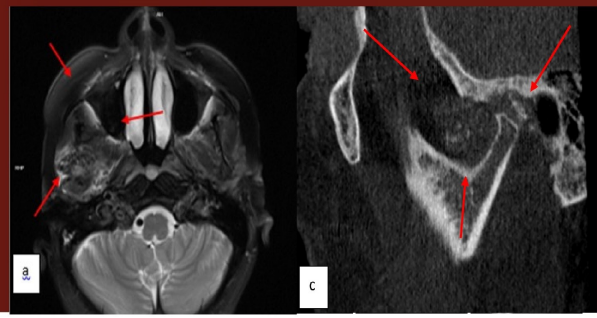
CUMHURIYET DENTAL JOURNAL

The Official Journal of Sivas Cumhuriyet University Faculty of Dentistry



Volume : 24 Issue : 3 2021
ISSN : 1302-5805 e-ISSN : 2146-2852

Cumhuriyet Dental Journal

The Official Journal of the Sivas Cumhuriyet University Faculty of Dentistry. The first issue was published in 1998 and journal's name was changed as Cumhuriyet Dental Journal in 2010. Issues are published quarterly since 2018.

Aims and Scope

Cumhuriyet Dental Journal (CDJ) is an international journal dedicated to the latest advancement of dentistry. The aim of this journal is to provide a platform for scientists and academicians all over the world to promote, share, and discuss various new issues and developments in different areas of dentistry.

CDJ publishes original research papers, reviews, and case reports within clinical dentistry, on all basic science aspects of structure, chemistry, developmental biology, physiology and pathology of relevant tissues, as well as on microbiology, biomaterials and the behavioral sciences as they relate to dentistry.

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ISSN 1302-5805

e-ISSN 2146-2852

Volume/24- Issue/3-2021

Secretary

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INDEXING

CUMHURIYET DENAL JOURNAL

AUTHOR GUIDELINE

Cumhuriyet Dental Journal (CDJ) is the Official Publication of the Cumhuriyet University, Faculty of Dentistry. CDJ accepts original experimental investigations and review articles concerning topics of clinical relevance to the general dental practitioner. Case reports and technique articles will be very critically reviewed in terms of interest to the general dental practitioner and the supporting data provided.

CDJ accepts articles in English. Submitting a paper to CDJ is free of charges. In addition, CDJ has not have article processing charges.

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CDJ is published using an open access publication model, meaning that all interested readers are able to freely access the journal online without the need for a subscription. Manuscripts will be reviewed by the editor, and at least two reviewers with expertise within the scope of the article.In addition, CDJ use double- blind review process (every effort is made to prevent the identities of the authors and reviewers from being known to each other)

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Manuscript Types Accepted

Original Research Article: Title, Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusions, Acknowledgements, References, Tables and Figure Legends

Review Articles: Although a Review article (particularly following a systematic review) may adhere to the format of the Original Research Article, both Review and Focus Articles need not contain Materials and Methods, Results or Discussion sections, and may instead employ other headings as relevant for the topic addressed.

Case Report: Title, Abstract, Introduction, Case Report, Discussion, Conclusions, Acknowledgements, References, Tables and Figure Legends

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ELEMENTS OF a MANUSCRIPT

1. Title Page

- ***Title page must be*** **uploaded apart from manuscript and should include;**
- -Title
- -Authors (first name, middle initial, surname) e.g. Burak Buldur, DDS, PhD,[a]
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- A running title, not exceeding 50 letters and spaces
- Corresponding Author details including name, complete address, phone, fax, and e-mail must be added.

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The main document includes, in a single electronic file (Word/text file, not pdf).

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- Should not exceed 300 words and should be presented under the following subheadings:

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4.Introduction

- This must be presented in a structured format, covering the following subjects, although not under subheadings: succinct statements of the issue in question; the essence of existing knowledge and understanding pertinent to the issue; and the aims and objectives of the research being reported.

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- The authors should describe the procedures and analytical techniques and identify names and sources of all commercial products e.g. magnetic attachment (Hyper Slim 5513, Hitachi Metals, Tokyo, Japan)

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- The authors should discuss the results of the study also state the agreement with other studies and identify the limitations of the present study and suggest areas for future research.

8. Conclusions

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9.Acknowledgements

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- Specify any potential conflict of interests, or state no conflicts of interest.

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- Colour and black-and-white photographs should be created and saved at a minimum of 300 dots per inch (dpi).
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For various reasons, this kind of free and unrestricted online availability, which we will call open access, has so far been limited to small portions of the journal literature. But even in these limited collections, many different initiatives have shown that open access is economically feasible, that it gives readers extraordinary power to find and make use of relevant literature, and that it gives authors and their works vast and measurable new visibility, readership, and impact. To secure these benefits for all, we call on all interested institutions and individuals to help open up access to the rest of this literature and remove the barriers, especially the price barriers, that stand in the way. The more who join the effort to advance this cause, the sooner we will all enjoy the benefits of open access.

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While the peer-reviewed journal literature should be accessible online without cost to readers, it is not costless to produce. However, experiments show that the overall costs of providing open access to this literature are far lower than the costs of traditional forms of dissemination. With such an opportunity to save money and expand the scope of dissemination at the same time, there is today a strong incentive for professional associations, universities, libraries, foundations, and others to embrace open access as a means of advancing their missions. Achieving open access will require new cost recovery models and financing mechanisms, but the significantly lower overall cost of dissemination is a reason to be confident that the goal is attainable and not merely preferable or utopian.

To achieve open access to scholarly journal literature, we recommend two complementary strategies.

I. Self-Archiving: First, scholars need the tools and assistance to deposit their refereed journal articles in open electronic archives, a practice commonly called, self-archiving. When these archives conform to standards created by the Open Archives Initiative, then search engines and other tools can treat the separate archives as one. Users then need not know which archives exist or where they are located in order to find and make use of their contents.

II. Open-access Journals: Second, scholars need the means to launch a new generation of journals committed to open access, and to help existing journals that elect to make the transition to open access. Because journal articles should be disseminated as widely as possible, these new journals will no longer invoke copyright to restrict access to and use of the material they publish. Instead they will use copyright and other tools to ensure permanent open access to all the articles they publish. Because price is a barrier to access, these new journals will not charge subscription or access fees, and will turn to other methods for covering their expenses. There are many alternative sources of funds for this purpose, including the foundations and governments that fund research, the universities and laboratories that employ researchers, endowments set up by discipline or institution, friends of the cause of open access, profits from the sale of add-ons to the basic texts, funds freed up by the demise or cancellation of journals charging traditional subscription or access fees, or even contributions from the researchers themselves. There is no need to favor one of these solutions over the others for all disciplines or nations, and no need to stop looking for other.

Open access to peer-reviewed journal literature is the goal. Self-archiving (I.) and a new generation of open-access journals (II.) are the ways to attain this goal. They are not only direct and effective means to this end, they are within the reach of scholars themselves, immediately, and need not wait on changes brought about by markets or legislation. While we endorse the two strategies just outlined, we also encourage experimentation with further ways to make the transition from the present methods of dissemination to open access. Flexibility, experimentation, and adaptation to local circumstances are the best ways to assure that progress in diverse settings will be rapid, secure, and long-lived.

The Open Society Institute, the foundation network founded by philanthropist George Soros, is committed to providing initial help and funding to realize this goal. It will use its resources and influence to extend and promote institutional self-archiving, to launch new open-access journals, and to help an open-access journal system become economically self-sustaining. While the Open Society Institute's commitment and resources are substantial, this initiative is very much in need of other organizations to lend their effort and resources.

We invite governments, universities, libraries, journal editors, publishers, foundations, learned societies, professional associations, and individual scholars who share our vision to join us in the task of removing the barriers to open access and building a future in which research and education in every part of the world are that much morefree to flourish. Submitting a paper to CDJ is free of charges. In addition, CDJ has not have article processing charges.

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The approval of the ethic committee, statement on the adherence to international guidelines mentioned above and that the patients'informed consent is obtained should be indicated in the "Materials and Methods" section and is required for case reports whenever data/media used could reveal identity of the patient. The declaration of the conflict of interest between authors, institutions, acknowledgement of any financial or material support, aid is mandatory for authors submitting manuscript and the statement should appear at the end of manuscript. Reviewers are required to report if any potential conflict of interest exists between reviewer and authors, institutions.

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e-ISSN 2146-2852

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Cumhuriyet Dental Journal: 2021; 24(3)
e-ISSN 2146-2852
Doi:10.7126/cumudj.815788

Original research

KNOWLEDGE AND ATTITUDES OF PAEDIATRIC DENTISTS IN TURKEY REGARDING CONE BEAM COMPUTED TOMOGRAPHY (CBCT)

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*Emre Cesur[2]
Tuğba Bezgin[3]
Kaan Orhan[4]

ABSTRACT

Objectives: The use of cone-beam computed tomography (CBCT) in dental practice has increased in recent years due to the lower radiation dose, fast scanning time, and superior imaging quality compared to conventional computed tomography (CT). Although CBCT is frequently preferred for the evaluation of the craniofacial region in areas such as orthodontics, orthognathic surgery, head trauma, and implant surgery, its use is still limited in paediatric dentistry. The aim of this study was to evaluate the knowledge and attitudes of paediatric dentists in Turkey regarding CBCT.

Materials and Methods: A self-administered anonymous questionnaire consisting of 17 questions was completed by 273 volunteer participants. Of these, 135 were postgraduate students in a paediatric dentistry program and 137 were PhDs or specialists. Relationships between categorical variables were evaluated using chi-square test.

Results: 237 (86.8%) of the 273 participants stated that they were knowledgeable about CT/CBCT, but 81% of the respondents said the information they received during dental education was insufficient. The only significant relationship among the studied variables was between the participants' self-rated knowledge about the use of CT and/or CBCT for the dentomaxillofacial region and the institution in which they worked (P =.001).

Conclusions: Although our results indicate that paediatric dentists have high levels of knowledge and positive attitudes towards digital imaging techniques and CBCT, it would be beneficial to update the dental curricula and enable dental students to gain practical experience in addition to theoretical knowledge.

Keywords: Pediatric dentistry, CBCT, digital imaging methods, knowledge, attitude.

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Received : 24.10.2020
Accepted : 17.05.2021

How to Cite Orhan AI, Cesur E, Bezgin T, Orhan K. Knowledge and Attitudes of Paediatric Dentists in Turkey Regarding Cone Beam Computerized Tomography (CBCT). Cumhuriyet Dent J 2021;24:3:206-215.
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INTRODUCTION

The use of cone-beam computed tomography (CBCT) in dental practice has increased in recent years for various reasons, ranging from the lower radiation dose and fast scanning time to the fact that it provides detailed images with fewer artefacts compared to conventional tomography. Although CBCT is frequently preferred for the evaluation of the craniofacial region areas such as orthodontics, orthognathic surgery, head trauma, and implant surgery, its use in paediatric dentistry is still limited.[1,2]

Dhillon and Kalra[1] stated that CBCT could be used in paediatric dentistry for applications such as evaluation of dental development, assessment of impacted/supernumerary teeth, evaluation of root morphology in endodontic procedures, detection of post-traumatic root fracture, and diagnosis of caries and lesions involving hard tissue. In contrast, Theodorakou *et al.*[3] recommended using CBCT in the paediatric population only when absolutely necessary and after optimization.

CBCT involves a higher radiation dose than panoramic and intra-oral imaging but a lower dose compared to conventional tomography.[4,7] When evaluating an individual's radiation risk, the effective doses recommended by ICRP (International Commission on Radiological Protection) should be kept in mind. Using anthropometric phantom models, Theodorakou *et al.*[3] determined that the lowest effective dose was achieved by selecting the smallest fields of view (FOV) and using small patient size settings. Vertical and horizontal collimation was recommended for CBCT units with only large FOV.

Despite the advantages mentioned above, ensuring the effective use of CBCT in dental practice is still crucial. Previous studies aiming to measure the attitudes and knowledge levels regarding the use of CBCT among students of dentistry and its various subfields revealed the need to improve knowledge and practices.[8-10] Especially considering the effects of X-rays on the DNA of growing individuals, the paediatric population deserves the most caution concerning the use of CBCT. Therefore, this study aimed to evaluate knowledge and attitudes regarding the use of CBCT among paediatric dentists in Turkey.

MATERIALS AND METHODS

A questionnaire including questions about demographic information as well as general knowledge about and attitudes toward CBCT was prepared by the researchers (A.I.O., K.O., E.C.). Before starting the study, the questionnaire was evaluated for comprehensibility and consistency by 3 experts (2 paediatric dentists and 1 biostatistician). From the initial 20 questions, 3 items evaluated as biased or confusing by the expert panel were omitted, resulting in a final questionnaire comprising 17 questions (Figure 1).

1. Age:

2. Gender: Female ( ) Male ( )

3. Title:

( ) Post-graduate student on a pedodontics program
( ) PhD or specialist

4. Institution:

( ) University
( ) Community oral and dental health center
( ) Private Practice
( ) Other:

5. How many years have you been working as a pediatric dentist (including the first year of the post-graduate pedodontics program)?

6. Do you use digital dental imaging methods?

( ) Yes ( ) No

7. Please indicate your reasons for using digital imaging methods (multiple choice is allowed):

( ) Reduced radiation dose
( ) Reduced time to obtain images
( ) Ease of the measurement and evaluation on images
( ) Reduced enviromental harm
( ) Other:

8. If you don't use digital imaging methods, please indicate the reasons (multiple choice is allowed):

( ) Expensive
( ) Poor image quality
( ) Difficulty to perform
( ) Lack of knowledge
( ) Lack of access to the necessary equipments in my institution
( ) Other:

9. Do you have information about CT and/or CBCT use for dentomaxillofacial region?

( ) Yes ( ) No

10. If you have information about CBCT, how did you obtain that information (multiple choice is allowed)?

( ) University education
( ) Seminar/ course/ congress
( ) Internet
( ) Written scientific documents
( ) Other:

11. Do you believe that you received sufficent information about CBCT during your dental education?

( ) Yes ( ) No

12. Do you have access to CBCT at your institution?

( ) Yes ( ) No

13. With the developing technology, do you think that the use of CBCT will become widespread in the field of pedodontics?

( ) Yes ( ) No ( ) No idea

14. Which method would you prefer for the visualization of the head and neck region in children if necessary?

( ) CBCT ( ) CT ( ) No idea

15. Please list the advantages of CBCT over CT from the most important (1) to the least important (4):

( ) Reduced radiation dose
( ) More affordable
( ) Achieving images in a shorter time
( ) Less space requiring
( ) Other:

16. Do you think the use of CBCT is necessary in the field of pedodontics?

( ) Yes ( ) No ( ) No idea

17. If you do, in what situations do you prefer to use CBCT in children?

( ) Evaluation of tooth development
( ) Diagnosis of caries and/or periapcal lesions
( ) Evaluation of impacted and/ or supernumerary teeth
( ) Evaluation of root resorbtions or root fractures
( ) Diagnosis and follow-up in traumas associated with dentoalveolar region
( ) Evaluation of defects and pathologies associated with maxilla and/or mandible
( ) Evaluation of deformities and syndromes involving the craniofacial region
( ) Other:

Figure 1. The questionnaire used in the survey.

Ethical approval for the study was obtained from İstanbul Medipol University Ethics Committee (IRB number: 973).

The survey was conducted between July 2019 and December 2019. The self-administered, anonymous questionnaire was sent by one of the researchers (T.B.) via e-mail and/or website (www.surveymonkey.com) to 490 paediatric dentists who were members of the Turkish Association of Paediatric Dentistry. Of those invited, 290 (59%) started the survey, but only the responses of the 273 participants (56%, mean age: 32 ± 7.5 years) who continued the questionnaire to the end were included in the analysis.

The evaluation of the participants' responses was carried out by all of the researchers. As participants were asked to answer the questions they wanted, the items they skipped were not taken into consideration in the evaluation. All questionnaires were recorded on the website (www.surveymonkey.com) and stored until evaluation. Data analyses were done using the IBM SPSS version 21 (SPSS Inc. Chicago, Illinois, USA) statistics package program. Relationships between categorical variables were evaluated using chi-square test. The significance level was accepted as P<.05.

RESULTS

Analysis of the demographic data showed that the respondents' mean age was 32 ± 7.5 years (Question 1 [Q1]; Table 1), and most respondents (87.5%) were women (Q2) (Table 2). Postgraduate students accounted for 49.6% of the participants (n=135), while the rest were PhDs or specialists (Q3). Most of the participants were employed in university hospitals (76.5%), followed by private practice (15.1%) and community oral and dental health centres (8.5%) (Q4; Table 2). The participants' experience in the field of paediatric dentistry was 7.2 ± 7.4 years on average, ranging from 36 years to the first year of a postgraduate paediatric dentistry program (Q5; Table 1)

Table 1. Distribution of age (Q1) and years of experience as a paediatric dentist (Q5)

	n	Mean	Median	Min.	Max.	±SD
Age (Q1)	273	32.0	29	21	60	±7.5
How many years have you been working as a paediatric dentist (including the first year of the post-graduate pedodontics program)? (Q5)	273	7.2	4	0	36	±7.4

Min: Minimum, Max: Maximum, SD: Standard deviation

Regarding the attitudes of the paediatric dentists towards the use of digital dental imaging methods, 91.5% stated that they utilized digital methods (Q6; Table 3). The most common reasons cited for using digital imaging were faster results (87.5%), ease of measurement and evaluation of images (82.3%), lower radiation dose (75.4%), less environmental harm (51.6%), and other (2.4%) (Q7; Table 2). Participants who marked "other" for Q7 explained their reasons as being able to visualize the images in 3 dimensions (n=1), that it was used in the institution where they work (n=2), and the high quality images (n=3). When participants who did not use digital imaging methods were asked why not, all (100%) cited the lack of access to the necessary equipment in their institutions. This option was followed by cost (95.7%), finding it difficult to perform (52.2%), lack of knowledge (47.8%), and poor image quality (39.1%) (Q8; Table 2).

Table 2. Demographic distribution of the participants and the distribution of their responses to Q7, Q8, and Q17.

		n	%
Gender (Q2)	Male	34	12.5
	Female	238	87.5
	Total	272	100.0
Title (Q3)	Post-graduate student on a pedodontics program	135	49.6
	PhD or specialist	137	50.4
	Total	272	100.0
Institution (Q4)	University	208	76.5
	Community oral and dental health centre	23	8.5
	Private Practice	41	15.1

Other	0	0
Total	272	100.0
Please indicate your reasons for using digital imaging methods (multiple choices are allowed) (Q7)		
Reduced radiation dose	187	75.4
Shorter imaging time	217	87.5
Ease of measurements and evaluation of images	204	82.3
Reduced environmental harm	128	51.6
Other	6	2.4
If you do not use digital imaging methods, please indicate the reasons (multiple choices are allowed) (Q8)		
Expensive	22	95.7
Poor image quality	9	39.1
Difficulty to perform	12	52.2
Lack of knowledge	11	47.8
Lack of access to the necessary equipment in my institution	23	100.0
If you do, in what situations do you prefer to use CBCT in children? (Q17)		
Evaluation of defects and pathologies associated with the maxilla and/or mandible	210	93.8
Evaluation of impacted and/or supernumerary teeth	196	87.5
Diagnosis and follow-up of traumas associated with the dentoalveolar region	167	74.6
Evaluation of deformities and syndromes involving the craniofacial region	167	74.6
Evaluation of root resorption or root fractures	146	65.2
Evaluation of tooth development	26	11.6
Diagnosis of caries and/or periapical lesions	17	7.6
Other	4	1.8

When the paediatric dentists were asked to rate their knowledge of CBCT, 237 (86.8%) of the 273 participants stated that they were knowledgeable about CBCT/CT (Q9; Table 3). Reported sources of this information included university education (68.4%), written scientific documents (14.8%), seminars/courses/congresses (12.1%), the internet (2.7%), and other (2%; observation, during PhD education, from experienced users) (Q10; Table 3).

Although most participants stated that they learned about CBCT during their dental education, 81% replied negatively when asked if sufficient information was provided during dental education (Q11; Table 3). While 64.9% of the participants (n=176) reported that they had access to CBCT in the institution they worked in, the remaining 95 (35.1%) stated that they did not (Q12; Table 3).

Table 3: Distribution of the participants' responses to Q6, Q9, Q10, Q11, Q12, Q13, Q14, and Q16.

		n	**%**
Do you use digital dental imaging methods? (Q6)	*Yes*	248	91.5
	No	23	8.5
	Total	271	100.0
Do you have information about CT and/or CBCT use for dentomaxillofacial region? (Q9)	*Yes*	237	86.8
	No	36	13.2
	Total	273	100.0
If you have information about CBCT, how did you obtain that information (multiple choices are allowed) (Q10)	*University Education*	175	68.4
	Seminar/ course/ congress	31	12.1
	Internet	7	2.7
	Written scientific documents	38	14.8
	Other	5	2.0
	Total	256	100.0
Do you believe that you received sufficient information about CBCT during your dental education? (Q11)	*Yes*	51	19.0
	No	218	81.0
	Total	269	100.0
Do you have access to CBCT at your institution? (Q12)	*Yes*	176	64.9
	No	95	35.1
	Total	271	100.0

With the developing technology, do you think that the use of CBCT will become widespread in the field of pedodontics? (Q13)	*Yes*	193	71.2
	No	38	14.0
	Not sure	40	14.8
	Total	271	100.0
Which method would you prefer for the visualization of the head and neck region in children if necessary? (Q14)	*CBCT*	225	82.4
	CT	17	6.2
	Not sure	31	11.4
	Total	273	100.0
Do you think the use of CBCT is necessary in the field of pedodontics? (Q16)	*Yes*	224	82.1
	No	20	7.3
	Not sure	29	10.6
	Total	273	100.0

When the participants currently practicing in the field of paediatric dentistry were asked whether CBCT use will be widespread in their fields, 193 participants answered "yes" (71.2%), 38 participants said "no" (14%), and the other 40 participants (14.8%) answered "not sure" (Q13, Table 3). When asked which method they would prefer for imaging of the head and neck in children if necessary, 225 participants (82.4%) selected CBCT, 17 (6.2%) chose CT, and 31 participants (11.4%) answered "not sure" (Q14; Table 3).

When participants were asked to list the advantages of CBCT over CT from the most important (1) to the least important (4), 218 participants (85.5%) ranked the reduced radiation dose first, while the second most selected option was shorter imaging time, with 67.2%. The third and fourth most often marked options were lower cost and less space required, respectively (Q15, Table 4).

Table 4. Distribution of the participants' responses to Q15.

		1		**2**		**3**		**4**		**Total**
		n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	
Please list the advantages of CBCT over CT from the most important (1) to the least important (4) (Q15)	*Reduced radiation dose*	218	85.49	12	4.71	3	1.18	0	0.00	228
	More affordable	6	2.47	48	19.75	129	53.09	50	20.58	233
	Shorter imaging time	8	3.16	170	67.19	50	19.76	8	3.16	236
	Requires less space	5	2.08	5	2.08	55	22.92	162	67.50	227
	Other	2	1.23	4	2.47	1	0.62	14	8.64	21
	Not sure	11	6.63	1	0.60	2	1.20	2	1.20	16

Most participants (n=224, 82.1%) considered CBCT use necessary in paediatric dentistry, but 20 (7.3%) stated that it was not necessary and 29 (10.6%) reported having no opinion on the subject (Q16, Table 3). When asked in which situations they preferred to use CBCT in children, the most selected option was evaluation of defects and pathologies associated with the maxilla and/or mandible (n=210, 93.8%), while the least selected option was diagnosis of caries and/or periapical lesions (n=17, 7.6%). Individuals who selected the "other" option stated that they used it to determine the prognosis of apexification and apexogenesis treatments (Q17, Table 2).

When inter-variable dependence was assessed using chi-square test between all questions, the only significant relationship detected was between responses to "Are you knowledgeable about the use of CT and/or CBCT for the dentomaxillofacial region?" and the participants' institution. Employees of community oral and dental health centres responded "yes" to this question significantly less frequently (60.9%) (P = .001) (Table 5).

