. *Balk J Stom*, 2007; 11:151-162.
4. Splieth CH, Ekstrand KR, Alkilzy M, Clarkson J, Meyer-Lueckel H, Martignon S, Paris S, Pitts NB, Ricketts DN, van Loveren C. Sealants in dentistry: outcomes of the ORCA Saturday Afternoon Symposium 2007. *Caries Res*, 2010; 44:3-13.
5. Ahovuo-Saloranta A, Hiiri A, Nordblad A, Mäkelä M, Worthington HV. Pit and fissure sealants for preventing dental decay in the permanent teeth of children and adolescents. *Cochrane Database Syst Rev*, 2008; 8(4):CD001830.
6. Kervanto-Seppälä S, Pietilä I, Meurman JH, Kerosuo E. Pit and fissure sealants in dental public health - application criteria and general policy in Finland. *BMC Oral Health*, 2009; 4; 9:5.
7. Corona SA, Borsatto MC, Garcia L, Ramos RP, Palma-Dibb RG. Randomized, controlled trial comparing the retention of a flowable restorative system with a conventional resin sealant: one-year follow up. *Int J Paediatr Dent*, 2005; 15:44-50.
8. Locker D, Jokovic A, Kay EJ. Prevention. Part 8: The use of pit and fissure sealants in preventing caries in the permanent dentition of children. *Br Dent J*, 2003; 195:375-378.
9. Higson JF. Caries prevention in first permanent molars by fissure sealing. A 2- year study in 6-8-year-old children. *J Dent*, 1976; 4:218-222.
10. Autio-Gold JT. Clinical evaluation of a medium-filled flowable restorative material as a pit and fissure sealant. *Oper Dent*, 2002; 27:325-329.
11. Feigal RJ. Sealants and preventive restorations: review of effectiveness and clinical changes for improvement. *Pediatr Dent*, 1998; 20:85-92.
12. Lobo MM, Pecharki GD, Tengan C, da Silva DD, da Tagliaferro EP, Napimoga MH. Fluoride-releasing capacity and cariostatic effect provided by sealants. *J Oral Sci*, 2005; 47:35-41.
13. Knobloch LA, Meyer T, Kerby RE, Johnston W. Microleakage and bond strength of sealant to primary enamel comparing air abrasion and acid etch techniques. *Pediatr Dent*, 2005; 27:463-469.
14. Pardi V, Sinhoreti MA, Pereira AC, Ambrosano GM, Meneghim Mde C. In vitro evaluation of microleakage of different materials used as pit-and-fissure sealants. *Braz Dent J*, 2006; 17:49-52.
15. Irinoda Y, Matsumura Y, Kito H, Nakano T, Toyama T, Nakagaki H, Tsuchiya T. Effect of sealant viscosity on the penetration of resin into etched human enamel. *Oper Dent*, 2000; 25:274-282.
16. Shah S, Roebuck EM, Nugent Z, Deery C. In vitro microleakage of a fissure sealant polymerized by either a quartz tungsten halogen curing light or a plasma arc curing light. *Int J Paediatr Dent*, 2007; 17:371-377.
17. Hevinga MA, Opdam NJ, Bronkhorst EM, Truin GJ, Huysmans MC. Long-term performance of resin based fissure sealants placed in a general dental practice. *J Dent*, 2010; 38:23-28.
18. Celiberti P, Lussi A. Penetration ability and microleakage of a fissure sealant applied on artificial and natural enamel fissure caries. *J Dent*, 2007; 35:59-67.
19. Grewal N, Chopra R. The effect of fissure morphology and eruption time on penetration and adaptation of pit and fissure sealants: A SEM study. *J Indian Soc Pedod Prev Dent*, 2008; 26:59-63.
20. Leskinen K, Ekman A, Oulis C, Forsberg H, Vadiakas G, Larmas M. Comparison of the effectiveness of fissure sealants in Finland, Sweden, and Greece. *Acta Odontol Scand*, 2008; 66:65-72.
21. Azarpazhooh A, Main PA. Pit and fissure sealants in the prevention of dental caries in children and adolescents: a systematic review. *J Can Dent Assoc*, 2008; 74:171-177.
22. Waggoner WF, Siegal M. Pit and fissure sealant application: updating the technique. *J Am Dent Assoc*, 1996; 127:351-361.
23. Beauchamp J, Caufield PW, Crall JJ, Donly K, Feigal R, Gooch B, Ismail A, Kohn W, Siegal M, Simonsen R. American Dental Association Council on Scientific Affairs. Evidence-based clinical recommendations for the use of pit-and-fissure sealants: a report of the American Dental Association Council on Scientific Affairs. *J Am Dent Assoc*, 2008; 139:257-268.
24. Hatibovic-Kofman S, Butler SA, Sadek H. Microleakage of three sealants following conventional, bur, and air-abrasion preparation of pits and fissures. *Int J Paediatr Dent*, 2001; 11:409-416.

25. Aleksejuniene J, Brondani MA, Pattanaporn K, Brukiene V. Best practices for dental sealants in community service-learning. *J Dent Educ*, 2010; 74:951-960.
26. Feigal RJ, Donly KJ. The use of pit and fissure sealants. *Pediatr Dent*, 2006; 28:143-150.
27. Welbury R, Raadal M, Lygidakis NA. EAPD guidelines for the use of pit and fissure sealants. *Eur J Paediatr Dent*, 2004; 5:179-184.
28. Burrow MF, Burrow JF, Makinson OF. Pits and fissures: etch resistance in prismless enamel walls. *Aust Dent J*, 2001; 46:258-262.
29. Zervou C, Kugel G, Leone C, Zavras A, Doherty EH, White GE. Enameloplasty effects on microleakage of pit and fissure sealants under load: an in vitro study. *J Clin Pediatr Dent*, 2000; 24:279-285.
30. Francescut P, Lussi A. Performance of a conventional sealant and a flowable composite on minimally invasive prepared fissures. *Oper Dent*, 2006; 31:543-550.
31. Geiger SB, Gulayev S, Weiss EI. Improving fissure sealant quality: mechanical preparation and filling level. *J Dent*, 2000; 28:407-412.
32. Lygidakis NA, Oulis KI, Christodoulidis A. Evaluation of fissure sealants retention following four different isolation and surface preparation techniques: four years clinical trial. *J Clin Pediatr Dent*, 1994; 19:23-25.
33. Salama FS, Al-Hammad NS. Marginal seal of sealant and compomer materials with and without enameloplasty. *Int J Paediatr Dent*, 2002; 12:39-46.
34. Yamada Y, Hossain M, Shimizu Y, Kimura Y, Masuda Y, Nakamura Y, Matsumoto K. Analysis of surface roughness and microleakage of fissure sealants following organic debris removal with Carisolv. *J Dent*, 2008; 36:130-137.
35. Garcia-Godoy F, de Araujo FB. Enhancement of fissure sealant penetration and adaptation: the enameloplasty technique. *J Clin Pediatr Dent*, 1994; 19:13-18.
36. Kolavic Gray S, Griffin SO, Malvitz DM, Gooch BF. A comparison of the effects of toothbrushing and handpiece prophylaxis on retention of sealants. *J Am Dent Assoc*, 2009; 140:38-46.
37. Smith Jr WP. Retention of Sealants. Letter. *J Am Dent Assoc*, 2009; 140:516-517.
38. Irish Oral Health Services Guideline Initiative. Pit and Fissure Sealants: Evidence-based guidance on the use of sealants for the prevention and management of pit and fissure caries. 2010. available at: <http://ohsrc.ucc.ie/html/guidelines.html>
39. Griffin SO, Jones K, Gray SK, Malvitz DM, Gooch BF. Exploring four-handed delivery and retention of resin-based sealants. *J Am Dent Assoc*, 2008; 139:281-289.
40. Blackwood JA, Dilley DC, Roberts MW, Swift EJ Jr. Evaluation of pumice, fissure enameloplasty and air abrasion on sealant microleakage. *Pediatr Dent*, 2002; 24:199-203.
41. Yazici AR, Kiremitçi A, Celik C, Özgünaltay G, Dayangaç B. A two-year clinical evaluation of pit and fissure sealants placed with and without air abrasion pretreatment in teenagers. *J Am Dent Assoc*, 2006; 137:1401-1405.
42. Moslemi M, Erfanparast L, Fekrazad R, Tadayon N, Dadjo H, Shadkar MM, Khalili Z. The effect of Er,Cr:YSGG laser and air abrasion on shear bond strength of a fissure sealant to enamel. *J Am Dent Assoc*, 2010; 141:157-161.
43. Ellis RW, Latta MA, Westerman GH. Effect of air abrasion and acid etching on sealant retention: an in vitro study. *Pediatr Dent*, 1999; 21:316-319.
44. Kanellis MJ, Warren JJ, Levy SM. Comparison of air abrasion versus acid etch sealant techniques: six-month retention. *Pediatr Dent*, 1997; 19:258-261.
45. Selecman JB, Owens BM, Johnson WW. Effect of preparation technique, fissure morphology, and material characteristics on the in vitro margin permeability and penetrability of pit and fissure sealants. *Pediatr Dent*, 2007; 29:308-314.
46. Bendinskaite R, Peciuliene V, Brukiene V. A five years clinical evaluation of sealed occlusal surfaces of molars. *Stomatologija*, 2010; 12:87-92.
47. Folke BD, Walton JL, Feigal RJ. Occlusal sealant success over ten years in a private practice: comparing longevity of sealants placed by dentists, hygienists, and assistants. *Pediatr Dent*, 2004; 26:426-432.
48. Lupi-Pegurier L, Muller-Bolla M, Bertrand MF, Ferrua G, Bolla M. Effect of sono-abrasion in the microleakage of a pit and fissure sealant. *Oral Health Prev Dent*, 2004; 2:19-26.
49. Lepri TP, Souza-Gabriel AE, Atoui JA, Palma-Dibb RG, Pécora JD, Milori Corona SA. Shear bond strength of a sealant to contaminated-enamel surface: influence of erbium: yttrium-aluminum-garnet laser pretreatment. *J Esthet Restor Dent*, 2008; 20:386-392.
50. Dental sealants. ADA Council on Access, Prevention and Interprofessional Relations; ADA Council on Scientific Affairs. *J Am Dent Assoc*, 1997; 128:485-488.
51. Gomes-Silva JM, Torres CP, Contente MM, Oliveira MA, Palma-Dibb RG, Borsatto MC. Bond strength of a pit-and-fissure sealant associated to etch-and-rinse and self-etching adhesive systems to saliva-contaminated enamel: individual vs. simultaneous light curing. *Braz Dent J*, 2008; 19:341-347.
52. Feigal RJ, Musherure P, Gillespie B, Levy-Polack M, Quelhas I, Hebling J. Improved sealant retention with bonding agents: a clinical study of two-bottle and single-bottle systems. *J Dent Res*. 2000; 79:1850-1856.
53. Borsatto MC, Thomaz MY, Contente MM, Gomes-Silva JM, Mellara Tde S, Galo R, Palma-Dibb RG. Bonding agent underneath sealant: shear bond strength to oil-contaminated. *Braz Dent J*, 2010; 21:50-54.
54. Hassall DC, Mellor AC. The sealant restoration: indications, success and clinical technique. *Br Dent J*, 2001; 191:358-362.
55. Feigal RJ, Hitt J, Splieth C. Retaining sealant on salivary contaminated enamel. *J Am Dent Assoc*, 1993; 124:88-97.
56. Straffon LH, Dennison JB, More FG. Three-year evaluation of sealant: effect of isolation on efficacy. *J Am Dent Assoc*, 1985; 110:714-717.
57. Collette J, Wilson S, Sullivan D. A study of the Isolite system during sealant placement: efficacy and patient acceptance. *Pediatr Dent*, 2010; 32:146-150.
58. Duangthip D, Lussi A. Effects of fissure cleaning methods, drying agents, and fissure morphology on microleakage and penetration ability of sealants in vitro. *Pediatr Dent*, 2003; 25:527-533.
59. Duangthip D, Lussi A. Effects of application techniques and fissure types on the in vitro performance of two fissure sealants. *Am J Dent*, 2004; 17:137-142.
60. Sundfeld RH, Mauro SJ, Briso AL, Sundfeld ML. Clinical/photographic evaluation of a single application of two sealants after eleven years. *Bull Tokyo Dent Coll*, 2004; 45:67-75.

61. Söderholm KJ. The impact of recent changes in the epidemiology of dental caries on guidelines for the use of dental sealants: clinical perspectives. *J Public Health Dent*, 1995; 55:302-311.
62. Celiberti P, Lussi A. Use of a self-etching adhesive on previously etched intact enamel and its effect on sealant microleakage and tag formation. *J Dent*, 2005; 33:163-171.
63. Guba CJ, Cochran MA, Swartz ML. The effects of varied etching time and etching solution viscosity on bond strength and enamel morphology. *Oper Dent*, 1994; 19:146-153.
64. Manton DJ, Messer LB. Pit and fissure sealants: another major cornerstone in preventive dentistry. *Aust Dent J*, 1995; 40:22-29.
65. Boksman L, McConnell RJ, Carson B, McCutcheon-Jones EF. A 2-year clinical evaluation of two pit and fissure sealants placed with and without the use of a bonding agent. *Quintessence Int*, 1993; 24:131-133.
66. Mascarenhas AK, Nazar H, Al-Mutawaa S, Soparkar P. Effectiveness of primer and bond in sealant retention and caries prevention. *Pediatr Dent*, 2008; 30:25-28.
67. Asselin ME, Sitbon Y, Fortin D, Abelardo L, Rompre PH. Bond strength of a sealant to permanent enamel: evaluation of 3 application protocols. *Pediatr Dent*, 2009; 31:323-328.
68. Asselin ME, Fortin D, Sitbon Y, Rompré PH. Marginal microleakage of a sealant applied to permanent enamel: evaluation of 3 application protocols. *Pediatr Dent*, 2008; 30:29-33.
69. Lygidakis NA, Dimou G, Stamataki E. Retention of fissure sealants using two different methods of application in teeth with hypomineralised molars (MIH): a 4 year clinical study. *Eur Arch Paediatr Dent*, 2009; 10:223-226.
70. Türkün SL. Clinical evaluation of a self-etching and a one-bottle adhesive system at two years. *J Dent*. 2003; 31:527-534.
71. Wadenya RO, Yego C, Blatz MB, Mante F. Bond strength and microleakage of a new self-etch sealant. *Quintessence Int*, 2009; 40:559-563.
72. Perdigão J, Fundingsland JW, Duarte S Jr, Lopes M. Microtensile adhesion of sealants to intact enamel. *Int J Paediatr Dent*, 2005; 15:342-348.
73. Venker DJ, Kuthy RA, Qian F, Kanellis MJ. Twelve-month sealant retention in a school-based program using a self-etching primer/adhesive. *J Public Health Dent*, 2004; 64:191-197.
74. Burbridge L, Nugent Z, Deery C. A randomized controlled trial of the effectiveness of a one-step conditioning agent in sealant placement: 6-month results. *Int J Paediatr Dent*, 2006; 16:424-430.
75. Gillet D, Nancy J, Dupuis V, Dorignac G. Microleakage and penetration depth of three types of materials in fissure sealant: self-etching primer vs etching: an in vitro study. *J Clin Pediatr Dent*, 2002; 26:175-178.
76. Ram D, Mamber E, Fuks AB. Clinical performance of a non-rinse conditioning sealant in three paediatric dental practices: a retrospective study. *Int J Paediatr Dent*, 2005; 15:61-66.
77. Fuks AB, Kupietzky A. Assessment of two curing systems in a self-etching primer/adhesive sealant: a preliminary study for a clinical trial. *Eur Arch Paediatr Dent*, 2007; 8:171-174.
78. Yazici AR, Karaman E, Baseren M, Tuncer D, Yazici E, Unlüer S. Clinical evaluation of a nanofilled fissure sealant placed with different adhesive systems: 24-month results. *Oper Dent*, 2009; 34:642-647.
79. dos Santos KT, Sundfeld RH, Garbin CA, de Alexandre RS, Sundfeld ML, Ceolim BN. Length of resin tags in pit-and-fissure sealants: all-in-one self-etching adhesive vs phosphoric acid etching. *Compend Contin Educ Dent*, 2008; 29:186-192.
80. Perdigão J, Geraldini S. Bonding characteristics of self-etching adhesives to intact versus prepared enamel. *J Esthet Restor Dent*, 2003; 15:32-41.
81. Dennison JB, Straffon LH, More FG. Evaluating tooth eruption on sealant efficacy. *J Am Dent Assoc*, 1990; 121:610-614.
82. Rethman J. Trends in preventive care: caries risk assessment and indications for sealants. *J Am Dent Assoc*, 2000; 131(Suppl):8S-12S.

Correspondence and request for offprints to:

Vasiliki Tzifa
 Solomou 50
 Sitia Crete
 E-mail: vasiatzifa@gmail.com

Effect of Post Material, Cement and Amount of Coronal Destruction on Stress Distribution: 3D FEA Study

SUMMARY

Introduction: The aim of this study was to assess the stress distribution of an endodontically treated maxillary incisor by 3-dimensional stress analyses using the Finite Element Analysis (FEA). The role of the post material and cement rigidity on reliability of endodontic restorations is discussed.

Material and methods: A 3D FEA model of a central maxillary incisor was created. The following parameters were studied: 2 levels of coronal destruction (total loss of coronal dentin, and partial loss of coronal dentin with 2 mm surviving dentinal walls); 3 loading conditions (mastication, bruxism and impact); 3 different luting cements (Zinc polycarboxylate cement; resin cement with low modulus of elasticity; resin cement with high modulus of elasticity); 4 post materials (steel, titanium, glass fibre, zirconium posts with composite cores) and composite restoration without post.

Results and Discussion: There were significant differences between post systems and cements. The stresses decreased with the post material in order of steel, zirconium, titanium, glass fibre. The presence of 2 mm coronal dentin decreased the maximum stress values in all the models. Minimum stress values were obtained with 2 mm coronal dentin with the glass fibre post and adhesive resin cement.

Keywords: Glass Fibre Post; Post Material; Finite Element Analysis

Burçin Vanlıoğlu¹, Yasemin Kulak Özkan¹,
Mert Uçankale¹, Erol Cansız²,
Oğuz Kayabaşı³

¹Department of Prosthodontics
Faculty of Dentistry, University of Marmara
Istanbul, Turkey

²Undergraduate Student, Faculty of Dentistry
University of Marmara, Istanbul, Turkey

³Gebze Institute of Technology
Department of Design & Manufacturing
Engineering, Gebze, Turkey

ORIGINAL PAPER (OP)

Balk J Stom, 2013; 17:18-25

Introduction

Special care is indicated when selecting the most efficient way to restore endodontically treated teeth because they have a higher risk of biomechanical failure than vital teeth¹. The fracture resistance of post-restored teeth has been the subject of numerous *in vitro* and *in vivo* studies²⁻⁶. A theoretical method for calculating stress distribution within complex structures is the finite element analysis (FEA), which allows the investigator to evaluate the influence of model parameter variation once the basic model have been correctly defined⁷.

In the case of incisors, when loaded transversely, the flexural behaviour of posts should be carefully considered⁸. The magnitude and the angle of incisal load greatly influence the long term success of restorative systems involving central incisors. A post with

biomechanical properties similar to those of dentin could reduce the risk of tooth root fractures⁹. A very stiff post working against the natural function of the tooth creates zones of tension and shear, both in the dentin and at the interfaces of the luting cement and the post. Some studies have been performed to evaluate the influence of post and luting material on stress distribution in dentin^{7,10}.

When a restorative system is loaded, it is able to absorb the applied forces, generating peculiar stress and strain distributions. The evaluation of such patterns, for example using the FEA, could be a reliable predictive parameter to forecast areas under risk of possible mechanical failures¹¹. It is possible to mechanically characterize not only enamel and dentin but also bone tissues, periodontal ligament and adhesive interfaces. Different loading conditions can be evaluated with FEA in order to evaluate stress distributions within the

restorations and hypothesize predictive areas under risk of clinical failure. Recently, many studies were related to biomechanical analyses with different post-and-core systems¹⁰⁻¹⁵. FEA can be performed with 2 dimensional (2D) and 3 dimensional (3D) models, and the consensus is that the results obtained with 3D models are more valid but also are more time-consuming and costly than 2D models^{7,12}.

The **aim** of this study was to analyse the stress distribution in a 3D FEA model of a central maxillary incisor with 2 levels of coronal destruction, restored with 3 different luting cements and 4 post materials, under 3 different loading conditions.

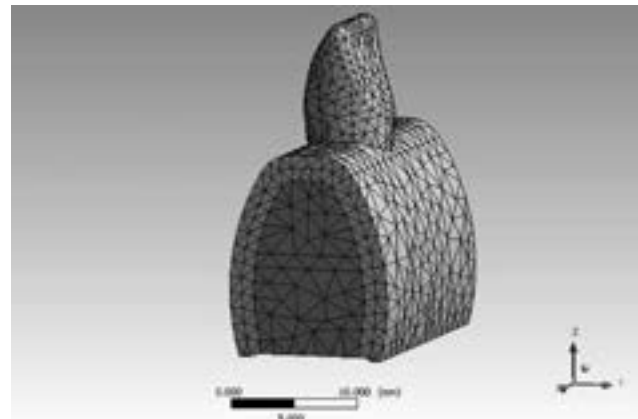


Figure 1a. 3D model of maxillary central incisor

Material and Methods

The solid model was generated using literature data and the dimensions of the tooth were modelled according to the ideal shape of a central incisor. A 3D FEA model of a central maxillary incisor and surrounding structures was generated by ANSYS software (Ansys, Inc. Houston), using IGES format. The volumes were redefined in the new environment and meshed with 8 nodes brick with 3 degree of freedom per node, finally resulting in a 3D FEA model with 85326 elements and 148787 nodes (Fig. 1a). All the nodes on the external surface of the root were constrained in all directions. Accuracy of the model was checked by convergence tests. Particular attention was devoted to the refinement of the mesh resulting from the convergence tests at the cement layer interfaces. Different material properties were coupled with the elements and geometries according to the volume material defined in fig. 1a (enamel, dentin, restored crown, core, cement, post, periodontal ligament, cortical bone, spongy bone). Due to the comparative aim of the structural evaluations, the given arbitrary commercially available post geometry has been used: 6% conicity; tip diameter 1.0 mm; 10 mm insertion depth (about 2/3 of the root length). All the nodes on the external surface of the root were constrained in all directions. Complete bonding between post and cement was considered. Mechanical properties of each component used in this study were summarized in table 1.

2 levels of coronal destruction were modelled: (1) Total loss of coronal dentin; and (2) Partial loss of coronal dentin with 2 mm surviving dentinal walls. The teeth were constructed with 5 different techniques: Group 1: Steel post with composite core; Group 2: Titanium post with composite core; Group 3: Glass fibre post with composite core; Group 4: Zirconium post with composite core; Group 5: Composite restoration without post. 3 luting cements with different elastic modules were discussed: (1) Zinc polycarboxylate cement; (2) Resin cement with low modulus of elasticity; (3) Resin cement with high modulus of elasticity.

Table 1. Mechanical properties used in this study

Material	Elastic modulus (Gpa)	Poisson ratio
Enamel	41	0,30
Pulp	0,002	0,45
Dentin	18,6	0,31
Periodontal Ligament	0,0689	0,45
Cortical bone	13,7	0,30
Spongy bone	1,37	0,30
Gingiva	0,003	0,45
Gutta-percha	0,00069	0,45
Porcelain crown	120	0,28
Composite core	12,0	0,30
Titanium post	112	0,33
Glass fibre post	40	0,26
Zirconium post	200	0,33
Steel post	210	0,30
Zinc phosphate cement	22,0	0,35
Resin cement (low modulus)	7,0	0,28
Resin cement (high modulus)	18,6	0,28

The structural efficiency of restorations have been evaluated under 3 different loading conditions: (1) 100N force inclined at 135° with respect to the tooth axis, 2 mm below the incisal edge in order to simulate mastication loads; (2) 100N vertical pressure in order to simulate axial loads; (3) 100N horizontal force to simulate an accidental impact force.

In order to identify areas of strain and stress concentration where possible fatigue failures are more expected to occur, the choice of the pertinent stress representation criterion was based on the evaluation of failure predictive potential of the analysis performed. Von Mises (equivalent stresses) energetic criterion was then chosen.

To standardize the results of the analysis, 22 different points were defined on the model and their analytical positions were determined (Fig. 1b)¹⁰.

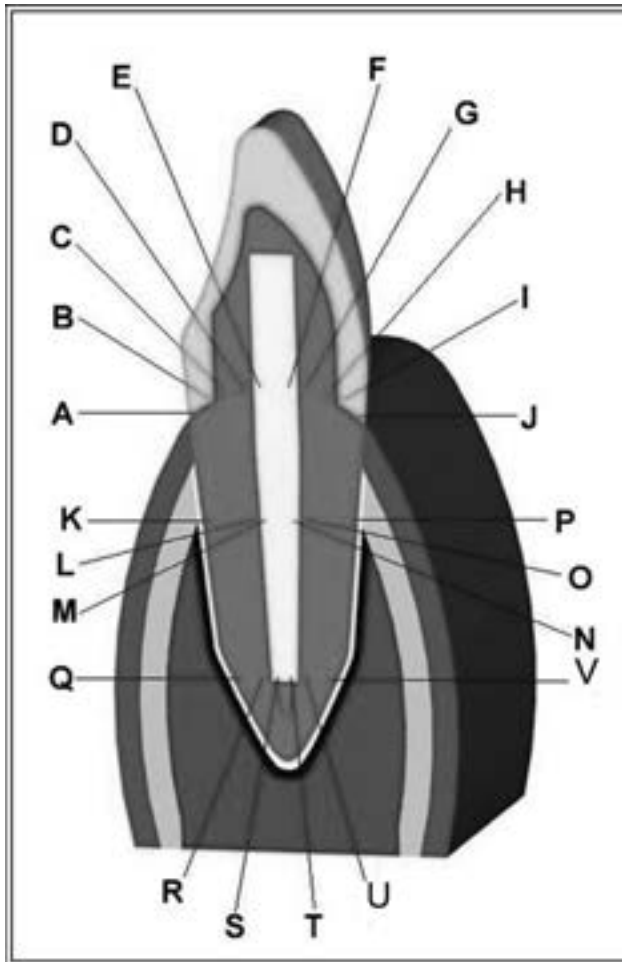


Figure 1b. Schematic presentation of the tooth model and local coordinate system

Results

The effect of coronal destruction, post materials, cements, and loading were shown in table 2. In functional loading, the presence of 2 mm coronal dentin tissue decreased the maximum stress values in all models. Horizontal loading from the buccal aspect increased and vertical loads decreased the maximum stress in all models. Minimum stress values were obtained with the glass fibre post bonded with adhesive resin cement having low elastic modulus in all loading conditions (Figs. 2-5).

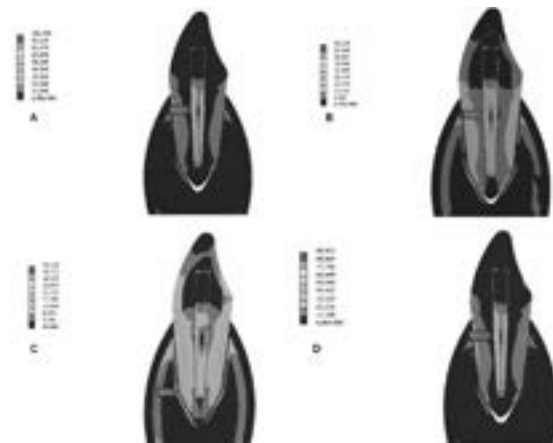


Figure 2. Effect of the post material bonded with adhesive cement (high) under functional loading in the model with 2 mm surviving dentinal walls (A) Steel post, (B) Titanium post, (C) Glass fibre post, (D) Zirconium post

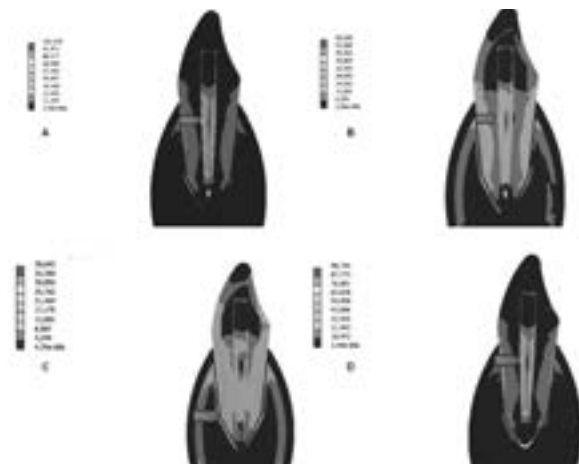


Figure 3. Effect of the post material bonded with adhesive cement (low) under functional loading in the model with 2 mm surviving dentinal walls (A) Steel post, (B) Titanium post, (C) Glass fibre post, (D) Zirconium post

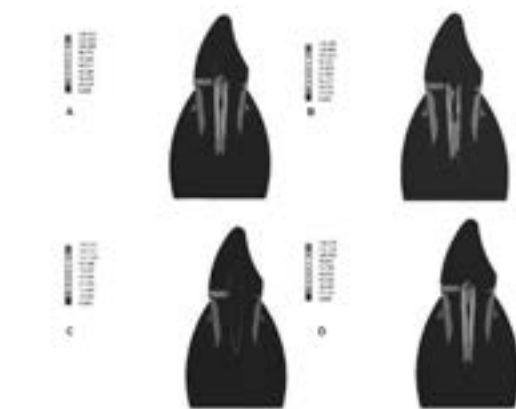


Figure 4. Effect of post material bonded with adhesive cement (high) under functional loading in the model with no surviving dentinal walls (A) Steel post, (B) Titanium post, (C) Glass fibre post, (D) Zirconium post

Table 2. Effect of coronal destruction, post material, cement and loading

	No coronal dentin	135	Vertical	Horizontal
Zinc Phosphate cement	Steel post	147,04	58,01	147,93
	Titanium post	181,65	54,43	150,16
	Glass fibre post	154,95	56,66	155,83
	Zirconium post	147,15	55,45	148,05
Adhesive cement low	Steel post	129,9	58,94	132,77
	Titanium post	74,26	34,94	75,8
	Glass fibre post	71,55	25,77	72,3
	Zirconium post	123,52	56,33	126,41
Adhesive cement high	Steel post	136,16	58,12	137,1
	Titanium post	138,38	50,27	139,38
	Glass fibre post	143,11	52,02	144,16
	Zirconium post	136,27	55,56	137,22
Adhesive restoration		40,56	17,28	40,17
	2 mm coronal dentin	135	Vertical	Horizontal
Zinc Phosphate cement	Steel post	101,08	51,41	108,69
	Titanium post	91,07	32,73	91,91
	Glass fibre post	92,55	33,05	93,41
	Zirconium post	96,45	49,35	103,78
Adhesive cement low	Steel post	103,47	52,3	110,47
	Titanium post	58,5	31,3	63,08
	Glass fibre post	38,64	26,07	37,24
	Zirconium post	98,74	50,21	105,48
Adhesive cement high	Steel post	104,75	59,42	111,21
	Titanium post	59,22	35,96	63,44
	Glass fiber post	39,11	26,23	37,68
	Zirconium post	99,97	56,99	106,19
Adhesive restoration		39,27	26,21	37,56

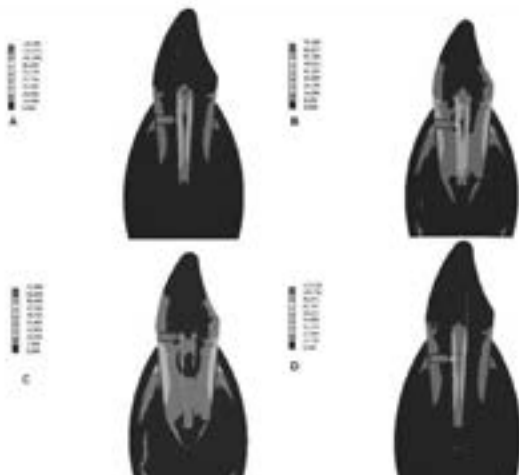


Figure 5. Effect of post material bonded with adhesive cement (low) under functional loading in the model with no surviving dentinal walls (A) Steel post, (B) Titanium post, (C) Glass fibre post, (D) Zirconium post

Regions of maximum stress changed for the different loading conditions. In functional loading, maximum equivalent stress mostly occurred at the vestibular side of the cement layer (interface between post and cement). As expected, in the bruxism case, the maximum stress area was located near the post apex. The area of maximum stress was located at the palatal surface in horizontal loading from the buccal aspect.

Under functional loading in the model without coronal dentinal tissue, the stress reached a maximum value of 181.65N for the titanium post and zinc phosphate cement, while it reached a significantly lower value of 71.55N for glass fibre posts cemented with dentin like

cements (low elastic modulus), and 143.11N for glass fibre posts cemented with softer cements (high elastic modulus). There was a significant difference in stress distribution at dentin interfaces for the glass post restored tooth cemented with materials of different rigidity. In the model with 2 mm coronal dentinal tissue, the difference between the stress values of glass fibre posts bonded with different cements was less.

However, even if significantly different load transfer characteristics from post to root occurred in the cases examined, no differences were evident at the level of external root structure either for stress distribution and intensities (Figs. 6-8).

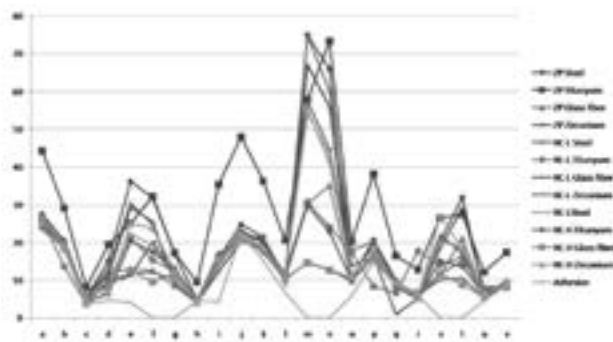


Figure 6a. Effect of post material and cement under functional loading in the model with no surviving dentinal walls

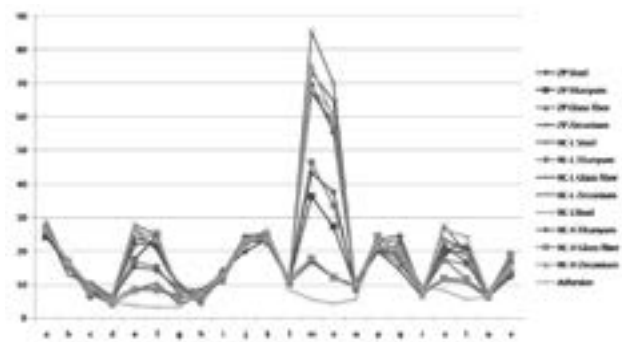


Figure 6b. Effect of post material and cement under functional loading in the model with 2 mm surviving dentinal walls

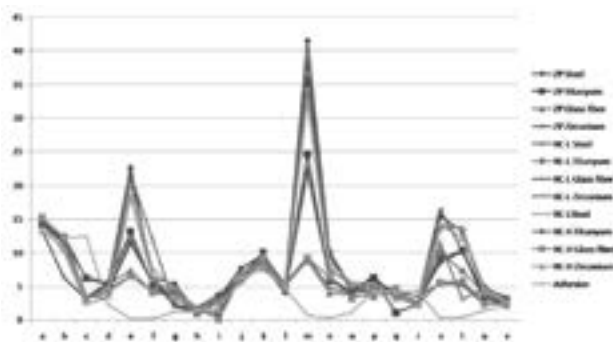


Figure 7a. Effect of post material and cement under vertical loading in the model with no surviving dentinal walls

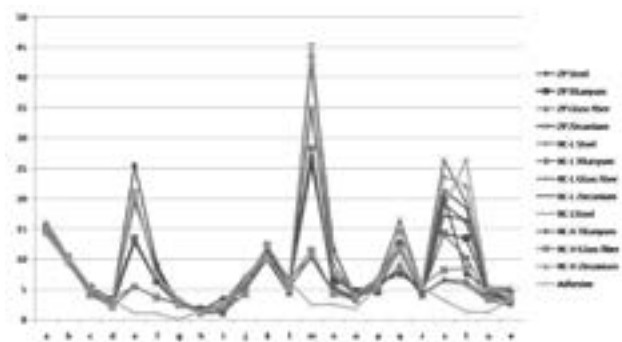


Figure 7b. Effect of post material and cement under vertical loading in the model with 2mm surviving dentinal walls

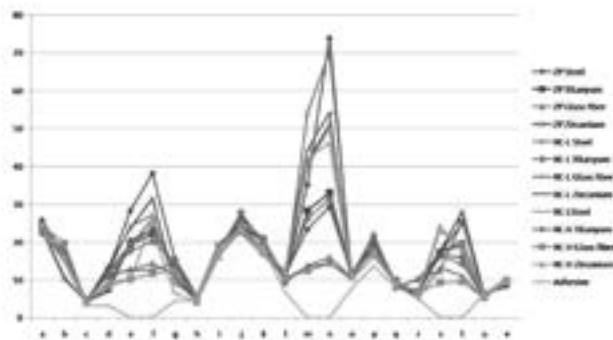


Figure 8a. Effect of post material and cement under horizontal loading in the model with no surviving dentinal walls

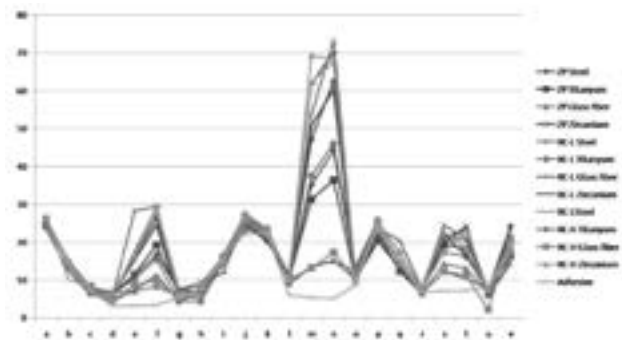


Figure 8b. Effect of post material and cement under horizontal loading in the model with 2 mm surviving dentinal walls

In particular, the investigation on central incisor tooth showed differences in load transfer capability of cast post when different cements were used. The stress was found to be 123.52 for zirconium posts cemented with dentine like cements (low elastic modulus) and 136.27 for glass fibre posts cemented with softer cements having high elastic modulus (Tab. 2, Fig. 5). Glass fibre posts exhibited the lowest stress values in the cervical part (Point f), middle part (Points m and n) and apical part of the post (Points s and t) in all loading conditions (Figs. 6-8). Steel posts exhibited higher stress values at the middle part of the root than glass fibre and titanium posts with all types of cements and under all loading conditions (Figs. 6-8).

The stresses decreased in order of steel, zirconium, titanium, glass fibre, with low modulus adhesive cement. The stresses decreased in order of glass fibre, titanium, zirconium, steel, with high modulus adhesive cement. Minimum stress values were obtained with the glass fibre post bonded with adhesive resin cement with low elastic modulus in all loading conditions (Tab. 2; Figs. 3c and 5c). The placement of an endodontic post created higher stress areas than adhesive restorations without posts.

Figures 6-8 show the stress patterns for the various post restorations considered here. The figures are grouped for each loading condition. Glass fibre restored system ensured enough uniformity of stress level (Figs. 6-8). It can be seen that the stress values in the post become less uniform as the stiffness of the post increased. The stress value at the middle of the steel post was 5 times as large as the stress at the glass fibre post. Steel posts and traditional cements, being no adhesive and also more rigid than fibre posts and resin cements, did not allow a homogeneous stress distribution (Figs. 6-8).

Critical areas of high stress concentration were the restoration-cement-dentin interface, both in the root canal and on the buccal and lingual aspects of the tooth-restoration interface. Regions of maximum stress changed for the different loading conditions. In functional loading, maximum equivalent stress mostly occurred at the vestibular side of the cement layer - interface between post and cement (Figs. 2-5, 6a and 6b). As expected, in the bruxism case, the stress near the post apex was higher than the other loading conditions (Figs. 7a and 7b). The stress values on the buccal aspect were higher in the horizontal loading (Figs. 8a and 8b).

Discussion

The 3D FEA showed significant changes in the stresses induced in the tooth-restoration complex when the investigated parameters varied. A post with biomechanical properties similar to those of dentin could be advantageous by reducing the risk of tooth

root fractures. A very stiff post, working against the natural function of the tooth, creates zones of tension and shear both in the dentin and at the interfaces of the luting cement and the post^{7,10}. Glass and carbon posts exhibit high fatigue and tensile strength, and they have a Young's modulus comparable to dentin². Moreover, these posts can be bonded in root canal with adhesive resin cement. Bonding agents transmit stress between the post and the root structure, reducing stress concentration and preventing fracture^{3,13}. Bonding between the post and the cement and between the cement and the dentin appears as an important parameter to achieve optimal behaviour of endodontic restorations. On the contrary, steel posts and traditional cements do not allow a homogeneous stress distribution. In this study, increased elastic modulus of the post was found to cause decreased dentin stress. This result is in agreement with the earlier *in vitro* investigations^{3,11,13}. The FEA study of Pegoretti et al¹⁴ concluded that glass fibre post resulted in lower stresses inside the root than did the carbon and metal post. Isidor et al⁴ found that teeth restored with a carbon fibre post were more resistant to fracture than teeth restored with a titanium post. Several *in vitro* studies have determined the resistance to fracture of post restored teeth under static loading and found smaller, the same or higher strength of teeth restored with fibre posts than those restored with metal posts^{6,9}.