Table 5. Cross-evaluation of Q4 and Q9. (Chi-square test; P=.05*, P=.01**, P=.001***)

		Institution (Q4)								Chi-Square	
		University		*Community oral and dental health centre*		*Private Practice*		*Total*			
		n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**Chi-Square**	**P**
Do you have information about CT and/or CBCT use for the dentomaxillofacial region? (Q9)	*Yes*	189	90.9	14	60.9	33	80.5	236	86.8		
	No	19	9.1	9	39.1	8	19.5	36	13.2	17.8	.0001***
	Total	208	100.0	23	100.0	41	100.0	272	100.0		

DISCUSSION

With advancing technology, CBCT has been widely adopted in nearly all areas of dentistry and is the most accepted 3D imaging method in current practice. As its use increases, the radiation-related effects of CBCT raise concerns about its use in the paediatric population. Aps[2] proposed three basic principles to protect children from unnecessary radiation. Radiographs should not be taken from paediatric patients unless there is an absolute indication, (justification principle), the radiation dose should be minimized (limitation principle), and it should be attempted to obtain the most appropriate diagnostic images (optimization principle). Oenning *et al*.[6] launched the DIMITRA (dentomaxillofacial paediatric imaging: an investigation toward low-dose radiation-induced risks) project and advocated the necessity to move from the principles of ALARA (as low as reasonably achievable) and ALADA (as low as diagnostically acceptable) toward ALADAIP (as low as diagnostically acceptable being indication-oriented and patient-specific) principles. Knowing about digital imaging methods and CT/CBCT is especially important for paediatric dentists and orthodontists, as they work more with the paediatric population. To our knowledge, there is only one study evaluating attitudes towards CBCT among paediatric dentists.[11] Therefore, in this study we aimed to investigate this issue in detail.

Most of the paediatric dentists included in our study stated that they used digital imaging methods. The reasons most commonly given for their use were the faster imaging and ease of measurement and image analysis, consistent with a previous study.[11] However, among individuals who did not use digital imaging, the main factor was identified as lack of access to the necessary equipment in their institution. Lavanya *et al*.[9] reported that limited CBCT use was associated with limited access to CBCT in the workplace, while Kamburoğlu *et al*.[8] reported in their survey study of Turkish dentistry students that their lack of knowledge about CBCT may be related to technical deficiencies in their institution and lack of practical experience. Our findings were consistent with these previous studies. The fact that academicians in our study reported greater knowledge of CBCT may be due to having more practical experience.

The majority of the participants (86.8%) answered positively when asked if they were knowledgeable about CT/CBCT. This result is higher than that reported by Eren Giray *et al*.[11], who stated that 30% of the paediatric dentists in their study did not have knowledge regarding CBCT. This discrepancy may be due to the inclusion of paediatric dentists from different countries in Eren Giray's study. The authors noted that more Turkish paediatric dentists reported having knowledge of CBCT than paediatric dentists from other countries.[11] Most of the participants in the present study stated that they learned this information during their university education. However, a significant number of participants still reported that they did not obtain sufficient information on this subject during dental training. The results of previous studies evaluating the competence of dentists, dentistry students, and orthodontists in education also demonstrated the inadequacy of dentistry education in this regard.[8-10,12] These results show that dental students should be provided more theoretical as well as practical information regarding CBCT.

In our evaluation of the relationships between variables assessed in the questionnaire, we observed that knowledge regarding the use of CT and/or CBCT differed significantly based on the participants' institutions. Self-reported knowledge was lowest among paediatric dentists practicing in community oral and dental health centres and highest among paediatric dentists working in universities. Geçgelen Cesur *et al.*[10] reported that CBCT use is more common at universities because of their status as 'leading-edge' technological institutions. Therefore, to increase knowledge of CBCT among paediatric dentists, undergraduate dentistry curricula should be modified to maintain, expand, and update education on this topic and provide access to this technology. The responses to our survey showed that most paediatric dentists use CBCT when evaluating the head and neck region and they think CBCT is necessary in the dentistry subfields, but our results also indicate that attained knowledge levels are not adequate.

One of the biggest advantages of CBCT in paediatric patients is that it reduces children's anxiety due to the decreased scan time. Furthermore, compared to conventional CT, CBCT images are higher quality with less distortion. However, the most important advantage of CBCT is the lower radiation dose.[2] Although the dose is still substantially higher than from intra-oral radiographs, a 51% to 96% reduction has been reported compared to conventional CT.[4] The paediatric dentists who participated in the current study prioritized the lower radiation dose and faster results as the main reasons they preferred CBCT. That said, the safe use of CBCT in children depends on it being applied for the correct indications and using the correct technique.

There are many reports and studies on the use of CBCTs in dentistry. In their review of studies documenting the use of CBCT in different areas of dentistry, de Vos *et al.*[13] reported that the use of CBCT was more common in the fields of maxillofacial surgery (41%), dentoalveolar problems (29%), orthodontics (16%), and dental implantology (11%). Its ability to provide detailed information about the anatomical structures of the region explains the popularity of CBCT in maxillofacial surgery in particular.[14-16] In addition, it is commonly used in orthodontics for guiding impacted teeth into alignment, evaluating syndromes/deformities involving the maxillofacial area, examining the airway, and planning orthognathic surgery and measurements of craniofacial morphology.[17,18] Although the use of CBCT for the dentoalveolar region is limited, some publications have described its use in caries evaluation.[19,20] According to Tyndall and Rathore[19], CBCT can also be beneficial in the examination of periodontal bone and endodontic applications. In a review evaluating the use of CBCT in the field of endodontics, it was stated that CBCT would be useful in preoperative procedures such as the evaluation of periapical pathologies, root fractures, and resorption and/or postoperative evaluations. In their systematic review, Horner *et al.*[7] stated that the use of CBCT for caries detection is not indicated in the paediatric population, but it may be indicated in cases such as acute infections and root resorption and fractures, where conventional radiographs are inadequate. According to the researchers, CBCT is indicated for the evaluation of developmental deformities and large bone defects. İşman *et al.*[21] reported that the most common indications of CBCT in the paediatric population and adolescents were dentomaxillofacial anomalies and localization of buried teeth.

When the paediatric dentists participating in the present study were asked for which purposes they used CBCT, the most common responses were evaluation of defects and pathologies associated with the maxilla and/or mandible, evaluation of impacted and/or supernumerary teeth, and the diagnosis and follow-up of trauma patients, while the least selected options were diagnosis of caries and/or periapical lesions and evaluation of tooth development. These findings are consistent with the results of previous studies, suggesting that paediatric dentists are knowledgeable about the indications of CBCT. Paediatric dentists are the front-line clinicians who diagnose and treat patients with trauma and dental/alveolar pathologies. Therefore, they should have current knowledge of the best diagnostic tools to help their patients. Although the results of this study showed

that paediatric dentists in Turkey generally know the correct indications for CBCT, this knowledge should be increased.

CONCLUSIONS

CBCT is one of the most important methods in current dental radiology due to advantages such as lower radiation dose and improved image quality. Our results indicate that paediatric dentists in Turkey have high levels of knowledge and positive attitudes regarding digital imaging techniques and CBCT. However, their knowledge could be further increased by updating the dental curriculum and providing practical experience in addition to theoretical knowledge. In addition, continuing education programs should be developed to inform and increase accessibility for paediatric dentists not working at universities.

ACKNOWLEDGEMENTS

None to declare

CONFLICT OF INTEREST

All authors declare no conflict of interest.

FUNDING

None to declare

ÖZ

Amaç: *Konik ışınlı bilgisayarlı tomografilerin (KIBT) diş hekimliğinde kullanımı son yıllarda oldukça yayılmaktadır. KIBT; ortodontide, ortognatik cerrahide, kafa travmalarında ve implant cerrahisinde, kraniyofasiyal bölgenin değerlendirilmesinde oldukça sık kullanılsa da, pedodontide kullanımı halen sınırlıdır. Bu çalışmanın amacı, Türkiye'de pedodontistler arasında KIBT kullanımına yönelik bilgi ve tutumun değerlendirilmesidir.* ***Gereç ve Yöntemler:*** *Bu çalışma 273 gönüllü katılımcı ile gerçekleştirilmiş olup, katılımcılardan kendi kendine yönetilen ve 17 sorudan oluşan bir anketi isimsiz olarak doldurmaları istenmiştir. Katılımcıların 135'ini doktora/uzmanlık öğrencileri oluştururken, 137'si pedodonti doktoru ve/veya uzmanıdır. Bu araştırmada, kategorik değişkenler arası ilişkilerin incelenmesinde chi-kare testi kullanılmıştır.* ***Bulgular:*** *237 (%86,8) katılımcı BT/KIBT hakkında bilgi sahibi olduğunu belirtmiştir. Katılımcıların %81'i diş hekimliği eğitimi sırasında aldıkları bilginin yetersiz olduğunu bildirmiştir. Değişkenler arası bağımlılık değerlendirildiğinde, anlamlı tek fark, katılımcıların BT/KIBT kullanımı konusunda bilgi sahibi olup olmadıklarına dair soru ile çalıştıkları kurum arasında bulunmuştur (P =,001).* ***Sonuçlar:*** *Çalışmamızın sonuçları, pedodontistlerin dijital görüntüleme yöntemleri ve KIBT kullanımına dair yüksek bilgiye sahip olduklarını ve olumlu tutum gösterdiklerini saptasa da, diş hekimliği müfredatının güncellenmesi ve öğrencilerin teorik bilginin yanı sıra pratik tecrübe edinmelerinin sağlanması, KIBT kullanımının yaygınlaşması ve yerinde kullanımının sağlanması açısından faydalı olacaktır.* ***Anahtar kelimeler:*** *Pedodonti, KIBT, dijital görüntüleme metodlaro, bilgi, tutum.*

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Cumhuriyet Dental Journal: 2021; 24(3)
e-ISSN 2146-2852
Doi:10.7126/cumudj.887101

Original research

REAL-TIME PCR TO IDENTIFY PORCINE DNA IN PROSTHODONTIC MATERIALS

ABSTRACT

Objectives: This study aimed to identify porcine DNA in prosthodontic materials using Real-Time PCR.

Materials and methods: Eighteen prosthodontic materials: three irreversible hydrocolloids, three elastomers, three denture adhesives, three soft denture linings, three temporary crowns, and three denture bases materials were used as the samples. It was conducted in two stages. First, extraction of dental material's DNA and the second was Real-Time PCR analysis based on amplification curve and Ct score in yellow and green channel.

Results: The sample analysis based on green channel demonstrated that all materials did not contain porcine DNA, however, 11 of 18 samples (DA-01, DA-02, DA-03, SDL-02, DB-01, DB-02, TC-03, IH-02, EM-01, EM-02, and EM-03) contained vertebrate DNA.

Conclusions: All prosthodontic materials tested were not containing porcine. The halal statues of the materials were still unclear.

Keywords: Pcr, Porcine DNA, prosthodontic materials.

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Received : 26.02.2021
Accepted : 15.05.2021

How to Cite Hutasoit I, Ramadhani Munthe F, Wiyah M, Nasution H, Pranata AW. Real-Time PCR to Identify Porcine DNA in Prosthodontic Materials. Cumhuriyet Dent J 2021;24:3:216-223.
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INTRODUCTION

Indonesia is the biggest Muslim-populated country worldwide where more than 200 million Indonesian people are Muslims.[1] Currently, halal product demands increase globally not only for food and beverages but also encompass pharmaceutical products such as vaccines, medicines, and dental materials.[2] A positive response regarding halal problems, primarily regarding food, medicines, and cosmetics, has been projected by the Indonesian government by publishing several legislations, one of which is Law No. 33 of 2014 on Halal Product Guarantee (UUJPH).[3,4]

Halal Product Guarantee (UUJPH) underlines that all products entering, circulating, and traded in Indonesia should have halal certificates. However, these regulations were made partially, inconsistently, overlapping, and non-systematically. Hence, technically, they could not be enforced as robust legal protection and specifically bound the halal product matter to producers (business actors) and provide a guarantee to customers. It contributed to the absence of legal certainty guarantee regulating halal products, whereas the need for halal product guarantee is urgent, particularly concerning customer protection and global trade scenes. Some of the products maybe contained non-halal product without listed by manufacturer, doesn't have halal certificate, and unclear information about ingredients of the product.[5,6] Halal status of dental materials traded in Indonesia remains to be doubtful if there is no guarantee from producers by the halal logo.[7]

Technological advances in the halal analysis field continued improving to detect halal materials contaminated by haram materials. Several plausible tests to distinguish various porcine content in a product are by identifying proteins, fats, and Deoxyribonucleic Acid (DNA).[8,9] The DNA analysis based on DNA can use conventional polymerase chain reaction (PCR) and Real-Time PCR. Deoxyribonucleic acid is nucleic acid polymers systematically arranged, a stable molecule in the extraction process found in all cell types of animals with identical genetic information. Deoxyribonucleic acid analysis in a product can be performed using Real-Time PCR by amplification DNA.[10,11,12] The advantages of Real-Time PCR are the samples can be analyzed in a small amount and produced rapidly and accurately.[13,14,15]

Studies concerning halal tests on dental materials have been conducted. In 2016, a study was conducted on periodontal materials. In the study, the researchers argued that medication and non-halal material usage were the requirements during emergency situations and only in particular conditions and limitations. As an alternative, BioGide®, Emdogain®, and Bio-Oss® products can be used in periodontal surgery treatments because they are halal medicines. However, the study suggested that periodontal specialists and fikh experts should improve their collaborations to understand fikh principles to be consulted in each legal issue, especially in modern dental treatments.[16] In 2017, a study was carried out on gelatin rapid testing in dental materials using the Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR). The study utilized 42 samples classified into homeostatic agents, restoration materials, prophylaxis materials, oral surgery materials, dental impressions, anesthetic agents, oral rinse, prosthodontic materials, and preventive materials. The study results asserted that none of the 42 samples were identified containing gelatin, with most samples were categorized as homeostatic agents. Several homeostatic agents used in dental services were sourced from bovine and porcine gelatin.[17] In 2017, a halal test on dental materials in orthodontics on 21 samples was performed. The results demonstrated no dental materials in orthodontics displaying a 100% resemblance with porcine gelatin spectrum tested. However, a further test should be employed for the halal test for these materials.[18] In 2020, a study on porcine gelatin identification on dental materials using the combination of Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR) and Chemometrics showed that four of 49 dental materials used in dental hospitals and clinics contained gelatin. One of them tended to resemble porcine gelatin, while the other three could not be

identified. The gelatin in the product acted as an emulsifier and added in a small amount.[19]

Dental materials are an integral part of every dental service.[21] Dental material is a study regarding materials used in dentistry which can be categorized into four classes, i.e., metals, ceramics, composites, and polymers.[21] Dental material studies, particularly in prosthodontics, have been studied vastly. However, to date, there are only limited identifications on porcine content in prosthodontic materials.[21,22] Therefore, the purpose of this study was to identify the porcine DNA in prosthodontic materials using Real-Time PCR.

MATERIALS AND METHODS

The study was carried out in Halal Science Center of Institut Pertanian Bogor. Three irreversible hydrocolloids, three elastomers, three denture adhesives, three soft denture linings, three temporary crowns, and three denture bases materials (n=18) were examined (Table 1).

Table 1. List of Samples

No	Code	Category	Manufacturer Country
1	DA-01	Denture adhesives	United Kingdom
2	DA-02	Denture adhesives	Austria
3	DA-03	Denture adhesives	Singapore
4	SDL-01	Soft denture lining	Japan
5	SDL-02	Soft denture lining	Germany
6	SDL-03	Soft denture lining	Germany
7	TC-01	Temporary crown	USA
8	TC-02	Temporary crown	Korea
9	TC-03	Temporary crown	USA
10	DB-01	Heat polymerized acrylic resin	Brazil
11	DB-02	Heat polymerized acrylic resin	Japan
12	DB-03	Heat polymerized acrylic resin	China
13	IH-01	Irreversible hydrocolloid	Italy
14	IH-02	Irreversible hydrocolloid	Japan
15	IH-03	Irreversible hydrocolloid	USA
16	EM-01	Elastomer	Japan
17	EM-02	Elastomer	Korea
18	EM-03	Elastomer	USA

Approval of the Scientific Research Committee of Universitas Sumatera Utara with ID: 205/KEP/USU/2020.

Each sample consists of 20 milligram/ material. The sampling technique used was the purposive sampling based on predetermined inclusion and exclusion criteria. All samples were analyzed using Real-Time PCR (Rotor-Gene Q D-40724, Qiagene, Hilden, Germany). The study was divided into two stages. First, extraction of dental material's DNA and the second was Real-Time PCR analysis based on amplification curve and Ct score in yellow and green channel.[23]

Before entering the initial stage, pre-running was carried out. All samples were extracted using EasyFast™ Extra Pharma I (Ph.Ext1, Progenus s.a., Gembloux, Belgium). DNA extraction products were detected using Kit Progenus EasyFast™ Pharma Pig/Suidae detection & quantification kit (EFPig50/100, Progenus s.a., Gembloux, Belgium) and analyzed using Real-Time PCR.[23]

The initial DNA extraction stage aimed to obtain a DNA solution. After obtaining DNA solution, the samples were added with a buffer on 65°C for 10 minutes, added with precipitation buffer in ice for 5 minutes, centrifuged for 2 minutes at 12.000 rpm, added with binding buffer and centrifuged for 1 minute at 12.000 rpm, added with washing buffer I at 12.000 rpm, heated in elution buffer on 65°C, and incubated on 65°C at 12.000 rpm. The second stage after obtaining the DNA solution was analyzed with repetition of 40 cycles. This stage was carried out using MIX solution (EFPig50, Progenus s.a., Gembloux, Belgium), Nuclease free water (EFPig50, Progenus s.a., Gembloux, Belgium) as the negative control, External Positive Control/EPC solution (EFPig50, Progenus s.a., Gembloux, Belgium) as the positive control with an initial temperature of 95°C,

followed by denaturation temperature of 95°C, and annealing temperature of 60°C. Data analyses were then carried out based on VIC (yellow channel)/ vertebrate and FAM (green channel)/porcine reporters. The yellow channel was used to detect the presence or absence of vertebrate DNA in the sample while the green channel was used to detect the presence or absence of porcine DNA in the sample.[23]

The sample was tested positive for porcine DNA if there was a Ct value; the sample curve line was above the threshold line on the green channel amplification graph and follows the positive control line on the amplification curve. The program of Real-Time PCR was set and the tubes containing the sample and control were inserted into the machine then run according to the Real-Time PCR program.[23] (Table 2)

Table 2. Set Program of RT – PCR

Step	**Time**	**Temperature**	**Comment**
Initial PCR activation step	5 min	95°C	Activation of HolStar Taq Plus DNA polymerase
3 Step cycling:			
Denaturation	15 s	95°C	
Annealing	15 s	60°C	Data collection of 60°C
Extension	10 s	72°C	
Number of cycles	40		
Detection	**Reporter**	**Excitation/ emission**	**Channel**
Target	FAM	495/520 nm	Green
Internal control	MAX	524/ 557 nm	Yellow

* FAM (fluorescein amidets)
MAX (Master Mix)

RESULT

The study results demonstrated that no porcine DNA was identified in the samples, where DNA amplification results based on the FAM (green channel) reporter did not report curve elevation on all tested samples, and no Ct values with porcine primers were found on each sample (Table 3). However, several tested dental materials contained vertebrate DNA.

Table 3. Ct value of sampels using yellow channel (identification of vertebrate DNA)

No	**Flour**	**Target**	**Samples**	**Ct Values**
1	VIC	Vertebrate	Negative Control	32,88
2	VIC	Vertebrate	Positive Control	30,08
3	VIC	Vertebrate	DA-01	39,53
4	VIC	Vertebrate	DA-02	32,41
5	VIC	Vertebrate	DA-03	35,17
6	VIC	Vertebrate	SDL-02	37,62
7	VIC	Vertebrate	DB-01	32,66
8	VIC	Vertebrate	DB-02	34,33
9	VIC	Vertebrate	TC-03	35,89
10	VIC	Vertebrate	IH-02	35,66
11	VIC	Vertebrate	EM-01	33,64
12	VIC	Vertebrate	Negative Control	
13	VIC	Vertebrate	Positive Control	28,11
14	VIC	Vertebrate	EM-02	34,08
15	VIC	Vertebrate	EM-03	33,51

* VIC (victoria)
FAM (fluorescein amidets)

The present study showed that 11 of 18 samples (DA-01, DA-02, DA-03, SDL-02, DB-01, DB-02, TC-03, IH-02, EM-01, EM-02, and EM-03) contained vertebrate DNA, as presented in Table 4.

Table 4. Contents of Vertebrate DNA

No	Code	Category	VIC
1	DA-01	Denture adhesives	+
2	DA-02	Denture adhesives	+
3	DA-03	Denture adhesives	+
4	SDL-01	Soft denture lining	-
5	SDL-02	Soft denture lining	+
6	SDL-03	Soft denture lining	-
7	TC-01	Temporary crown	-
8	TC-02	Temporary crown	+
9	TC-03	Temporary crown	-
10	DB-01	Heat polymerized acrylic resin	+
11	DB-02	Heat polymerized acrylic resin	+
12	DB-03	Heat polymerized acrylic resin	-
13	IH-01	Irreversible hydrocolloid	-
14	IH-02	Irreversible hydrocolloid	+
15	IH-03	Irreversible hydrocolloid	-
16	EM-01	Elastomer	+
17	EM-02	Elastomer	+
18	EM-03	Elastomer	+

* + (Vertebrate DNA)
- (No vertebrate DNA)

Detection results based on the VIC reporter (yellow channel) showed that DA-01 (Ct 39,53), DA-02 (Ct 32,41), DA-03 (Ct 35,17), SDL-02 (Ct 37,62), DB-01 (Ct 32,66), DB-02 (Ct 34,33), TC-03 (Ct 35,89), IH-02 (Ct 35,66), EM-01 (Ct 33,64), EM-02 (Ct 34,08) and EM-03 (Ct 33,55) have significantly elevated curves over the threshold line (Figure 1).

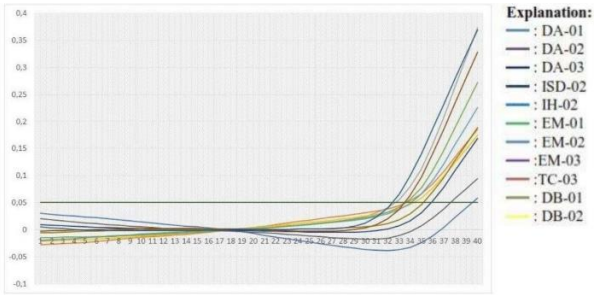

Figure 1. Sample amplification based on the VIC reporter (vertebrate detection)

These Ct values were obtained from the control positive curve passing over the threshold line and the number of cycle.

DISCUSSION

The halal status of prosthodontics materials is a necessity for Muslim patient. In the present study, porcine DNA was not detected in all samples, where the DNA amplification based on FAM reporter (green channel) did not discover curve elevation and Ct values with a porcine primer on each sample. However, 11 samples were having vertebrate DNA.