The maximum shear stresses were found to be primarily located at post cement/dentin interface. Cohen et al¹⁵ reported that micro-movement of a cemented post results in disintegration of the cement and the concentration of stress at the apical end of the post over time. The fracture strength of dental cements is less than that of dentin or post material. Thus, failure of the system usually occurs cohesively within the cement or at its interface with dentin.

There was a significant difference in stress distribution at dentin interfaces for the post restored tooth cemented with materials of different rigidity. The results lead to the conclusion that the more flexible the posts are the less rigidity of the cementing medium is relevant. In the model with 2 mm coronal dentinal tissue, the difference between stresses values of posts bonded with different cements was less. Clinically, both carbon and glass posts are subjected to de-bonding/loosening phenomena¹⁶. Tough cement systems could improve the restoration reliability by opposing to mechanical progression of failure and crack growth.

Recently, several papers supported the use of a direct restoration without placing any post for restoring endodontically treated teeth¹⁷. Krejci et al⁵ showed no significant differences between teeth restored with and without posts at fracture strength and fracture patterns. Moreover, some studies pointed out that mechanical resistance to fracture of endodontically treated teeth could be affected by the presence of posts and the risk

of damage could increase^{3,9,18}. In endodontically treated teeth, occlusal loads could be transferred intra-radically by post restorations, increasing the occurrence of vertical root fractures. On the contrary, other authors noticed that fibre posts reduced the risk of root fractures¹⁹. Actual consensus in restorative dentistry indicates that de-cementation or failure of posts is preferable than fracture of residual tooth structure². In this study, the stress values and location of maximum stress in glass fibre post restored teeth were less. The less residual tooth structure the more important the physical properties of post-and-core systems.

This study confirmed that the reconstructed tooth is subject to most stress in the cervical region under a functional load. Pierrisnard et al¹⁸ mentioned that the absence of a cervical ferrule was a negative factor, giving rise to considerably higher stress levels. They also concluded that the cervical area of a root canal of treated and restored teeth was the area most subject to stress under a simulated occlusal load.

The magnitude of deflection and peak stress generated in the reconstructed tooth with horizontal loading was greater than that with vertical loading. Load direction has a greater effect than post material on maximum stress and displacement.

It clearly appears that the oblique load is more critical than the vertical one, both for restoration structures and residual dentin. Moreover, the results' analysis shows a deeply different behaviour between post-core restorations and adhesive restoration without post. As far as residual dentin is concerned, a significant reduction of the mean stress level is obtained at the middle/cervical root zone, whereas high peaks of the average stress are induced at the root apex. Furthermore, high stress concentrations at the post-dentin interface appear.

Finally, the choice of the posts' material and type of cement appears very important when the stress values and dentin-interfaces were considered. A post with biomechanical properties to those of dentin could be advantageous by reducing the risk of tooth root fractures. The ideal root canal post must be sufficiently elastic to accompany the natural flexural movements of the structure of the tooth, something that a very rigid metal post cannot do.

Conclusions

FEA is a powerful tool in calculating stress distributions in complex structures. As stated earlier, the method provides results without variation. The validity of the study, however, depends on the extent to which the model approaches reality.

Within the limitations of this FEA study, the following conclusions were drawn:

- The placement of an endodontic post creates an unnatural restored structure since it fills the root canal space with a material that has a defined stiffness unlike the pulp. Hence it is not possible to recreate the original stress distribution of the tooth. Steel posts are the most dangerous for the root, potentially leading to its fracture. Even working on the cement layer, stress absorbing capability by using less rigid cements cannot possibly improve the stress arising in the system because of the high rigidity of the steel post. Using a fibre post reconstruction, the elastic modulus of the cement layer strongly influences the stress absorbing capability of the system;
- In functional loading, maximum equivalent stress mostly occurs at the vestibular side of the cement layer;
- The presence of 2 mm coronal dentin tissue decreased the maximum stress values in all models;
- Horizontal loading from the buccal aspect increased and vertical loading decreased the maximum stress in all models;
- Minimum stress values were obtained with the glass fibre post bonded with adhesive resin cement with low elastic modulus;
- The stress decreased with the post material in order of steel, zirconium, titanium, glass fibre;
- The ideal root canal post must be sufficiently elastic to accompany the natural flexural movements of the tooth structure, something that a very rigid metal post cannot do;
- Bonding between the post and the cement and between the cement and the dentin appears an important parameter to achieve optimal behaviour of endodontic restorations.

Acknowledgements: A pilot study of this research was rewarded the Top Prize at the Student Clinician Programme at the Annual Meeting of the Turkish Dental Association in 2006. It was also published as a lecture in the 147th Annual Session of the American Dental Association at Las Vegas, NV, in October 2006 as a VIP guest of Dentsply Company.

References

1. Tamse A, Fuss Z, Lustig J, Kaplavi J. An evaluation of endodontically treated vertically fractured teeth. *J Endod*, 1999; 7:506-508.
2. Ferrari M, Vichi A, Mannocci F, Mason PN. Retrospective study of the clinical performance of fiber posts. *Am J Dent*, 2000; 13:9-13.
3. Fokkinga WA, Kreulen CM, Vallittu PK, Creugers NHJ. A structured analysis of in vitro failure loads and failure modes of fiber, metal and ceramic post-and-core systems. *Int J Prosthodont*, 2004; 17:476-482.

4. Isidor F, Brondum K, Ravnholt G. The influence of post length and crown ferrule length on the resistance to cyclic loading of bovine teeth with prefabricated titanium posts. *Int J Prosthodont*, 1999; 12:78-82.
5. Krejci I, Duc O, Dietschi D, de Campos E. Marginal adaptation, retention and fracture resistance of adhesive composite restorations on devital teeth with and without posts. *Oper Dent*, 2003; 28:127-135.
6. Raygot CG, Chai J, Jameson DL. Fracture resistance and primary failure mode of endodontically treated teeth restored with a carbon fiber-reinforced resin post system in vitro. *Int J Prosthodont*, 2001; 14:141-145.
7. Lanza A, Aversab R, Rengob S, Apicellaa D, Apicella A. 3D FEA of cemented steel, glass and carbon posts in a maxillary incisor. *Dent Mater*, 2005; 21:709-715.
8. Heydecke G, Butz F, Strub JR. Fracture strength and survival rate of endodontically treated maxillary incisors with approximal cavities after restoration with different post and core systems: an in vitro study. *J Dent*, 2001; 29:427-433
9. Akkayan B, Gulmez T. Resistance to fracture of endodontically treated teeth restored with different post systems. *J Prosthodont*, 2002; 87:431-437.
10. Genovese K, Lamberti L, Pappalettere C. Finite element analysis of a new customized composite post system for endodontically treated teeth. *J Biomech*, 2005; 38:2375-2389.
11. Zarone F, Apicella D, Sorrentino R, Ferro V, Aversa R, Apicella A. Influence of tooth preparation design on the stress distribution in maxillary central incisors restored by means of alumina porcelain veneers: a 3D-finite element analysis. *Dent Mater*, 2005, 21:1178-1188.
12. Ko C, Chu C, Chung K, Lee M. Effects of posts on dentin stress distribution in pulpless teeth. *J Prosthodont*, 1992; 68:421-427.
13. Asmussen E, Peutzfeldt A, Sahafi A. Finite element analysis of stresses in endodontically treated, post-restored teeth. *J Prosthodont*, 2005; 94:321-329.
14. Pegoretti A, Fambri L, Zappini G, Bianchetti M. Finite element analysis of a glass fibre reinforced composite endodontic post. *Biomaterials*, 2002; 23:2667-2682.
15. Hsu ML, Chen CS, Chen BJ, Huang HH, Chang CL. Effects of post materials and length on the stress distribution of endodontically treated maxillary central incisors: a 3D finite element analysis. *J Oral Rehab*, 2009; 36:821-830.
16. Cohen BI, Pagnillo MK, Condos S, Deutsch AS. Four different core materials measured for fracture strength in combination with five different designs of endodontic posts. *J Prosthodont*, 1996; 76:487-495.
17. Baratieri LN, De Andrada MA, Arcari GM, Ritter AV. Influence of post placement in the fracture resistance of endodontically treated incisors veneered with direct composite. *J Prosthodont*, 2000; 84:180-184.
18. Strub JR, Pontius O, Koutayas S. Survival rate and fracture strength of incisors restored with different post and core systems after exposure in the artificial mouth. *J Oral Rehab*, 2001; 28:120-124.
19. Pierrisnard L, Bohin F, Renault P, Barquins M. Coronoradicular reconstruction of pulpless teeth: A mechanical study using finite element analysis. *J Prosthodont*, 2002; 88:442-448.

Correspondence and request for offprints to:

Burçin Vanlıoğlu
Marmara University
Güzelbahçe, Büyükciftlik Sokak, No: 6
34365, Nişantaşı, İstanbul
Turkey
E-mail: drburcinakoglu@hotmail.com

Preventive and Etiological Aspect of Oral Candidiasis

SUMMARY

Introduction: Oral candidiasis is an indivisible part of everyday oral medicine, although the treatment of candidial infection often remains temporary, with frequent recurrences. Microbiological proof as a diagnostic method does not seem to be appropriate enough for successful treatment outcome. Therefore, the need for enlightening the factors that exaggerate the pathogenicity of *Candida albicans* becomes necessary. The aim of this study was to follow up patients with diagnosed oral candidiasis as to obtain better knowledge of reasons that favour clinical manifestation of oral candidiasis and its frequent recurrences.

Material and Method: At the Clinic for Oral Pathology and Periodontology we examined 50 patients with previously diagnosed oral candidiasis. Subjects were selected randomly, from different geographic areas of the state, and of different age and gender (67% females and 33% males).

Results: The results revealed that the most usual cause of oral candidiasis was long-lasting antibiotic treatment, xerostomia, medications for cardiovascular diseases, sedation and hyperglycemia. Other predisposing factors were anaemia, age and fissured tongue.

Conclusion: Adequate antimycotic therapy and removing of favouring factors resulted in positive treatment outcome and rapid reduce of recurrences.

Keywords: *Candida albicans*; Oral Candidiasis

Z. Belazelkoska¹, M. Popovska¹,
L. Zendeli-Bedzeti²

¹ Faculty of Dentistry

Department for Oral Pathology and Periodontology

² University Dental Clinic

Department for Oral Pathology and Periodontology
Skopje, FYROM

ORIGINAL PAPER (OP)

Balk J Stom, 2013; 17:26-30

Introduction

The incidence of candidial infection in the last decade increased according to the higher incidence of diabetes mellitus, malignant diseases, abuse of antibiotic treatment and the increasing number of AIDS-affected persons. This was the reason that *Candida albicans* and the candidial infection attract even more attention for the last 10 years. The number of *Candida* species is about 300, and 7 of them are considered as very pathogenic^{2,3}.

Candida albicans in the oral cavity is present as a saprophyte, but under some circumstances gets pathogenic feature, causing inflammation and other pathologic changes in the mouth. The reasons for the pathogenic changes of the oral mucosa can be local or systemic, as well as drug-related.

In certain conditions and certain circumstances, some patients with oral candidiasis are treated inadequately and that results in temporary improvement of the condition, but not with definitely recovering, so the appearance of recurrences is very frequent⁹. The use of antimycotic therapy, as well as diagnosing oral candidiasis with microbiological evidence, seems not to be enough for the successful treatment outcome. The need for discovering the factors that enhance the pathogenicity of *Candida albicans* becomes necessary and was a reason more for us to pose the aim of our study, through examination of patients with diagnosed oral candidiasis to obtain our own understandings for reasons that enhance and support the clinical manifestation of the disease.

Material and Method

To realize our aim, 50 patients with diagnosed oral candidiasis were included in our study. Patients, randomly selected, from different geographic regions of the state, from various age groups and gender, were examined at baseline, after 6 month and a year after the treatment. The participant's age was between 20 and 70 years, all Caucasians, and with diagnosed oral candidiasis through verified diagnostic procedures, without prior treatment, but with frequent recurrences. Diagnose was established with oral mucous swabs and slides observed under optic microscope in the laboratory of the University Dental Clinical Centre in Skopje.

All the participants through thorough dental and medical history, gave information about: oral hygiene habits, especially in older patients with dentures, smoking habits, food consumption (consistency, quality, carbohydrate contents, etc.), presence of diabetes mellitus, cardiovascular diseases, blood disorders (iron deficiency anaemia or megaloblastic anaemia, acute leukaemia), the use of immunosuppressive drugs, broad spectrum antibiotics, sedatives, cardiotonics, antihypertensive drugs, radiotherapy, , pregnancy and the use of contraceptive treatment, HIV infection and other immunodeficient conditions, TBC, etc.

With clinical examination and use of plaque index (PI), described by Sillnes- Løe, we determined the oral hygiene status on teeth and fixed and removable dentures. Also, in all participants, the following conditions were noted: state of the dentition, dryness of the oral cavity, pH of the saliva, painful pathological conditions in the oral cavity, fissured tongue, haematological disorders (iron deficiency anaemia and megaloblastic anaemia,

acute leukaemia), diabetes mellitus, as well as local microbiological investigation. In all participants differential blood analysis with automatic counter, and determination of salivary pH with pH-meter (Iskra MA 5706) were done.

After recording anamnesis data and conducting clinical examination, we prescribed the usual therapy for oral candidiasis i.e. antimycotic therapy, rinsed the oral cavity with benzydanine hydrochloride in the form of solution or spray 0.15%, recommended artificial saliva in patients with xerostomia, and gave instructions for further oral hygiene and hygiene of the dentures, adequate dental treatment, establishing dietetic-nutritional regime in everyday life, preventing measures for patients that have to use any kind of treatment that could help the pathologic action of *Candida albicans*.

Results

Candidial infection was more present in female population (67%) than in male population (33%). Table 1 shows the distribution of oral candidiasis according to the obtained anamnesis data of the participants involved in the study. Candidiasis was diagnosed in 22 smokers (44%), 37 participants using soft and carbohydrate food (74%), 7 subjects under immunosuppressive medication (14%), 33 subjects taking antibiotics (66%), 7 (14%) patients undergoing radiotherapy, and 41 patients (82%) using sedatives, cardiotonics or antihypertensive drugs. Also, oral candidiasis was detected in 15 patients with blood disorders (30%), 13 (26%) with diabetes mellitus and 40 (80%) with cardiovascular diseases. Candidial infection was lowest in pregnant women, only 3%.

Table 1. Prevalence of oral candidiasis in accordance with anamnestic data

Anamnestic data	Number of cases	%	
Smokers	22	44	
Food mushy carbohydrate	37	74	
Drugs	Immunosuppressive drugs	7	14
	Antibiotic	33	66
	Radiation therapy	7	14
	Sedatives, antihypertensive drugs, cardiotonics	41	82
Age over 50 years	45	90	
Hematologic diseases, diabetes, Cardiovascular diseases	15	30	
Pregnancy and contraceptive use	3	6	

Table 2 presents the distribution of oral candidiasis in accordance with clinical examination. It can be concluded that PI with score 3 was present on teeth in all subjects,

and PI score 2 was present in all patients with fixed and removable dentures. 45 participants with poor oral hygiene status, especially in older subjects with dentures, also in

those with untreated dentition (100%) candidiasis was determined, as well as in 45 patients with xerostomia or (90%) and 43 (86%) participants with fissured tongue. In 40 (80%) participants with low pH of the saliva (high acidity) and 50 (100%) participants with coated tongue was found with candidial affection. Candidial infection was also found in 25 (50%) haematological patients and 20 (40%)

diabetic patients. From all of the participants with painful pathological conditions in the oral cavity candidial infection was found in 7 patients (14%).

Correct and persistent treatment of candidial infection didn't recurred in 86% patients (43) and recurred in only (7%) patients (Fig. 1, and 2).

Table 2. Prevalence of oral candidiasis based on objective findings

Objective findings	IDP	Study group	%
Teeth/plaque index	3	50	100
Fixed prosthetic constructions	2	50	100
Mobile prosthetic constructions	2	30	60
Unsatisfactory oral hygiene especially with old patients with prostheses		45	90
Unrepaired dentition		50	100
Xerostomia		45	90
Lingua plicata		43	86
Acidic saliva reaction		40	80
Painful pathological states in the oral cavity		7	14
Tongue coating		50	100
Hematologic findings Ferodeficiency		25	50
Diabetes		20	40



Figure 1. Candidiasis on the hard palate, showing red surfaces with white plaque



Figure 2. Candidiasis of the tongue, showing white layers on the dorsum of the tongue

Discussion

Colonisation of oral cavity by *Candida albicans* appears in conditions of specific retention or interaction between the oral mucous membranes and *Candida albicans*. *Candida albicans* may colonize the tongue, buccal and palatal mucosa, dental plaque, dental caries, subgingival flora, dentures, etc^{4,5}. Our results disclose the important connection between clinical manifestation of oral candidiasis and xerostomia, low salivary values of pH, the use of medicaments (cardiotonics, sedatives, antihypertensives, being used by older population and the most present participants in our study), diabetics, and patients' poor oral hygiene status, especially in patients with removable dentures. Oral candidiasis is a condition of secondary candidial infection of the tissues previously being changed by continuous expose of the painful moving of the denture, and is always connected with poor oral hygiene.

This condition is clinically similar to chronic erythema and oedema of the part of the denture bearing mucosa, beneath an upper denture. The affected mucosa is clearly marked and distinguished from the rest mucosa out of the denture. Palatal mucosa is most often affected because of the firm contact with the bases of the denture and thus favours the growth of *Candida albicans*. The space between the mucous and upper denture, poor oral hygiene of those surfaces, carrying the dentures during the night, and sleeping, supports the growth of *Candida albicans*, resulting in misbalance of the host-yeast relation. Uncontrolled use of antibiotics, due to suppression of bacteria, has important role in clinical manifestation of oral candidiasis, especially of the pseudomembranous form, presented as whitish membranes at the dorsal site of the tongue, accompanied with burning sensation²⁰.

In our study there wasn't any HIV positive patient, although AIDS is being closely associated with every clinical form of oral candidiasis.

Our results revealed that antimycotic treatment with removing of predisposing factors favouring candidial infection resulted in successful cure of oral candidiasis, without recurrence. Treatment of oral candidiasis includes removing of local factors, acting against *Candida albicans*, and relieving of symptoms.

Removing of local factors includes: giving instructions for maintaining of oral hygiene, removing of dental calculus, dental plaque and soft layers; root planning and scaling of periodontal pockets if the patients have periodontitis: treating carious lesions; making new dentures; balancing of the salivary pH; and if the patient is taking immunosuppressive therapy to view the possibility of reducing the dose. The way of living should be in concordance with the therapy, without use of alcohol and tobacco smoking, but with consumption of food rich in vitamins etc.

Conclusion

The detailed dental and medical history, clinical examination, laboratory results, microbiologic evidence for the presence of *Candida albicans*, the effect of the administered antimycotic therapy, rinsing with benzydanine hydrochloride, and removing of the favouring factors for *Candida albicans* overgrowth, as well as follow-up of patients after the treatment, allow us to conclude that it is not enough only to detect the presence of *Candida albicans*, but also to note and carefully disclose all possible predisposing factors that enhance the pathogenic action, and eliminating them during the treatment, in the aim of achievement a complete recovering, without recurrence.

References

1. Akpan A, Morgan R. Candidiasis. *Postgrad Med J*, 2002; 78:455-459.
2. Alexio RQ, Scherma AP, Guimarães G, Cortelli JR, Cortelli SC. DMFT index and oral mucosal lesions associated with HIV infection: cross-sectional study in Porto Velho, Amazonian region, Brazil. *Braz J Infect Dis*, 2010; 14(5):449-456.
3. Neville BW, Damm DD, White DK. Color Atlas of Clinical Oral Pathology. 2nd Ed. USA: Williams & Wilkins, 1999.
4. Belazelkoska Z, Nakova M. Oral Pathology. Skopje: Faculty of Dentistry, 2003.
5. Bunetel L, Bounnaure MM. Oral pathoses caused by *Candida albicans* during chemotherapy. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 1996; 82:161-165.
6. Cannon RD, Holmes AB, Mason AB, Monk BC. Oral candida: clearance colonization of candidiasis. *J Dent Res*, 1995; 74:1152-1161.
7. Chi AC, Neville BW, Krayner JW, Gonsalves WC. Oral manifestations of systemic disease. *Am Fam Physician*, 2010; 82(11):1381-1388.
8. Flemmig TF, Beikler T. Control of oral biofilms. *Periodontol* 2000, 2011; 55(1):9-15.
9. Giannini PJ, Shetty KV. Diagnosis and management of oral candidiasis. *Otolaryngol Clin North Am*, 2011; 44(1):231-240.
10. González GH, González de ME, Zambrano O, Lozano CM, Rodríguez de VS, Robertis S, Mesa L. Oral candidiasis in children and adolescents with cancer. Identification of *Candida* spp. *Med Oral Patol Oral Cir Bucal*, 2007; 12(6):E419-23.
11. Magaldi S, Mata S, Hartung C, et al. In vitro susceptibility of 137 *Candida* sp. isolates from HIV positive patients to several antifungal drugs. *Mycopathologia*, 2001; 149(2):63-68.
12. Reboli AC, Rotstein C, Pappas PG, Chapman SW, Kett DH, Kumar D, Betts R, Wible M, Goldstein BP, Schranz J, Krause DS, Walsh TJ. Anidulafungin Study Group. Anidulafungin versus fluconazole for invasive candidiasis. *N Engl J Med*, 2007; 356(24):2472-2482.
13. Reichart PA, Philipsen HP, Schmit-Westhausen A, Samaranayake LP. Pseudomembranous oral candidiasis in HIV infection: ultrastructural findings. *J Oral Pathol Med*, 1955; pp 276-281.

14. Reichart PA. Clinical Management of selected oral fungal and viral infections during HIV-disease. *Int Dental J*, 1999; 49:251-259.
15. Rippon RJ, Alley MR, Castro I. Candida albicans infection in free-living populations of hihi (stitchbird; *Notiomystis cincta*). *N Z Vet J*, 2010; 58(6):299-306.
16. Sarifakioglu E, Gunduz C, Gorpelioglu C. Oral mucosa manifestations of 100 pregnant versus non-pregnant patient: an epidemiological observational study. *Eur J Dermatol*, 2006; 16(6):674-676.
17. Silverman S Jr. Mucosal lesions in older adults. *J Am Dent Assoc*, 2007; 138(Suppl):41S-46S.
18. Soames JV, Southam JC. Oral Pathology. 3rd Ed. Oxford, New York, 1999.
19. Williams DW, Kuriyama T, Silva S, Malic S, Lewis MA. Candida biofilms and oral candidosis: treatment and prevention. *Periodontol 2000*, 2011; 55(1):250-265.
20. Wingeter MA, Guilhermetti E, Shinobu CS, Takaki I, Svidzinski TI. Microbiological identification and in vitro sensitivity of Candida isolates from the oral cavity of HIV positive individuals. *Rev Soc Bras Med Trop*, 2007; 40(3):272-276. (in Portuguese)

Correspondence and request for offprints to:

Prof. Z. Belazelkoska
Faculty of Dentistry
Department for Oral Pathology and Periodontology
Vodnjanska 17, Skopje
FYR Macedonia
E-mail: belazlatanka@yahoo.com

Effects of Various Rates of Polyacrylic Acid and Fluoride on *Streptococcus Mutans* and *Actinomyces Viscosus*

SUMMARY

Glass ionomer cements have been shown to possess antimicrobial activity. Fluoride release from these materials and initial acidity have been reported with regard to this. The purpose of this study was to investigate antimicrobial effects of various concentrations of fluoride and polyacrylic acid mixtures and to evaluate whether or not fluoride is responsible for this antibacterial activity. The antimicrobial properties of various fluoride and polyacid mixtures were tested against *Streptococcus mutans* and *Actinomyces viscosus*. The fluoride and polyacid mixtures; 5%, 10% and 20% polyacrylic acid were mixed with 1.000 ppm, 2.500 ppm, 5.000 ppm, and 10.000 ppm F. The role of fluoride ions and low pH in the antibacterial activity were also studied by using agar diffusion assay method measuring inhibition zones of the mixtures. 6 samples were prepared for each experimental group. Kruskal-Wallis test (ANOVA) was performed for statistical analysis.

The findings showed that there were significant differences between 0 ppm and 5.000 ppm F; 0 ppm and 10.000 ppm F; 1.000 ppm and 10.000 ppm F when fluorides were mixed with 5% or 10% or 20% polyacrylic acid to determine the inhibition of *S. Mutans* ($p < 0.02$). For *Actinomyces viscosus* significant differences were found between 0 ppm and 10000 ppmF; 1000 ppm and 10000 ppm F when fluorides were mixed with 5% or 10% or 20% polyacrylic acid ($p < 0.004$). It is concluded that not only fluoride was directly responsible for the antibacterial activity, but the low pH of the medium appeared to play even more important role.

Keywords: Polyacrylic Acid; Fluoride; *Streptococcus mutans*; *Actinomyces viscosus*

Yasemin Benderli¹, Kağan Gökçe²

¹Istanbul University, Faculty of Dentistry
Department of Operative Dentistry

²Yeditepe University, Faculty of Dentistry
Department of Operative Dentistry
Istanbul, Turkey

ORIGINAL PAPER (OP)

Balk J Stom, 2013; 17:31-36

Introduction

The fluoride releasing property of glass ionomers is considerable for decreasing enamel solubility and secondary caries related to increased fluoride uptake by adjacent enamel¹⁻⁵. On the other hand, fluoride ions affect metabolic activations of some microorganisms that are responsible for plaque accumulation and tooth caries in the mouth⁶⁻¹¹.

Due to the high frequency of recurrent caries after restorative treatment¹²⁻¹⁴, much attention has been paid to the therapeutic effects revealed by direct filling

materials¹⁵⁻¹⁷. Brannström¹⁸ has found that pulpal damage caused by bacteria under the restoration is not related with the restorative material itself. This may indicate that restorative materials may be used for beneficial purposes, such as the prevention of secondary caries and subsequent pulpal infection. To serve this purpose, some products containing antibacterial agents have been produced by manufacturers^{19,20}. Some of these products are glass ionomer cements, whereas some others are composite resins and bonding resins which have fluoride release capacity^{19,21-23}. After determining the antimicrobial properties of glass ionomer cements, the number of

investigations evaluated ion release from the material^{24,25}, and the effects of fluoride ion release increased^{2-4,16,26-28}. On the other hand, one adhesive system containing the antibacterial monomer MDPB was added to these materials. Fluoride and MDPB containing composites strongly contributed to elimination of residual bacteria in cavities^{23,29,30}.

In attempts to maintain oral hygiene and prevent secondary and recurrent caries, it is not only that elimination of microorganisms plays a significant role. Another major aim is prevention of caries that might likely occur adjacent to the restoration²⁸. Thus provision of acid resistance and remineralisation of dental tissues necessitate a detailed evaluation of the significance of fluoride releasing materials³¹⁻³⁴.

Recently, it has been noticed that the antimicrobial nature of glass ionomer materials appears to be caused not only by fluoride release but also by low pH levels on the setting of the material³⁵⁻³⁸. Results have shown that polyalkenoic acids appear to be a key factor in the antibiosis^{7,35}. The acidity of glass ionomer cements is caused by polyalkenoic acids and other acidic components. Polyalkenoic acids are present in all formulations of these cements, in either the liquid or the powder components. Polyalkenoic acids not only furnish a very acidic environment but also initiate the setting reaction that releases fluoride from the glass ionomer. In addition to this, some researchers have shown that release of fluoride was increased by lowering the pH of the environment or saliva^{2,26}. Furthermore, it has been stated that low pH potentiates the antimicrobial effect of fluoride³⁹. The antimicrobial effects of low pH and fluoride of glass ionomer cements, and also the same effects of MDPB of bonding agents, have been investigated by several researchers^{7,23,35,40-42}.

Even though adhesive possess these antibacterial properties, there are situations where glass ionomer cements are the material of choice owing to their beneficial properties, such as chemical adhesion to dentinal tissues³². In such cases, a necessity arises to increase the antibacterial properties of glass ionomer cements⁴³. Therefore, interactions need to be examined by altering the polyalkenoic acid and fluoride ratios within the structure of the material. The **aim** of this study was, therefore, to investigate the synergistic effects of low pH and fluoride ions on the inhibition of microbial growth related to the different polyacid and fluoride concentrations.

Materials and Methods

Various mixtures of fluoride and polyacrylic acid were prepared. These mixtures, which contained different

NaF ions and polyacrylic acid in different concentrations, were used as test materials. So, 1000 ppm, 2.500 ppm, 5.000 ppm and 10.000 ppm fluoride ions were mixed with 5%, 10% and 20% polyacrylic acids to obtain different concentrations and various mixtures of these materials. All of the above materials were produced by the Laboratory of the Government Chemist of UK. Distilled water (0 ppm F and 0% polyacid) was used as a control. The antibacterial activities of the above materials were investigated against *Streptococcus mutans* Ingbritt NCTC 10449 and *Actinomyces viscosus* NCTC 9935.

After producing fluoride and polyacrylic acid mixtures, the antibacterial activities of these solutions were evaluated using the standard agar diffusion method. Agar plates were inoculated with each species of microorganism. All plates were divided into 2 groups. Group 1 was inoculated with *Streptococcus mutans* Ingbritt and Group 2 with *Actinomyces viscosus* bacteria. Using a sterile inoculating loop, the appropriate culture was transferred into a glass tube containing approximately 10 ml of sterile water. According to 0.5 number McFarland turbidity, the concentration of each bacteria was 1.5×10^8 cfu/ml. A cotton wool swab was dipped into the tube and then streaked across the surface of the plate in 2 directions, spreading the bacteria over the agar medium. After inoculation of the plates, wells (5 mm in diameter) were made in the agar using a sterile cork borer. After preparation of the wells (5 x 3 mm), the test materials ($\cong 59 \text{ mm}^3$) were placed into each well in the agar plates. The materials were dispensed into the wells with a sterile syringe. 6 samples were prepared for each experimental group. All of the procedures were performed using an aseptic technique.

After placing the materials into the wells, the plates were incubated in a 10% CO₂ atmosphere, at 37°C, for 48 h. After the incubation period, the plates were observed for zones of bacterial inhibition surrounding each well. The criteria for establishing the susceptibility of the microorganisms and antibacterial power of the materials were based on the size of the inhibition zone surrounding the material. The sizes of the zones were measured after 48 hours with a dial calliper. The zones that contained inhibition zones surrounding the material and wells in the centre of the zones were measured between the edge of the inhibition zone and the opposite edge of the same inhibition zone. To compensate for any irregularity in the shape of the zone, the width was measured twice, the second measurement being taken at right angles to the first.

The antibacterial effects of different concentrations of fluoride and polyacrylic acid on *S. mutans* and *A. viscosus* were evaluated and compared one another. All of the results were analyzed by the 2-way ANOVA and "a linear regression analysis of diameter" tests, with the value of statistical significance being set at $p < 0.05$.

Results

The findings of *S. mutans* showed that there were significant differences between 5.000 ppm and other concentrations of fluoride (0, 1.000, 2.500); 10.000 ppm and other concentrations of Fluoride (0, 1.000, 2.500), when fluorides were used separately (unless

addition of polyacrylic acid) but in addition to this, it was found that there were significant differences ($p < 0.001$) for all multiple comparisons when different concentrations (5%, 10%, 20%) of polyacrylic acid were mixed with 0, 1.000, 2.500, 5.000 and 10.000 ppm fluoride to determine the inhibition of *Streptococcus mutans* (Tab. 1; Fig. 1).

Table 1. The arithmetic means, standard deviations and statistical analyzes of inhibition zones (diameter, cm) of *Streptococcus mutans* by different concentrated polyacrylic acid and fluoride (ppm) mixtures

Fluoride Conc. Polyac. Conc.	0 ppm F	1000ppm F	2500 ppm F	5000 ppm F	10000 ppm F	X, p
0%	0	0	0	1.00 ± 0.02	1.16 ± 0.02	5.33; 0.02
5%	1.04 ± 1.29	1.10 ± 0.01	1.27 ± 0.02	1.33 ± 1.01	1.59 ± 0.02	18.31; 0.001
10%	1.25 ± 0.03	1.34 ± 0.02	1.47 ± 0.02	1.61 ± 0.03	1.97 ± 0.05	18,29; 0.001
20%	1.67 ± 0.01	1.76 ± 0.02	1.83 ± 0.01	1.99 ± 0.01	2.14 ± 1.02	18.33; 0.001
X, p	9.88; 0.07	9.88; 0.007	9.88; 0.007	14.14; 0.003	14.14; 0.003	

Table 2: The arithmetic means, standard deviations and statistical analyzes of inhibition zones (diameter, cm) of *Actinomyces viscosus* by different concentrated polyacrylic acid and fluoride (ppm) mixtures.

Fluoride Conc. Polya. Conc.	0 ppm F	1000ppm F	2500 ppm F	5000 ppm F	10000 ppm F	X, p
0%	0	0	0	0	0	0
5%	1.15 ± 0.02	1.15 ± 0.02	1.16 ± 0.01	1.21 ± 0.01	1.36 ± 0.01	15.38; 0.004
10%	1.33 ± 0.01	1.34 ± 0.01	1.36 ± 0.01	1.50 ± 0.01	1.61 ± 0.01	17.36; 0.002
20%	1.74 ± 0.01	1.73 ± 0.01	1.76 ± 0.01	1.91 ± 0.02	2.03 ± 0.02	16.05; 0.003
X, p	9.84; 0.007	9.91; 0.007	9.88; 0.007	9.95; 0.007	9.91; 0.007	

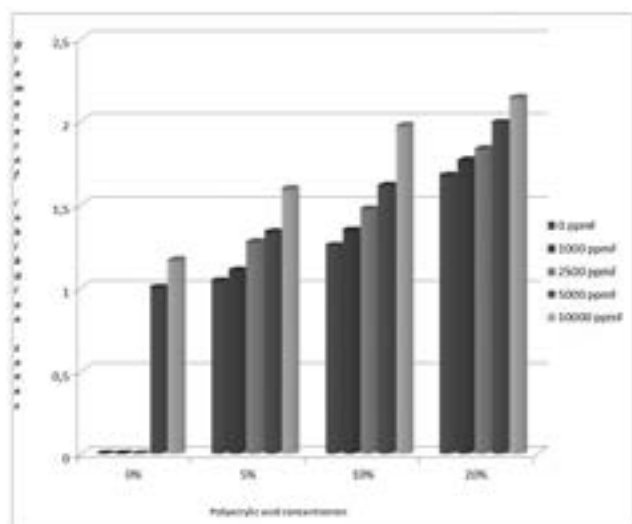


Figure 1: The schematic view of the arithmetic means of inhibition zones (diameter, cm) of *Streptococcus mutans* by different concentrated polyacrylic acid and fluoride (ppm) mixtures

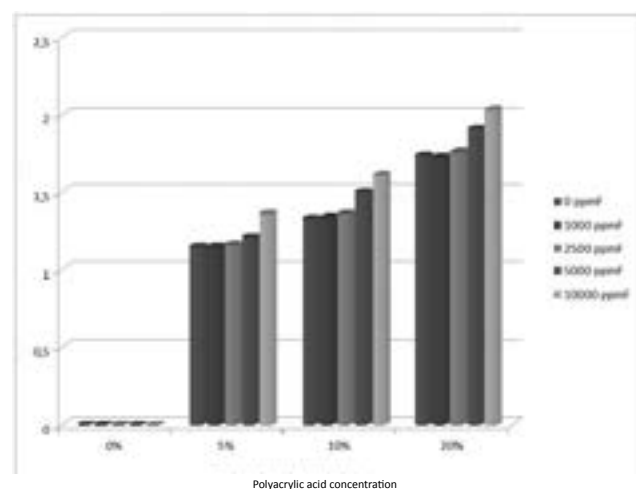


Figure 2 : The schematic view of the arithmetic means of inhibition zones (diameter, cm) of *Actinomyces viscosus* by different concentrated polyacrylic acid and fluoride (ppm) mixtures

For *Actinomyces viscosus*, significant differences were found between 5.000 ppm and other concentrations of fluoride (0, 1000, 2500); 10.000 ppm and other concentrations of fluoride (0, 1.000, 2.500), when fluorides were mixed with 5%, 10%, 20% polyacrylic acid (Tab. 2; Fig. 2).

The results showed that *Actinomyces viscosus* was more acid sensitive than *Streptococcus mutans*, and *S. mutans* was more fluoride sensitive than *A. viscosus*.

Discussion

In the present study, determination of antibacterial properties depended upon good contact between the material and the adjacent agar gel, as well as the ability of the antibacterial agent to diffuse readily through the agar gel. Both factors would greatly influence the amount of antibacterial effect observed^{44,45}. As a result, materials were prepared and put into the wells immediately after very careful mixing.

The present study aimed to correlate fluoride or polyacrylic acid contents with antibacterial activity. Concentrations of polyacrylic acid and fluoride ions were determined according to biocompatibilities of the ratios. Therefore, it seemed more appropriate to use the agar diffusion method employed by previous studies, which reported a correlation between fluoride release and antibacterial activity^{35,36,39,46}. Furthermore, agar diffusion assay method served as a screening method for comparison of dental materials because it is quick, inexpensive and, to some extent, reproducible.

In this study, results of the inhibition zone showed that *A. viscosus* was more acid sensitive than *S. mutans*, and *S. mutans* was more fluoride sensitive than *A. viscosus*. On the other hand, according to these results, it was determined that the increase of fluoride concentration was influential on the increase of antimicrobial property of polyacid solution for *A. viscosus*. In addition to this, the increase of polyacid concentration also affected the antimicrobial property of fluoride for *S. mutans*.

Scherer et al³⁶ noticed that the glass ionomer cement restorative materials exhibited large zones of inhibition as a group of *Actinomyces viscosus*, compared to glass ionomer liner/base materials. It may be thought that the glass ionomer cement restorative materials contain more acid in their structure than liner/base materials.

Recently, after cavity preparation, some solutions or materials were applied into the cavity for cavity disinfection. With glass ionomer cements as a lining or restorative material, the application of cavity disinfection solution need not be necessary and, if added polyacrylic acid and fluoride concentrations into the material were proper, their synergistic effects can be benefit for

inhibition of microorganisms that are important for tooth caries and residual caries.

Some researchers compared the antimicrobial activities of freshly mixed glass ionomers with pre-set materials. They noticed that all freshly mixed materials showed some degree of antibacterial activity, though the effects varied between microorganisms^{7,47}. Thus, it was reported that low pH of the cement before setting is an important factor of the antibacterial property of glass ionomer cement. In addition, the fluoride content of glass ionomer cements is responsible for antimicrobial properties of these cements according to earlier studies because of the fluoride release from glass ionomer cements^{6,48,49}. All of these results support the findings of this study. We found that either fluoride or polyacrylic acid were effective for inhibition of *S. mutans* and *A. viscosus*, but the degree of antibacterial activity varied between microorganisms because it was determined that polyacid and fluoride mixtures in various concentrations affected *S. mutans* and *A. viscosus* differently.

If long term results are considered, the pH of glass ionomer cements tends to increase, while the fluoride release decreases^{40,50}. On the other hand, according to earlier studies, large amounts of fluoride released during the early life of the cement and the initial pH were found to be very low. So, the synergistic effects of polyacrylic acid and fluoride can be adequate for the inhibition of some microorganisms at the early stages of setting reactions when the cements are put into a prepared cavity. It can be concluded that the synergistic effects of polyacrylic acid and fluoride contained in glass ionomers can be effective on oral microorganisms in the early stages of the setting reactions of glass ionomer cements but, after setting, fluoride release from the materials and the antibacterial activity of fluoride against cariogenic microorganisms continues in relation to the fluoride uptake property of glass ionomer cements^{27,51}.

When resin-modified glass ionomers were considered, these cements have low water content. Part of the water in these cements has been replaced by HEMA (hydroxy-ethyl methacrylate). Although their setting reaction is a dual one, consisting of a photo-induced polymerization process and the conventional glass ionomer cement acid-base setting reaction, this lack of water in the system reduces the rate of the acid-base setting reaction. On the other hand, previous studies have shown that the high antibacterial activity was exhibited by resin-modified glass ionomer materials^{6,46,52}. So, this may be caused by the presence of free monomers in the freshly-mixed materials, and toxic effects of HEMA⁵³. In addition to this, due to slow rate of the acid-base setting reaction of resin-modified glass ionomers, free polyacrylic acid can be available for release over a longer period of time than in the case of conventional glass ionomer cements^{6,53}, which may be effective on the high antibacterial activity.