Amplifications using PCR Real-Time in the study was conducted for 40 cycles to obtain accurate results. During the amplification, the probe's fluorescence signal increased, and therefore, the signal significantly increased over the threshold line. This value was recorded as Ct (cycle threshold), where the larger the target DNA initial quantity, the lower the Ct value, and vice versa. Ct values aimed to discover the PCR Real-Time validity according to the detection parameter

and discovered whether study samples contain vertebrate or porcine DNA.[23,24]

Positive and negative controls demonstrated valid results according to the kit, where Cq FAM and VIC + 30 for the positive control and Cq FAM and VIC >38 for the negative control. Based on amplification results that shown in Figure 1, in sample tests conducted, 11 of 18 samples contained vertebrate DNA. The External Positive Control (EPC), as the study's positive control, experienced a significantly increased amplification curve over the threshold line. Besides EPC, there was the Non-Template Control (NTC) as the negative control.[31] Negative control utilization showed that utilized reagents were not contaminated by target DNA to be amplified. Theoretically, the negative control curve did not show curve elevation; however, nine of 18 samples (DA-01, DA-02, DA-03, TC-02, TC-3, DB-03, IH-01, IH-03, and EM-03) experienced curve elevation due to unknown external contaminations. Contaminations might be caused by incautious pipetting techniques or during tube insertion into the PCR Real-Time equipment, or from the analyst DNA adhering in the study site and carried by aerosols. Nevertheless, the study was declared valid because only the VIC reporter (yellow channel) experiencing contaminations while the FAM reporter (green channel) to detect porcine DNA had no Ct values indicating contaminations.[23,24,25]

Similar result were also obtained by Aziz in 2017, a study was carried out to detect the presence of gelatin in orthodontic dental materials for halal authentication.[7] The detection of gelatin was done using Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR). In the study, 21 samples were included and the spectrums were generated by OMNIC software of Nicolet iS50 FTIR. All data were subjected to similarity match (SM) using TQ Analyst software. From the study, it was found that no samples exhibit similar spectra as the gelatin tested and SM found that no samples showed 100% similarities with porcine gelatin.[7] On the other hand, present study was not in line with the research conducted by Dewi N (2020) where the examination of pork gelatin was carried out using Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR) and Chemometrics.[19] The obtained results were four of the 49 dental materials used in hospitals and clinics contained gelatin, one of which was included in pork gelatin while the three could not be identified.[19]

There are many analysis methods currently used, including Enzyme-Linked Immunosorbent Assay (ELISA), Real-Time Polymerase Chain Reaction DNA (RT-PCR), Gas Chromatography (GC), Gas Chromatography-Mass Spectrophotometry (GC-MS), Fourier Transform-Infrared. Spectroscopy (FTIR). Several methods have been developed with many methods using protein, fat and DNA. Analysis based on protein content in porcine can use porcine detection kits with an immunochromatography method, i.e., a combination of enzyme-linked immunosorbent assay (ELISA) and chromatography.[26,27] However; this method has a disadvantage because the protein will lose its biological activity after the animal dies. In addition, the ineffective nature of protein after meat undergoes high processing caused by denaturation at high temperatures and high pressures hinders the analysis process.[28,29] Infrared spectroscopy, differential scanning calorimetric, gas chromatography, high-performance liquid chromatography (HPLC), and electronic odor detector can be used for analysis based on fat content.[10,11] While the PCR method has a target, namely DNA, this method that is considered the most valid at this time. PCR is one of the most studied and widely used in vitro nucleic acid amplification techniques. PCR is used to multiply the number of DNA molecules on a specific target by analyzing new DNA molecules that complement the target DNA molecule via enzymes and oligonucleotides as primers in a thermo cycle.[10] Molecular biology analysis, especially DNA by using PCR, has advantages compared to proteins or other molecules, because of the fact that DNA is more stable.[10,11]

In Real-Time PCR, the amount of DNA product formed can be monitored in real time, i.e., during the course of the reaction, with accuracy and high sensitivity over a dynamic range.[9] This monitoring can be assisted by the use of fluorescent dyes or probes applied into the reaction. The

fluorescence intensity of these probes will be proportional to the amount of DNA product formed.[9] Moreover, in Real-Time PCR the number of amplification cycles required to obtain a particular amount of DNA molecules is recorded.[10,11] Assuming a certain amplification efficiency, which is typically close to double the number of molecules per amplification cycle, the number of DNA molecules carrying the target sequence that were initially present in the sample can be calculated.[11] A number of highly efficient chemistry detection, sensitive instrumentation, and optimized assays are now available that allow quantification of the number of DNA molecules of a particular sequence in a complex sample with unprecedented accuracy and sensitivity that can detect a single molecule.[10,11]

The study should be carried out with caution, and performed repetitively to ensure that the results were correct. The researchers only tested 18 prosthodontic materials in the market, and thus, the study results could not represent prosthodontic materials in general. In this study, the species of the vertebrate DNAs were not known. Further study was required to examine the halal status of dental materials.[28]

CONCLUSIONS

The study concluded that no porcine DNA was detected in all samples. However, 11 of 18 samples were containing vertebrate DNA (non-porcine). The halal status of the materials tested remained unclear.

ACKNOWLEDGEMENTS

The authors gratefully acknowledges for Mr. Agy Wirabudi and Mrs. Lulu Dwiana at the Halal Study Center of Institut Pertanian Bogor for assisting this research.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Cumhuriyet Dental Journal: 2021; 24(3)
e-ISSN 2146-2852
Doi:10.7126/cumudj.943229

Original research

EFFECT OF TIDEGLUSIB WITH BONE GRAFTING ON NEW BONE FORMATION

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ABSTRACT

Objectives: The goal of this study was to observe the regenerative potential of Tideglusib in combination with autogenous and xenograft mandibular defects in rats.

Material Methods: Our study consists of five groups: one control and four experimental. In 40 Wistar albino rats, 5-mm-diameter critical bone defects were created at the angle of the mandible. In the control group, the defect was not filled. The defects were grafted only Xenograft in Group 1, with Xenograft and tideglusib in Group 2, and with only autogenous bone graft in Group3, and with autogenous bone graft mixed with tideglusib in Group 4.

Results: Sterological analyses revealed that enhanced new bone formation in the Group 4 compare to Control and Group 1. Immunohistochemically marked expressions of BMP-2 and VEGF were observed in Group 4.

Conclusions: Our results demonstrated that Tideglusib, in combination with bone grafting has an adjuvant effect on BMP-2 and VEGF-A expressions that may accelerate bone regeneration.

Keywords: Autogenous Bone Graft, BMP-2, Bone healing, Tideglusib, VEGF-A.

Received : 29.05.2021
Accepted : 01.07.2021

How to Cite: İyilikci B, Taşdemir U, Özmen Ö, Kızıldağ A. Effect of Tideglusib with Bone Grafting on New Bone Formation. Cumhuriyet Dent J 2021;24:3:224-231.
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INTRODUCTION

A bony defect is defined as an acquired deformity from trauma, surgery, or congenitally. Different materials, including autogenous, xenogenous, allogenic, and alloplastic bone grafts, are available for the management of bony defects.[1] However, none of these materials are perfect because of harvesting problems, preparation difficulties, antigenicity, and consumption.[2] Therefore, researchers have focused on alternative biomaterials and applications, especially with the goal of accelerating bone formation and reducing healing time. Drugs or recombinant products have been combined with bone grafts, especially, to perform osteoinductive tasks such as growth factor expression.

Bone morphogenetic proteins (BMPs) are important factors in bone formation and regeneration Among the BMPs, BMP-2 has strong osteoinductive activity.[3] Regardless of the material, the development of a supporting vasculature concomitant with graft maturation is well documented and is a key determinant in optimizing bone regeneration at grafted sites. Vascular endothelial growth factor (VEGF) is a critical regulatory factor new vascular formation which is the most researched factor that has the strongest association with angiogenesis.

Tideglusib is a glycogen synthase kinase 3 (GSK-3) inhibitor used to treat neurological diseases such as Alzheimer's disease[4] and it has been reported to have a positive impact on the formation of dentin.[5]

Embryologically, tooth and bone are mineralized tissues derived from the neural crest with similar organic and inorganic matrices.[6] The organic matrix of dentin is composed of type I collagen (90%) similar to that of bone. The remaining 10% of the dentin matrix consists of biopolymers, lactate, citrate, lipid, osteocalcin, osteonectin, sialoprotein, phosphoprotein, which play a role in bone calcification and growth factors, including bone morphogenetic protein and insulin-like growth factor.

This study aimed to evaluate tideglusib mixed with bone grafts effect on formation and to evaluate the expression of Bone Morphogenetic Protein-2 and Vascular Endothelial Growth Factor by using immunohistochemical analysis.

MATERIAL AND METHOD

The study was approved by the Institutional Animal Care-Use Committee of Pamukkale University (protocol No PAUHADYEK- 2018/05). All experimental procedures on the animals complied with the Guidelines of Pamukkale University Care and Use of Laboratory Animals.

Study Design

Our study consists of five groups: one control and four experimental. Bony defects of 5 mm in diameter were created in the angle of the mandibles of 40 Wistar albino rats (250-300 g). The study groups were as follows: Control group: defects not filled, Group 1: xenograft material applied to the defect area, Group 2: xenograft and Tideglusib applied to the defect area, Group 3: autogenous graft material applied to the defect area, Group 4: autogenous graft and Tideglusib applied to the defect area (Figure 1).

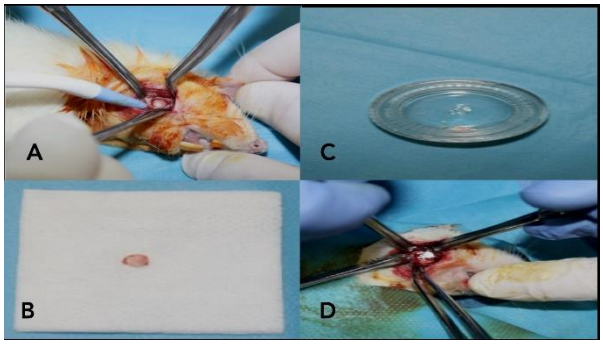

Figure 1.
A. Five-mm diameter bone defect made in angle of mandible.
B. Autogenous bone graft taken from rat's mandible.
C. Ideal sized autogenous graft material for grafting procedure.
D. Autogenous graft and Tideglusib application to defect area.

Tideglusib Preparation

Before the surgery, 50 nM Tideglusib (SIGMA AQ) was dissolved and diluted in dimethyl sulfoxide (DMSO) and mixed with the graft material.

Autogenous Graft Preparation

Autogenous bone graft material was taken from each rat's mandibular defect. The material was put into the sterile chamber of a grinding machine (KometaBio Dentin Grinder; Tidal Tech Dental, Turkey) that crushed and sterilized the material and

prepared particles (mean 0.75 mm in size; range 0.3-1.2 mm) suitable for the grafting procedure.

Surgical Procedures

The animals were anesthetized intraperiteonally with 1% ketamine–(Ketalar; Eczacibasi-Warner Lambert, Istanbul, Turkey) and 2% xylazine (Rompun 2%; Bayer, Istanbul, Turkey). The mandible was shaved and then scrubbed with sterile gauze soaked with an iodine solution. With sterile instruments and an aseptic technique, a 4- to 5-cm-horizontal incision was made in the right angle of the mandible. The subcutaneous tissue, muscles, and periosteum were dissected and reflected to expose the angle of the mandible. Only one bone defect (5 mm in diameter) was made in the mandibular bone of each rat with a trephine drill under constant irrigation with a 0.9% saline solution. No grafting material was used in the control group. Autologous mandibular onlay grafts were harvested from the right mandibular region, which was ground and used in Groups 3 and 4. Xenograft material (Bonefill, Bionnovation, Brasil) was used in Groups 1 and 2. The soft tissues were firmly repositioned and sutured (Resorbable 4.0 polyglycolic acid, Pegalak; Dogsan, Istanbul, Turkey). After the surgery, each rat was medicated with an intramuscular injection of Ceftriaxone, 50 mg/kg Rocephin; Deva, Istanbul, Turkey) and Carprofen, 4 mg/kg (Rimadyl; Pfizer, New York, NY) once daily for three days. The animals were euthanized 28 days after the surgery. The bones were dissected, and any signs of abnormal healing were documented.

Histopathological and Sterological Analysis

Specimens of the mandible with the bony defect were fixed in a 10% buffered formalin solution, after decalcification with a ready to use decalcification solution (Osteofast 1, Biognost, Zagreb, Croatia) for two weeks. Then the defective area was trimmed and tissue samples were washed under tap water during the 8 hours. After the routine tissue processing using an automatic tissue processor (Leica ASP300S, Leica Microsystems, Wetzlar, Germany), the specimens were embedded in paraffin. Then paraffin blocks were cut 5-µm thickness with a rotary microtome (Leica RM2155, Leica Microsystems, Wetzlar, Germany), Two different serial sections taken from each defective area and, stained with hematoxylin and eosin (HE).

Sections were coverslip and examined under a light microscope. Histomorphometric parameters were calculated for the total augmented area (TIA; mm^2), and the residual material area (RMA; mm^2). Osteoblasts and osteoclasts were counted in a 1.23 mm^2 area at ×400 magnification. Whole defective areas were examined at two dimensionally. All calculations were made 5 different areas of each sections of the defective areas.

For the stereological estimation of volume of newly formed bone area (VNFB; mm^3) light microscopic images via using point counting test grids were used. The point density of the point counting grids was designed to obtain an appropriate coefficient of error for interesting area in images of the serial sections in the 1mm thickness. Coefficient of error and coefficient of variation were estimated according to Gundersen and Jensen' formula. The test grid with systematic array of points was randomly placed on the screen of PC. For to estimation of the volume of each interesting area in all sections was used with following formula:

$$\text{Volume} = t \times a/p \times \sum p$$

('t', section thickness; 'a/p', representing area of each point on the point counting grid; '$\sum p$', (total number of the points hitting the interesting area) (Gundersen and Jensen, 1987) Mean values of each group results statistically examined. For histomorphometric and sterological analysis the Database Manual CellSens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan) was used. Histopathological changes were evaluated in a blinded manner by a specialized pathologist from another university who was unaware of the study design.

Immunohistochemical Method

For the immunohistochemical examination, the specimens were stained with BMP-2 (Anti-BMP2 antibody bs-1012R, 1/100 dilution) and VEGF-A (Anti-VEGF-A antibody bs-1957R, 1/100 dilution), by streptavidin–biotin complex peroxidase technique. Primary antibodies were purchased from Bioss (AQ USA). The sections were incubated with the primary antibodies for a period of 60 minutes, and

the immunohistochemistry was evaluated using a biotinylated secondary antibody and a streptavidin–alkaline phosphatase conjugate. As a secondary antibody, Expose Mouse and rabbit specific HRP/DAB Detection IHC Kit (AQ ab80436), and DAB (3,3-diaminobenzidine) (Abcam, UK) was used as a chromogen. The phosphate-buffered saline (PBS; pH: 7,2) solution was used for negative controls instead of primary antiserum. The evaluated by a specialized pathologist by manner. The immunohistochemical expressions were semiquantitatively scored from 0 to 3 (0=negative, 1=slight expression, 2=medium expression, 3=severe expression). After the microscopic examination (Olympus CX41 AQ), the Database Manual CellSens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan) was used for microtomographic and morphometric evaluation. Scores were statistically analyzed and the differences between the groups were determined.

Statistical Analysis

Statistical analysis of the immunohistochemical scores was calculated with the one-way ANOVA test by using the SPSS 15.00 statistical program. Significant differences between groups were detected with the DUNCAN test (α=0.005).

RESULTS

Histopathological Findings

The histopathological examination of the mandibular specimens revealed that the defect area was usually filled with connective tissue in all groups. However, in some groups, cartilage and bone were observed. Especially in the Control group, the center of the defect was empty. Maximum ossification was observed for the groups 3 and 4. Minimum ossification was observed in the Control group (Figure 2).

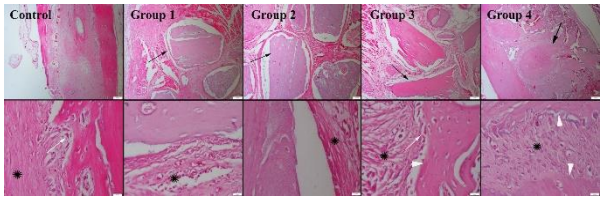

Figure 2.
Histopathological appearance of the bone defect area between the groups. (Upper row) graft materials (thin arrows) and new bone formation (thick arrow), HE, Bars= 200µm. (Below row) higher magnification of the fibrous tissue formations (stars), newly bone formations (white arrow heads) and osteoclasts (white arrows), HE, Bars= 20µm.

Sterological analyses showed that the total augmented area of the defects was significantly different. The marked amelioration was observed in Group 4. The new bone area was also higher in this group than in the Control and Group 1. The defect closure rate was also significantly higher in Group 4. The sterological findings and immunohistochemical expressions of BMP-2 and VEGF-A are shown in (Figure 3-5).

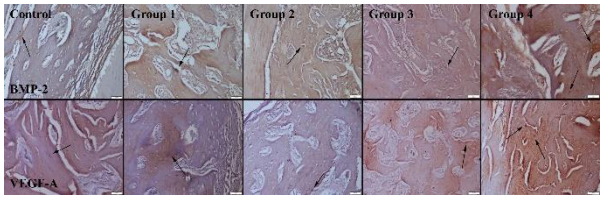

Figure 3.
BMP-2 and VEGF-A immunoexpressions (arrows) between the groups, the most marked increase in Group 4, streptavidin biotin peroxidase method, Bars= 20µm.

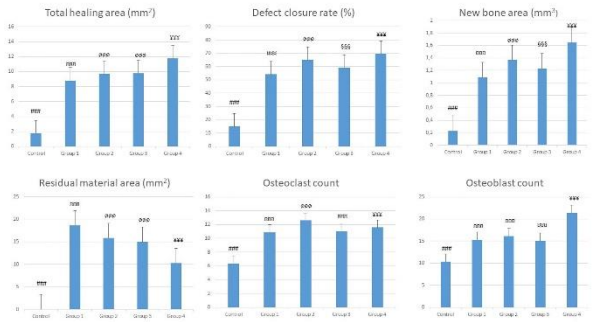

Figure 4
Statistical analysis results of the bone healing markers between the groups. Groups with superscripts of same letter are similar, different ones are statistically significantly different compared the control group (P<0.001).

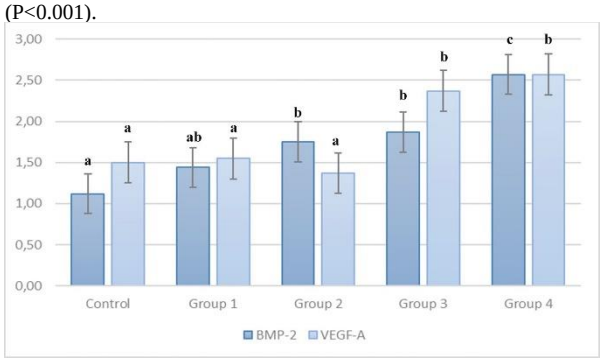

Figure 5
Graph of the statistical analysis results of BMP-2 and VEGF- A immunohistochemical scores. * One-way Anova Duncan test was used in statistical analysis.
** Groups with superscripts of same letter are similar, different ones are statistically significantly different for each marker (P<0.001).

DISCUSSION

If a graft has osteoinductive and osteoconductive effects, the healing rate and regeneration is improved compared with other materials.[7] Recently researchers have focused on enhancing the osteoconduction and osteoinduction capacity

with improved preparation design or by adding drugs that express growth factors.[8] Growth factors are chemical signals which regulate pathways involved in bone regeneration[8], which, at any given time, may not be observed under physiologic conditions. Growth factors effects are associated with significant changes in their local concentration and do not depend only on their presence or absence.[9] In our study, Tideglusib was combined with grafts to increase the local concentration of growth factors, especially BMP-2 and VEGF.

Combining bone grafts with other materials commonly focuses on enhancing regeneration via osteoinductive and vascularization processes. Studies have demonstrated that some materials that promote bone formation are LLP (lipid-lowering agent)[10,11], antioxidant molecules[12,13] or recombinant BMP-2.[14,15]

Growth factors bind to specific receptors and activate intracellular events by specific biochemical pathways and increase the chemotaxis of the same cellular elements in the regeneration area. Growth factors act on target cells by using specific receptors via specific biochemical pathways and also participate in the chemotaxis of the same cellular elements. The pathways have been reported in the literature. The Wnt pathway[16,17], the TGF-β/BMP superfamily[18], notch signaling[19], hedgehog proteins[20] and fibroblast growth factors (FGFs)[21] have all been identified in the molecular signaling of osteogenesis. Although the exact mechanisms of osteogenesis are complex and only partially elucidated, advances have been made regarding the initiation and molecular control of this process. The Wnt/β-catenin pathway organizes the postnatal bone acquisition by checking the differentiation of both osteoblasts and osteoclasts.[22] Wnt/β-catenin signaling is also essential for skeletal formation and development in the fetus and is known to be responsible for both osteoblast and chondrocyte differentiation.[23] This pathway not only plays a critical role in growth and development but in the maintenance of the mature skeleton.[24] Tideglusib is a non-competitive irreversible inhibitor of GSK-3ß and a member of the thiazolidinediodine family.[25] This drug activates the WNT/ß-catenin signaling pathway by inhibiting GSK-3ß.[26]

Our results demonstrated that Tideglusib increased BMP-2 expression. As Tideglusib has a positive effect on dentin regeneration by the Wnt pathway, we concluded that the same effect was seen in our study and may have been by the same pathway.

Chen *et al.*[27] reported in an in vivo study that BMP-2 induced the Wnt/ß-catenin pathway during ectopic endochondral ossification, chondrogenesis, and osteogenesis. Yuan *et al.*[28] reported that synchronous stimulation of BMP and Wnt/ß-catenin pathways indicated a scheduled function of BMP and Wnt signaling in coordination early tooth formation. Another study reported that suitable interference between BMP and Wnt signaling pathways was necessary for tooth formation.[29] According to these studies BMP-2 and WNT signaling pathways works in sync, and this condition is consistent with our results.

Therapeutic molecules reported to have a positive effect on BMP expression for enhanced bone healing include lithium chloride, sclerostin antibodies, strontium ranelate, LLP, and AZD2858. The effect of these drugs may be via the same pathway or others. Recently, Galli *et al.*[30] reported that activation of the canonical WNT/ß catenin pathway via lithium chloride increased osteoblast differentiation on hydrophilic modSLA surfaces. Also, Arioka *et al.*[26] reported that local application of lithium chloride and other GSK-3 inhibitors may expedite bone healing by activating osteoblastogenesis and suppressing osteoclastogenesis. LiCl increases implant osseointegration, implant fixation, and bone formation in osteoporotic conditions, so LiCl may be a promising curative material for avoiding implant failure and bone loss in patients with osteoporosis.[31]

Local drug application has the advantages of decreasing adverse systemic effects and systemic toxicity. Our study demonstrated that bone has a high tolerance for Tideglusib, and the dose and concentration were selected with the guidance of Neves *et al.*[5]

Vascularization is a critical factor for bone regeneration. Our results demonstrated that Tideglusib enhanced VEGF-A expression, which is associated with bone regeneration. In addition, this condition stimulated new vessel production and circulation and increased osteogenic cell formation, increasing regenerative capacity. Some drugs like LLP[32] and Link si-RNA[33] are both VEGF and BMP-2 releasing. Similarly, our results showed that Tideglusib promotes the release of both BMP-2 and VEGF. If used together with BMP-2, VEGF, and bFGF, it might synergistically support osteogenic differentiation, with low concentrations. BMP-2, and VEGF-A influence each other during bone regeneration; therefore, both BMP-2 and VEGF-A release is anticipated to increase bone healing compared with each alone.[34,35,36] Similarly, in our study, the synergistic effect of BMP-2 and VEGF might increase bone regeneration. Tideglusib is a drug used for Alzheimer therapy that has been shown to contribute to dentin regeneration.[5]

CONCLUSIONS

These study findings revealed that this drug effective for new bone and vessel formations that support to healing. BMP-2 and VEGF have pivotal role for bone and tissue remodelling. Our results demonstrated that this drug has a positive impact on bone regeneration owing to the similarity of bone and dentin. Additional studies are necessary to determine the mechanism of this drug.

ACKNOWLEDGEMENTS

This study was supported by the Scientific Research Project Fund of Pamukkale University. (Project Number: 2018DİŞF011)

DECLARATIONS OF INTEREST

"No potential conflict of interest was reported by the authors."

ÖZ

Bu çalışmanın amacı, otojen ve ksenojen kemik greftlerle karıştılarılan Tideglusib'in ratlarda oluşturulan kemik defektlerindeki rejeneratif potansiyelinin incelenmesidir. Çalışma bir kontrol ve dört deney grubu olmak üzere beş gruptan oluşmaktadır. Rat mandibula angulus bölgesinde, 5 mm çapında kritik kemik defekti oluşturulmuştur. Kontrol grubunda defektler boş bırakılmıştır. Grup 1 de defektlere sadece Ksenojen kemik greft, Grup 2 de Ksenojen kemik greft ve Tideglusib, Grup 3 de sadece otojen kemik greft ve Grup 4 de ise otojen kemik greft tideglusib ile karıştırılarak uyugulanmıştır. Sterelojik analiz sonuçlarına göre yeni kemik formasyonu, Grup 1 ve Kontrol Grubu ile karşılaştırıldığında Grup 4' de daha fazla oluşmuştur. Grup 4 de immunohistokimyasal olarak daha fazla hacimde BMP-2 ve VEGF ekspresyonu gözlemlenmiştir. Çalışmamızın sonuçları; Tideglusib ile karıştılan kemik greftlerin BMP-2 ve VEGF ekspresyonununu artırdığını ve bu durumunda kemik rejenerasyonunu geliştirdiğini göstermektedir.

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Cumhuriyet Dental Journal: 2021; 24(3)
e-ISSN 2146-2852
Doi:10.7126/cumudj.910642

Original research

INFLUENCE OF DIFFERENT CAVITY DISINFECTION SOLUTIONS AND LASER SYSTEMS ON REPAIR BOND STRENGTH OF SILORANE BASED COMPOSITE

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ABSTRACT

Purpose: To evalute the effects of Nd:YAG-laser, Er:YAG-laser, chlorhexidine and ozonated water application used for antibacterial effects on repair bond strength of silorane based composite.

Materials and Methods: 100 cavities (2 mm deep, 3 mm diameter) prepared in acrylic blocks were filled with silorane composite and subjected thermal cycle and divided into 5 groups (N=20). Group 1: Chlorhexidine; Group 2: Ozonated-water; Group 3: Nd:YAG-laser; Group 4: Er:YAG-laser; Group 5 (control): untreated. And disinfection method treated according to the groups. Composite repair procedure was implemented with the same silorane based composite. Groups divided into 2 subgroups (n=10). One of the subgroups for each group was subjected second thermal cycle. All of the samples tested by a universal test device. Data were statistically analyzed and significance test of the difference between the two means, the variance analysis, and Student-t Test used.