In this study, it was found that 0 ppm, 1.000 ppm and 2.500 ppm fluoride produced very similar inhibition zones for *A. viscosus* when they were mixed with 5% or 10% or 20% polyacids. 5.000 ppm fluoride solutions produced larger zones than 0 ppm, 1.000 ppm and 2.500 ppm fluoride solutions with all polyacid concentrations. The largest inhibition zones were obtained with 10.000 ppm fluoride solution with all polyacid concentrations for *A. viscosus*. After comparing 5%, 10%, and 20% polyacrylic acid concentrations in relation to the production of inhibition zones and the antibacterial effect on *A. viscosus*, results showed that the diameters of the inhibition zones increased in relation to the increasing percentage of polyacid. But, an inhibition zone could not be created with 0% polyacrylic acid solution in all fluoride concentrations for *A. viscosus*.

On the other hand, the mixture of 0% polyacrylic acid and some fluoride concentrations created inhibition zones for *S. mutans*. Although no inhibition zone was observed with 0 ppm, 1.000 ppm and 2.500 ppm fluoride and 0% polyacid mixtures; 5.000 ppm and 10.000 ppm fluoride and the same percentage (0%) polyacid mixtures created inhibition zones. The inhibition zone of 10.000 ppm fluoride solution was found to be larger than for 5.000 ppm fluoride solutions. For *S. mutans*, more regular results were determined in relation to increasing polyacid and fluoride concentrations. After comparing inhibition zone sizes that were produced by increased fluoride concentrations, it was seen that inhibition zones grew in relation to the increase of fluoride concentrations in each polyacid concentrations (5%, 10%, 20%).

References

1. Phillips RW, Swartz ML, Norman RD. Zinc silico phosphate cement: influence of composition on the acid solubility and fluoride content of enamel. *J Prosthet Dent*, 1973; 29:628-631.
2. Rezk-Lega F, Ogaard B, Rolla G. Availability of fluoride from glass ionomer luting cements in human saliva. *Scand J Dent Res*, 1991; 99:60-63.
3. Modesto A, Chevitaese O, Cury JA, Vieira AR. VariGlass fluoride release and uptake by an adjacent tooth. *Am J Dent*, 1997; 1:123-127.
4. Eronat N, Kocatas N, Alpoz AR. A comparative study of fluoride uptake from dentin bonding agents and glass-ionomer cements in permanent and primary tooth enamel. *Quintessence Int*, 1999; 30:496-500.
5. Han L, Abu-Bakr N, Okamoto A, Iwaku M. Study of the fluoridated adhesive resin cement-fluoride release, fluoride uptake and acid resistance of tooth structures. *Dent Mater J*, 2001; 20:114-122.
6. Benderli Y, Ulukapı H, Balkanlı O, Külekçi G. In vitro plaque formation on some dental filling materials. *J Oral Rehab*, 1997; 24:80-83.
7. Seppa L, Torppa-Saarinen E, Luoma H. Effect of different glass ionomers on the acid production and electrolyte metabolism of *Streptococcus mutans* Ingbritt. *Caries Res*, 1992; 26:434-438.
8. Van Miller EJ, Donly KJ. Enamel demineralization inhibition by cements at orthodontic band margins. *Am J Dent*, 2003; 16:356-358.
9. Donly KJ, Segura A. Fluoride release and caries inhibition associated with a resin-modified glass-ionomer cement at varying fluoride loading doses. *Am J Dent*, 2002; 15:8-10.
10. Burke FM, Ray NJ, McConnell RJ. Fluoride containing restorative materials. *Int Dent J*, 2006; 56:33-43.
11. Okuyama K, Murata Y, Pereira PN, Miguez PA, Komatsu H, Sano H. Fluoride release and uptake by various dental materials after fluoride application. *Am J Dent*, 2006; 19:123-127.
12. Poorterman JH, Weerheijm KL, Aartman IH, Kalsbeek H. Radiographic dentinal caries and its progression in occlusal surfaces in Dutch 17-year-olds: a 6-year longitudinal study. *Caries Res*, 2003; 37:29-33.
13. Trachtenberg F, Maserejian NN, Tavares M, Soncini JA, Hayes C. Extent of tooth decay in the mouth and increased need for replacement of dental restorations: the New England Children's Amalgam Trial. *Pediatr Dent*, 2008; 30:388-392.
14. Al-Negrish AR. Composite resin restorations: a cross-sectional survey of placement and replacement in Jordan. *Int Dent J*, 2002; 52:461-468.
15. Gandolfi MG, Chersoni S, Acquaviva GL, Piana G, Prati C, Mongiorgi R. Fluoride release and absorption at different pH from glass ionomer cements. *Dent Mater*, 2006; 22:441-449.
16. Exterkate RA, Damen JJ, ten Cate JM. Effect of fluoride releasing filling materials on underlying dentinal lesions in vitro. *Caries Res*, 2005; 39:509-513.
17. Han L, Okamoto A, Fukushima M, Okiji T. Evaluation of a new fluoride-releasing one-step adhesive. *Dent Mater J*, 2006; 25:509-515.
18. Brännström M. Communication between the oral cavity and the dental pulp associated with restorative treatment. *Oper Dent*, 1984; 9:57-68.
19. Marczuk-Kolada G, Jakoniuk P, Mystkowska J, Luczaj-Cepowicz E, Waszkiel D, Dabrowski JR, Leszczynska K. Fluoride release and antibacterial activity of selected dental materials. *Postepy Hig Med Dosw* (Online), 2006; 60:416-420.
20. Frencken JE, Imazato S, Toi C, Mulder J, Mickenautsch S, Takahashi Y, Ebisu S. Antibacterial effect of chlorhexidine-containing glass ionomer cement in vivo: a pilot study. *Caries Res*, 2007; 41:102-107.
21. Markovic DLj, Petrovic BB, Peric TO. Fluoride content and recharge ability of five glass ionomer dental materials. *BMC Oral Health*, 2008; 8:21-28.
22. Hoszek A, Ericson D. In vitro fluoride release and the antibacterial effect of glass ionomers containing chlorhexidine gluconate. *Oper Dent*, 2008; 33:696-701.
23. Lobo MM, Gonçalves RB, Pimenta LF, Bedran-Russo AB, Pereira PNR. In vitro evaluation of caries inhibition promoted by self-etching adhesive systems containing antibacterial agents. *J Biomed Mater Res, Part B: Appl Biomater*, 2005; 75B:122-127.
24. Jost-Brinkmann PG. Effect of air polishing on the fluoride release of (resin-modified) glass ionomer cements and of a polyacid-modified composite resin. *Clin Oral Invest*, 1998; 2:91-95.

25. Cildir SK, Sandalli N. Fluoride release/uptake of glass ionomer cements and polyacid-modified composite resins. *Dent Mater J*, 2005; 24:92-97.
26. Forsten L. Short- and long-term fluoride release from glass ionomers and other fluoride containing materials in vitro. *Scan J Dent Res*, 1990; 98:179-185.
27. Forsten L. Resin-modified glass ionomer cements: Fluoride release and uptake. *Acta Odontol Scand*, 1995; 53:222-225.
28. Cenci MS, Tenuta LM, Pereira-Cenci T, Del Bel Cury AA, ten Cate JM, Cury JA. Effect of microleakage and fluoride on enamel-dentine demineralization around restorations. *Caries Res*, 2008; 42:369-379.
29. Imazato S, Kinomoto Y, Tarumi H, Ebisu S, Tay FR. Antibacterial activity and bonding characteristics of an adhesive resin containing antibacterial monomer MDPB. *Dent Mater*, 2003; 19:313-319.
30. Imazato S, Torii Y, Takatsuka T, Inoue K, Ebi N, Ebisu S. Bactericidal effect of dentin primer containing antibacterial monomer methacryloyloxydodecylpyridinium bromide (MDPB) against bacteria in human carious dentin. *J Oral Rehabil*, 2001; 28:314-319.
31. Tambirojn D, Feigal RJ, Ko CC, Versluis A. Remineralized dentin lesions induced by glass ionomer demonstrate increased resistance to subsequent acid challenge. *Quintessence Int*, 2006; 37:273-281.
32. Wiegand A, Buchalla W, Attin T. Review on fluoride-releasing restorative materials - fluoride release and uptake characteristics, antibacterial activity and influence on caries formation. *Dent Mater*, 2007; 23:343-362.
33. Barlow AP, Sufi F, Mason SC. Evaluation of different fluoridated dentifrice formulations using an in situ erosion remineralization model. *J Clin Dent*, 2009; 20:192-198.
34. Lin R, Hildebrand T, Donly KJ. In vitro remineralization associated with a bioerodible fluoridated resin and a fluoride varnish. *Am J Dent*, 2009; 22:203-205.
35. De Schepper EJ, White RR, von der Lehr W. Antibacterial effects of glass ionomers. *Am J Dent*, 1989; 2:51-56.
36. Scherer W, Lippman N, Kaim J. Antimicrobial properties of glass ionomer cements and other restorative materials. *Oper Dent*, 1989; 14:77-81.
37. Botelho MG. Inhibitory effects on selected oral bacteria of antibacterial agents incorporated in glass ionomer cement. *Caries Res*, 2003; 37:108-114.
38. Sanders BJ, Gregory RL, Moore K, Avery DR. Antibacterial and physical properties of resin modified glass-ionomers combined with chlorhexidine. *J Oral Rehabil*, 2002; 29:553-558.
39. Hamilton IR, Bowden G. Effect of fluoride on oral microorganism. In: Ekstrand J, Fejerskov O, Silverstone LM (eds): Fluoride in Dentistry. Copenhagen: Munksgaard, 1988; pp 77-103.
40. Smith DC, Ruse D. Acidity of glass ionomer cements during setting and its relation to pulp sensitivity. *J Am Dent Assoc*, 1986; 112:654-657.
41. Perez CR, Hirata R Jr, Sergio PP. Evaluation of antimicrobial activity of fluoride-releasing dental materials using a new in vitro method. *Quintessence Int*, 2003; 34:473-477.
42. de Carvalho FG, Puppini-Rontani RM, Soares LE, Santo AM, Martin AA, Nociti-Junior FH. Mineral distribution and CLSM analysis of secondary caries inhibition by fluoride/MDPB-containing adhesive system after cariogenic challenges. *J Dent*, 2009; 37:307-314.
43. De Moor RJ, Stassen IG, van't Veldt Y, Torbeyns D, Hommez GM. Two-year clinical performance of glass ionomer and resin composite restorations in Xerostomic head- and neck-irradiated cancer patients. *Clin Oral Invest*, 2009; Dec 8 [Epub ahead of print] DOI 10.1007/s00784-009-0355-4.
44. Orstavik D. Antibacterial properties of root canal sealers, cements and pastes. *Int Endodont J*, 1981; 14:125-133.
45. Cobankara FK, Altinoz HC, Ergani O, Kav K, Belli S. In vitro antibacterial activities of root-canal sealers by using two different methods. *J Endod*, 2004; 30:57-60.
46. Friedl KH, Schmalz G, Hiller KA, Shams M. Resin-modified glass ionomer cements: fluoride release and influence on *Streptococcus mutans* growth. *Eur J Oral Sci*, 1997; 105:81-85.
47. Charlton DG, Moore BK, Swartz ML. Direct surface pH determinations of setting cements. *Oper Dent*, 1991; 16:231-238.
48. Meryon SD, Johnson SG. The modified model cavity method for assessing antibacterial properties of dental restorative materials. *J Dent Res*, 1989; 68:835-839.
49. De Schepper EJ, Berry EA, Cailleteau JG, Tate WH. A comparative study of fluoride release from glass ionomer cements. *Quintessence Int*, 1991; 22:215-220.
50. Shaw AJ, Carrick T, Mc Cabe JF. Fluoride release from glass ionomer and compomer restorative materials: 6-month data. *J Dent*, 1998; 26:355-359.
51. Strother JM, Kohn DH, Dennison JB, Clarkson BH. Fluoride release and re-uptake in direct tooth colored restorative materials. *Dent Mater*, 1998; 14:129-136.
52. Seppa L, Korhonen A, Nuutinen A. Inhibitory effect on *S. mutans* by fluoridated conventional and resin-reinforced glass ionomer cements. *Eur J Oral Sci*, 1995; 103:182-185.
53. Wilson AD. Resin-modified glass ionomer cements. *Int J Prosthodont*, 1990; 3:425-429.

Correspondence and request for offprints to:

Kağan Gökçe
 Yeditepe Üniversitesi, Diş Hekimliği Fakültesi
 Bağdat Caddesi No:238
 81060, Güztepe
 İstanbul, Turkey
 E-mail: kagangokce@hotmail.com

Oral Clinical Findings in Patients with Chronic Renal Failure

SUMMARY

Aim: To make comparative evaluation of objective oral clinical findings and subjective oral symptoms in patients with chronic renal failure (CRF) undergoing various therapeutic treatments, and to find possible link between subjective symptoms and objective clinical findings.

Material and Methods: We examined 90 patients with CRF, divided into 3 groups: patients with CRF undergoing haemodialysis, patients with CRF without haemodialysis and serum creatinine $<120\mu\text{mol/L}$, and patients with renal transplantation. Swab for *Candida albicans* was taken from oral mucosa. Oral changes were followed on the entire mucosal surface of the oral cavity and were classified into subjective and objective findings.

Results: Certain oral changes showed a predisposition to a particular group of patients, such as petechiae and ecchymoses in the dialysis group and gingival enlargement in the transplant group. Coated tongue, thirst, pale oral mucosa and dry fissured lips were the most frequent oral symptoms and changes among all CRF patients independently the study group. Significant association was found between xerostomia and coated tongue, as well as between unpleasant taste and coated tongue in all the studied patients.

Conclusion: The stadium and consequently severity of CRF, as well as the type of treatment, have influenced the severity of oral clinical findings.

Keywords: Chronic Renal Failure, Oral Changes, Dialysis, Renal Transplantation

A. Belazelkovska¹, M. Popovska¹,
G. Spasovski², V. Radojkova-Nikolovska¹,
A. Minovska³, Z. Belazelkoska¹, K. Mitic¹

¹Ss. Cyril and Methodius University
Department of Oral Pathology and Periodontology,
Skopje, FYROM

²Ss. Cyril and Methodius University
Department of Nephrology, Skopje, FYROM

³University "Goce Delcev"
Faculty of Dental Medicine
Department of Oral Pathology and Periodontology
Stip, FYROM

ORIGINAL PAPER (OP)

Balk J Stom, 2013; 17:37-43

Introduction

Chronic renal failure (CRF) represents an important worldwide health problem with a tendency for annual progression⁹, and diabetic nephropathy is considered to be the most common cause of the end stage of renal disease. Patients, due to residual renal function and adaptation mechanisms of glomerular filtration rate, may pass through a long asymptomatic period. But, with progression of renal disease, through 5 stages and finally to irreversible bilateral renal destruction, comes to an increased occurrence of morbidities associated with this condition and rich symptomatology due to impact of many organs and organ systems. Under such circumstances there are also repercussions in the oral cavity.

Approximately 90% of all the affected patients²⁷ have oral manifestations that originate from soft tissues,

jaw bones and salivary glands. Which of the systemic complications and oral changes will appear in patients with CRF depends not only on the etiological factors, but also on the type of treatment they receive, ranging from usual measures of dietary restriction^{13,26}, various forms of dialysis, and finally to renal transplantation^{4,29}. Despite advantages of renal replacement therapy, some oral abnormalities, as uremic smell, xerostomia, unpleasant taste and mucosal pain are irreversible and further persist regardless the adequate medical treatment.

The aims of this study were to make comparative evaluation of objective oral clinical findings and subjective oral symptoms in patients with CRF undergoing various treatments, and to find possible link between subjective symptoms and objective clinical findings.

Material and Methods

A total of 90 patients with CRF were included in this study. Selection of the patients was made at the University Nephrology Department in Skopje, and the eponymous haemodialysis centre. Complete case histories and clinical examinations were performed at the University Department of Oral Medicine and Periodontology; laboratory investigations were done at the Institute for Microbiology, Medical Faculty in Skopje.

All participants included in this study were divided into 3 groups:

- The first group (group A) consisted of 30 patients with CRF and serum creatinine level less than 120 $\mu\text{mol/L}$;
- The second group (group B) consisted of 30 patients with CRF undergoing haemodialysis;
- The third group (group C) consisted of 30 patients with renal transplants.

All patients regardless to the group were from both genders, aged 18 to 65 years. In patients undergoing haemodialysis, the treatment was performed 3 times a week, lasting 3 hours per session. Patients with renal transplants, in their main therapy, were receiving Cyclosporine in a daily dosage of 125 mg (Neoral; 6 -8mg/kg).

All subjects were informed about the procedure and agreed to participate in the study. For all patients included in the study were noted information about their oral health status from the anamnesis and clinical examination.

Oral changes were followed on the entire mucosal surface of the oral cavity and were classified into subjective and objective findings. Through anamnestic data, the most common subjective oral symptoms and signs were recorded, as follows: uremic foetor, unpleasant taste, thirst, xerostomia and burning tongue. Uremic foetor was recorded as a urine-smell breath, and unpleasant taste due to loss of sensation of different tastes in food. Diagnosis of xerostomia was made when the patients reported dry mouth and during oral inspection dental instrument was sticking to the oral mucosa.

Oral lesions were registered according to acknowledged clinical diagnostic criteria^{2,26}. Dry and fissured lips were recorded when smaller or larger squamous formations on mildly erythematous vermilion surface were observed. Coated tongue was recorded as dirty white plaque formations on the dorsal surface, which could be easily removed, and also elongated filiform papillae were present. Uremic stomatitis was registered as a form of irregular mildly erythematous areas covered with greyish white pseudo-membranes localized on lateral borders and dorsum of the tongue or buccal mucosa, accompanied with painful sensations. Gingival enlargement was observed in the region of marginal gingiva and interdental papilla.

Detection of *Candida Albicans*

Material from oral mucosa was taken with swab stick by rotational movements from each patient. It was placed in a sterile tube and within 2 hours distributed to the Institute of Microbiology where it was cultivated on Sabouraud agar or selective agar surface. The sample was kept in agar for 48-72 hours prior to determination of the results.

Statistical Analysis

The obtained data were presented as percentages of total and were statistically processed using the program Statistica 7.1. Kruskal-Wallis-test was used to assess the significance of differences in distribution of oral lesions and symptoms among the studied groups. The degree of difference between 2 groups was assessed using Mann-Whitney U-test. Intra-group association between oral symptoms and oral lesions was examined with Wilcoxon Signed Rank test.

Results

The results are presented in tables 1-3.

Table 1. Oral symptoms and signs in study groups

Oral symptoms and signs	Group A		Group B		Group C		p(a:b)	p(a:c)	p(b:c)
	Cases	%	Cases	%	Cases	%			
Uremic foetor	8	26.66	17	56.66	9	30	0.019	0.576	0.072
Unpleasant taste	8	26.66	10	33.33	9	30	0.576	0.713	0.851
Thirst	23	76.66	24	80	18	60	0.756	0.227	0.132
Xerostomia	22	73.33	20	66.66	12	40	0.501	0.079	0.040
Burning tongue	0	0	9	30	2	6,66	0.001	0.154	0.021

Table 2. Oral lesions in study groups

Oral lesion	Group A		Group B		Group C		p(a:b)	p(a:c)	p(b:c)
	Cases	%	Cases	%	Cases	%			
Pale oral mucosa	16	53.33	25	83.33	23	76.66	0.013	0.060	0.522
Dry fissured lips	22	73.33	27	90	8	26.66	0.098	0.000	0.000
Coated tongue	23	76.66	30	100	24	80	0.005	0.756	0.010
Candidiasis	0	0	3	10	0	0	0.013		
Petechiae or ecchymoses	0	0	27	90	4	13.33	0.000	0.040	0.000
Uremic stomatitis	0	0	4	13.33	4	13.33	0.040	0.040	1.000
Erythema	23	76.66	20	66.66	12	40	0.394	0.004	0.040
Angular cheilitis	14	46.66	19	63.33	6	20	0.198	0.030	0.001
Gingival enlargement	0	0	0	0	14	46.66	0.001		

Table 3. Intra-group associations between oral symptoms, signs and oral lesions in all study groups

Groups	Symptoms, signs	Oral lesions	Wilcoxon Signed Ranks Test	p
Group A	Uremic fetor	Uremic stomatitis	Z=1.897	0.058
	Unpleasant taste	Saburral tongue	Z=3.873	0.000
	Thirst	Dry fissured lips	Z=1.732	0.083
	Xerostomia	Saburral tongue	Z=2.714	0.007
	Xerostomia	Angular cheilitis	Z=1.871	0.061
	Xerostomia	Erythema	Z=2.714	0.007
	Burning tongue	Uremic stomatitis	Z=0.000	1.000
Group B	Uremic fetor	Uremic stomatitis	Z=3.606	0.000
	Unpleasant taste	Saburral tongue	Z=4.472	0.000
	Thirst	Dry fissured lips	Z=2.01	0.005
	Xerostomia	Saburral tongue	Z=3.276	0.001
	Xerostomia	Angular cheilitis	Z=0.180	0.857
	Xerostomia	Erythema	Z=0.474	0.635
	Burning tongue	Uremic stomatitis	Z=1.667	0.096
Group C	Uremic fetor	Uremic stomatitis	Z=2.828	0.005
	Unpleasant taste	Saburral tongue	Z=3.128	0.002
	Thirst	Dry fissured lips	Z=2.500	0.012
	Xerostomia	Saburral tongue	Z=2.558	0.011
	Xerostomia	Angular cheilitis	Z=2.449	0.014
	Xerostomia	Erythema	Z=0.000	1.000

Statistically significant differences were found in the distribution of uremic foetor ($Z=2.337$, $p=0.019$) and subjective feeling of burning tongue ($Z=3.227$, $p=0.001$) between patients with CRF in pre-dialysis stadium and patients with CRF undergoing haemodialysis. There was no statistically significant difference in the prevalence of xerostomia ($Z=2.053$, $p=0.040$) and burning tongue ($Z=2.316$, $p=0.021$) between haemodialysis patients and renal transplant patients. Statistically significant difference has not been found in the prevalence of unpleasant taste and subjective feeling of thirst among the different groups of patients (Tabs. 1 and 3).