Results: No statistical difference was observed among groups after first thermal cycle ($p>0.05$). After the composite repair, there were no statistically significant difference between groups that were subjected to second thermal cycle ($p>0.05$). When each group was evaluated in themselves on comparing before and after the thermal cycle after the repair operation; as no statistically significant difference between Ozonated-water, Nd:YAG, Er:YAG and Control groups ($p>0.05$), a statistically significant decrease was observed only in the Chlorhexidine group after thermal cycle ($p<0.05$).

Conclusions: Ozonated-water, Nd:YAG-laser and Er:YAG-laser applications can be used in the repair of silorane-based composite restorations as an alternative antibacterial application, since the application of chlorhexidine reduces the repair bonding strength.

Key words: Er:YAG laser, composite repair, Chlorhexidine, Nd:YAG laser, ozonated water.

Received : 06.04.2021
Accepted : 03.06.2021

How to Cite Kaptan A, Hürmüzlü F. Influence of Different Cavity Disinfection Solutions and Laser Systems on Repair Bond Strength of Silorane Based Composite. Cumhuriyet Dent J 2021;24:3:232-243.

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INTRODUCTION

In recent years, composite resins have become the main preferred material for dental restorations. Continuous efforts are being made to improve the properties of these restorative materials. Composite restorative materials are traditionally based on methacrylate technology and these materials are constantly being developed to provide better physical properties and optimal aesthetic appearance.[1,2] In recent years new restorative materials such as silorane-based composite resins have been developed as alternatives to traditional composite resins.[3] These restorative materials have been produced to cope with polymerization shrinkage in polymeric composite resins during dental applications and polymerization stress afterwards.[4]

It is reported that secondary caries and marginal fractures are the main reasons for the failure of composite restorations.[5] Defective restorations are traditionally removed and the restoration is renewed. This operative approach leads to more loss of healthy tooth structure and requires a wider cavity preparation than before. For this reason, a minimally invasive approach has recently been proposed, which results in less material loss as a repair of the old restoration rather than the replacement of unsuccessful restorations, which increases the life of the original restoration.[6]

It is stated in the composite restorations that bonding between the two resin layers can be due to the non-polymerizable resin that inhibited by oxygen.[7] Since the unfinished restorations do not contain an unpolymerized layer on their surface, bonding with the new composite can occur with the compound effect of micromechanical retention and physico-chemical bonding.[8] Various in-vitro studies reveal that the composite-composite bond strength is adequate.[7-9]

Removal of small amounts of caries and discolorations at the tooth -restoration interface does not mean that all pathogenic bacteria are completely removed[10] and the continuity of the pathogenic bacteria may lead to repetition of the reprocessing caries which may lead to failure of the repair restoration.[11] Therefore, additional methods for disinfection of the repair area can be considered. Some preparations containing chlorhexidine are often recommended for cavity disinfection. However, studies have reported that chlorhexidine administration affects the binding strength of adhesive systems adversely.[12] Recently, the use of ozone treatment for cavity disinfection has been on the rise.[13,14]

Ozone has entered the practice of dentistry because of its antimicrobial effect against oral pathogens.[15] Gaseous ozone has been investigated for the treatment of occlusal caries[16] and root caries[17] while the liquid form has been proposed as an alternative for the treatment of periodontal disease due to its biocompatibility and anti-inflammatory potential.[18] Ozone gas and ozonated water are also used as cavity disinfection agents before bonding processes.[19,20]

Lasers in restorative dentistry used in cavity preparation, elimination of dentin sensitivity and preparation of the dentin surface prior to the application of adhesive systems.[21] Er:YAG laser[22,23] and Nd:YAG laser[24] which are frequently used in cavity and root canal disinfection due to their antibacterial effects on *streptococcus mutans (S. mutans)*, *lactobacilli*, *Enterococcus faecalis (E. faecalis)* in dentistry.

The purpose of this study is to investigate the effects of chlorhexidine, ozonated water, Nd:YAG laser and Er:YAG laser application using for antibacterial effects on the repair bond strength of silorane based composite restorative materials.

The null hypothesis of the study was that the all cavity disinfection methods would not effect the repair bond strength of silorane based composite.

MATERIALS AND METHODS

Ethics

Ethical approval was obtained from the Health Ethics Committee of Sivas Cumhuriyet University in Turkey (ID: 2013-11/02).

Sample Size Calculation and Experimental Groups

Sample size was calculated using a sample size calculator (Sample Size Determination in Health Studies, World Health Organization) as follows: power at 80%, α at 5%, β at 20%, and the sample

size was determined to be 20 teeth in each group. Thus, a total of 100 samples was prepared for the study.

Preparation of Experimental Specimens

In the preparation of the composite specimens, a metal block (15 mm diameter, 20 mm height) with cylindrical cavities (2 mm depth and 6 mm diameter) were prepared. A silicone mold were obtained from this metal block with silicone-based impression material (Bonasil, DMP Ltd., USA). The prepared silicon molds were filled with acrylic and waited until polymerize. Following the polymerization, the acrylic blocks were removed from the silicon mold. With this method, 100 acrylic blocks with a cavity of 2 mm in depth and 6 mm in diameter on one surface were obtained. A silorane-based composite (Filtek Silorane, 3M Espe, St Paul, MN, USA) was placed in a single layer to the cavities. After placement of the composite resin in the cavities, polymerization was carried out using a LED light device (Valo Cordless, Ultradent Products Inc, SJ, Utah) with a power output of 1400 mW/cm² and a distance of 1 mm for 20 seconds in accordance with the manufacturer's instructions. In this way 100 composite samples were obtained.

Artificial aging of specimens

All the samples prepared were aged for 5,000 cycles in the thermal cycling to simulate oral environment conditions. The thermal cycling was carried out in 5°C and 55°C (±2°C) temperature baths, with a transfer time of 5 seconds and a dwell time of 30 seconds respectively.

Samples group design

After aging procedures, composite surfaces were ground with 400 μm grit silicon carbide paper to obtain a homogeneous surface. And then all specimens were divided into 5 groups with 20 composite samples in each group (N=20).

Group 1: Each sample surface treated 2% chlorhexidine with a disposable brush for 30 s and gently dried with air sprey. Afterwards, the self-acidic primer of the Silorane Adhesive System (Filtek Silorane Primer, 3M Espe, St Paul, MN, USA) was applied to the surface of the composite samples with a disposable brush for 15 seconds. It was slightly air-dried and polymerized for 10 s with an LED light device (Valo Cordless, Ultradent Products Inc, SJ, Utah) at a distance of 1 mm. Then the adhesive of the system in the second bottle (Filtek Silorane Bond, 3M Espe, St Paul, MN, USA) was applied with a different disposable brush and slightly thinned with air spray. The polymerization of the bonding agent was achieved for 10 s with the LED light device. The intensity of the light source was measured with a radiometer at every 5 samples, and the light intensity level was tried to be kept constant in all samples.

Following the adhesive application, a cylindrical transparent pipe with a diameter of 3mm and a height of 2 mm prepared previously was placed in the restoration center as a matrix to the repair composite material. Silorane-based composite (Filtek Silorane, 3M Espe, St Paul, MN, USA) was placed inside this transparent pipe with the help of hand instrument and polymerized from a distance of 1 mm for 20 s. Following polymerization, the transparent matrix was carefully cut away with a lancet.

Group 2: Ozonated water was used as a disinfectant in the samples in this group. Ozonated water were obtained using ozone producing generator (tekno3zo to, Izmir, Turkey). With the help of the ozone measurement probe in the reactor tank where distilled water is placed, the ozone density is displayed on the digital display on the device.

Ozonated water at a concentration of 4 ppm (mg/lt) was applied to the exposed surfaces of each of the 20 composite samples in the group with a disposable brush for 15 s and dried slightly with air spray and silorane adhesive system (Prmer-Bond) was applied and the repair process was carried out in Group 1. It was carried out with a silorane-based composite (Filtek Silorane, 3M Espe) as described above.

Group 3: The parameters of the Nd:YAG laser device were set to pulse at a wavelength of 1.064 nm, a power of 1.5W, an energy level of 100 mJ, and a frequency of 15 Hz. It was applied with a fiber optic tip with a diameter of 300 μm from a distance of 1 mm from the composite surface, so that the entire surface was treated. The repair procedure was carried out as in the previous

groups using a silorane adhesive system and a silorane-based composite (Filtek Silorane, 3M Espe).

Group 4: Er: YAG Laser was used as a disinfectant in this group. Er: YAG laser was applied at a wavelength of 2940 nm, 1.5W power, 150 mJ energy level, 10 Hz frequency, 700 ms long pulse to scan the entire composite surface from a distance of 10 mm (Figure 3.13). Repair process was carried out using silorane adhesive system and silorane based composite (Filtek Silorane, 3M Espe) as in the other groups.

Group 5 (Control group): 20 composite samples were repaired with a silorane adhesive system and a silorane-based composite (Filtek Silorane, 3M Espe) without any disinfection method.

Second aging procedure

Each group was divided into two subgroups (n=10). In order to evaluate the long-term performance of the restorations, a subgroup of each group was re-stored in the thermal cycle device for 5000 cycles. Thermal cycle application was carried out in a temperature of 5°C and 55°C (±2°C), respectively, with a transfer time of 5 s and a waiting time of 30 s. Thus, it was ensured that the composite restorations were exposed to the temperature changes in the oral environment after the repair. (Figure 1)

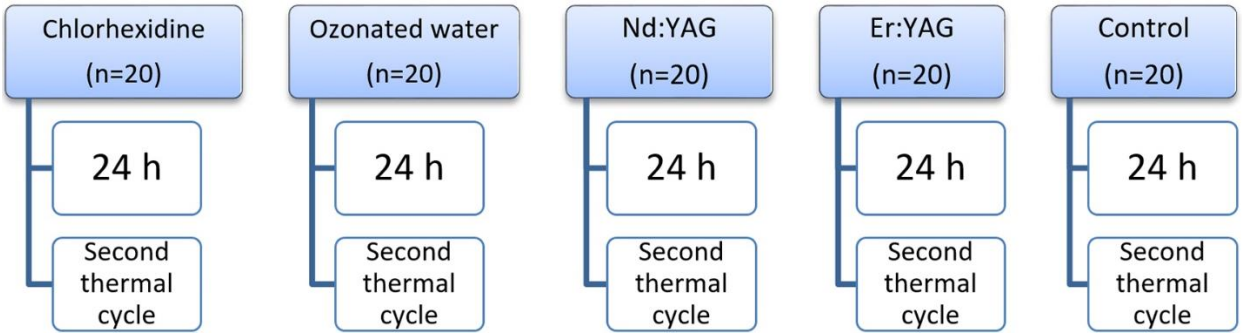

Figure 1. Schematic view of group design

Half of the samples (n_1=10) in the groups were subjected to the shear bond strength test immediately after being kept in distilled water at 37ºC for 24 hours. The other half of the samples (n_1=10) were subjected to shear bond strength test after a second aging in thermal cycle.

Shear Bond Strength Test

The shear bond strength test was carried out using a Universal Testing machine (LF Plus, LLOYD Instruments, Ametek Inc. England). The shear aparatus utilized 90º load application angle to the repair composite with a 1 mm/min crosshead speed and load cell of 1 kN until fracture occured and shear bond strengths were calculated in MPa.

After shear bond strength test, the fracture surfaces of all specimens were examined under a self-luminous stereomicroscope (SMZ 800, Nikon, Tokyo, Japan) at 32X magnification and the types of failures were categorized as A) adhesive at the interface, B) cohesive in the substrate, C) cohesive in the repair composite, D) mix type (adhesive + cohesive in the repair composite)

Statistical analysis

Statistical analysis was performed using SPSS (ver: 22.0) software. When the parametric assumptions were fulfilled (Kolmogorov-Simirnov) variance analysis was used to investigate whether there was a difference between experimental groups before thermal cycling and after thermal cycling shear bond strength values. While investigating whether there is a difference between the shear bond strength values before and after the second thermal cycle in each group, the significance test (Student t) of the difference between the two means in independent groups was used and the level of error was taken as 0.05.

RESULTS

As a result of the statistical analyses, no statistically significant difference was observed between all groups in terms of shear bond strength in the evaluation after the first thermal cycle application (p>0.05). Table 1 shows the mean values and standard deviations of the repair shear bond strength test of the control and experimental groups.

Table 1. Statistical comparison of the mean repair shear bond strength values before and after the second thermal cycle in the groups

Groups	24 h		Second thermal cycle		*P value*
	Mean(MPa)	SD	Mean(MPa)	SD	
Chlorhexidine	16.86	± 2.39^{a,A}	12.82	± 3.21^{b,A}	t=3.18 P=0.005 *
Ozonated water	16.37	± 2.84^{a,A}	13.99	± 3.39^{a,A}	t=1.69 P=0.107
Nd:YAG	17.57	± 2.38^{a,A}	15.15	± 3.65^{a,A}	t=1.75 P=0.096
Er:YAG	17.41	± 2.43^{a,A}	15.83	± 2.85^{a,A}	t=1.33 P=0.199
Control	16.96	± 4.09^{a,A}	16.12	± 3.68^{a,A}	t=0.48 P=0.635
	F=0.28 *p*=0.885		F=1.69 *p*=0.180		

*(p<0.05); ** In each row, groups with the same lower case letter superscripts are not significantly different, and in each column, groups with the same upper case letter superscripts are not significantly different

A statistically significant difference was not observed in terms of repair shear bonding strength values between all groups that were subjected to the second thermal cycle after the composite repair procedure was applied (p>0.05) (Table 1).

When each group was evaluated within itself in the comparison before and after the second thermal cycle after repairing all samples; A statistically significant decrease was observed in the repair shear bond strength values after thermal cycle application in the chlorhexidine group. (p<0.05) (Table 1).

Failure types results

When the failure types of the experimental samples were examined under the stereomicroscope, adhesive failure, cohesive failure at the restoration material, cohesive failure at the repair material and mixed failure types were determined. The predominant type of failure in all groups was found to be mixed type failure. The distribution of failure types is shown in Figure 2 and SEM images of failure types of groups shown in Figure 3.

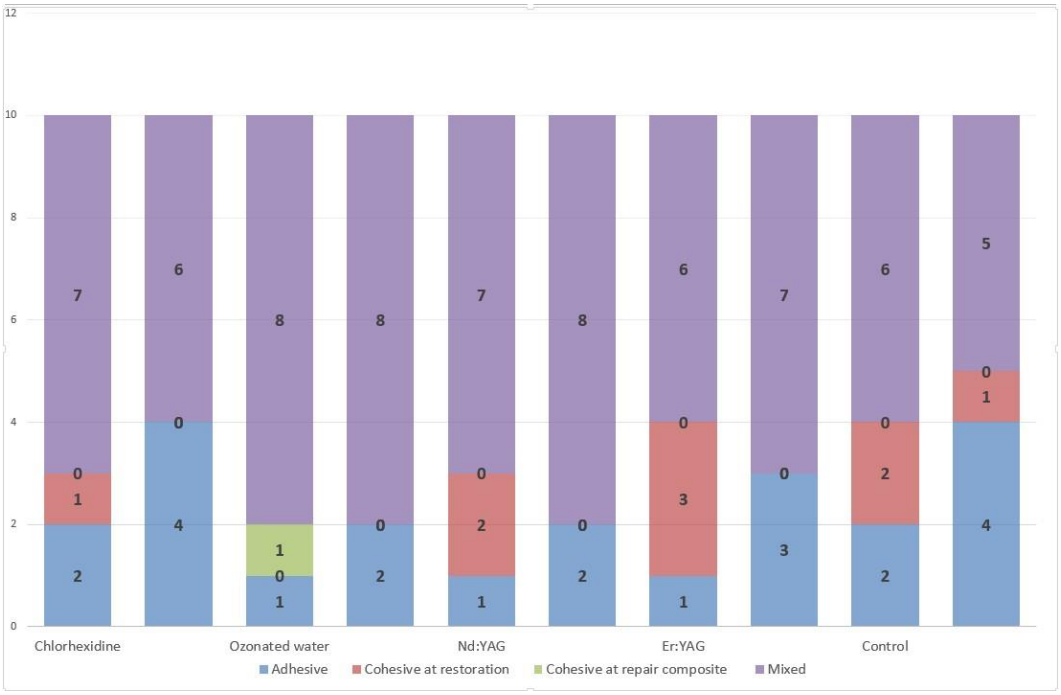

Figure 2. Distribution of failure types

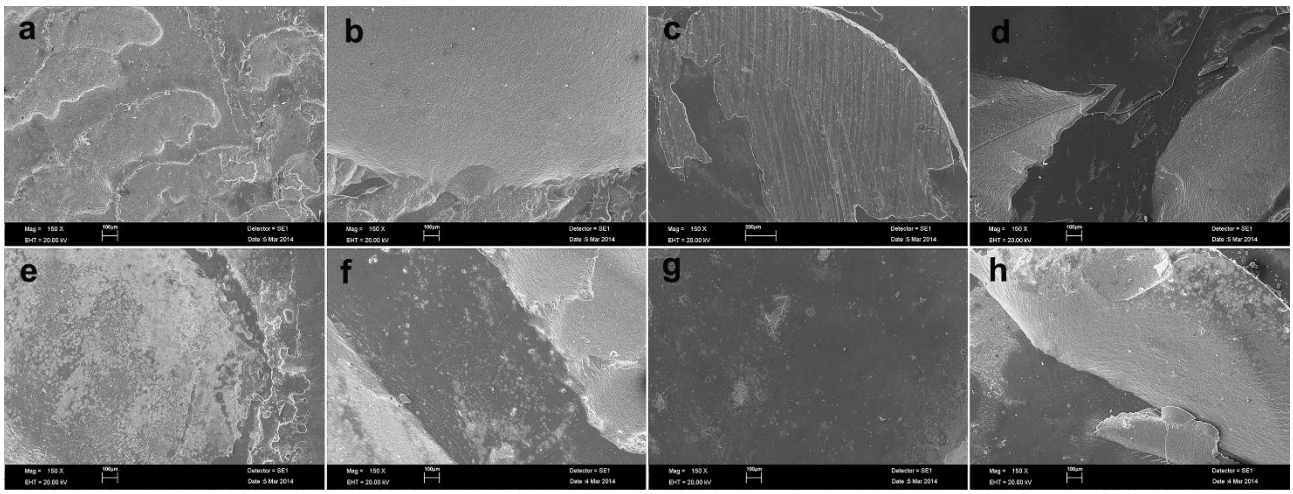

Figure 3. SEM images of failure types, **a:** Adhesive type failure belongs to Er:YAG laser group, **b:** Cohesive type failure belongs to ErYAG laser group, **c:** Adhesive type failure belongs to chlorhexidine group, **d:** Mixed type failure belongs to chlorhexidine group, **e:** Adhesive type failure belongs to Nd:YAG laser, **f:** Mixed type failure belongs to Nd:YAG laser, **g:** Adhesive type failure belongs to ozonated water group, **h:** Mixed type failure belongs to ozonated water group

DISCUSSION

The repair of a failed restoration rather than replacement is a good alternative to avoid the unnecessary loss of tooth structure. When secondary caries and discolored restoration margins are removed prior to repair, the possibility of there being cariogenic bacteria in the repair site becomes a concern. In addition to lesion removal, the disinfection of the cavity has been recommended to eliminate the risk for caries recurrence due to the presence of residual bacteria under the restoration.[11,25] The feature desired in a cavity disinfectant, besides its antimicrobial activity, is the lack of any detrimental effect on adhesive processes during the restoration repair. According to previous studies, chlorhexidine is one of the most effective chemotherapeutic agent against mutans streptococci and dentin caries, and is widely used.[26] Common disinfectants, such as chlorhexidine or sodium hypochlorite, have also been reported to have a negative effect on adhesion[27], leading to research into new products.[25] There are encouraging previous studies regarding the use of ozone application in dental hard tissues prior to adhesive procedures.[20]

In the present study the null hypothesis was accepted. All cavity disfection methods used in the study did not affect repair bond strength of silorane based composite. Chlorhexidine group did not show any statistically significant difference in repair shear bond strength values, both before and after thermal cycling, compared to other groups (Table 1). The findings of this study are consistent with those of previous studies using etch-and-rinse and two-step self-etch adhesive systems.[28-30] In such studies, the authors suggested that the lack of any negative effect of chlorhexidine application on the bond strength of adhesive systems was due to the compatibility of the adhesive resin used with chlorhexidine. It has been reported that the effect of disinfectant agents, such as chlorhexidine, on the bonding of composite restorations depends on the type of adhesive resin and the interaction of the adhesive resin with disinfectant agents.[31]

The present study examined the effect of 4 ppm ozonated water application on the repair strength of the silorane-based restorative material and the ozonated water group did not show any statistically significant difference in the repair shear bond strength values, both before and after thermal cycling, compared to other groups (Table 1). The findings of this study are consistent with those of bond strength studies using ozonated water application in dental hard tissues.[19,32,33]

Pithon and Santos.[32] have demonstrated that ozonated water did not have a negative effect on the bond strength of resin-modified glass ionomer cements. Ozonated water, when compared with other disinfectant agents such as sodium hypochlorite (2.25%), chlorhexidine (2%) and gaseous ozone, did not show any statistical difference.[34] Garcia *et al.*[19] examined the effect of gaseous ozone and ozonated water on the composite resin-dentin bond strength of two-step adhesive systems (Adper Single Bond 2, XP

Bond), and reported that ozone water does not have a negative effect on the bond strength.

Papacchini *et al.*[35] have shown that hydrogen peroxide has a detrimental effect on composite repair bond strength, especially when an adhesive is used as an intermediate bonding agent. The authors suggested that this result was due to the undesired interaction of residual hydrogen peroxide and oxygen on the composite surface to be repaired, and reported that oxygen, which could diminish the polymerization of the intermediate bonding agent used in the repair, originated from the atmosphere [35]. It could be argued that the oxidative effect of ozone does not lead to the formation of critical amounts of oxygen by-products on the composite surface. The fact that the ozone, applied directly or following thermal cycling has no effect on the micromechanical properties of the composite (Table 1), supports the hypothesis that chemical modification of the composite surface by ozone application is unlikely [36]. On the other hand, there are reports of commonly used bleaching agents such as hydrogen or carbamide peroxide affecting certain physical properties of the composite.[37-39]

Ozone application to resin-dentin interfaces can be compared with applications of other oxidants such as hydrogen peroxide used for bleaching and sodium hypochlorite used for storage.[40-43] For instance, these two products cause deterioration in the adhesive interface;[40-42] however, the present study, as shown in Table 1, observed no difference between ozone-treated groups and the control group. This may be due to the low concentration of ozone and the short contact time.[19]

There are several studies showing that the Nd:YAG laser causes changes and modifications on enamel and dentin surfaces.[44,45] Oskoee *et al.*[46] examined the effect of Nd:YAG (3W, 150 mJ, 20 Hz) laser on the repair bond strength of a silorane-based composite and reported that the Nd: YAG laser significantly increases the repair bond strength compared to the control group. Türkmen *et al.*[47] reported that the Nd:YAG laser application to the composite resin surface results in crater formations, microcracks and porosities on the composite surface. We consider that the increased bond strength in these studies[46-48] may be due to the microretention caused by the use of Nd:YAG laser in high modes.

In the present study repair SBS were not affected by the Nd:YAG laser application (Table 1). Compared to ozonated water and chlorhexidine groups, there was no statistically difference in bond strength, although the bond strength values were slightly higher in the Nd: YAG group ($p>0.05$).

The Er:YAG laser causes ablation on the composite surface through explosive vaporization and subsequent hydrodynamic ejection. During this process, the rapid softening and consequent change in the volume of molten materials create strong suspension masses. The interaction between the masses and the composite resin structure creates protrusions on the surface and the molten materials are removed from the surface in droplets. This microretentive morphology formed on the composite resin surface increases the surface area.[49] The increased surface area results in an increase in the bonding surface area and modifies the stress distribution at the interface of the two bonded materials.[50] All these events lead to an increase in the repair bond strength.

In present study Er: YAG laser was used (power, 1.5W; energy level, 150 mJ; frequency, 10 Hz) as a cavity disinfectant due to its bactericidal effect.[51] In the present study repair SBS values were not affected by the Er:YAG laser application ($p>0.05$) (Table 1). Compared to ozonated water and chlorhexidine groups, there was no statistically significant difference in bond strength, although the bond strength values were slightly higher in the Er:YAG group. Findings of this study are similar to those of previous studies that evaluated the composite repair bond strength using the Er:YAG laser.[52,53]

The variation in the studies' findings may be related to the type of composite used because the content of the composite resin could affect the efficacy of mechanical surface treatments.[54] While various components of the resin-based parts of the composites absorb the laser energy, the filler particles of dental composites scatter the laser energy.[55] Lizarelli *et al.* investigated the ablation rate and morphological impact of the Er:YAG

laser on different types of composite resin such as microfiller, hybrid, and condensable, and reported that micromorphological aspects, penetration rate and ablation rate were dependent on the structure and chemical composition of composite resin as well as laser parameters.

In the present study predominant fracture type in all groups was determined as mixed-type fracture (Figure 2). When silorane composites are used with a compatible adhesive during repair, a more stable interface is obtained, which positively affects bonding . However, the dentist may not always be able to identify the original restoration and select the correct repair composite and the correct adhesive.[56]

The repair of composite restorations is usually required months or years after their insertion. During aging, various changes occur in composites, such as water absorption, chemical degradation, and leakage of some components, all of which affect the success of the repair procedure.[50] Thus, the age of the repaired restoration plays a fundamental role in the bond strength of composite repairs.[57]

Thermal cycling is frequently used in laboratory settings to mimic the stress caused by temperature changes at the interface between materials with different thermal expansion coefficients due to environmental conditions. Papacchini *et al.*[9] examined the hydrolytic stability of different composite repair procedures by subjecting them to thermal cycling and found a significant decrease in composite-composite repair strength after thermal cycling only in the group in which an etch-and-rinse adhesive system was applied together with a non-prehydrolyzed silane as an intermediate bonding agent. The present study, when each group was evaluated within itself in the comparison before and after thermal cycling, found no statistically significant difference in the repair shear bond strength values between ozonated water, Nd:YAG, Er:YAG and the control groups (Table 1). This demonstrated that the adhesive intermediate bonding layer also formed a stable bonding in the repair of the new restorative material.[36]

The data of this study are in agreement with those of previous studies.[9,36,56,58] However, it has also been reported that the composite bond strength decreases after performing a higher number of thermal cycles.[59] Present study established a statistically significant decrease in the repair shear bond strength values after thermal cycling only in the chlorhexidine group (Table 1).

Due to the scarcity of findings in the literature on this subject, it was not possible to compare the findings of the present study, which was planned to reveal the effect of chlorhexidine and ozonated water applications for cavity disinfection purposes on the repair shear bond strength values when used with the compatible adhesive of silorane-based composites. However, we believe that this study will be a step for further studies on this matter.

CONCLUSIONS

Applications of ozonated water, Nd:YAG laser and Er:YAG laser did not affect the repair bond strength of the tested silorane-based composite when compatible adhesive was used. However, the bond strength values were found to be higher in the laser groups. It was established that the application of chlorhexidine significantly reduced the repair bond strength of the silorane-based composite after thermal cycling, while the repair bond strength of the ozonated water, Nd:YAG laser and Er:YAG laser groups was not affected by thermal cycling. Since the chlorhexidine application reduced the repair bond strength of silorane-based composites after aging procedure, it is believed that ozonated water, Nd:YAG laser and Er:YAG laser can be used as disinfectants in the repair of silorane-based composite restorations as an alternative antibacterial treatment following the removal of secondary caries in the margins of failed composite restorations.

Farklı Antimikrobiyal Solüsyonların ve Lazer Sistemlerinin Siloran Bazlı Kompozitlerin Tamir Dayanımı Üzerine Etkisi

ÖZ

Amaç: *Antibakteriyel etkileri sebebiyle kullanılan ozonlu su, klorheksidin, Er:YAG lazer ve Nd:YAG lazer uygulamalarının yaşlandırma öncesi ve sonrası siloran bazlı kompozit restoratif materyallerinin tamir dayanımı üzerine incelenmesi* ***Gereç ve yöntem:*** *Akrilik bloklar üzerine 2 mm derinlik ve 3 mm çapında 100*

kavite hazırlandı ve siloran bazlı kompozit ile doldurularak polimerize edildi. Tüm örnekler tamir işlemi öncesi yaşlandırma işlemine tabi tutuldu ve sonrasında 5 gruba ayrıldı (N=20). Grup 1: Klorheksidin; Grup 2: Ozonlu su; Grup 3: Nd:YAG-lazer; Grup 4: Er:YAG-lazer; Grup 5 (kontrol): İşlem uygulanmayan. Dezenfeksiyon işlemi gruplar doğrultusunda yapılarak siloran bazlı bir kompozitle tamir işlemi gerçekleştirildi. Sonrasında gruplar iki altgruba ayrılarak (n=10) bir alt gruba termal yaşlandırma işlemi uygulandı. Bütün örnekler üniversal test cihazında bağlanma dayanımı testine tabi tutuldu. Elde edilen verilerin istatistiksel analizi varyans analizi ve Student-t testi ile gerçekleştirildi. ***Bulgular:*** *Yaşlandırma öncesi gruplar arasında istatistiksel olarak fark bulunmamıştır. (p>0,05). Yaşlandırma sonrası da gruplar arası istatistiksel bir fark görülmezken (p>0,05) gruplar yaşlandırma öncesi ve sonrası kendi içerisinde karşılaştırıldığında ozonlu su, Nd:YAG laze, Er:YAG lazer ve kontrol gruplarında istatistiksel olarak anlamlı bir fark bulunmazken (p>0,05), klorhekzidin grubunda yaşlandırma sonrası tamir bağlanma kuvveti anlamlı bir şekilde azalmıştır (p<0,05).* ***Sonuç****: Ozonlu su, Nd:YAG-lazer ve Er:YAG-lazer uygulamaları siloran bazlı kompozitlerin tamirinde alternatif dezenfektan olarak uygulanabilir. Ancak klorheksidin uygulaması yaşlanma sonrası tamir bağlanma kuvvetini azaltmaktadır.* ***Anahtar kelimeler:*** *Er:YAG lazer, kompozit tamiri, klorhekzidin, Nd:YAG lazer, ozonlu su.*

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Cumhuriyet Dental Journal: 2021; 24(3)
e-ISSN 2146-2852
Doi:10.7126/cumudj.925752

Original research

EVALUATION OF FACTORS AFFECTING DENTAL ANXIETY IN ADOLESCENTS

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ABSTRACT

Objectives: Dental anxiety determines the frequency of dental treatment availed with long-term implications in oral health maintenance. The aim of this study is to investigate the effects of sociodemographic data, dental anxiety levels of parents, family functionality, perceived family support and other associated factors on dental anxiety of adolescents.

Materials and Methods: 213 adoloscents who applied to the Pediatric Dental Clinic and their parents were included in the study and their sociodemographic data were recorded. The Corah Dental Anxiety Scale was applied to the adolescents and their parents to determine dental anxiety and the Perceived Family Support Scale was applied to measure the perceived family support of the adolescents. Regarding the family functionality, the parents answered the Family Assessment Device.

Results: The avarage dental anxiety level of the adolescents was 8.87±3.10. The family functions were unhealthy in terms of roles, and affective involvement. According to the multiple regression analysis, the predictors of dental anxiety levels were family functionality, maternal educational level, previous application to the dentist, having negative experience in the past, the choosing of a dental treatment center, and training regarding dental treatment.

Conclusions: Clinicians should consider that socio-cultural characteristics, family functionality and support may affect dental anxiety, and the improvement of the physical environment may be effective in reducing dental anxiety during dental treatment of adolescents.

Keywords: Adolescents, dental anxiety, family functionality, perceived family support, sociodemographic factors.

Received : 23.04.2021
Accepted : 01.07.2021

How to Cite Arat Maden E, Maden Ö, Karabulut B, Güven Polat G. Evaluation of Factors Affecting Dental Anxiety in Adolescents. Cumhuriyet Dent J 2021;24:3:244-255.
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INTRODUCTION

Dental anxiety is defined as the fear and concern an individual feel against any dental process without being associated with private external stimulant.[1] Dental anxiety is known to be related with multiple psychosocial factors, i.e. it is a problem in which social factors and individual thoughts and behaviors are related to each other.[2] Although it is seen at all ages, it generally appears in childhood and adolescent periods. Dental anxiety affects 10 to 20% of adults and 43% of children and adolescents.[3,4] Anxiety is an important problem in the dental care of children and adolescents. Dental anxiety often causes avoidance of dental care, and can cause serious deterioration in oral and dental health. Such a deterioration can significantly increase dental care costs. For this reason, reducing anxiety is important in terms of patients, doctors and costs.

Many factors like age, gender and sociodemographic factors were reported to be effective in dental anxiety.[5-8] Some authors recommend a classification for the reasons of children's dental anxiety - intrinsic factors (i.e. age, temperament), external family factors (i.e. family anxiety, socioeconomic status), and external dental factors (i.e. dental and medical experience).[2] One of the most important internal factors regarding children's dental anxiety is age. Some authors reported that although there was a decrease in anxiety levels, it was not linear.[9,10]

The role of families is also important in the formation of anxiety in children and adolescents. Epstein and Bishop defined families that can solve problems as those coming together, those being emotionally connected to each other and those not preventing each other's freedom, everyone can effectively perform the role expected of him/her, control each other's behaviors in a way that does not overdo, and families with a comfortable and direct communication functions as healthy families.[11] Families who perform their functions at the expected level are functional families. Families who cannot perform their functions because of impaired domestic interaction were defined as non-functional families. The most important characteristic of a "non-functional" family is that the communication function is corrupted, and interpersonal relations are broken, and are subject to rules.[12] It was determined that adolescents in families who engage in negative attitudes and behaviors have high anxiety levels.[13] To more fully understand adolescent dental anxiety, examination of family functionality is critical.

Social support that is perceived from the family makes individuals feel good in spiritual terms, increase their confidence, and help them develop positive self-esteem and selfawareness. Social support also prevents stress and anxiety, and also has an effect on the problem-solving skills of the individual.[14] In this context, it can be speculated that it is important to investigate the relations between social support and family functionality perceived from the family, and the effects of these on dental anxiety.

In general, children and adoescents who have dental anxiety try all means to avoid or delay dental treatment, which causes deterioration of oral health.[15] In addition, they also show poor cooperation during dental visits, which jeopardizes the treatment outcomes, creating occupational stress on the dentist and a dispute between the dentist and the parents.[16] Dental anxiety in childhood and puberty continue in adulthood, leading to the avoiding of dental treatment; and as a result, oral-dental health is adversely affected.[7,17] Adolescents with dental anxiety had negative thoughts regarding their dental and general health.[18]

Adolescence may represent a developmental period in which dental fear/anxiety can have a disproportionate effect on future dental attendance or avoidance behavior. Dental anxiety in adolescence is a critical period for the development of independence in decision-making and control emerged as an important factor of dental fear / anxiety in this period[19], and it is worth examining as it complicates its etiology and potential future impact. Considering the not so adequate and contradictory evidence, in the present study it was aimed to determine whether there is a difference between the dental anxiety levels of the adolescents according to the sociodemographic data of the participants and whether there is a relationship between the dental anxiety levels of the families and the dental anxiety levels of the adolescents, and familial factors such as family functionality and

perceived family support in relation to the previous experiences in dental treatments. In addition, it was aimed to determine whether these variables are predictors of adolescents' dental anxiety levels.

MATERIALS AND METHODS

This study was conducted with 213 parents and adolescents between the ages of 13 and 16 who applied to Taksim Education and Research Hospital, Gumussuyu Children's Dentistry department, and Health Sciences University, Sultan Abdulhamid Khan II, Education and Research Hospital between January 2019 and July 2019. Simple random sampling method was preferred in the selection of the study group. Ethical permission required for the study to be carried out was obtained from Health Sciences University, Medical Research Local Ethics Board (decision no: 18/69- decision date: 26/10/2018). All procedures of the presented research were performed based on the Declaration of Helsinki. After two pedodontists informed the participants about the study and obtained the required permissions, the forms and scales that were prepared by the researchers were given in line with the desired information, and the participants were asked to complete these forms in full. Those with any immediate treatment requirement, and those with mental physical and/or systemic disease were not included in the study. The questionnaires took about 20 minutes to complete.

Data Collection

Socio-demographical Data Form

It was developed by the researcher and filled in by the parents to determine the sociodemographic characteristics of the participants, such as age, gender, education and income status of their parents. This form also included questions about previous application to the dentist, having negative experience in the past, choosing a dental treatment center, and training regarding dental treatment.

Corah Dental Anxiety Scale (DAS)

The translation of this scale into Turkish and its validity and reliability study were conducted by Seydaoglu et al.[20] This scale was filled by adolescents and their families. The scale consists of four questions and is scored between 1-5 points. The total score obtained from the scale ranges from 4-20. According to this scale, a total score of less than 13 points show low anxiety, 13-14 show moderate anxiety, and 15 and above show high anxiety.

Family Assessment Device (FAD)

The McMaster Family Assessment Device (FAD), a questionnaire developed to evaluate families according to the McMaster Model, consists of seven scales that measure problem solving (PS), communication (CM), roles (RL), affective responsiveness (AR), affective involvement (AI), behavior control (BC) and general functioning (GF). Its validity and reliability study was done by Epstein et al.[21] and adaptation to Turkish was done by Bulut.[22] This scale was filled in by the parents. The total score of the scale consisting of 60 items varies between 1-4 points. If the family's score in an area exceeds 2, it is considered to be a problem in that area and indicates that the family function in that area is unhealthy. The scale does not have a total score and subscales are evaluated separately.

Perceived Family Support Scale (PFSS)

This scale, which is used to evaluate perceived family support, was developed by Procidano and Heller, and was adapted to Turkish by Eskin.[23] This scale was filled by the adolescents. The scale consists of 20 questions which are answered as "Yes", "No", and "Partly". The 3rd, 4th, 16th, 19th and 20th questions of the scale are scored with 2 (Yes), 0 (No), and 1 (Partly), and all other questions are scored as 0 (No), 2 (Yes), and 1 (Partly). The total score of the scale varied between 0-40. Increased scores show good family support.

Statistical analysis

The statistical evaluations were made by using the SPSS (ver. 22.0. Chicago. II. USA) program. The resulting data were transferred to computer medium. While evaluating the findings obtained in the study, mean, standard deviation, and frequency were used in descriptive statistics. Kolmogorov-Smirnov test for the normal distribution fit of quantitative data, Chi-square test for comparison of categorical variables, Independent sample t-test for comparison of means of numeric variables, and Pearson correlation analysis for determining the relationships between variables were used. Multiple regression analysis was used to determine the factors predicting the dependent variable. Results were

evaluated at 95% confidence interval and significance level of $p<0.05$.

RESULTS

Most of the adolescents participating in the study (55.9%, n = 119) were girls, and the mean age was 14.36±1.12. When the socio-demographical data of the parents and the adolescents were evaluated, the mean age of the mothers was 40.00±5.07, the mean age of the fathers was 44.39±4.84 and the mean age of the adolescents was 14.25±1.13. A majority of the mothers 32.9% (n=70) had 1-4 years of education, and a majority of the fathers 32.4% (n=69) had 9-12 years of education. A large portion of the family income (83.6%) could be described as families having middle income. 93.4% of the adolescents (n=199) had previously referred to dentists, and 12.7% (n=27) had negative experience in the past. Of the adolescents, 53.5% (n=114) had recent toothache. 35.7% of the adolescents (n=76), applied to a state hospital, and 98.1% had received training on dental treatment (Table 1).

Table 1. Distribution of socio-demographic data and comparison with adolescents' dental anxiety levels (n=213)

	Data value			
	Number (%)	Me.±S.D.	Statistical Analyzes	
			χ^2	p*
Sex				
Boy	94 (44.1)	9.00±3.36	16.907	>0.05
Girl	119 (55.9)	8.70±2.74		
Groups of age				
13	76 (35.7)	8.50±2.91		
14	47 (22.1)	9.43±3.44	67.190	=0.001
15	51 (23.9)	9.12±2.92		
16	39 (18.3)	8.59±3.24		
Maternal age				
29-37	63 (29.6)	8.57±3.28		
38-46	121 (56.8)	9.18±3.07	37.041	<0.05
47 and upper	29 (13.6)	8.21±2.70		
Paternal age				
32-39	46 (21.6)	9.41±3.32		
40-47	125 (58.7)	8.65±3.10	53.234	=0.001
48 and upper	42 (19.7)	8.93±2.81		
Maternal education level				
<1 year	6 (2.8)	7.67±3.39		
1-4 year	70 (32.9)	8.60±3.10		
5-8 year	48 (22.5)	9.75±2.89	37.041	<0.05
9-12 year	56 (26.3)	9.45±3.15		
13 year and upper	33 (15.5)	7.39±2.68		
Paternal education level				
<1 year	5 (2.3)	9.40±4.10		
1-4 year	54 (25.4)	8.78±3.41		
5-8 year	32 (15.0)	8.16±3.11	53.234	=0.001
9-12 year	69 (32.4)	9.61±3.10		
13 year and upper	53 (24.9)	8.38±2.51		
Family income				
Low	26 (12.2)	8.92±2.31		
Moderate	178 (83.6)	9.03±3.18	57.765	<0.001
High	9 (4.2)	5.56±0.53		
Previous application to the dentist				
Yes	199 (93.4)	8.79±2.99	29.380	=0.003
No	14 (6.6)	9.93±4.34		
Having negative experience in the past				
Yes	27 (12.7)	9.89±2.93	21.711	<0.05
No	186 (87.3)	8.72±3.10		
Toothache				
Yes	114 (53.5)	8.81±3.36	20.010	>0.05
No	99 (46.5)	8.94±2.78		
Choosing a Dental Treatment Center				
PDC	20 (9.4)	6.65±2.68	94.493	=0.003

CBDC	41 (19.2)	8.80±3.30		
PHDC	16 (7.5)	8.94±3.75		
SHDC	76 (35.7)	9.00±2.93		
T/RHDC	33 (15.5)	8.82±3.10		
UHDC	27 (12.7)	10.26±2.36		
Training regarding dental treatment				
Yes	153 (71.8)	9.22±3.27	29.731	=0.003
No	60 (28.2)	8.50±4.12		

*p<0.05. t: Independent sample t-test, F: One way ANOVA. Me.: Mean, S.D.: Standard Derivation. PDC: Private Dental Clinic, CBDC: Community Based Dental Clinic, PHDC: Private Hospital Dental Clinic, SHDC: State Hospital Dental Clinic, T/RHDC: Training and Research Hospital Dental Clinic, UHDC: University Hospital Dental Clinic.

The comparison of dental anxiety levels of adolescents according to sociodemographic data is presented in Table 1. According to the results of the chi-square test, a statistically significant difference ($p<0.05$) was found in the dental anxiety levels of the adolescents in terms of adolescents' age, maternal age, paternal age, maternal education level, paternal education level, family income, previous application to the dentist, having negative experience in the past, choosing a dental treatment center, and training regarding dental treatment (Table 1).

The average of the adolescents' dental anxiety level was 8.87±3.10, and the average of the family's dental anxiety level was 8.97±3.24. In addition, the average of perceived family support level by the adolescents was 28.84±7.04. The average of the family assessment device subscale scores were problem solving 1.81±0.59, communication 1.78±0.52, roles 2.08±0.43, affective responsiveness 1.84±0.55, affective involvement 2.47±0.43, behavior control 1.93±0.42, and general functioning 1.84±0.45. According the results of the FAD, it was stated that mean scores above 2.0 are indicative of a trend towards "unhealthy" in family functions. Accordingly, the family functions of the adolescents included in study were unhealthy only in terms of "roles" and "affective involvement", and healthy in terms of other variables. The results of the independent sample t-test regarding whether C-DAS, F-DAS, PFSS, and FAD subscale scores differ significantly according to gender were presented in Table-2. Adolescents' "Family Assessment Affective Responsiveness Subscale" scores differed significantly according to gender ($t=2.288$, $p<0.05$, Table 2).

Table 2. The independent sample t-test results of A-DAS, F-DAS, PFSS, and FAD subscale scores by gender

	Sex	n	Me.±S.D.	Statistical Analyze t	Statistical Analyze p*
A-DAS	Boy	94	8.70±2.74	-.713	.477
	Girl	119	9.00±3.36		
F-DAS	Boy	94	8.81±3.16	-.634	.527
	Girl	119	9.09±3.31		
PFSS	Boy	94	28.64±7.15	-.363	.717
	Girl	119	28.99±6.98		
FAD					
PS	Boy	94	1.79±0.53	-.514	.608
	Girl	119	1.83±0.63		
CM	Boy	94	1.80±0.47	.458	.648
	Girl	119	1.77±0.56		
RL	Boy	94	2.09±0.47	.055	.956
	Girl	119	2.08±0.40		
AR	Boy	94	1.93±0.51	2.288	.023
	Girl	119	1.76±0.57		
AI	Boy	94	2.47±0.43	-.002	.999
	Girl	119	2.47±0.43		
BC	Boy	94	1.93±0.39	.163	.870
	Girl	119	1.92±0.44		
GF	Boy	94	1.83±0.45	-.334	.738
	Girl	119	1.85±0.45		

*p<0.05, t: Independent sample t-test, Values are as given range (Mean±Standard Deviation).
†A-DAS: Adolescent Dental Anxiety Scale.

‡F-DAS: Family Dental Anxiety Scale.
§PFSS: Perceived Family Support Scale.
#FAD: Family Assessment Device Scale, PS: Problem Solving, CM: Communication, RL: Roles, AR: Affective Responsiveness, AI: Affective Involvement, BC: Behavior Control, GF: General Functioning.

The results of the correlation between adolescents' dental anxiety scale and sociodemographic data (adolescents' ages, maternal age, father's age, family income, maternal education level, paternal education level, previous admission to the dentist, having negative experience in the past, choosing a dental treatment center, training related to dental treatment, toothache) are presented below (Table 3). When the relationship between the dental anxiety levels of the adolescents and sociodemographic data was evaluated, a statistically significant positive correlation was found between the dental anxiety levels of the adolescents and the choice of a dental treatment center, and training regarding dental treatment (Table 3).

Table 3. Pearson correlations (rs) between A-DAS† and adolescents' sociodemographic data‡

	A-DAS	Sex	A-Age	M-Age	P-Age	INC	MEL	PEL	PaTd	NEP	DTC	TRDT	T-Ache
A-DAS	1	.05	.03	.00	-.05	-.12	-.09	.02	-.09	.13	.214**	.173*	-.02
Sex		1	.11	.07	-.01	-.08	.01	.00	-.01	-.12	-.05	-.05	.08
A-Age			1	.09	.156*	.11	.149*	.03	-.143*	-.10	.01	-.01	-.154*
M-Age				1	.577**	-.11	.11	-.04	.11	.01	.06	-.11	.180**
P-Age					1	.01	.10	.10	.228**	.10	-.10	-.07	.05
INC						1	.178**	.05	-.10	.01	-.155*	-.12	.03
MEL							1	.12	-.169*	.07	-.02	-.167*	-.13
PEL								1	-.07	-.04	-.06	.01	-.07
PaTd									1	.10	.03	-.143*	.13
NEP										1	-.09	.03	.07
DTC											1	-.144*	.01
TRDT												1	.00
T-Ache													1

*p<0.05, **p<0.01.
†A-DAS: Adolescent Dental Anxiety Scale.
‡Socio-demographic data (A-Age: Adolescents' ages, M-Age: Maternal age, P-Age: Paternal age, INC: Family income, MEL: Maternal education level, PEL: Paternal education level, PaTd: Previous application to the dentist, NEP: Having negative experience in the past, DTC: Choosing a dental treatment center, TRDT: Training related to dental treatment, T-Ache: Toothache).

The results of the correlation between adolescents' dental anxiety scale and family's dental anxiety scale, PFSS scores and sub-scale of FAD are presented below (Table 4). When the relationship between adolescents' dental anxiety levels and F-DAS, PFSS, and subscale scores of FAD was evaluated, a statistically significant positive correlation was found between adolescents' dental anxiety levels and F-DAS mean scores and FAD general functions subscale mean scores (Table 4).

Table 4. Pearson correlations (rs) between A-DAS† and F-DAS‡, PFSS§, subscale scores of FAD#

	A-DAS	F-DAS	PFSS	PS	CM	RL	AR	AI	BC	GF
A-DAS	1	.346**	-.10	.06	.00	.06	.04	-.10	.11	.135*
F-DAS		1	-.194**	.07	.141*	.13	.12	.12	.13	.03
PFSS			1	-.178**	-.158*	-.282**	-.08	.01	-.169*	-.251**
FAD										
PS				1	.621**	.519**	.491**	.154*	.489**	.681**
CM					1	.567**	.550**	.312**	.537**	.706**
RL						1	.469**	.325**	.528**	.622**
AR							1	.409**	.532**	.655**
AI								1	.207**	.279**
BC									1	.601**

GF	1

* p<0.05, ** p<0.01.
†A-DAS: Adolescent Dental Anxiety Scale.
‡F-DAS: Family Dental Anxiety Scale.
§PFSS: Perceived Family Support ScalE.
#FAD: Family Assessment Device Scale, PS: Problem Solving, CM: Communication, RL: Roles, AR: Affective Responsiveness, AI: Affective Involvement, BC: Behavior Control, GF: General Functioning.