We observed statistically significant difference in the prevalence of pale mucosa ($Z=2.477$, $p=0.013$), coated tongue ($Z=2.791$, $p=0.005$), petechiae and/or ecchymoses ($Z=6.948$, $p=0.000$) and uremic stomatitis ($Z=2.053$, $p=0.040$) between pre-dialysis patients and haemodialysis patients (Tabs. 2 and 3). Between pre-dialysis patients and renal transplant patients we observed statistically significant difference in the prevalence of dry, fissured lips ($Z=3.585$, $p=0.000$), and the presence of petechiae and/or ecchymoses ($Z=2.053$, $p=0.040$), uremic stomatitis ($Z=2.053$, $p=0.040$), erythema ($Z=2.856$, $p=0.004$) and angular cheilitis ($Z=2.173$, $p=0.030$). Between haemodialysis patients and renal transplant patients we observed statistically significant difference in the prevalence of xerostomia ($Z=4.934$, $p=0.000$), coated tongue ($Z=2.560$, $r=0.010$), presence of petechiae and/or ecchymoses ($Z=5.892$, $p=0.000$), erythema ($Z=2.053$, $r=0.040$) and angular cheilitis ($Z=3.376$, $p=0.001$).

The Kruskal -Wallis test showed statistically significant difference in the prevalence of gingival enlargement and candidiasis ($\chi^2=11.09$; $df=2$; $p<0.01$) among all the studied groups.

In the group of pre-dialysis patients, we found a significant association between following subjective and objective clinical oral findings: unpleasant taste and coated tongue, xerostomia and coated tongue, xerostomia and erythema. In the group of haemodialysis patients, we found a significant association between: uremic foetor and uremic stomatitis, unpleasant taste and coated tongue, thirst and dry fissured lips, xerostomia and coated tongue. In the group of renal transplant patients, we found a significant association between: uremic foetor and uremic stomatitis, unpleasant taste and coated tongue, thirst and dry fissured lips, xerostomia and coated tongue, xerostomia and angular cheilitis.

Discussion

To our knowledge, this is the first study in FYROM in this field - investigating oral manifestations in CRF patients undergoing different treatments. Based on the findings from this study, as we expected, the highest

prevalence of oral symptoms and lesions was recorded in haemodialysis patients, and the lowest percentage was recorded in renal transplant patients. We believe that this is due to the fact that haemodialysis patients are not very suitable for routine dental treatment, their dependence on dialysis centres, and their lack of motivation and less priority to maintain oral health due to the severity of their primary disease. On the other hand, transplant patients are aware that maintaining oral health at high level is necessary to prevent oral infection, which can jeopardize the success of the transplantation, but nevertheless, certain oral manifestations occur as a side effect of post-transplantation immunosuppressive therapy.

In our study, most frequent oral symptom among patients from each group was subjective feeling of thirst, symptom present in 65 out of 90 patients (72.22%). Xerostomia and uremic foetor followed, which is consistent with findings in other studies^{5,6,14,17,20,21,25}. We think that thirst in haemodialysis patients appears as a result from the fluid restriction implemented in order to prevent fluid overload between dialysis sessions, and as a consequence of the present hyposalivation. We believe that thirst in renal transplant patients is a complex phenomenon, predominantly due to a synergistic side effect of immunosuppressive and corticosteroid therapy that these patients receive. After thirst, as a second most frequent oral symptom in all the patients in our study was xerostomia, present in 54% out of 90 patients. Some higher rates have recorded Junn et al¹⁴ in their study, where xerostomia was present among 68.9% of 90 haemodialysis patients. According to Hamid et al¹⁰, xerostomia is common among patients with CRF. Patients included in this study, apart from their main treatment, also received ACE-inhibitors, antidepressants and sedatives. This additional medical treatment worsened the symptoms, which is probably the most responsible factor for xerostomia as a side-effect. In our study, we found an association between xerostomia and coated tongue in all the patients, and also association between xerostomia and erythema in patients of group A, as well as xerostomia and angular cheilitis in patients of group C. The latter association was expected because angular cheilitis occurs in persons with present oral dryness, individuals under immunosuppressive therapy, or among dehydrated patients. However, the number of registered cases with angular cheilitis among renal transplant patients was significantly lower compared to pre-dialysis and haemodialysis patients.

In a way, xerostomia is an additional cause for the uremic bad foetor and unpleasant taste, which were more prevalent in groups A and B. Postorino et al²⁵ registered dry mouth associated with unpleasant metallic taste in patients with terminal stage of CRF who had diabetes. Similarly, our findings showed almost equal distribution of unpleasant taste among all 3 groups, which supports the

opinion that in all uremic patients, regardless the type of treatment, there is a distortion of taste perception⁷.

Low oral hygiene status and dental plaque accumulation due to de-motivation in patients who are in this condition are additional factors that jeopardize the obtained clinical finding. In our study, we noted association between unpleasant taste and the appearance of coated tongue among each of the investigated groups. According to our study, the coated tongue was the most common oral change of chronic renal patients. Similar results were presented in previous reports^{5,6,17}. Coated tongue in our study was present in all haemodialysis patients (group B). Retention of residues of food, desquamated epithelial cells and bacterial accumulation due to the filiform papillae enlargement, aggravated maintenance of oral hygiene and decreased amount of saliva, being all the main reasons for the appearance of this common oral manifestation³². In this context, quite logical is the obtained strong association between the coated tongue and xerostomia among all the examined patients with CRF.

The uremic foetor was the third most frequent oral symptom among the CRF patients, present in highest percentage in patients of the group B, and with the least prevalence in patients of the group A. The uremic foetor found in 56.66% patients on haemodialysis is similar to that reported by Kao et al¹⁵ and Estela De La Rosa et al⁵. The uremic foetor in patients with CRF is considered to be a consequence of the high concentration of urea in saliva and its posterior transformation into ammonium^{5,26,27}. Investigating the intra-group association between oral symptoms and oral changes, we have found association between uremic foetor and uremic stomatitis among haemodialysis patients and renal transplant patients.

No association was found between burning tongue and any of the oral manifestations, which is in agreement with findings in the study of De La Rosa et al⁵. Predominant reasons for the appearance of burning tongue are dried oral mucosa, xerostomia due to several reasons, the presence of candidiasis, prolonged clearance of medications, as well as vitamin deficit. This oral symptom was not detected among patients with CRF in pre-dialysis phase.

Frequent observation among all participants was pale mucosa, present in 64 of a total of 90 CRF patients, and its prevalence in group B was 90%. The appearance of pale mucosa in renal patients we explain by anaemia, which as a complication appearing in the early stadium of chronic renal disease and progressing with further loss of renal function.

We registered high prevalence of dry fissured lips, which were recorded among 27 patients of group B (90%), 22 patients from group A and only in 8 patients of group C. De la Rosa et al⁵ reported the presence of dry mouth in 28.3% patients with terminal renal disease and absence of any association with the other oral symptoms

and changes. In contrast, in our study, we have found strong association between dry fissured lips and thirst in dialysis and renal transplant patients.

Despite the fact that candidiasis is presented as a common oral manifestation in patients with renal transplants, in which usually occurs in the first few months of post-transplantation period, *Candida albicans* was determined in only 3 patients on haemodialysis. According to the data in the literature^{8,32}, the prevalence of oral candidiasis in patients with CRF who are on haemodialysis ranges from 5.7% to 37%. We consider that in patients on haemodialysis, who are immunocompromised due to suppression of cellular-induced immunity and dysfunction of granulocytes caused by persisting uraemia^{22,24,27}, their poor oral hygiene, xerostomia and diabetes as most often etiological factor for the CRF, make this group of patients high susceptible for oral infections. Therefore, the presence of oral candidiasis is not surprising, as *Candida albicans* presents an integral part of the normal oral microflora, and so represents endogenous infection¹². The absence of oral candidiasis among transplant patients in this study could be explained by the fact that most of the patients were young, with optimal level of oral hygiene, and the rest of the patients were old, with medical history of several transplant graft rejections and occurrences of oral candidiasis in the past.

Gingival enlargement, as one of the most known oral manifestation among transplanted patients, whose prevalence according to data from different reports^{18,30} ranges from 22% to 81%, in our research was detected in 14 (46.66%) renal transplant patients from group C. The undisputed fact is that cyclosporine treatment leads to this kind of oral alteration, but it raises the question of whether the dose of cyclosporine or duration of the therapy has more important role in the development of gingival enlargement. Regarding this issue, opinions are very controversial^{3,11,16,23}. Still, it has generally been accepted that pathogenesis of cyclosporine-induced gingival enlargement represents complex mechanism, which includes number of cellular, local and hereditary factors. We suppose that this clinical finding can additionally be exacerbated by antihypertensive drugs.

From the conducted researches, we were not able to find an association between the gingival enlargement and any of the noted oral symptoms, but we found an association with uremic stomatitis, or precisely, among 4 out of 14 total transplant patients with gingival enlargement, simultaneously was evidenced the presence of uremic stomatitis as well. The uremic stomatitis was also evidenced among 4 patients undergoing haemodialysis and not even in 1 case among CRF patients in pre-dialysis phase. The uremic stomatitis was diagnosed as painful erythematous area on the buccal and labial mucosa, covered with greyish exudates. Leão et al¹⁹ and Ross and Salisbury²⁸ came out to the same

result in their researches. The results of the obtained statistical analysis showed association between uremic stomatitis and uremic foetor, which was expected due to their identical causal factor - rapid increase in serum and salivary urea concentration¹.

Erythematous appearances on the oral mucosa surface had highest prevalence among patients from group A, at whom we discovered a strong association between erythematous areas and oral dryness. Petechial bleeding in oral mucosa represents relatively common oral clinical finding in patients with CRF. In our study, this clinical finding was detected in 90% of patients on haemodialysis treatment. In contrast, Kho et al¹⁷ inform for 12.2% and De la Rosa et al⁵ for 15.2% prevalence of petechiae in patients on haemodialysis. We suppose that this clinical finding refers to the impaired platelet aggregation as a consequence of the uremic syndrome and accumulation of inhibitory factors in blood that cannot be removed with the process of dialysis. Heparin and other anticoagulants are additional causal factors. The petechiae and ecchymoses in transplant patients have been reported as a secondary consequence of the side-effects of immunosuppressive therapy^{2,31}.

Conclusion

The stadium and consequently severity of CRF, as well as the type of treatment, have influence on the severity of oral clinical finding in patients with CRF. Patients with CRF who were on the treatment with haemodialysis had higher prevalence of oral manifestations compared with CRF patients in pre-dialysis phase and renal transplant patients. Coated tongue, thirst, pale oral mucosa and dry fissured lips were the most frequent oral symptoms and changes among all CRF patients, independently to which group they have belonged.

Monitoring of the patients with CRF, local preventive and curative oral treatment, as well as communication between nephrologists and dentists, are just a part of the measures for maintaining and improvement of oral health among patients with CRF.

References

1. Antoniadis DZ, Markopoulos AK, Andreadis D, Balaskas I, Patrikalou E, Grekas D. Ulcerative uremic stomatitis associated with untreated chronic renal failure: report of a case and review of the literature. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 2006; 101(5): 608-613.
2. Cohen SG. Entermedades renales. In: Lynch MA, Brightman VJ, Greenberg MS (eds). *Medicina bucal de Burket*. Mexico: McGraw-Hill Interamericana Eds, 1996; pp 492-514.
3. Daley TD, Wysocki GP. Cyclosporine therapy: its significance to the periodontist. *J Periodontol*, 1984; 55:708-712.
4. Davidovich E, Davidovitch M, Eidelman, et al. Pathophysiology, Therapy and Oral Implications of Renal Failure in Children and Adolescents: An Update. *Pediatric Dentistry*, 2005; 27(2):98-106.
5. De la Rosa-García E, Mondragón-Padilla A, Aranda-Romo S, Bustamante-Ramírez MA. Oral mucosa symptoms, signs and lesions, in end stage renal disease and non-end stage renal disease diabetic patients. *Med Oral Patol Oral Cir Bucal*, 2006; 11:467-473.
6. Dirschnabel AJ, Martins AS, Dantas SA, et al. Clinical oral findings in dialysis and kidney-transplant patients. *Quintessence Int*, 2011; 42(2):127-133.
7. Fernström A, Hylander B, Rössner S. Taste acuity in patients with chronic renal failure. *Clin Nephrol*, 1996; 45(3):169-174.
8. Galvada C, Bagan J, Scully C, et al. Renal hemodialysis patients: oral, salivary, dental and periodontal findings in 105 adult cases. *Oral Dis*, 1999; 5:299-302.
9. Greenwood M, Meechan JG, Bryant DG. General medicine and surgery for dental practitioners. Part 7. *Braz Dent J*, 2006; 17(2):170.
10. Hamid MJAA, Dummer CD, Pinto LS. Systemic conditions, oral findings and dental management of chronic renal failure patients: General considerations and case report. *Braz Dent J*, 2006; 17:166-170.
11. Hefti AF, Eshenaur AE, Hassel TM, Stone C. Gingival overgrowth in cyclosporine A treated multiple sclerosis patients. *J Periodontol*, 1994; 65:774-749.
12. Holmstrup P, Axell T. Classification and clinical manifestations of oral yeast infections. *Acta Odontol Scand*, 1990; 48:57-59.
13. Mandayam S, Mitch WF. Dietary protein restriction benefits patients with chronic kidney disease. *Nephrology (Carlton)*, 2006; 11(1):53-57.
14. Junn MS, Shin CK, How RG, Shu FC, Szu YL, Jeng JH. Decreased salivary flow rate as a dispogenic factor in hemodialysis patients: Evidence from an observational study and a pilocarpine clinical trial. *J Am Soc Nephrol*, 2005; 16:3418-3429.
15. Kao CH, Hsieh JF, Tsai SC, Ho YJ, Chuang HR. Decreased salivary function in patients with end stage renal disease requiring hemodialysis. *Am J Kidney Dis*, 2000; 36:1110-1114.
16. Karpinia KA, Matt M, Fennel RS, Hefti AF. Factors affecting cyclosporine-induced gingival overgrowth in pediatric renal transplant recipients. *Pediatric Dentistry*, 1996; 18(7):450-455.
17. Kho HS, Lee SW, Chung SC, Kim YK. Oral manifestations and salivary flow rate, pH, and buffer capacity in patients with end-stage renal disease undergoing hemodialysis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 1999; 88:316-319.
18. King GN, Fulliafaw R, Higgins TJ, Walker RG, Francis DMA, Weisenteld D. Gingival hyperplasia in renal allograft recipients receiving cyclosporine A and calcium antagonists. *J Clin Perodontol*, 1993; 20:286-293.

19. Leão JC, Gueiros LAM, Segundo AVL, Carvalho AT, Barret W, Porter SR. Uremic stomatitis in chronic renal failure. *Clinics*, 2005; 60:259-262.
20. Martins C, Siqueira WL, de Oliveira E, Primo LS, Nicolau J. Salivary analysis of patients with chronic renal failure undergoing hemodialysis. *Spec Care Dentist*, 2006; 26:205-208.
21. Nandan RK, Sivapathasundharam B, Sivakumar G. Oral manifestations and analysis of salivary and blood urea levels of patients undergoing haemodialysis and kidney transplant Indian J Dent Res. 2005 Jul-Sep;16(3):77-82.
22. Naylor GD, Fredericks MR. Pharmacologic considerations in dental management of the patient with disorders of the renal system. *Dent Clin North Am*, 1996; 40:665-683.
23. Nurmenniemi PK, Pernu HE, Knuutila LE. Mitotic activity of keratinocytes in nifedipine and immunosuppressive medication-induced gingival overgrowth. *J Periodontol*, 2001; 72:167-173.
24. Oksala E. Factors predisposing to oral yeast infections. *Acta Odontol Scand*, 1990; 48:71-74.
25. Postorino M, Catalano C, Martorano C, Cutrupi S, Marino C, Cozzupoli P, et al. Salivary and lacrimal secretion is reduced in patients with ESRD. *Am J Kidney Dis*, 2003; 42:722-728.
26. Proctor R, Kumar N, Stein A, Moles D, Porter S. Oral and dental aspects of chronic renal failure. *J Dent Res*, 2005; 84:199-208.
27. Rossi SS, Glick M. Dental Considerations for the patient with renal disease receiving hemodialysis. *J Amer Dent Assoc*, 1996; 19:127-211.
28. Ross WF, Salisbury PL. Uremic stomatitis associated with undiagnosed renal failure. *Gen Dent*, 1994; 9:410-412.
29. Sobrado MJS, Carmona TI, Loureiro A, et al. Oral health status in patients with moderate-severe and terminal renal failure. *Med Oral Patol Oral Cir Bucal*, 2007; 12(4):305-310.
30. Tyldesley WR, Rotter E. Gingival hyperplasia induced by cyclosporin A. *Br Dent J*, 1984; 157:305-309.
31. Wynn RL, Mellier TF, Crossley HL. Drug information. Handbook for Dentistry. 12th ed. Hudson (OH): Lexi-Comp Inc, 2006; pp 168-169.
32. Ziccardi VB, Saini J, Demas PN, Braun TW. Management of the oral and maxillofacial surgery patient with end-stage renal disease. *J Oral Maxillofac Surg*, 1992; 50:1207-1212.

Correspondence and request for offprints to:

Prof. M. Popovska
Ss. Cyril and Methodius University
Department of Oral Pathology and Periodontology
Skopje, FYR Macedonia

The Effect of a New Topical Agent on Recurrent Aphthous Stomatitis

SUMMARY

Background: Recurrent aphthous stomatitis (RAS) is a common condition, which is characterized by multiple recurrent small, round or ovoid, ulcers with circumscribed margins, erythematous haloes, and yellow or grey floors typically presenting in childhood or adolescence. The treatment of RAS is principally directed towards reducing pain and duration of each episode of ulceration. The aim of this study was to determine the efficacy of a new agent Stomatovis®, which is a unique combination of ingredients of moloha, xylitol, aloe vera, vitamin A, vitamin E, and d-panthenol.

Methods: 42 patients with minor RAS were included in the study (13 male, 29 female, mean age: 37.07 years, range 18-62 years). The diagnosis of RAS was based on accepted clinical criteria. The patients with RAS were randomly separated in 2 groups: group 1 with 21 patients treated by Stomatovis® oral pomade (Moloha, xylitol, aloe vera, vitamin A, vitamin E, d-panthenol 5ml - Genesis İlaç Sağlık Ürünleri, Istanbul) and the group 2 with 21 patients treated by Kenacort-A orabase® oral pomade (triamcinolone acetonide 5g - Bristol-Myers Squibb Inc, Istanbul). Both remedies were used 3 times a day during 2 months. RAS status was estimated by the Ulcer Severe Score (USS), concerning symptoms, such as ulcer number, ulcer duration, the disease-free period measured. USS values of 2 groups were compared using independent-sample t test and the non-parametric Mann-Whitney U test.

Results: Before the treatment, USS values were 11 and 13 for the group 1 and 2, respectively, and 7 (for both groups) after the treatment. There was no significant difference between 2 groups with respect to USS values ($p>0.05$) at the entry and at the end of the study. There was a statistically significant difference decrease between the USS values before and after the treatment ($p<0.001$). No side effects were observed in none of the patients.

Conclusions: Similar effects have observed between 2 groups. Stomatovis® seems to be an effective and safe agent which can be used in symptomatic treatment of patients with RAS. It effectively reduces pain symptoms of RAS.