In the first multiple logistic regression analysis, the extent to which sociodemographic data predicted dental anxiety levels in adolescents was examined. This regression analyses showed that maternal education level, previous application to the dentist, having negative experience in the past, choosing a dental treatment center, and training related to dental treatment were associated with adolescents' dental anxiety levels ($F_{(12, 200)}$=2.855, p<0.001) and the independent variables in the model explained 14.6% of the unique variance in the adolescents' dental anxiety levels. According to the standardized regression coefficients, the order of importance of predictive variables on adolescents' dental anxiety levels is training related to dental treatment (β=-0.207), choosing a dental treatment center (β=0.198), having negative experience in the past (β=0.191), previous application to the dentist (β=-0.171), and maternal education level (β=-0.143). Considering the significance tests of the regression coefficients, it was seen that only training related to dental treatment, choosing a dental treatment center, having negative experience in the past, previous application to the dentist, and maternal education level (p<0.05) were significant predictors on the adolescents' dental anxiety levels (Table 5).

Table 5. The multiple regression analysis results between A-DAS† and adolescents' sociodemographic data‡

	Unstandardized Coefficients		Standardized Coefficients	t	p*	Correlations	
	B	Std. Error	β			Zero-order	Partial
(Constant)	2.963	3.766		.787	.432		
Sex	.399	.418	.064	.956	.340	.048	.067
A-Age	.101	.192	.037	.527	.599	.026	.037
M-Age	-.005	.063	-.008	-.080	.937	-.021	-.006
P-Age	-.004	.068	-.006	-.055	.956	-.025	-.004
INC	-.818	.538	-.105	-1.521	.130	-.119	-.107
MEL	-.380	.185	-.143	-2.053	.041	-.088	-.144
PEL	.128	.177	.049	.724	.470	.015	.051
PaTd	-2.126	.886	-.171	-2.400	.017	-.091	-.167
NEP	1.777	.625	.191	2.843	.005	.126	.197
DTC	.408	.139	.198	2.930	.004	.214	.203
TRDT	1.421	.471	.207	3.019	.003	.173	.209
T-Ache	-.204	.430	-.033	-.476	.635	-.021	-.034

*p<0.05. R=0.382, R^2=0.146, Adjusted R^2= 0.01, $F_{(12\text{-}200)}$=2.855, p<0.001.
†A-DAS: Adolescent Dental Anxiety Scale (Dependent variable).
‡Socio-demographic data (A-Age: Adolescents' age, M-Age: Maternal age, P-Age: Paternal age, INC: Family income, MEL: Maternal education level, PEL: Paternal education level, PaTd: Previous application to the dentist, NEP: Having negative experience in the past, DTC: Choosing a dental treatment center, TRDT: Training related to dental treatment, T-Ache: Toothache).

In the second multiple logistic regression analysis, the extent to which F-DAS, PFSS and FAD sub-scale scores predicted dental anxiety levels in adolescents were examined. This regression analysis showed that F-DAS score, and sub-scale scores FAD (communication, affective involvement and general functions) were associated with adolescents' dental anxiety levels, and the independent variables in the model explained 20.4% of the unique variance in the adolescents' dental anxiety levels. According to the standardized regression coefficients, the order of importance of predictive variables on adolescents' dental anxiety levels were F-DAS scores (β=0.401), FAD-General functions (β=0.395), FAD-Communication (β=-0.229), and FAD-Affective involvement (β=-0.158). Considering the significance tests of the regression coefficients, it was seen that only family's dental anxiety level, and family functions, such as general functions, communication, and affective involvement

($p<0.05$) were significant predictors on the adolescents' dental anxiety levels (Table 6).

Table 6. The multiple regression analysis results between A-DAS† and F-DAS‡, PFSS§ and subscale scores of FAD#

	Unstandardized Coefficients		Standardized Coefficients	t	p*	Correlations	
	B	Std. Error	B			Zero-order	Partial
(Constant)	5.957	1.853		3.215	.002		
F-DAS	.383	.063	.401	6.070	.000	.346	.392
PFSS	.014	.030	.032	.467	.641	-.101	.033
FAD							
PS	-.185	.474	-.035	-.392	.696	.064	-.027
CM	-1.361	.574	-.229	-2.373	.019	.004	-.164
RL	-.161	.629	-.022	-.257	.798	.059	-.018
AR	-.430	.511	-.076	-.842	.401	.039	-.059
AI	-1.143	.516	-.158	-2.214	.028	-.104	-.154
BC	.397	.622	.054	.637	.525	.113	.045
GF	2.728	.804	.395	3.394	.001	.135	.232

*$p<0.05$. $R=0.452$, $R^2=0.204$, Adjusted $R^2= 0.17$, $F_{(9\text{-}203)}=5.786$, $p<0.001$.
†A-DAS: Adolescent Dental Anxiety Scale (Dependent variable).
‡F-DAS: Family Dental Anxiety Scale.
§PFSS: Perceived Family Support Scale.
#FAD: Family Assessment Device Scale, PS: Problem Solving, CM: Communication, RL: Roles, AR: Affective Responsiveness, AI: Affective Involvement, BC: Behavior Control, GF: General Functioning.

DISCUSSION

Excessive dental fear and anxiety is a universal problem affecting the great majority of the population.[24] It was reported in previous studies that the dental anxiety in childhood continues in adult period, and therefore, determining dental anxiety at an early stage is important.[25,26]

Although it was reported that gender has an effect on dental anxiety levels, and in general, females have higher levels of dental anxiety than males[27,28], there are several other studies reporting that dental anxiety is not gender-related.[5,8,29] Foloyan et al.[5] reported that gender alone is not effective on dental anxiety; however, it is also known that it may cause a predisposition to dental anxiety by interacting with other variables. We found that in adolescents, gender differences in the report of dental anxiety were not as robust as in some other studies, and indeed non-significant in the present study. The specific age cohorts in the current study may have affected these results.

The effect of close surroundings, especially that of the family is important in children's behaviors in dental settings.[30,31] In the study of Neverlien, it was reported that there was a positive correlation between the socioeconomic status and cultural structures of the family and the behavior of children in clinics.[32] Tuuti[33] reported that children between the ages of 7 and 10 who had fathers with high educational status exhibited better and harmonious behaviors during dental treatments. Stabholz and Peretz[34] reported that people with high levels of education had developed the ability to deal with stressful conditions more easily. However, there are also several studies that did not report any relation between the level of education and dental anxiety.[35,36]

When the relation between the income status of the family and dental anxiety level was evaluated, the lowest dental anxiety in adolescents was determined in high-income families. Similarly, Wright and Alpern[37] found that families with high socioeconomic status showed better co-operation during dental treatment for their 3-5-year-old children. Higher income families pay more attention to oral and dental health; hence, one can talk about more time and budget allocation. According to the results of this study, the dental anxiety level was found to be higher in the adolescents with mothers having low education levels and the level of dental anxiety was higher in the adolescents belonging to low-income families.

There is no consensus on the relations between children and their parents' dental anxiety. Wu et al.[38] reported a weak relation between the dental anxiety of children and their parents, and for

this reason, they suggested that children might not have learned dental anxiety from their parents. However, in some studies, the mother's dental anxiety affected the dental anxiety of children because children spent longer time with their mothers during the period of social learning, and it was suggested that it adversely affected children's behaviors during dental treatments.[39,40] One of the most interesting research findings was that, when accounted for separately and concurrently, fathers' but not mothers' dental fear predicts adolescent dental fear/anxiety.[41] In this study, a relation was not detected between parents' dental anxiety and their adolescents' dental anxiety. Additional research is needed to comprehensively understand the parents' dental anxiety role in adolescens' dental anxiety.

It is often stated that the primary cause of anxiety is negative dental treatment experiences, and numerous studies support the importance of conditioning in anxiety.[42,43] However, repeated asymptomatic visits play a prophylactic role for anxiety, and children participating in preventive programs show lower levels of fear symptoms. Nicolas et al.[44] state that children who have had their teeth filled before have less fear than those who have never had dental treatment. The first dentist appointment is an important factor in how the child will take a position regarding a dentist and dental treatments in the future. Patients should be encouraged to ask questions about their treatment and be fully informed about what to do before starting the procedure, and also, during the procedure. According to results of a study by Töredi et al.[45], it can be said that the disclosure of the treatment is important for reducing the anxiety of the children. Similarly, the dental anxiety level of adolescents who have received information related to their dental treatment was lower than the dental anxiety level of uninformed adolescents in this study. In previous studies, it was found that there were no significant relations between anxiety and restorative treatment history in 5-year-old children; however, there was a significant relation between anxiety and restorative treatment experience in adolescents between 12 and 15 years of age.[46,47] In this study, dental anxiety levels were lower in adolescents who had a previous dental examination and higher in adolescents who had negative experiences during their dental treatments in the past.

Dental health center ambience can play a significant role in initiating dental anxiety.[48] The office atmosphere can be made calm and unthreatening. Importantly, anxious patients should not be made to wait too long so that they have less time to absorb negative experiences; additionally, a longer waiting time gives them time to recall the threatening stimuli.[49] In a previous study by Fux-Noy et al.[50], dental anxiety was significantly higher in patients who had longer waiting time prior to treatment. In this study, the dental anxiety of adolescents applied to a state hospital was found to be higher due to the atmosphere and the long waiting time in these institutions.

This is truly the first study to address the unique roles of family functionality in dental anxiety especially in a sample of adolescents. It was reported that preschool anxiety had effects on the way the family functioned, especially on the parental compliance, and thus, highlighting family dysfunction acierated with early anxiety.[47] The study provides support for this phenomenon for dental anxiety occurring not only in childhood, but also, in adolescence. In the current study, a positive and statistically significant relation was detected between communication, affective involvement and general functions scores of the families and the dental anxiety levels of the adolescents. The dental anxiety levels of the adolescents of families with disrupted family functionality were found to be higher. The communication and role distribution within the family, problem-solving skills of the family, and conflicts in exhibiting due attention cause behavioral and emotional reflections in children and adolescents. Being less aware of the problems within the family or having difficulties in talking about such problems increase the anxiety levels of children and adolescents.

Limitations

There are limitations to the study. Firstly, the scales used in the study were filled based on the individuals' self-assessment. For this reason, the

biases people can make while evaluating themselves cannot be controlled. The biases and evaluation mistakes that may be made by the individuals may have affected the results of the study. Secondly, the findings of the study may not be generalizable to older adolescents as differences in psychological and physical status exist between early adolescents and late adolescents. Additional research is needed to comprehensively understand factors affecting dental anxiety in adolescants.

CONCLUSIONS

When the reasons underlying dental anxiety are evaluated, the patient should be considered in a holistic manner. Determining the dental anxiety/fear level will be the first step in establishing cooperation and a healthy dentist-patient relation in the treatment process. Family functionality plays a role in dental anxiety development in adolescents. In this sense, activities and trainings should be organized for parents to acquire the skill of detecting problems in their families and talking about them.

ACKNOWLEDGMENT

No funding was received for this study.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ÖZ

Amaç: *Dental kaygı, ağız sağlığının korunmasında uzun vadeli etkilerle birlikte yararlanılan diş tedavisinin sıklığını belirler. Bu çalışmanın amacı, sosyodemografik veriler, ebeveynlerin dental kaygı düzeyleri, aile işlevselliği, algılanan aile desteği ve diğer ilişkili faktörlerin ergen dental kaygısı üzerindeki etkilerini araştırmaktır.* ***Gereç ve Yöntemler:*** *Çocuk Diş Kliniği'ne başvuran 213 ergen ve ebeveyn çalışmaya dahil edildi ve sosyodemografik verileri kaydedildi. Dental kaygıyı belirlemek için, ergenlere ve ebeveynlerine Corah Dental Anksiyete Ölçeği ve ergenlerin algılanan aile desteğini belirlemek için, ergenlere Algılanan Aile Desteği Ölçeği uygulandı. Aile işlevselliği ile ilgili olarak, ebeveynler Aile Değerlendirme Ölçeği'ni cevapladı.* ***Bulgular:*** *Ergenlerin ortalama dental kaygı düzeyi 8.87 ± 3.10'dur. Aile işlevleri roller ve duygusal katılım açısından sağlıksızdı. Çoklu regresyon analizine göre, dental kaygı düzeylerinin yordayıcıları; aile işlevselliği, annenin eğitim düzeyi, daha önce diş hekimine başvurma, geçmişte olumsuz deneyime sahip olma, diş tedavi merkezi seçimi ve diş tedavisi ile ilgili eğitimdir.* ***Sonuçlar****: Klinisyenler, sosyo-kültürel özelliklerin, aile işlevselliğinin ve aile desteğinin, dental kaygıyı etkileyebileceğini ve fiziksel çevrenin iyileştirilmesinin ergenlerin diş tedavisi sırasında dental kaygıyı azaltmada etkili olabileceğini göz önünde bulundurmalıdır.* ***Anahtar Kelimeler:*** *Ergenler; diş kaygısı, aile işlevselliği, algılanan aile desteği, sosyodemografik faktörler.*

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Cumhuriyet Dental Journal: 2021; 24(3)
e-ISSN 2146-2852
Doi:10.7126/cumudj.912285

Original research

IN VITRO COMPARISON OF THE ANTIMICROBIAL EFFECTS OF DIFFERENT ROOT CANAL MEDICAMENTS ON *ENTEROCOCCUS FAECALIS* AND *CANDIDA ALBICANS*

*Burçin Arıcan[1]
Hesna Sazak Öveçoğlu[2]

ABSTRACT

Objectives: To evaluate the antimicrobial effects of experimental root canal medicaments on *Enterococcus faecalis* and *Candida albicans* on day 3 and day 7 using quantitative polymerase chain reaction (Q-PCR) analyses.

Materials and Methods: 240 single-rooted, single-canalled human teeth were used. Root canals were shaped mechanically and embedded in acrylic blocks, then sterilized in an autoclave. The samples were divided into two groups and infected with *E. faecalis* and *C. albicans*, and then divided into subgroups (n=10); calcium hydroxide ($Ca(OH)_2$), triple paste (TAP), double paste (DP), modified double paste (MDP), lactoferrin (Lf), negative and positive groups. At the end of 3 and 7 days, paper points containing the root canal samples were placed in empty Eppendorf tubes, and DNA was isolated. Real-time Q-PCR was applied and the data were analyzed statistically.

Results: The antimicrobial effects of each medicament increased from days 3 to 7. $Ca(OH)_2$ and TAP groups showed the similar eradication rates for *E. faecalis* and *C. albicans* on day 3 and 7 ($P>0.05$). There was no significant difference between DP, MDP and Lf for the eradication of microorganisms at both experimental days ($P>0.05$), except the amount of eradicated *E. faecalis* by DP at day 7 in which DP caught the similar percentages with TAP and $Ca(OH)_2$ ($P<0.05$).

Conclusions: Experimental medicaments demonstrated antimicrobial efficiancy similar to those used routinely in endodontic clinic. It is promising that lactoferrin which is a very biocompatible material can be used in different combinations as an intracanal medicament.

Key words: Antibiotic paste, *Candida albicans*, *Enterococcus faecalis*, Lactoferrin, Root canal medicament.

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Received : 09.04.2021
Accepted : 05.08.2021

How to Cite Arıcan B, Sazak Öveçoğlu H. In Vitro Comparison of the Antimicrobial Effects of Different Root Canal Medicaments on Enterococcus Faecalis and Candida Albicans. Cumhuriyet Dent J 2021;24:3:256-265.
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INTRODUCTION

The central aim of root canal treatment is to eliminate bacteria from the infected root canal and prevent a subsequent reinfection.[1] Microorganisms and their byproducts are the main etiological factors in dentinal, pulpal, and periapical pathosis. *Enterococcus faecalis* and *Candida albicans* are the most common microorganisms isolated during root canal treatments that can best adapt easily to environmental changes.[2] Intracanal medicaments should be used to eliminate these factors following mechanical preparation.

Several root canal medicaments are in use to successfully eliminate microorganisms and infected tissues. $Ca(OH)_2$ is commonly used as an effective intracanal agent; however, some recent studies have questioned the ability of $Ca(OH)_2$ to kill some resistant bacterial species in root canals.[3,4] The combination of metronidazole, ciprofloxacin, and minocycline (TAP) has been developed to disinfect oral lesions.[5] However, this combination of medicaments has some disadvantages, such as the inability to remove the antibiotics from the root canal[6] and crown discoloration.[7] Thus, new medicaments and antibiotic combinations should be developed.

In 1979, *Streptococcus faecalis* was reported to be resistant to aminoglycosides. However, using the aminoglycosides with penicillin caused a synergistic effect against the same microorganism.[8] This combination has been advised for use as a treatment option against infective endocarditis by the European Society of Cardiology since 2009.[9] Accordingly, it may show antimicrobial effects against persistent microorganisms isolated from the root canal and may be used as a new generation intracanal medicaments in endodontics.

Another potential medicament could be lactoferrin (Lf), which is a multifunctional iron glycoprotein in human secretions, such as milk, amniotic fluid, vaginal mucus, synovial fluid, and seminal plasma.[10] Lf exhibits non-iron-dependent antibacterial, antioxidant, antifungal, antiviral, antitumor, anti-inflammatory, and immunoregulatory activities.[11,12] Lf inhibits the initial stage of infection by destroying the cell membrane structure or blocking adhesion to the host cell.[13] Moreover, it was reported that Lf severely prevent the formation of bacterial biofilm.[14]

Although there are several studies about the antimicrobial activity of $Ca(OH)_2$ and TAP against endodontic pathogens[3,7], to the best of authors' knowledge there are no studies in the literature regarding antimicrobial effect of the mentioned novel antibiotic combinations and lactoferrin. Therefore, in the present study, it was aimed to compare the antimicrobial activities of these new age medicaments and traditional agents towards *E. faecalis* and *C. albicans* using quantitative polymerase chain reaction (Q-PCR) analyses.

MATERIAL AND METHODS

All patients signed informed consent to permit use of their extracted teeth in this study. A total of 240 intact teeth which were freshly extracted for periodontal or orthodontic reasons were selected for study. The inclusion criterion was single-rooted and single-canalled teeth. The exclusion criteria were teeth from patients who received antibiotic therapy in the previous 3 months, teeth with a root fracture, previous endodontic therapy, oval shaped canal, curved canal, and carious lesions.

Preparation of root canals

A total of 240 roots from freshly extracted human teeth were used. The bone, calculus, and periodontal tissues on the root surface were gently removed with periodontal curettes. The teeth remained in saline solution until they were used. Each tooth was decoronated horizontally at the cement to enamel junction, and root lengths were standardized to 15 mm. After preparing the access cavity, root canal patency was defined with a 15 K file. The Protaper Universal System (Dentsply Maillefer, Ballaigues, Switzerland) was used as rotary instrumentation. An SX file was used followed by S1 and S2 files in the coronal part of the canal. The canals were finished using F1, F2, and F3 files to full working length. After using each instrument, 5.25% NaOCl was used for irrigation for 1 minutes. A final rinse with 10 mL of 5.25% NaOCl was used followed by 10 mL irrigation with 17% EDTA to remove the dentin particles.[7] The apical foramen was closed with composite resin and the outer surfaces of the specimens were covered with nail varnish to prevent possible

contamination from the external surface. The roots were embedded in acrylic blocks and sterilized by autoclaving for 30 min at 120°C.

Sample contamination

Ten teeth from each microorganism group were used as a negative control (Figure 1).

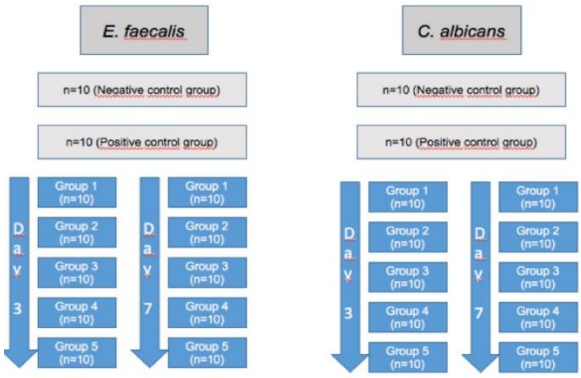

Figure 1: Distribution of the 240 extracted teeth according to the groups

The 220 teeth were randomly divided into two groups; 110 teeth were contaminated with freshly subcultured (24 h) *E. faecalis* ATCC 29212, which was grown in brain heart infusion (BHI) agar and BHI broth. The other 110 teeth, contaminated with freshly subcultured (24 h) *C. albicans* ATCC 10231, were grown in Sabouraud dextrose (SD) agar and SD broth. The cell suspensions were adjusted to 0.5 McFarland units. Each tooth was inoculated with microorganisms using a micropipette and agitated with a #15 K file. This procedure was repeated every 24 h for 2 days using freshly prepared 24 h cultures. The root canal orifices were sealed with temporary filling material each time. The teeth were always kept in a humid environment at 37°C.

Dressing of the canals

At the end of the 48h inoculation period, the temporary filling material was removed and the root canals were irrigated with 10 mL saline solution. Ten teeth from each microorganism group were used as a control to check for contamination of the canal. Three sterile paper points were placed in the canal and kept in the place for 60 s to sample the microbes. These paper points were placed in sterile Eppendorf tubes.

In total, 100 teeth from each microorganism group were divided into five groups, according to the intracanal medicaments used, as follows:

- Group 1 (n: 10 + 10): Calcium hydroxide ($Ca(OH)_2$) (Vision, Frankfurt, Germany) was mixed with sterile saline in a ratio of 2:1 (liquid/power) to obtain a paste-like consistency.
- Group 2 (n: 10 + 10) (TAP): Equal weights (200 mg) of metronidazole (Flagyl, Eczacıbaşı, Turkey), ciprofloxacin (Cipro, Biofarma, Turkey), and minocycline (Minocin, Teofarma, Italy) (1:1:1) were mixed with sterile saline[5] to a final concentration of 0.1 mg/mL. A paste-like consistency was delivered to canal using a lentulo.
- Group 3 (n: 10 + 10) (DP): Ampicillin (Alfasilin, Actavis, Istanbul, Turkey) (200 mg) was mixed with gentamicin (Genta, İE Ulagay, Istanbul, Turkey) (125 µL) to obtain a paste-like consistency.
- Group 4 (n: 10 + 10) (MDP) Ampicillin (200 mg) was mixed with gentamicin (125 µL) and 2% LF suspension (50 µL) to obtain a paste-like consistency,
- Group 5 (n: 10 + 10): A 2% suspension of Lf (Sigma Aldrich, USA) in sterile saline was prepared in an Eppendorf tube. The tube was covered with aluminum foil to design a dark medium. F3 paper points soaked in this suspension were placed into the root canal.

A total of 12 pilot studies were conducted to determine the proportions of the experimental root canal medicaments. Of these pilot studies, 2 are on the agar diffusion method, 8 on the culture method and 2 on the testing of different PCR methods.

The prepared medicaments were placed in the root canals by using lentulo until the excess medicaments were seen from the coronal access. Then F3 paper point was inserted into the root canal 2mm beyond the working length and thus it was aimed to ensure the contact of the medicament with the dentin wall. Then this space was filled with the medicament again. The root canal orifices were sealed with temporary filling material after applying the intracanal medicaments, which was day 0. All root canals were kept in a humid environment at 37°C. At the end of day 3, the temporary filling material from 50 specimens (10 specimen from each group) was removed. Excess medicament was removed with a #30 K file, and each root canal was irrigated with sterile saline

solution. Microbiological samples were collected using three sterile paper points which were held in the root canal for 1 min and stored in sterile Eppendorf tubes. The same procedure was applied for the other 50 specimens (10 specimen from each group) at the end of day 7.

DNA isolation

Dead cells were removed from the samples via a DNase-I treatment.[15] A 200 µL aliquot of 0.1 U/mL DNase-I was added to the samples and incubated for 15 min at 37°C. The samples were homogenized at 3,000 rpm for 1 min. A 400 µL aliquot of binding buffer (6 M guanidine thiocyanate, 20 mM Tris-HCl, pH 8) was combined with the samples and incubated at 98°C for 10 min. The extracted DNA was combined with 400 µL 2-propanol and captured on a silica column. The column was washed twice with a buffer containing 20 mM NaCl and 2 mM Tris-HCl, pH 7.5; 80% v/v ethanol. The DNA was eluted in 100 mM Tris-HCl pH 8.0 and stored at –20°C.

Quantitative real-time PCR

The Biospeedy™ Real-Time PCR EvaGreen Master Mix (Bioeksen Ltd., Co., Istanbul, Turkey) was used for all reactions. *E. faecalis* cells were quantified using the BactF 5'-AGA GTT TGA TCC TGG CTC AG-3' and BactR 5'-AAG GAG GTG ATC CAG CCG CA-3' primers that target bacterial DNA coding 16S Rrna.[16] *C. albicans* cells were quantified using the FungF 5'- TCC TCC GCT TAT TGA TAT GC-3' and FungR 5'-GGA AGT AAA AGT CGT AAC AAG G-3' primers that target the fungal internal transcribed spacer region.[17] The reaction mixtures contained 25 mg template DNA, 6 mg/mL bovine serum albumin, 5 mg/mL PEG 400, 0.25% Tween 20, 20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 0.1 U Proof Reading Hot-Start DNA Polymerase, 1× EvaGreen and 200 nM of each primer. The following thermocycling program was applied: 98°C, 3 min; 35 cycles of 10 s at 95°C, 5 s at 52°C and 20 s at 72°C. Melting curve analyses were performed from 65°C to 95°C to determine if only one amplified product was generated during Q-PCR. The Q-PCR runs were analyzed using CFX Manager Software 3.0. The $2^{-\Delta Ct}$ method was used for relative quantification of the targets in the different samples.[18]

Statistical analyses

Eradication of *E. faecalis* and *C. albicans* were reported as a number (percentage) and the differences were detected using the two-tailed *t*-test. MINITAB 17 software (Minitab Ltd., London, England) was used for all calculations. Degrees of freedom (df) and alpha level (p) were 18 and 0.05, respectively. All the tests and analyses were executed with p=0.05.

RESULTS

Relative eradication (%) of microorganisms compared to day 0 was represented in Table 1.

Table 1. Relative eradication (%) of microorganisms compared to day 0. Values for the groups marked with different superscript letters in the same column were significantly different ($P < 0.05$).

		E. faecalis		***C. albicans***	
		day 3	**day 7**	**day 3**	**day 7**
Group 1	Mean	97.618^{d}	98.389^{D}	99.849^{a}	99.986^{A}
	Std dev.	2.033	1.854	0.182	0.004
Group 2	Mean	96.947^{d}	96.918^{D}	99.915^{ab}	99.988^{AB}
	Std dev.	2.082	2.157	0.023	0.003
Group 3	Mean	91.673^{e}	96.798^{DF}	99.892^{a}	99.988^{A}
	Std dev.	6.121	2.111	0.056	0.004
Group 4	Mean	89.505^{e}	94.062^{EF}	99.898^{ac}	99.986^{AC}
	Std dev.	8.880	4.114	0.020	0.003
Group 5	Mean	89.943^{e}	92.509^{E}	99.900^{a}	99.986^{A}
	Std dev.	7.794	4.674	0.016	0.003

(Std dev: Standart deviation)

E. faecalis and *C. albicans* present in the root canals decreased in all study groups on days 3 and 7. All medicaments eliminated at least 99.849% of *C. albicans* and 89.505% of *E. faecalis* on day 3 compared to day 0. At least 99.986% of the *C. albicans* and 92.509% of the *E. faecalis* were eradicated on day 7 compared to day 0 (Table 1). $Ca(OH)_2$ and TAP groups showed similar the eradication rates for *E. faecalis* and *C. albicans* on day 3 and 7 (P>0.05). There was no significant difference between DP, MDP and Lf for the eradication of microorganisms at both experimental days (P> 0.05), except the amount of eradicated *E. faecalis* by DP at day 7 in which DP caught the similar percentages with TAP and $Ca(OH)_2$ (P<0.05).Beside, adding Lf to DP did not change its efficiancy against the tested pathogens (P>0.05).

Differences in eradication rates on days 3 to 7 was shown in Table 2. The antimicrobial effects of each medicament increased from days 3 to 7. However, no significant difference was observed between the antimicrobial effects of the medicaments (P>0.05) (Table 2).

Table 2. Differences in eradication rates on day 3 to day 7.

	E. faecalis	***C. albicans***
Group 1	0.38717406*	0.04071924**
Group 2	0.97614066*	0.00000335**
Group 3	0.02914106**	0.00042520**
Group 4	0.16525757*	0.00000017**
Group 5	0.39266484*	0.00000002**
P value	>0.05	>0.05

P > 0.05 indicates *, P < 0.05 indicates **

Q-PCR DNA amplification and melt peak of *E. faecalis* (Figure 2) and *C. albicans* (Figure 3) on experimental days were also shown. The same melting peaks revealed both the accuracy of the application of the method and no contamination.

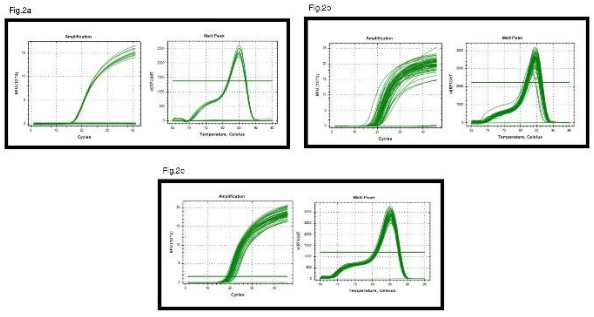

Figure 2: Q-PCR DNA Amplification and Melt peak of Enterococcus faecalis on day 0 (A), day 3 (B) and day 7 (C)

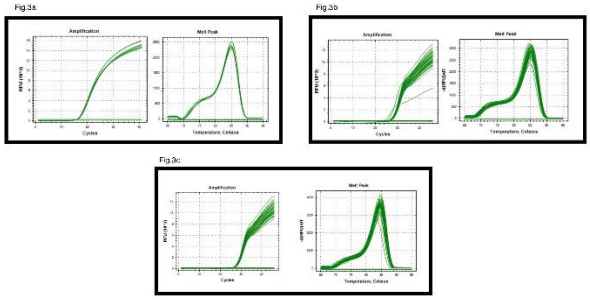

Figure 3: Q-PCR DNA Amplification and Melt peak of Candida albicans on day 0 (A), day 3 (B) and day 7 (C).

DISCUSSION

Adequate elimination of microorganisms from root canals is the golden key of a successful endodontic treatment. Although calcium hydroxide is routinely used in endodontics, it is insufficient for this purpose in some cases. Therefore, the creation of new drugs with better antimicrobial activity and more biocompatible than calcium hydroxide could open a new era in endodontic therapy. From this point of view, it was aimed to test new intracanal medicament combinations against endodontic pathogens in this study.

The agar diffusion test, direct contact test, culture and molecular methods have been widely used to determine the effectiveness of removing microorganisms from root canals.[4,19-22] Because of being an extensive investigation, we performed several pilot studies. We used agar diffusion test, culture methods, Reverse transcription polymerase chain reaction (RT-PCR) and Q-PCR method. When compared with DNA-based methods, RT-PCR is an excellent indicator of cell viability. By using this method, we also succeeded to obtain double-chained DNA. However, we did not prefer

to use this method because of being very complex, sensitive, time consuming and not reproducible.[23]

Under harsh conditions, such as insufficient nutrient supply and environmental stress including extremes in temperature, pH, UV irradiation, and fluctuating levels of toxic chemicals and oxygen concentrations, microorganisms enter a viable but non-cultivable (VBNC) state.[21] When optimum conditions are realized, these microorganisms recover from this phase.[22] Molecular methods are useful for determining which microorganisms enter the VBNC phase. Q-PCR is the sensitive and reliable method. However, Song reported several major practical problems that yield false results, including inadequate removal of PCR inhibitors from the sample, ineffective release of microbial DNA from the cells, poor DNA recovery after the purification step, presence of compounds derived from extracted substrates that inhibit the reaction, and contamination of the assay by background DNA leading to false results.[19]

In light of these studies and our pilot studies, samples were taken from the canals using three paper points to increase sample volume. The samples were placed in empty sterile Eppendorf tubes to avoid any excess substrates that could inhibit the PCR reaction, and DNA was isolated immediately. DNA-based methods detect total amounts of DNA but cannot differentiate living or dead cells.[13] Thus, DNA of viable cells was distinguished from dead microorganisms using DNase I followed by the real-time Q-PCR. The same melting peaks revealed no contamination except for the sample with a melting peak of 81.5°C, number 9 in group 5 on day 7, which was removed from the study.

According to previous studies, $Ca(OH)_2$ and TAP are the most popular medicaments used in microbiota studies.[3,4,23] However, the evidence for these medicaments is paradoxical. Chua *et al*.[24] evaluated the antimicrobial effects of several medicaments on *C. albicans*, and no difference was detected between TAP and the $Ca(OH)_2$ groups, which were prepared in saline solution. The antimicrobial effects of $Ca(OH)_2$ and TAP were evaluated at the end of a 7-day period in another study in which root blocks were contaminated with *E. faecalis*. According to that study, TAP was more effective than $Ca(OH)_2$ for eliminating the microorganisms from the root canal wall.[3] These results are in accordance with Mozayeni *et al*.[4] In our study, no significant difference between the groups was found. The vehicle could have been a cause for this difference. Polyethylene glycol, which has an antimicrobial effect[27], was used as the liquid vehicle in those investigations. Saline solution was chosen as the vehicle in the present study to evaluate the antimicrobial effects of newly designed medicaments. Saline solution has no antimicrobial effect, so using this liquid may have decreased the efficacy of the paste. In addition, the extracted teeth were mechanically shaped instead of using dentine blocks to mimic routine root canal therapy. Furthermore, a Glidden drill was used to take samples from the root canals. In light of pilot studies, paper points, which were used by Siqueira and Roças, were used to take samples from the canal.[28]

E. *faecalis* has high capacity to form biofilm.[29] Several studies reported that 100% E. *faecalis* strains had the ability to form biofilm.[30,31] However, it is an open-secret that creating biofilm is not an easy procedure in *in-vitro* conditions. Several factors such as surface energy of the substrate, temperature, pH, flow rate of the fluid passing over the surface, virulance factors, contact time, surface hydrophobicity, and nutrient availability affect the biofilm formation.[32] Although genetic structure of the biofilm formation is unclear, virulance factor such as enterococcal surface protesin (esp), surface aggregating protein (asa), cytolysin A (cylA), aggregation subtance (agg) were also shown to be associated with weak, medium, or strong biofilm formation of E. *faecalis*.[29,33] Beside this, the adhering capacity of E. *faecalis* in VBNC state reduces. For this reason, they are unable to form measurable biofilms, but still have the ability to form polymeric matrix and maintain its pathogenicity.[34]

In our pilot studies, we obtained variable and contradictory data using culture methods because of the VBNC phase of the *E. faecalis*. Therefore, our aim in this study was to examine the eradication of these microorganisms which are

capable of living in any environment changes such as VBNC state in which microorganism still have the ability to synthesize polymetric matrix. For this purpose, the root canals and the lumen were infected with experimental microorganisms for 2 days rather than creating strong and mature biofilms.

Shen *et al.* evaluated the susceptibility of biofilm formation at different growth phases for time periods ranging from 2 days to 12 weeks. They showed that although biofilm formation could occur even at 2 days with the average thickness of 57 μm, mature biofilms at 3 weeks were more resistant to chlorhexidine solution.[35] Kristich *et al.*[33] studied the esp-independent biofilm formation by several E. *faecalis* strains and reported a dense biofilm within 24 h of growth. Zheng *et al.*[29] also used 1 day biofilm formation in their study in which they analyzed the association between virulance factor and biofilm formation of E. *faecalis* strains by using RT-QPCR. In the present study, each tooth was infected with microorganisms every 24 h for 2 days using freshly prepared 24 h cultures. According to our results, all medicaments eradicated at least 90% microorganisms from the root canal at both day 3 and day 7. The possible reason for this good result could be the creation of young and fresh biofilm on the root surface.

New-generation root canal medicaments should be developed because of the poor antimicrobial effects of routinely used medicaments. Several antibiotics and their combinations have been used against resistant microorganisms, such as *E. faecalis* in *in vivo* and *in vitro* studies. Pinheiro *et al*.[36] evaluated the root canal microorganisms isolated from teeth with endodontic failure and their antimicrobial susceptibility. All species isolated from these failed canals were susceptible to penicillin-type antibiotics. The results of that study were compatible with Aksoy's study.[19] Aslangül *et al.*[37] proposed the use of a combination of penicillin and glycopeptide against *E. faecalis*. Wang *et al*.[38] also evaluated drug resistance in outpatients over 8 years and showed that the combination of ampicillin and gentamicin was very effective against enterococcal infections. The synergistic effects of a penicillin type of ampicillin and a kind of glycopeptide, gentamicin, was an option in microbiological studies in the dental sciences and clinical practice. In our *in-vitro* study, groups 3 and 4 showed considerable antimicrobial activities, which tended to increase from days 3 to 7. Further investigations should be conducted to consider the timing interval.

Dental studies based on Lf are generally conducted on treating oral dryness[39], anti-biofilm efficacy[40], toothpaste content[28], effects on cariogenic microorganisms[41], and irrigation solutions.[42] Gudipaneni *et al.*[28] showed that the tooth pastes containing Lf, lysozyme and lactoperoxidase reduced the salivary levels of *Streptococcus mutans* and L*actobacillus acidophilus* in children with severe early childhood caries. Mizuhashi *et al.*[39] reported that the oral dryness was related with the level and flow rate of Lf in the saliva. Alves *et al.* used lactoferrin as a root canal irrigant in different concentrations and compared its effect with xylitol, farnesol and salicylic acid on the biomass of bacterial biofilms. They reported that the combination of farnesol, xylitol and lactoferrin was the most effective against *E. faecalis* MB35 and this combination also reduced the biomass of *Staphylococcus epidermis* biofilms.[42] Surprisingly, no study has used Lf or the aminoglycoside-penicillin combination as intracanal medicaments despite their antimicrobial activities. In the present study, Lf showed antimicrobial affect for both experimental microorganisms when used in alone or combination with antibiotics. The antimicrobial effect of Lf against both *E. faecalis* and *C. albicans* had tendency to increase from day 3 to day 7 and almost caught the performance of other experimental groups. When taking into consideration the results of this study, Lf could be used in several forms as intracanal medicament by means of its biocompatibility. However, new product should be tested not only about their antimicrobial activity but also their effect on dentinal tissue[43] and its microhardness for various time intervals.[44] Further studies conducted with different forms of Lf and focused on dentinal

effects of it could make a new epoch in endodontics.

CONCLUSIONS

Under the limitation of this study, all intracanal medicaments resulted in significant reductions in *E. faecalis* and *C. albicans* compared to day 0. Although no significant differences were observed between medicaments in both microorganism groups, DP and MDP displayed increases from days 3 to 7. As a result of all these results, the use of new experimental combinations in the endodontics clinic is promising. However, it is not clear how many days the use of these new generation intracanal medicaments will give better results in the clinic. For this reason, further investigations using different time intervals and concentrations are needed.

FUNDING

This study was supported by the Marmara University Scientific Research Projects Committee (Research No: SAG-C-DRP 150513–0154).

ETHICAL APPROVAL

The Ethics Committee of the Marmara University Institute of Health Science approved the protocol. (Number of approval: 05-04-2013-9)

CONFLICT OF INTEREST

The authors deny any conflicts of interest related to this study.

Farklı Kök Kanal Medikamentlerinin Enterococcus Faecalis ve Candida Albicans Üzerine Antimikrobiyal Etkilerinin in Vitro Olarak Karşılaştırılması

ÖZ

Amaç: *Bu çalışmanın amacı deneysel kök kanal medikamentlerinin 3 ve 7 günlük süreçlerde Candida albicans ve Enterococcus faecalis üzerindeki antimikrobiyal etkisini gerçek zamanlı kantitatif polimeraz zincir reaksiyonu (PZR) ile araştırmaktır.* ***Gereç ve yöntem:*** *Çalışmamızda 240 adet tek kök tek kanallı insan dişi kullanıldı. Kök kanalları şekillendirildikten ve akrilik bloklara gömüldükten sonra otoklavda steril edildi. Örnekler iki gruba ayrılarak birinci grubun kök kanallarına E. faecalis diğerine ise C. albicans süspansiyonu inoküle edilmiş ve alt gruplara ayrıldı; kalsiyum hidroksit ($Ca(OH)_2$), üçlü antibiyotik patı (TAP), ikili antibiyotik patı (DP), modifiye üçlü pat (MDP), laktoferrin (Lf), negatif ve pozitif kontrol grupları. Üçüncü ve yedinci gün sonunda kök kanallarından paper point ile alınan örnekler boş eppendorf tüplere alınıp bir seri işlemden geçirildikten sonra DNA izolasyonu gerçekleştirildi. Daha sonra da Gerçek Zamanlı PZR işlemi uygulandı. Veriler istatistiksel olarak analiz edilmişti.* ***Bulgular:*** *Tüm medikamentlerin antimikrobiyal etkinliği 3. günden 7. güne yükseldi. $Ca(OH)_2$ ve TAP grupları, 3. ve 7. günlerde E. faecalis ve C. albicans için benzer eradikasyon oranlarını gösterdi ($P>0,05$). DP'nin yedince günde E. faecalis'i eredike etme oranının TAP ve $Ca(OH)_2$ ile benzerlik göstermesinin dışında, DP, MDP ve Lf arasında her iki deney gününde de mikroorganizmaların yok edilmesi için anlamlı bir farklılık bulunmadı ($P<0,05$).* ***Sonuçlar:*** *Bu çalışmanın sonuçlarına göre deneysel olarak kullanılan kök kanal medikamentleri endodonti kliniği rutininde olarak kullanılanlan CH'ye benzer antimikrobiyal etkinlik göstermiştir. Biyouyumlu bir malzeme olan laktoferrinin kanal içi bir ilaç olarak farklı kombinasyonlarda kullanılabileceği umut vericidir.* ***Anahtar kelimeler:*** *Antibiyotik patı, C. albicans, E. faecalis, Kök kanal medikamenti, Laktoferrin.*

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Cumhuriyet Dental Journal: 2021; 24(3)
e-ISSN 2146-2852
Doi:10.7126/cumudj.920058

Original research

COMPARISON OF TWO AEROSOL-FREE CARIES REMOVAL METHODS: A SPLIT-MOUTH RANDOMIZED CLINICAL TRIAL

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ABSTRACT

Objectives: The management of deep caries lesions in immature permanent molars can be challenging in clinical practice, but minimally invasive caries removal methods can maintain apexogenesis by preventing extensive tissue loss. Here we compare a chemo-mechanical caries removal (CMCR) gel and polymer bur in terms of time spent on caries removal, patient acceptability, and clinical success.

Materials and Methods: The teeth of 30 children were randomly divided into two groups. The duration of each method, the level of cooperation during each method, and the child's choice of caries removal method were recorded. Patients were followed at six-month intervals for at least two years.

Results: The difference between the patients' preferences was not statistically significant, while the average caries removal time of the polymer bur method was significantly shorter ($p<0.05$) than the CMCR method. The rates of apical closure without pathology in the CMCR and polymer bur groups were 63.2% and 73.7%, respectively; 10% of each group underwent further treatment due to their clinical and/or radiographic pathology.

Conclusions: These methods were thought to serve as an interim treatment in managing immature permanent teeth with deep caries. Furthermore, these methods, which do not involve water cooling, can minimize the risk of contamination and cross-infection.

Keywords: Dental atraumatic restorative treatment; Permanent; Pain; Papain; Polymer bur.

Received : 19.04.2021
Accepted : 15.07.2021

How to Cite Duman C, Kalaoglu EE, Sirinoglu Capan B, Egil E. Comparison of Two Aerosol-Free Caries Removal Methods - A Split Mouth Randomized Clinical Trial. Cumhuriyet Dent J 2021;24:3:266-273.

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INTRODUCTION

Dental caries is a disease with a high prevalence worldwide.[1] According to Takao Fusayama's 1980 description, the carious lesion consists of two layers. The 'outer layer' is an acidic, irreversibly demineralized, touch sensitive layer that contains high levels of bacteria, and therefore can be removed without the need for local anaesthesia. The 'inner layer' is a partially demineralized, less contaminated layer that contains collagen fibrils in the dentinal tubules. Because the dentine preserves the inner layer's structural integrity, it cannot be removed without local anaesthesia. The use of rotary instruments in the treatment of dental caries results in the removal of extra tooth tissue.[2] Traditional caries removal methods have other disadvantages as well, such as decreased patient comfort due to pressure and heat and the need for local anaesthesia during the procedure.[3] Furthermore, the need for water coolant to prevent heat during the use of a high speed hand piece increases the aerosol transmission and the risk of contamination with bacteria, fungi, and viruses, including COVID-19.[4]

Pain, heat, and pressure felt during treatment with traditional methods can cause dental anxiety, especially in paediatric patients, and the persistence of this anxiety may cause people to avoid dental treatment in adulthood.[5]

Contemporary restorative dentistry has moved away from the traditional, surgical method of managing dental caries based on the operative concepts of G.V. Black of more than a century ago and towards a 'minimally invasive' approach.[6] The minimally invasive principle aims to preserve the maximum of healthy tooth tissue with remineralization capacity by removing as little tooth tissue as possible, thus allowing pulp integrity to be preserved.[1] Minimally invasive dentistry continues to gain importance, especially in the treatment of permanent teeth with deep caries lesions and immature roots. Advanced endodontic treatments in these patients include complex root tip closure treatments such as apexification.[7] It has been reported that the apical closure of vital young permanent teeth can be successfully completed with appropriate indirect vital pulp treatments[8] and that avoiding the complete removal of carious lesions close to the pulp reduces the risk of pulp exposure.[9]

The chemo-mechanical caries removal (CMCR) method, one of the minimally invasive caries removal methods, was first described in 1975 by Habib *et al.*, who used 5% sodium hypochlorite. In the following years, GK-101, Caridex systems, and Carisolv systems were developed and used. Due to these system's disadvantages, such as short shelf life, high corrosion effect, the requirement of special equipment, and high cost, in 2003 a new formulation was created in Brazil by Bassadori *et al.*[10] Subsequently, a new CMCR agent, containing papain, chloramine, toluidine blue, water, salt, and thinner, was introduced with the commercial name Brix3000 (Brix Srl Argentina).[11] Papain is a proteolytic enzyme that consists of the pulp of the fruit, leaves, and rubber of the Carica papaya tree, which is grown in tropical regions such as Brazil, India, South Africa, and Hawaii. It is similar to human pepsin and has bactericidal, bacteriostatic, and anti-inflammatory properties. With its antibacterial effect, it can prevent the proliferation of both Gram negative and Gram positive organisms. Papain also acts as an anti-inflammatory, debriding agent that does not damage healthy tissue and accelerates the cicatricle process. Chloramines, which are amines containing at least one chlorine atom bonded directly to a nitrogen atom, are formed during the reaction between chlorine and ammonia. They have bactericidal and disinfecting properties and are used chemically to soften carious dentine. Toluidine blue is an antimicrobial agent. Papain, chloramine T, and toluidine blue, which form the papain-containing gel, create a synergistic effect and facilitate the removal of caries.[12] In sum, papain-containing gel is a biomaterial that allows the protection of maximum healthy tooth tissue, is easy to apply, and does not require special equipment.[10]

In 2003, polymer burs with the commercial name SmartBur (SS White, Lakewood, N.J., USA) were developed in Boston as an alternative to traditional caries removal methods. Used with low-

speed rotary tools, these single-use burs that specifically remove infected dentine and abrade when it comes to affected dentine.[2]

The aim of this study was to preserve pulp vitality and provide apical closure physiologically by using minimally invasive methods in vital permanent teeth with incomplete root development. The two minimally invasive methods, CMCR (BRIX 3000) and the polymer bur (SmartBur), were compared in terms of time spent on caries removal, patient acceptability, and clinical success.

MATERIAL AND METHODS

This prospective study received approval from the Human Research Ethics Committee of Biruni University (Turkey) under process number 2015-KAEK-43-18-08 and was conducted in the Department of Pediatric Dentistry of the same university.

The sample size was calculated by G*Power 3.1.9.2 software (Dusseldorf, Germany), keeping alpha at 0.05 and power at 80%. A total number of 30 healthy children in the age group 7-11 years old were selected for this randomized and controlled clinical trial with a 'split-mouth' design. The investigation was designed, analysed, and interpreted according to the Consolidated Standards of Reporting Trials (CONSORT).

Inclusion Criteria:

- Bilateral class 1 deep carious lesion on permanent molars with incomplete roots
- No pulpal involvement as evident on a radiograph
- No clinical signs or symptoms of irreversible pulpitis.