Keywords: Recurrent Aphthous Stomatitis, treatment; Pain

Meltem Koray¹, Merve Özgül¹, Duygu Ofluoğlu¹, Alp Saruhanoğlu¹, Halim İşsever², Hakkı Tanyeri¹

¹Istanbul University, Faculty of Dentistry
Department of Oral Surgery

²Istanbul University, Faculty of Medicine
Department of Public Health
Istanbul, Turkey

ORIGINAL PAPER (OP)

Balk J Stom, 2013; 17:44-47

Introduction

Recurrent aphthous stomatitis (RAS) is a common condition, which is characterized by multiple recurrent small, round or ovoid, ulcers with circumscribed margins, erythematous haloes, and yellow or grey floors

typically presenting in childhood or adolescence⁹. RAS is seen worldwide and the prevalence is about 20% of the population⁴. RAS can be clinically characterized into 3 forms, such as minor, major and herpetiform^{9,13}. Minor aphthous ulceration form is the most common form⁹.

The aetiology of RAS remains unknown. Suggested aetiological factors include a family history of RAS, idiopathic haematinic deficiency, food sensitivities, immune defects, menstrual cycle variations. Also, some patients suffer from an increase in RAS episodes caused by cessation of tobacco, due to trauma of oral mucosa or psychological stress^{6,11}.

RAS is a type of lesion of the oral mucosa consisting of sudden acute, painful loss of normal mucosal tissue, being recurrent and fulsome. No efficient treatment has been introduced and medication is usually challenging^{4,10}. Reducing pain and healing time for RAS restores the ability to eat, swallow and talk, improving the quality of life of those who suffers from this condition^{4,8}. The treatment goals are a decrease in symptoms, reduction in ulcer number and size, and an increase in the disease-free period^{8,10}.

Topical steroids have been shown to decrease severity and duration of ulcers, implicating deregulation of the immune system as the causative process^{2,7}. However, there is no proper medication that has been proven to be effective in all RAS patients, and most common treatment choice remains symptomatic.

Stomatovis® is an oral pomade agent and consist of 6 ingredients including: moloha, xylitol, aloe vera, vitamin A, vitamin E, and d-panthenol (Genesis İlac Saglik Urunleri, Istanbul). The topical use of d-panthenol, the stable alcoholic analogue of pantothenic acid, is based on good skin penetration and high local concentrations of d-panthenol when administered in an adequate vehicle, such as water-in-oil emulsion. Topical d-panthenol acts like a moisturizer, improving stratum corneum hydration, reducing trans-epidermal water loss and maintaining skin softness and elasticity. Activation of fibroblast proliferation, which is of relevance in wound healing, has been observed both *in vitro* and *in vivo* with d-panthenol. Accelerated re-epithelization in wound healing, monitored by means of the trans-epidermal water loss as an indicator of the intact epidermal barrier function, has also been seen. Also, d-panthenol has been shown to have an anti-inflammatory effect on experimental ultraviolet-induced erythema³.

Antioxidant vitamins and trace elements counteract potential damage caused by reactive oxygen species to cellular tissues and modulate immune cell function through regulation of redox-sensitive transcription factors, and affect production of cytokines and prostaglandins. Adequate intake of vitamins B(6), folates, B(12), C, E, and of selenium, zinc, copper, and iron, supports a Th1 cytokine-mediated immune response with sufficient production of pro-inflammatory cytokines, which maintains an effective immune response. Vitamins A and D play important roles in both cell-mediated and humoral antibody response and support a Th2-mediated anti-inflammatory cytokine profile¹⁴. Vitamin A deficiency impairs both innate immunity (mucosal epithelial

regeneration) and adaptive immune response to infection, resulting in an impaired ability to counteract extracellular pathogens. Overall, inadequate intake and status of these vitamins and minerals may lead to suppressed immunity, which predisposes to infections and aggravates malnutrition¹⁴.

The aim of this study was to determine the efficacy of a new topical agent Stomatovis® and to compare the outcomes with Kencaort-A orabase®, as a topical corticosteroid.

Materials and Methods

Patient Selection

The sample consisted of 42 patients (13 male, 29 female, mean age: 37.07 years, range 18-62 years) suffering from minor RAS. Study subjects were chosen from patients presenting for treatment at the Istanbul University, Faculty of Dentistry, Oral Surgery Department. The diagnosis of RAS based on the accepted clinical criteria. Patients were at least 18 years old at the time of enrolment, and did not have RAS associated with other conditions such as anaemia, vitamin deficiencies, inflammatory bowel disease, celiac disease, Behcet's disease, Reiter's disease or HIV-associated immunosuppression. Exclusion criteria also included a history of allergy to ingredients of the products used in the study.

Study Design

The patients with RAS were randomly separated in 2 groups: group 1, with 21 patients, was treated by Stomatovis® oral pomade (Moloha, xylitol, aloe vera, vitamin A, vitamin E, d-panthenol 5ml - Genesis İlac Saglik Urunleri, Istanbul) and the group 2, with 21 patients, was treated by Kenacort-A orabase® oral pomade (triamcinolone acetonide 5g - Bristol-Myers Squibb Inc, Istanbul). Both remedies were used 3 times a day, during 2 months. Patients were asked not to use any other product for prevention or treatment of RAS while participating in the study.

RAS status was estimated by the Ulcer Severe Score (USS). The rating was assigned as follows: **number** - 0 = no ulcer, 1 = minor ulcer, 2 = major ulcer; **duration** - 0=zero day, 1= less than 7 days, 2=7 days, 3= more than 7 days; **ulcer-free period**: 0 = more than 30 days, 1 = 15-29 days, 2 = 1-14 days, 3 = no ulcer-free period; **site** - 0= no ulcer, 1= 1 site of oral mucosa, 2 = 2 sites of oral mucosa, 3 = more than 2 sites of oral mucosa; **pain** - 0 = no pain, 1 = mild pain, 2 = moderate pain, 3 = severe pain. USS scores differed between 0-14 to identify the severity of lesions for each patient with RAS - 14 was the highest severity score, 0 was the minimum severity score. According to the Helsinki

declaration, a witness assisted the patients before signing the informed consent form.

Statistical Methods

USS values of 2 groups were compared using independent-sample t-test and the non-parametric Mann-Whitney U test.

Results

Of the 42 patients enrolled in the study, all patients completed at least 2 month of follow-up. Overall, the compliance rate in this study was 100%. Before the treatment, USS values were 11 and 13 for the group 1 and 2, respectively, and 7 (for both groups) after the treatment. There was no significant difference between 2 groups with respect to USS values ($p>0.05$) at the entry and at the end of the study. There was a statistically significant decrease between the USS values before and after the treatment ($p<0.001$) (Table1). No side effects were observed in none of the patients.

Table 1. USS (Ulcer Severity Score) values of study groups

	Before the treatment	After the treatment	P value
Group 1*	11	7	$P<0.001$
Group 2**	13	7	$P<0.001$

*Stomatovis®

**Kenacorte-A Orabase®

Discussion

RAS has been described as presenting in 3 different forms including minor, major, and herpetiform³. Minor form of RAS accounts for 80-85% of all RAS cases and can involve any non-keratinized part of the oral cavity mucosa¹. The characteristic lesions are smaller than 10 millimetres and heal within 7-14 days without scarring. Major form of RAS, comprising 10-15% of RAS cases, are larger, of longer healing time. They are more severe than minor form and tend to leave scars after healing. The herpetiform ulcers represent only 5-10% of RAS cases and consist of multiple 1-3 millimetres resembling herpes simplex, but involving non-keratinized mucosa^{4,10}. In our study, all of the subjects were diagnosed with minor form of RAS.

The aetiology of RAS is still unknown; thus, there is no safe and conclusive treatment that can decrease the frequency of ulceration episodes in a patient. There is no gold standard on how to evaluate relief for this

condition in the literature; therefore, in this study, we aimed to evaluate the success which included decrease in symptoms, reduction in pain, ulcer number, duration, size, and an increase in the disease-free period. The most significant outcome of this study was to compare USS values before and after treatment. The data showed that there was a significant reduce between USS values registered before and after treatment in both group ($p<0.001$). Data revealed that USS values of the group 1 (Stomatovis®) were 11 before the treatment and that were reduced to 7 after treatment; on the other hand, in group 2, USS values were reduced from 13 to 7. These data indicate that both products can be used for improving discomfort associated with RAS. The other significant outcome of this study was that no significant differences were found between 2 groups with respect to USS values ($p>0.05$). It seems that both products have a positive effect on symptomatic treatment of RAS, without any significant differences.

Many topical treatments (corticosteroids, benzydamine, chlorhexidine, amlexanox, triclosan, tetracyclines, low intensity ultrasound, dentifrices, barrier techniques, laser and etc.) have been used to improve discomfort associated with RAS¹². Topical medications appear to be the first choice for symptomatic treatment of RAS. The topical agents have limitations respect to drug application and retention on the oral mucosa⁵. Although none of our patients complained about the taste, local tolerability, or way of application of the product, we think these features probably impact our study.

The dosage of Stomatovis® was 3 times a day in this study, in accordance to manufacturer advice. Further studies can challenge with a different types of regime to figure the best adequate.

In our study no side effects were observed in none of the patients. The present data indicate that Stomatovis® safely and conveniently reduces the pain of RAS.

One of the limitations of this study was a short follow-up time (2 months) to detect stronger statistical analysis to evaluate the ulcer free-period.

We hope that a larger scale study will allow for stronger statistical trends to be identified. While a larger sample size is necessary, on the basis of our data showed in this study, Stomatovis® seems to be an effective and safe agent, which can be used for symptomatic treatment in patients with RAS.

References

1. Akintoye SO, Greenberg MS. Recurrent aphthous stomatitis. *Dent Clin North Am*, 2005; 49(1):31-47.
2. Barrons RW. Treatment strategies for recurrent oral aphthous ulcers. *Am J Health Syst Pharm*, 2001; 58(1):41-50.

3. Ebner F, Heller A, Rippke F, Tausch I. Topical use of dexpanthenol in skin disorders. *Am J Clin Dermatol*, 2002; 3(6):427-433.
4. Femiano F, Lanza A, Buonaiuto C, Gombos F, Nunziata M, Piccolo S, Cirillo N. Guidelines for diagnosis and management of aphthous stomatitis. *Pediatr Infect Dis J*, 2007; 26:728-732.
5. Kaiser AB, Kernodle DS. Synergism between poly-(1-6)-beta-D-glucopyranosyl-(1-3)-beta-D-glucopyranose glucan and cefazolin in prophylaxis of staphylococcal wound infection in a guinea pig model. *Antimicrob Agents Chemother*, 1998; 42(9):2449-2451.
6. McCullough MJ, Abdel-Hafeth S, Scully C. Recurrent aphthous stomatitis revisited; clinical features, associations, and new association with infant feeding practices? *J Oral Pathol Med*, 2007; 36:615-620.
7. Natah SS, Konttinen YT, Enattah NS, Ashammakhi N, Sharkey KA, Hayrinen-Immonen R. Recurrent aphthous ulcers today: a review of the growing knowledge. *Int J Oral Maxillofac Surg*, 2004; 33:221-234.
8. Porter S, Scully C. Aphthous ulcers (recurrent). *Clin Evid*, 2005; 13:1687-1694.
9. Preeti L, Magesh KT, Rajkumar K, Karthik R. Recurrent aphthous stomatitis. *J Oral Maxillofac Pathol*, 2011; 15:252-256.
10. Scully C, Gorsky M, Lozada-Nur F. The diagnosis and management of recurrent aphthous stomatitis: a consensus approach. *J Am Dent Assoc*, 2003; 134:200-207.
11. Scully C. Aphthous ulceration. *N Engl J Med*, 2006; 355:165-172.
12. Scully C, Porter S. Oral mucosal disease: Recurrent aphthous stomatitis. *Br J Oral Maxillofac Surg*, 2008; 46:198-206.
13. Stanley HR. Aphthous lesions. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 1972; 30:407-416.
14. Wintergerst ES, Maggini S, Hornig DH. Contribution of selected vitamins and trace elements to immune function. *Ann Nutr Metab*, 2007; 51(4):301-323.

Correspondence and request for offprints to:

Assoc. Prof. Meltem Koray
Istanbul Universitesi
Diş Hekimliği Fakültesi
Ağız, Diş, Çene Hastalıkları ve Cerrahisi Anabilim Dalı 6. Kat
Fatih/Istanbul
E-mail: mkoray@istanbul.edu.tr

Profile Convexity Changes after Maxillary Protraction Therapy in Patients with Class III Malocclusion

SUMMARY

The purpose of this study was to determine profile convexity changes in Class III growing patients after maxillary protraction therapy.

This study comprised a sample of 11 patients with Class III malocclusion (4 females and 7 males), ranging from 6 years 4 months to 12 years of age. Changes in sagittal jaw relationship and facial profile were detected by using lateral cephalograms before and after therapy. Mean treatment time was 11 months, and the effect of maxillary protraction treatment was improvement of skeletal sagittal jaw relationship and increase of profile convexity angles, mostly as a result of forward displacement of the maxilla ($SNA\ p < 0.05$), mandibular retro-position ($SNB\ p < 0.05$) and clockwise rotation of the mandible, which significantly increased vertical cephalometric angles and measurements $SN/GoGn, SN/Gn, Sna\ to\ Me$).

These findings indicate that concave facial profile in Class III patients can be improved after maxillary protraction treatment, which is very important in this period of life for building self confidence and normal socializing in the community.

Keywords: Profile Convexity; Maxillary Protraction; Growth; Lateral Cephalograms

Sofija Carceva Salja

University "St.Cyril and Methodius"
Faculty of Dentistry
Department of Orthodontics
Skopje, FYROM

ORIGINAL PAPER (OP)

Balk J Stom, 2013; 17:48-53

Introduction

Skeletal Class III malocclusions are usually growth-related discrepancies, and these patients usually have components of maxillary size deficiency, maxillary retro-positioning, true mandibular excess, mandibular forward positioning, or any combination of these^{1,2}. In many patients with Class III malocclusion, the mandible appears large because of a deficient maxillary growth^{3,4}. With the limited ability to influence mandibular growth, treatment modalities for influencing mild to moderate Class III alveolar base discrepancies have shifted to a maxillary protraction treatment. Ellis and McNamara⁵ showed that combination of mandibular protrusion and maxillary retrusion existed in their sample of Class III patients, while Jacobson et al¹ found that 25% of class III malocclusion had components of maxillary deficiency.

In all of these cases the treatment of choice would be facial mask treatment or more precisely protraction of the maxilla downward and forward, inhibition of the mandibular forward growth, and clockwise rotation of the mandible.

The effects of maxillary protraction have been perhaps best studied on Japanese subjects⁶⁻¹⁰ because of their higher incidence of Class III malocclusion^{11,12}. It is good to know that longitudinal cephalometric data on untreated Class III subjects to which the treatment effects produced by the facial mask can be contrasted are deficit. Much of information about the skeletal effects of protraction forces still derives from animal studies, which show that orthopaedic forces are able to provide significant sagittal changes in growing craniofacial complex, by stimulating the anterior displacement of the entire maxilla¹³⁻¹⁵, with significant increase of cellular activity at the circum-maxillary sutures, and at the

tuberosity, at the bone surfaces through the periosteal influence and even at the deeper cranial structures^{16,17}.

As clinical, experimental, and biomechanical studies certify, the orthopaedic approach to the maxilla for Class III correction, when well indicated, enables a morphologic and functional conditions that favours the ensuing normal facial growth, in addition to creating more acceptable aesthetics through improvement of facial profile in the early stages¹⁸.

Most cases with Class III malocclusion are characterized by an average of a 60% maxillary deficiency³. In this sense, it becomes logical to alter aberrant growth patterns promoting maxillary advancement in the same physiologic maxillary displacement direction by using protraction face mask treatment. This movement can be facilitated by rapid maxillary transverse expansion,

which disrupts the sutural articulation of the maxilla to 9 bones of craniofacial complex, allowing for a more positive reaction to protraction forces^{19,20}.

As we said before, Class III individuals are well-recognized and perceived to be abnormal by the lay public, as well as by health care practitioners. Concave facial profile is one of the most unattractive facial look, which has a negative impact to normal socializing of those individuals in the community. The facial mask that has been popularized by Delaire is very common at our clinic and, because of all these reasons and big frequency of class III patients, the purpose in our study was to determine sagittal and vertical behaviour of the jaws, and profile convexity changes in Class III growing patients after maxillary protraction treatment.

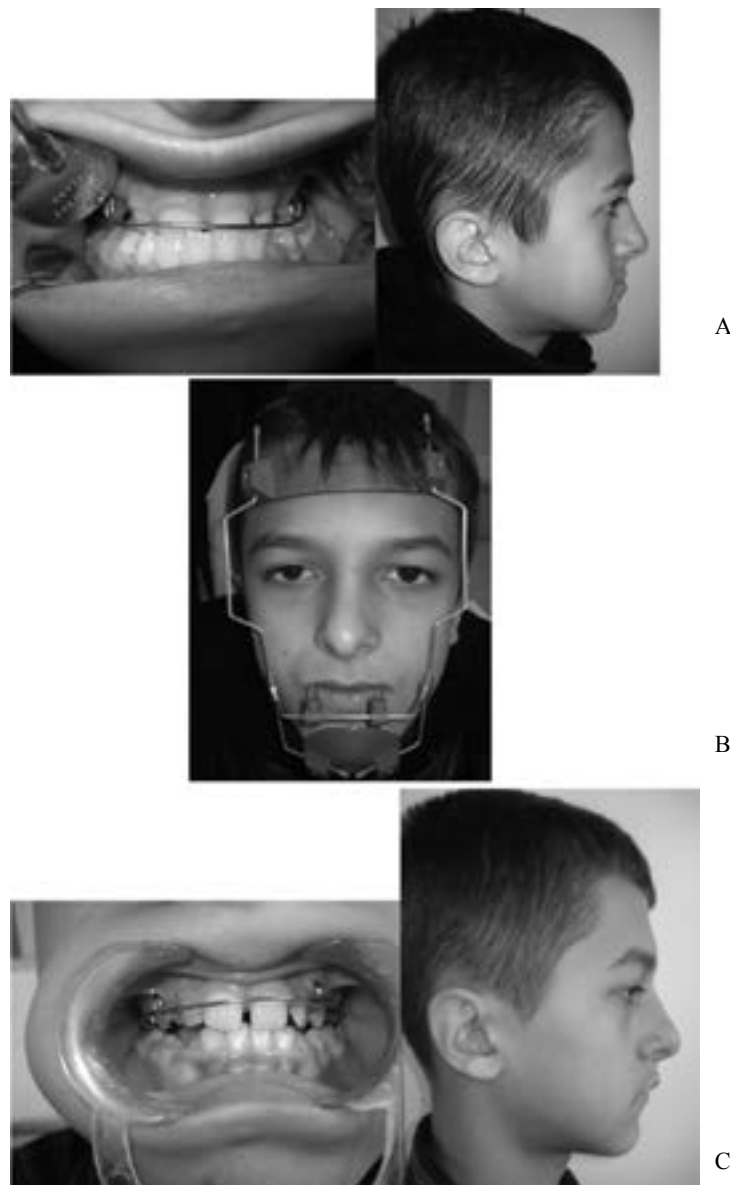


Figure 1. A. Patient before treatment; B. Delaire facial mask; C. Patient after treatment

Material and Method

The sample consisted of 11 patients (4 females and 7 males), ranging from 6 years 4 months to 12 years of age, who had Class III malocclusion with an anterior cross-bite and a component of maxillary deficiency. The evaluation of the facial profile was 1 of the most important items in our differential diagnosis. Flat or concave profiles, retrusive maxillas, and prominent mandibles were included. Cephalometric values were also used, but bearing in mind that those measurements are more realistic in older children with a limited value in younger ones. Generally, children up to 10 years of age present positive angular and linear measurements, which could mislead the practitioner into postponing treatment¹⁸.

2 radiographs were evaluated, the first was taken before the beginning of the treatment and the second was taken immediately after face mask treatment. The treatment time varied as a result of patients' compliance, severity of the problem and individual response of the patient to treatment. Mean treatment time was 11 months. The maxillary protraction was performed through a Delaire facial mask (Fig. 1), using elastics with a force delivering of about 350 gm per side. Bands were fitted on first permanent molars and first primary molars in early mixed dentition, or on first premolars in late mixed dentition or early permanent dentition. These

bands were joined by a heavy wire to the palatal plane or hyrax rapid maxillary expansion appliance. Another wire was soldered bilaterally to the buccal aspects of the molar bands and first primary molars or first premolars; a hook for elastic traction was extended into the canine region.

Facial mask was adjusted to rest on the forehead and the chin of the patient (Fig. 1). The patients were instructed to wear the protraction mask at bed time for children under the age of 9 and for 14 hours for children over the age of 9. Treatment was discontinued when positive overjet was achieved and no more changes were noted after 3 months. In some patients with posterior cross-bite, before protracting maxilla, rapid maxillary expansion appliance was used, and it was activated every day until achieving correction of the bite posteriorly.

The results obtained with this therapy have been evaluated through pre-treatment and post-treatment lateral radiographs (Figs. 2 and 3). Cephalometric measurements that were used included evaluation of maxillary sagittal relationships (SNA, Co-A point mm), mandibular sagittal relationships (SNB, Co-Gn mm), facial convexity angles and measurements (ANB, N-Pg to A - Fig. 2), and vertical behaviour of the jaws (SN/GoGn, SN/Gn, Sna to Me - Fig. 3).

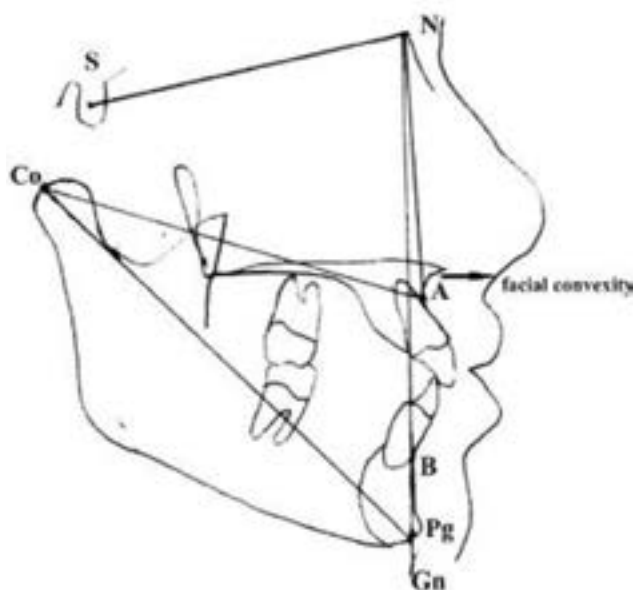


Figure 2. Cephalometric angles and measurements for sagittal jaw relationship and profile convexity

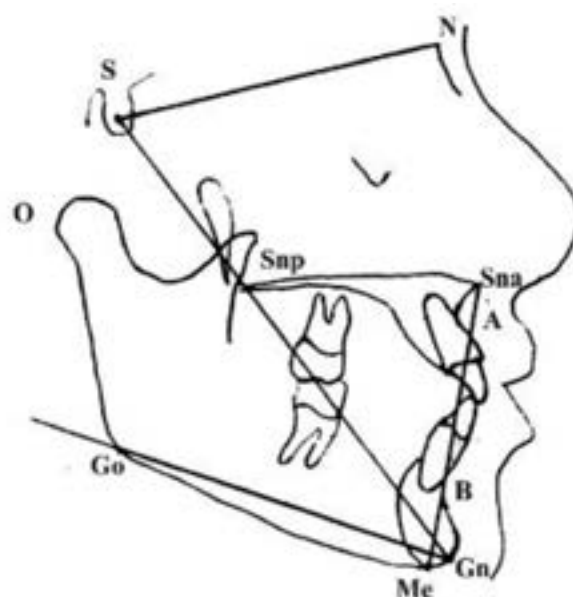


Figure 3. Cephalometric angles and measurements for vertical jaw relationship

Results and Discussion

All cephalometric measurements used in this study showed significant changes in sagittal relationships of jaws (Tab. 1), facial convexity angles (Tab. 2) and vertical behaviour of the jaws (Tab. 3).

Changes in the angle between the anterior part of the maxilla and the base of the skull (SNA), when compared pre- and post-treatment cephalograms, showed significant

increase ($p < 0.001$) as a result of forward displacement of the maxilla after protraction treatment. Statistically significant difference was noticed in Co-A measurements as well. It is good to know that these cephalometric measurements do not usually change in normal conditions²¹. The forward displacement of the maxilla with the use of facial mask has proved to be efficient clinically and experimentally^{15,22,23} in many studies, which confirm our findings.

Table1. Sagittal relationship of the jaws before and after face mask treatment

Difference in angular and linear measurements before and after the treatment									
Angle	Before the treatment		After the treatment		σ	D	σ_D	t test	p
	N	M	N	M					
SNA	16	78.6250	16	81.56250	1.54600	-2.93750	0.54728	-5.36741	<0.001***
Co-A		77.5625		80.31250	1.62700	-2.75000	0.57596	-4.77465	<0.001***
SNB		80.0625		79.37500	1.44095	0.68750	0.51009	1.34778	>0.005
Co-GN		101.4375		104.37500	1.82300	-2.93750	0.64534	-4.55185	<0.001***

Table2. Profile convexity changes before and after face mask treatment

Difference in angular and linear measurements before and after the treatment									
Angle	Before the treatment		After the treatment		σ	D	σ_D	t test	p
	N	M	N	M					
ANB	16	-0.0625	16	1.81818	1.48300	-1.88068	0.52498	-3.58237	<0.001***
N-Pg to A		23.3125		26.18182	0.53737	-2.86932	0.53737	-5.33954	<0.001***

Table 3. Vertical jaw relationship before and after face mask treatment

Difference in angular and linear measurements before and after the treatment									
Angle	Before the treatment		After the treatment		σ	D	σ_D	t test	p
	N	M	N	M					
SN/GoGN	16	29.2500	16	31.54545	1.59100	-2.29545	0.56321	-4.07563	<0.001***
SN/GN		62.1250		64.63636	3.09742	-2.51160	1.09649	-2.29038	<0.005*
Sna-Me		56.6875		59.18182	2.36612	-2.49432	0.83761	-2.97791	<0.005*

Changes in the angle between the anterior part of the mandible and the base of the skull (SNB) showed significant decrease after face mask treatment ($p < 0.005$), which is reflection of downward and backward mandible rotation diagnosed by statistically significant increases with Steiner's analysis²⁴ of angles (SN/GoGn and SN/Gn) and McNamara's measurements²⁵ (Sna-Me). From this, we can conclude that it is desirable if patients have deficit in anterior high when protracting maxilla because with downward and forward mandible rotation, as a result of protraction therapy, there is an increase of facial high, which in other case can worsen malocclusion.

After maxillary protraction, significant positive changes were seen in the ANB angle ($p < 0.001$) and profile convexity measurements N-Pg to A ($p < 0.001$),

suggesting the fact that forward displacement of the maxilla and mandible retro-position increase facial convexity, thereby improving the facial profile.

Facial attractiveness is very important component in human communication and can also have positive influence in all areas of civilized society. Physical attractiveness or more precisely, facial balance and symmetry, are considered as parameters of how people are perceived by the public, as well as how their own perception is. Negative psychological consequences as a result of facial non-attractiveness from skeletal malocclusions have been documented long time ago²⁶. A study designed to survey facial profile preferences in a sample of 1189 Asian teenagers found that mandibular prognathism was the least favoured of all profiles.

Most Class III malocclusions can be detected early, but still there are many clinicians who are delaying treatment because there is a hope that with growth the problem will cease, there is a fear of treating young children, and also a possibility of relapse exists. The recommended treatment for these patients is usually to wait until the end of the growth period for the combined orthodontic treatment with orthognathic surgery. But aside of this, in Class III patients there is a big motivation for orthodontic treatment because their dentofacial appearance deviates from socio-cultural norms²⁶. Therefore, an important objective of accepting maxillary protraction treatment in Class III malocclusion is providing nonsurgical alternative in the treatment and improving the physico-social wellbeing and appearance of the patients, especially during their teenage years.

Conclusion

In summary, results from this study showed sagittal and vertical skeletal changes and improvements in facial profile after maxillary protraction therapy in Class III patients. Mean treatment time was 11 months, and the effect of maxillary protraction treatment was improvement of skeletal sagittal jaw relationship and increase of profile convexity angles, mostly as a result of forward displacement of the maxilla (SNA $p < 0.001$), mandibular retroposition (SNB $p < 0.005$) and clockwise rotation of the mandible, which significantly increases vertical cephalometric angles and measurements (SN/GoGn, SN/Gn, SNA to Me). These findings indicate that concave facial profile in class III patients can be improved after maxillary protraction treatment, which is very important in the teenage period of life for building self confidence and normal socializing in the community.

Even though, the management of Class III malocclusion remains 1 of the most challenging problems in the clinical practice today; the results from this study support maxillary protraction for correction of Class III malocclusion with deficient maxillary growth.

References

1. Jacobson A, Evans WG, Preston CB, Sadowsky PW. Mandibular prognathism. *Am J Orthod*, 1974; 66:140-171.
2. Sanborn RT. Differences between the facial skeletal patterns of Class III malocclusion and normal occlusion. *Angle Orthod*, 1955; 25:208-222.
3. Guyer EC, Ellis EE, McNamara JA Jr, Behrents RG. Components of Class III malocclusion in juveniles and adolescents. *Angle Orthod*, 1986; 56:7-30.
4. McNamara JA Jr. An orthopedic approach to the treatment of Class III malocclusion in your patients. *J Clin Orthod*, 1987; 21:598-608.
5. Ellis E, McNamara JA Jr. Components of adult Class III open bite malocclusions. *Am J Orthod*, 1984; 86:277-290.
6. Wendell PD, Nanda R, Sakamoto T, Nakamura S. The effects of chin cup therapy on the mandible: a longitudinal study. *Am J Orthod*, 1985; 87:265-274.
7. Ishii H, Morita S, Takeuchi Y, Nakamura S. Treatment effect of combined maxillary protraction and chin cup appliance in severe skeletal Class III cases. *Am J Orthod Dentofac Orthop*, 1987; 92:304-312.
8. Ngan P, Wei SHY, Hagg U, Yiu CKY, Merwin D, Stickel B. Effect of protraction headgear on Class III malocclusion. *Quintessence Int*, 1992; 23:197-207.
9. Takada D, Petdachai S, Sakuda M. Changes in dentofacial morphology in skeletal Class III children treated by a modified maxillary protraction headgear and a chin cup: a longitudinal cephalometric appraisal. *Eur J Orthod*, 1993; 15:211-221.
10. Ngan P, Hagg U, Yiu C, Merwin D, Wei SHY. Treatment response to maxillary expansion and protraction. *Eur J Orthod*, 1996; 18:151-168.
11. Irie M, Nakamura S. Orthopedic approach to severe skeletal Class III malocclusion. *Am J Orthod*, 1975; 67:377-392.
12. Allwright WC, Burndred WH. A survey of handicapping dentofacial anomalies among Chinese Hong Kong. *Int Dent J*, 1964; 14:505-519.
13. Massler M, Frankel JM. Prevalence of malocclusion in children aged 14-18 years. *Am J Orthod*, 1951; 37:751-768.
14. Thilander B, Myrberg N. The prevalence of malocclusion in Swedish children. *Scand J Dent Res*, 1973; 81:12-20.
15. Jackson GW, Kokich VG, Shapiro PA. Experimental and postexperimental response to anteriorly directed extraoral force in young *Macaca nemestrina*. *Am J Orthod*, 1979; 75:318-333.
16. Mills LF. Epidemiologic studies of occlusion IV. The prevalence of malocclusion in a population of 1455 school children. *J Dent Res*, 1966; 45:332-336.
17. Mitani H, Sakamoto T. Chin cup forces to a growing mandible. Long term clinical reports. *Angle Orthod*, 1984; 54:93-122.
18. Silva F, et al. Early treatment of the Class III malocclusion with rapid maxillary expansion and maxillary protraction. *Am J Orthod*, 1998; 113:196-203.
19. Haas AJ. Palatal expansion: just the beginning of dentofacial orthopedics. *Am J Orthod*, 1970; 57:219-255
20. Bell RA. A review of maxillary expansion in relation to the rate of orthopedics. *Am J Orthod*, 1981; 80:638-650
21. Riolo ML, Moyers RE, McNamara Jr JA, Huntewr WS. An atlas of craniofacial growth: Cephalometric standards from the University school growth study. The University of Michigan. Monograph 2. Craniofacial Growth Series. Ann Arbor Michigan: Center for Human Growth and Development. The University of, 1974.

22. *Dellinger EL*. A preliminary study of anterior maxillary displacement. *Am J Orthod*, 1973; 63:509-516
23. *Kambara T*. Dentofacial changes produced by extra oral forward force in the *Macaca irus*. *Am J Orthod*, 1977; 71:249-277
24. *Steiner C*. Cephalometrics for you and me. *Am J Orthod*, 1953; 39:729-755.
25. *McNamara J*. A method of cephalometric evaluation. *Am J Orthod*, 1984; 86:449-469.
26. *Adams GR*. The Effects of Physical Attractiveness on the Socialization Process. *Psychological Aspects of Facial Form*. Ann Arbor, Michigan: Center for Human Growth and Development, 1980; pp 25-47.

Correspondence and request for offprint:

Sofija Carceva Salja
University "St.Cyril and Methodius"
Faculty of Dentistry, Department of Orthodontics
Skopje, FYR Macedonia
E-mail: sofijacarcevasalja@yahoo.com

Avulsed Tooth Revascularization after 13-Hour Extra-Oral Time

SUMMARY

Background: Tooth avulsion is often the result of trauma in young ages and its management remains a clinical challenge. Tooth reimplantation refers to the repositioning of the avulsed tooth into the alveolar socket from which was violently removed.

Case Report: The paper describes the management of a reimplantation case, done after 13 hours of extra-oral time.

Conclusions: Revascularization ability of immature teeth, which allows reimplantation to be successful even when prognosis is poor, is illustrated. Also, it is pointed that guidelines that strictly reject reimplantation as a treatment plan when extra-oral time exceeds 60 minutes may need to be reconsidered and that critical consideration of the clinical signs is needed.

Keywords: Reimplantation; Revascularization; Tooth Avulsion, Freezer

K. Tolidis¹, P.G. Liokatis², C. Boutsiouki², D. Michailidou³

¹Aristotle University, School of Dentistry
Dept. of Operative Dentistry

²Aristotle University, School of Dentistry
Thessaloniki, Greece

CASE REPORT (CR)

Balk J Stom, 2013; 17:54-56

Introduction

Tooth avulsion is a complex injury that affects dental hard tissues, alveolar bone, gingiva, the pulp tissue and the periodontal ligament. Following tooth reimplantation surface resorption of cementum and dentin takes place⁹. In larger trauma cases, the replanted tooth could face replacement resorption or inflammatory related resorption⁹. During the replacement resorption, dentin and cementum are replaced by bone^{1,7}, leading to ankylosis⁹. The inflammatory related resorption of the alveolar bone is the result of infection of the periodontal area, due to the diffusion of remnants from the necrotic pulp^{1,7}. Trauma extent and thus factors affecting the outcome of tooth reimplantation, is affected by: extraoral time and storage media before tooth reimplantation, and the extent of mechanical tissue injury⁹.

Case Report

An 8-year-old girl sustained facial trauma inside her house, which resulted in upper left central incisor

(#21) avulsion. The tooth had fully shaped root with open apex (>1 mm). Avulsed tooth was left for 2 hours in dry conditions on the floor and after it was found, it was maintained for 11 hours in a glass full of cubes in a freezer.

Table 1 and figures 1-7 summarize the case management.



Figure 1. Clinical image 13 hours after accident

Table 1. Case Management ⁴

Examination Time	Clinical and Radiographic Findings	Clinical Steps
1h after the accident		Wound care, lip stitching and tetanus prophylaxis at a public hospital
13 hours later (first visit)	#21 tooth avulsion, #11 tooth fracture (Fig. 1)	Alveolar socket and avulsed tooth rinsing with sterile saline, #21 reimplantation, semi-rigid splinting, antibiotic prescription (amoxicillin for 7 days)
2 weeks recall	Increased tooth mobility Positive vitality test	Clinical examination Splinting removal
4 weeks recall	<u>Clinical Examination</u> Increased tooth mobility	
6 weeks recall	Positive vitality test	
3 months recall	<u>Clinical & Radiographic Examination</u> (Figs. 2 and 3) Increased tooth mobility Positive vitality test Radiographic signs of possible root resorption	
6 months recall	<u>Clinical & Radiographic Examination</u> (Figs. 4 and 5) Increased tooth mobility Positive vitality test Radiographic signs of possible root resorption	
12 months recall	<u>Clinical & Radiographic examination</u> (Figs. 6 and 7) Normal tooth mobility Positive vitality test Radiographic signs of possible root absorption are stable	



Figure 2. Clinical image at 3 months recall



Figure 4. Clinical image at 6 months recall

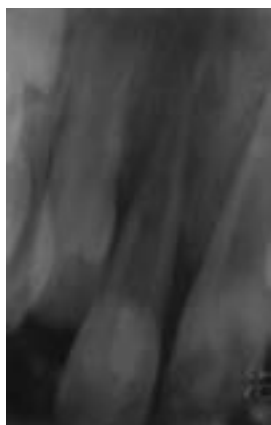


Figure 3. Radiographic image at 3 months recall



Figure 5a and b. Radiographic images at 6 months recall



Figure 6. Clinical image at 12 months recall



Figure 7. Radiographic image at 12 months recall

Accurate instructions were given to the patient and to the parents for soft diet, use of soft toothbrush and chlorhexidine mouth rinse (0.1%)³. The case is still ongoing reviews and it is in author's opinion that, beside the positive behaviour of the avulsed tooth in the first 12 months, a longer recall process is needed in order to establish full success.

Discussion

Avulsed and replanted teeth may be lost as early as 2 months after reimplantation or survive for many years even when ankylosis and replacement resorption take place. In the case presented, avulsed tooth was replanted after 13 hours of total extra-oral time in spite of the unfavourable conditions. This case report indicates the revascularization ability of permanent teeth with open apices^{7,9}. It looks that the need for endodontic treatment, even several hours after the accident, does not look always mandatory^{3,5}. At 2 week recall tooth vitality test was positive and for this reason it was decided not to perform endodontic treatment⁵.

Preservation in a suitable solution (saliva, milk and physiologic saline and tooth rescue boxes), seems to improve the probability of pulp healing^{2,9}. Freezing

of avulsed teeth might be indicated as a potential means of preservation. Socket was rinsed with saline⁸ before reimplantation, but no mechanical scraping or chemical root surface treatment was performed in order to avoid causing additional mechanical trauma.

The most practical method that allows normal tooth movement and acts as a functional stimulus for periodontal healing is the use of wire splints and composite resin for 1-2 weeks⁶.

In conclusion, in cases of avulsed permanent teeth with prolonged extra-oral storage time, reimplantation should be the first possible treatment, although the risk of replacement resorption and tooth loss may be highly probable. If managed properly, avulsed teeth can remain functional for many years and minimize the need of applying other prosthetic restorations, or in worse apply them in later time with more options available.

References

1. *Andreasen JO, et al.* Relationship between surface and inflammatory resorption and changes in the pulp after replantation of permanent incisors in monkeys. *J Endod*, 1981; 7(7):294-301.
2. *Blomlof L, Lindskog S, Andersson L, Hedstrom KG, Hammarstrom L.* Storage of experimentally avulsed teeth in milk prior to replantation. *J Dent Res*, 1983; 62:912.
3. *Flores MT, Andersson L, Andreasen JO, Bakland LK, Malmgren B, Barnett F, et al.* International Association of Dental Traumatology. Guidelines for the management of traumatic dental injuries. II. Avulsion of permanent teeth. *Dent Traumatol*, 2007; 23(3):130-136.
4. *Flores MT, Andreasen JO, Bakland LK.* Guidelines for the evaluation and management of traumatic dental injuries. *Dent Traumatol*, 2001; 17:193-196.
5. *Hinckfuss SE, Messer LB.* An evidence-based assessment of the clinical guidelines for replanted avulsed teeth. Part 1: Timing of pulp extirpation. *Dent Traumatol*, 2009; 25:32-42.
6. *Hinckfuss SE, Messer LB.* Splinting duration and periodontal outcomes for replanted avulsed teeth: a systematic review. *Dent Traumatol*, 2009; 25:150-157.
7. *Ne RF, Witherspoon DE, Gutmann JL.* Tooth resorption. *Quintessence Int*, 1999; 30(1):9-25.
8. *Panzarini SR, Gulinelli JL, Poi WR, Sonoda CK, Pedrini D, Brandini DA.* Treatment of root surface in delayed tooth replantation: a review of literature. *Dent Traumatol*, 2008; 24:277-282.
9. *Pohl Y, Wahl G, Filippi A, Kirschner H.* Results after replantation of avulsed permanent teeth. III. Tooth loss and survival analysis. *Dent Traumatol*, 2005; 21:102-110.

Correspondence and request for offprints to:

Christina Boutsouki
12 Kallidopoulou Str.
54642, Thessaloniki
Greece
E-mail: christinaboutsouki@gmail.com

Instructions to authors

The BALKAN JOURNAL OF STOMATOLOGY 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 Todorovic, 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 by 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 precede 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 Francisco: 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.