Exclusion Criteria:

- Pulpal exposure or bleeding during the excavation procedure
- Presence of underlying systemic diseases
- Lack of compliance.

One researcher completed the clinical procedures, while another researcher recorded the data. Follow-up evaluations were performed by a third researcher who was blind.

Procedure:

The procedure was explained in detail to the parents, and written informed consent was obtained before the study.

The teeth were randomly divided into two groups: Group I (CMCR agent) and Group II (polymer bur). Randomization of the groups was performed by lots, using numbered tiles to determine the tooth and the treatment that would be done first. The other tooth in the same subject was automatically submitted to the other form of treatment. During the clinical procedure, the duration of each method and the child's choice of caries removal method were recorded. The cooperation levels of the patients were evaluated according to the Frankl Behavior Rating Scale.[13]

Group I: The teeth with a sufficient amount of hard tissue were isolated with a rubber dam, while for the teeth with extensive tissue loss and the teeth of uncooperative patients, relative moisture control using cotton wool rolls and suction was performed. BRIX 3000 gel was applied to the cavity and allowed to work for 120 seconds. The softened carious dentine was then removed with a round tip excavator, as recommended by the manufacturer. The application was repeated until the colour of the gel did not change. Finally, the cavity was washed with water spray and dried with moisture- and oil-free air.

Group II: The teeth with a sufficient amount of hard tissue were isolated with a rubber dam, while for the teeth with extensive tissue loss and the teeth of uncooperative patients, relative moisture control using cotton wool rolls and suction was performed. After isolation, the caries excavation was done with a Smart Bur using a low speed hand piece (500–800 rpm) in circular movements starting from the centre to the periphery of the carious lesion. Visibly abraded Smart Burs were replaced. The procedure was continued until complete caries excavation was achieved.

The restorations were performed with glass ionomer cement (Equia Forte, GC®). All patients were followed clinically and radiographically at 6-month intervals until the root development was completed and for at least 2 years. Teeth whose

root development was completed without any pathology were recorded as successful treatments. Root canal treatment was applied to teeth with pain, intraoral or extra oral abscess formation, fistula formation, or periapical lesions on radiography during follow-up and were recorded as unsuccessful treatments.

IBM SPSS Statistics 22 for statistical analysis (SPSS IBM, Turkey) programs were used for statistical analysis, and the compliance of the parameters to the normal distribution was evaluated with the Shapiro-Wilk test. While evaluating the study data, in addition to descriptive statistical methods (mean, standard deviation, and frequency), the Mann-Whitney U test was used for comparing parameters between two groups. Fisher's exact chi-squared and Fisher-Freeman-Halton tests were used to compare qualitative data. Significance was evaluated at the $p < 0.05$ level.

RESULTS

This split-mouth designed study was conducted on 60 teeth of 28 children aged between 7 and 11 years old. Nineteen females (63.3%) and 11 males (36.7%) with a mean age of 7.93±1.14 years participated in the study. The majority of the teeth (90%) were the first permanent molars of the patients aged from 7 to 9 years old. The second permanent molars of three patients who were 10 or 11 years old were included. The research flow chart is shown in Figure 1.

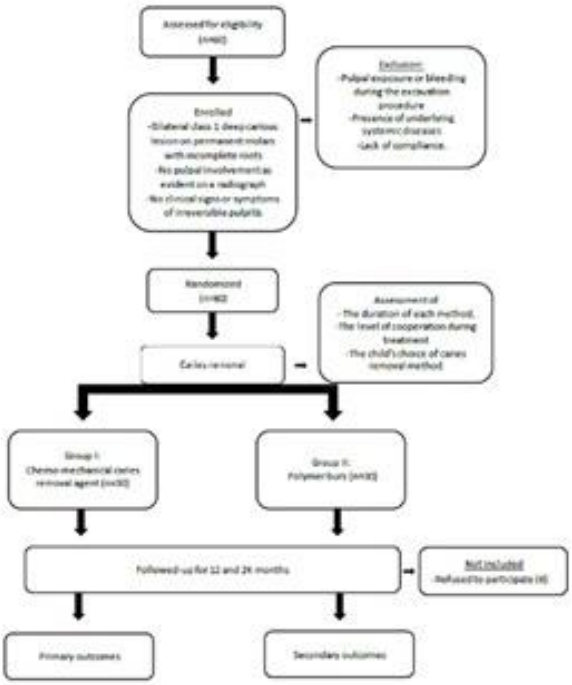

Figure 1. Research flow chart

Subsequent to the procedure, when patients were asked about their favourite between the two methods, 46.7% of the participants chose the polymer bur, while 43.3% preferred the CMCR agent. The proportion of children who were undecided was 10%.

The average caries removal time of the polymer bur method was statistically significantly shorter than the CMCR agent method ($p<0.05$) (Table 1).

Table 1. The time required for caries removal

	Time (second)	
Method	**Mean±SD**	**Median**
CMCR gel	476.3±223.4	425
Polymer bur	100.8±54.2	81
p	0.000*	

Mann Whitney U Test ** p<0.05*

No statistically significant difference was found between the caries removal method and the level of cooperation. The method used and the level of cooperation are shown in Table 2.

Table 2. Frank Behavior Rating Scores of Patients

		Method		p
		CMCR Gel	**Polymer Bur**	
		n (%)	**n (%)**	
Cooperation Level	Definitely negative	5 (16.7%)	6 (20%)	[1]0.985
	Negative	9 (30%)	8 (26.7%)	
	Positive	6 (20%)	6 (20%)	
	Definitely positive	10 (33.3%)	10 (33.3%)	

[1]*Fisher Freeman Halton Test p<0.05*

At the end of the first year, the failure rates of both study groups were 10%, and apical closure was detected in 33.3% of each group. Details of the first year clinical evaluation are shown in Table 3. Eight patients (16 teeth) were excluded due to nonattendance at the second year appointments due to the COVID-19 pandemic. Table 3 shows the data from the second year follow-up appointments.

Table 3. 12- Month and 24- Month Clinical and Radiographic Evaluation

		Method					
		CMCR Gel 12 Month	**CMCR Gel 24 Month**	**Polymer Bur 12 Month**	**Polymer Bur 24 Month**	**P 12 month**	**P 24 Month**
		n (%)	**n (%)**	**n (%)**	**n (%)**		
Pain	No	27 (90%)	19 (100%)	27 (90%)	19 (100%)	[1]1.000	-
	Yes	3 (10%)	0 (0%)	3 (10%)	0 (0%)		
Infection	No	29 (96.7%)	19 (100%)	27 (90%)	17 (89.5%)	[1]0.612	[1]0.486
	Yes	1 (3.3%)	0 (0%)	3 (10%)	2 (10.5%)		
Radiographic Pathology	No	27 (90%)	17 (89,5%)	27 (90%)	18 (94.7%)	[1]1.000	[1]1.000
	Yes	3 (10%)	2 (10.5%)	3 (10%)	1 (5.3%)		
Apical Closure	No	20(66.7%)	7(36.8%)	20(66.7%)	5(26.3%)	[1]1.000	[1]0.728
	Yes	10(33.3%)	12(63.2%)	10(33.3%)	14(73.7%)		

[1]*Fisher's Exact Test*

DISCUSSION

The management of deep carious lesions in paediatric patients can be challenging for clinicians. The maintenance of pulp vitality should be a priority in permanent teeth which are asymptomatic; however, conventional caries removal methods lead to the loss of healthy tooth structure and may result in pulp exposure and the need for endodontic treatment.[6,14] Furthermore, in the case of performing apexification procedures, proper root development cannot be achieved, and the tooth may be more susceptible to fracturing.[15]

Previous studies have reported numerous minimally invasive caries removal methods with the purpose of reducing noise, vibration, fear, excessive removal of uninfected dentine, and pain.[14,16–18] For example, carbon steel, tungsten carbide, or polymer burs, hand instruments (excavators, chisels), air abrasion, air polishing, ultrasonics, sono-abrasion, CMCR agents, lasers, photo-active disinfection (PAD), or ozone were used for selective caries removal.[19] Papain-based CMCR agents were reported as an effective method for caries removal by previous studies.[11,12,20] Similarly, studies investigating the efficiency of polymer burs were conducted.[2,21–23] Although there are few in vitro and in vivo studies comparing these two methods in the previous literature, to our knowledge no clinical study has been conducted on young permanent molars regardless the patients' level of cooperation.[2,24–26] To evaluate the effect of vibration sensation on patient acceptability, these two methods, one of which involves the use of a rotary instrument, were chosen.

A split-mouth design was used due to its reported advantages in paediatric dentistry research.

A study's inconsistency or random error can be considerably reduced by performing within-patient instead of between-patient comparisons. In addition, a split-mouth design decreases the majority of inter-subject variability in terms of the treatment effect, thus increasing research accuracy and power to detect real differences with fewer participants.[27]

The duration of the procedures in paediatric dentistry practice has critical importance in terms of patient acceptance. As found in this study, the time taken for caries removal was less with a polymer bur (100.80 seconds) than with the CMCR gel (476.30 seconds). These results were consistent with those reported in the previous literature.[25,28] The duration of caries removal with polymer burs was reported as 147.5 and 208.4 seconds in previous in vitro studies.[29,30] Unlike in Divya *et al.*[2], the statistically significant difference in the duration of the two methods may be due to the 120-second waiting time of the CMCR agent we used. That the CMCR agent caries removal time was found to be 300 seconds in another study using the same product supports this idea.[20]

In the current study, children's choice rates were almost equal between the two caries removal methods. In the majority of the studies evaluating patients' selection or pain during the procedure, minimally invasive methods were compared with traditional methods, and conventional drilling methods were found to be less acceptable and more painful.[22,31] Studies which compared patient acceptance of the two methods reported on in this study found that the polymer bur was more painful than the CMCR agent.[25,28] In this context, although there is no study comparing the two methods in terms of patient choice, that 46.7% of the patients in this study had a 'definitely negative' or 'negative' level of cooperation for both methods may indicate that both methods are acceptable to the patients, especially since we had the opportunity to carry out the treatments in clinical conditions.

When we evaluate the methods in terms of clinical success, 10% of both groups underwent further treatment due to the clinical and/or radiographic pathology. On the other hand, apical closure was ensured properly in 50% of each group. This apical closure rate, low when compared to a study that reported 96.8% success after a 24-month follow-up period, may be due to the differences in the developmental stage of roots at the time of treatment.[32] The previous studies comparing the efficiency of these methods regarding the remaining amounts of bacteria after treatment reported that both methods were efficient and that there were no significant differences among groups.[25,28]

The major limitation of this study was the high number of patients not attending the controls due to the pandemic. Additionally, standardizing root developmental levels would be useful for the clinical success assessment.

CONCLUSIONS

The use of a CMCR agent or polymer bur is recommended as a solution for the treatment of patients seeking an alternative to conventional methods. Additionally, these methods may serve as an interim treatment during the apexogenesis process in the management of immature permanent teeth with deep caries. Another advantage of these methods is that, since they do not involve water cooling, they can also minimize the risk of contamination and cross infection.

ACKNOWLEDGMENTS

This prospective study received approval from the Human Research Ethics Committee of Biruni University (Turkey) under process number 2015-KAEK-43-18-08 and was conducted in the Department of Pediatric Dentistry of the same university.

All authors gave final approval and agree to be accountable for all aspects of the work. The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

Aerosol İçermeyen İki Farklı Çürük Temizleme Yönteminin Karşılaştırılması-Bölünmüş Ağız Randomize Klinik Çalışma

ÖZ

Amaç: *Kök gelişimini tamamlamamış daimi azı dişlerinde görülen derin çürük lezyonlarının tedavisi klinik pratikte zorlayıcı olabilmektedir. Minimal girişimsel çürük temizleme yöntemleri, gereksiz doku kaybını önleyerek apeksogenezin sürdürülmesine*

olanak vermektedir. Bu çalışmada, kemo-mekanik çürük temizleme jeli ve polimer frez, çürüğün tamamen uzaklaştırılması için harcanan süre, hasta kabul edilebilirliği ve klinik başarı açısından karşılaştırılmaktadır. ***Gereç ve Yöntemler:*** *Otuz çocuk hastanın çift taraflı çürük azı dişleri rastgele iki gruba ayrılmıştır. Klinik prosedürlerin uygulanma süresi, işlem sırasındaki kooperasyon düzeyi ve çocuğun çürük temizleme yöntemi arasındaki seçimi kaydedilmiştir. Hastalar 6 aylık aralıklarla en az 2 yıl olacak şekilde takip edilmiştir.* ***Bulgular:*** *Hastaların tercihleri arasındaki fark istatistiksel olarak anlamlı bulunmamıştır, polimer frez ile çürük temizleme yönteminin ortalama süresi anlamlı olarak daha kısadır (p<0,05). Çürük temizleme jeli ve polimer frez gruplarında patoloji görülmeksizin kök ucu kapanma oranları sırasıyla % 63,2 ve % 73,7 iken, her grubun % 10'una klinik ve/veya radyografik patoloji nedeniyle ek tedaviler uygulanmıştır.* ***Sonuçlar:*** *Bu yöntemlerin, derin çürük kavitesine sahip ve kök ucu kapanmamış kalıcı dişlerin tedavisinde geçici bir tedavi görevi görebileceği düşünülmektedir. Ayrıca, su soğutması içermeyen bu yöntemler çapraz enfeksiyon riskini anlamlı derecede azaltmaktadır.* ***Anahtar Kelimeler:*** *Dental artavmatik restoratif tedavi, Azı dişi, Ağrı, Papain, Polimer frez.*

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Cumhuriyet Dental Journal: 2021; 24(3)
e-ISSN 2146-2852
Doi:10.7126/cumudj.893008

Case Report

REPETITIVE REGENERATIVE ENDODONTIC PROTOCOL TREATMENT FOR MATURE TEETH WITH DENS INVAGINATUS AND SEVERE PERFORATED INTERNAL ROOT RESORPTION: A CASE REPORT WITH 24-MONTH FOLLOW-UP

ABSTRACT

The association of internal root resorption (IRR) with dens invaginatus is an extremely rare finding, and its incidence may be underestimated, adding to the difficulty of endodontic treatment. A 9-year-old boy was admitted with complaints of swelling and pain. Radiological examination revealed closed apex Oehlers type II dens invaginatus and severe IRR, as well as apical third of two lateral root perforation and lesion. Calcium hydroxide applied in the regenerative endodontic protocol (REP) as intracanal medicament. Differently, bleeding was achieved by irritating the lateral perforation area. However, the tooth showed spontaneous pain and sensitivity to percussion after 2 months. The same REP was performed, but a triple antibiotic paste was used as the intracanal medicament. After 2 years, the tooth was asymptomatic, and radiographic examination revealed the decrease was cared on the previous resorption sites, and one of the openings on the lateral root surface had closed.

Key words: Cone-beam computed tomography, dens invaginatus, internal root resorption, regenerative endodontic treatment, triple antibiotic paste.

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Received : 08.03.2021
Accepted : 15.06.2021

How to Cite Yigit T, Aslan H. Repetitive Regenerative Endodontic Protocol Treatment for Mature Teeth with Dens Invaginatus and Severe Perforated Internal Root Resorption: A Case Report with 24-Month Follow-Up. Cumhuriyet Dent J 2021;24:3:274-280.
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INTRODUCTION

Regenerative endodontic protocol (REP) is a valuable therapeutic treatment alternative to increase tooth survival of necrotic and immature teeth.[1] Recently, REP has been the choice to treat necrotic pulp and apical periodontitis in mature teeth.[2,3] The disinfected canals are filled with biocompatible, nonvital foreign materials in root canal therapy, while in REP, different from this classic treatment, the canals are filled with vital tissue.

Dens invaginatus is a developmental structure pathology caused by the progression of enamel and dentin toward pulp before the completion of tooth calcification. Tooth invagination is limited by tooth crown or can extend to the apex of the root.[4] It is thought that trauma, infection, growth retardation in specific cells, the disruption of factors that enable enamel development, and genetic factors influence dens invaginatus etiology.[5] The phenomenon's prevalence varies from 0.3% to 10%, most frequently affecting the maxillary lateral and central incisors, respectively, and may occur bilaterally.[6] The clinical importance of dens invaginatus involves increased caries, periodontal inflammation, and pulp necrosis risk due to abnormal morphological plaque retention in the invagination area.[7] Treatment options include canal treatment; however, irregularly shaped canal structures may cause additional problems.

Odontoclastic activity related to chronic pulp inflammation and trauma cause a resorptive defect in the internal root known as internal root resorption (IRR). Other etiological factors may be continuous pulpotomy, pathogenic irritation and infections, transplantation procedures, cracks, and orthodontic treatment.[8,9] Maintaining the integrity of the tooth is important in the early diagnosis and accurate management of IRR.[9] The treatment process could grow more difficult if the resorption area starts to perforate the root canal system, though recalcification treatment and/or surgical approaches using calcium hydroxide ($Ca(OH)_2$) have been suggested as an alternative treatment.[10]

In IRR cases, REP may be a sufficient treatment[11,12], as it is more conservative and may significantly contribute to tooth retention. Therefore, the target of this case report is to feature REP as used to treat a mature permanent tooth with dens invaginatus and perforated IRR.

Case Report

A 9-year-old boy was admitted to the Department of Pediatric Dentistry at XXX University with complaints of swelling and pain. His medical history was not clear. Clinically, the crown of the maxillary left lateral incisor (#10) was without caries. There was swelling of the palatinal region. The type 2 mobile tooth was sensitive to percussion and palpation. Electric (Vitality Scanner; Analytic Technology, Glendora, CA, USA) and thermal (Endo-Ice; The Hygenic Corporation, OH, USA) pulp sensitivity testing was negative only for tooth #10, while the adjacent teeth presented normal responses. There were no pathological periodontal pockets in any of the teeth in the left maxillary region. Radiological examination revealed closed apex Oehlers type II dens invaginatus and severe IRR, as well as apical third of two lateral root perforation and lesion. (Figure 1A).

Figure 1 Preoperative (A), 1 month recall

The patient's parent was informed of all the possible modalities, risks, and benefits of the recommended REP treatment. Under local anesthesia (infiltration of 2% Lidocaine with 1:100,000 epinephrine), a rubber dam was inserted in the patient's mouth. After the invagination area was passed with a diamond bur, suppurative yellowish fluid was drained. No instrumentation was performed. For gentle root canal irritation, 20

ml 1.25% sodium hypochlorite (NaOCl) was used, and the area was dried with sterile paper points. A calcium hydroxide powder (Sultan Chemists Inc., Englewood, NJ, USA) and sterile water mixture was placed in the pulp chamber with an ISO #25 K-file. A glass ionomer restoration was inserted for temporary use (Fuji IX; GC America, Alsip, IL, USA). After 4 weeks, the tooth was asymptomatic and anesthetized with 2% mepivacaine, then isolated with a rubber dam. The dressing paste was irrigated with 10 ml sterile saline for removal, dried with sterile paper points, irrigated again with 20 ml 17% ethylenediaminetetraacetic acid (EDTA, Merck, Darmstadt, Germany), and dried again. The periapical tissues of the lateral perforation area were irritated without touching the apical area, as performed with an ISO #25 K-file. Bleeding reached a minimum of 3 mm below the cementoenamel junction in 5 minutes. A mineral trioxide aggregate (MTA; MTA-A; Angelus, Londrina, Brazil) plug was gently placed on the blood clot. The MTA was protected with a moist sterile cotton pellet and the entrance sealed with a temporary glass ionomer restoration Cavit G (3M ESPE Dental-Medizin GmbH Co, Seefeld, Germany). One week later, FujiIX glass ionomer cement (Fuji Corporation, Osaka, Japan) and a composite (Filtek Z250; 3M ESPE) were placed for the final restoration.

However, the tooth showed spontaneous pain and sensitivity to percussion after 2 months. Cone beam computed tomography (CBCT) scans generated a three-dimensional image of the tooth. A severe perforation region on the root surface was observed in axial, sagittal, and coronal CBCT cross-sections (Figs. 2A–C).

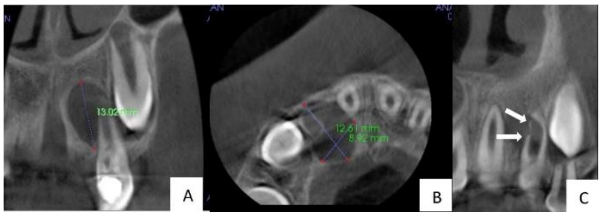

Figure 2 3-month recall CBCT of the patient (before the repetitive REP)

The mesial and distal surfaces of the tooth had resorbed, and the resorption process distally occupied the periodontal ligament of the central tooth (Figure 2A). The cortical bone was completely resorbed in the tooth's palatinal region as well (Figure 2A). The resorption area measured 8.92x12.61x13.02 mm with a software measurement tool (CS 3D Imaging Software version 3.1.9; Carestream Dental LLC, Atlanta, GA, USA; Figs. 2A, B). Also, apical third of two lateral root perforation were detected (Figure 2C).

The initial regenerative stimulation was considered ineffective, and for this reason, REP was repeated. A rubber dam was placed in the patient's mouth under local anesthesia. The composite resin restoration and MTA were removed with a diamond bur. The same REP was performed, but a triple antibiotic paste was used as the intracanal medicament. Triple antibiotic paste, clindamycin, ciprofloxacin, and metronidazole were mixed equally (all from Sigma- Aldrich, St. Louis, MO, USA) with sterile water to generate triple antibiotic paste (TAP) at a concentration of 1 mg/ml. In addition, in this second REP appointment, the antibiotic paste was removed via copious irrigation with 1.25% NaOCl and thereafter with 10 mL sterile distilled water. The root canals were slowly irrigated with 20 mL 17% EDTA but first dried with sterile paper points.

Healthy soft tissues were noted at the patient's 6-, 12-, 18-, and 24-month follow-up appointments (Figs. 1B–E).

Figure 1. (B), 6-month recall (C), 12-month recall (D), and 18-month recall (E) radiographs of the patient.

The tooth had a negative response to vitality testing. Especially the lateral lesion's healing after 6 months was observed via periapical radiographic examination (Figure 1C). When the CBCT images taken in the third and the twenty-fourth months were compared, it was observed that the lesion had been surrounded by sclerotic borders, demonstrating the healing of all tooth surfaces; the decreasing area was very excited compared to the previous resorption sites. Measurements demonstrated that the resorption area had decreased to 2.40x5.21x5.31 mm. The thickness of

the lateral walls also increased relatively, and one of the openings on the lateral root surface had closed (Figure 3A, B).

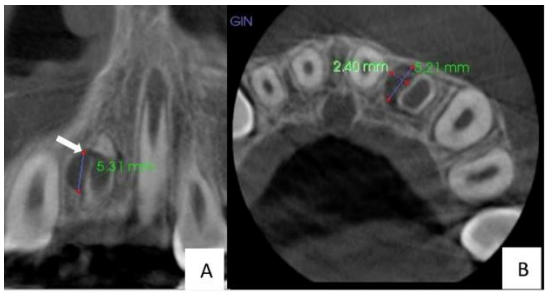

Figure 3 24-month recall CBCT of the patient

DISCUSSION

Dens invaginatus is a common dental occurrence; however, it cannot be easily diagnosed due to a lack of specific clinical signs. It is still clinically important because it serves as an ideal harbor for plaque and microorganisms from where focal periodontitis can begin. Clinicians have had difficulty determining a clinical approach after diagnosing dens invagination.[13] Current dens invaginatus treatment options include non-surgical endodontic treatment of the infected invagination,[14–16] treatment of the entire root canal system,[17–19] combined endodontic and surgical therapy[20–22] and REP.[23,24] Although there are no clinical studies on REP treatment for dens invaginatus, there are a limited number of case reports. The success of REP can be evaluated as reaching primary, secondary, and tertiary goals. Of course, the primary goal is healing the bone and eradicating symptoms. The secondary goal, then, is to increase root wall thickness/length, and the tertiary goal is the positive response to vitality testing. In the presented case, treatment success was limited to the primary and secondary goals.

Wedenberg and Lindskog[25] reported that IRR can be a transient or progressive event and researchers damaged the root canal predentin of 32 primate incisors. While some of the cavities had closed, those remaining were left open in the oral cavity. Multinucleated clastic cells in the closed teeth showed a temporary colonization, that is, temporary internal resorption. There was no bacterial contamination or active hard tissue resorption. In the group associated with the oral cavity, the damaged dentin surface showed signs of long-term clastic cell colonization and progressive IRR. Bacterial contamination was also seen in the pulp tissue and dentinal tubules. According to Wedenberg and Lindskog[25], internal resorption does not progress when bacterial stimulation is blocked. In fact, with REP treatment, bacteria are removed from the canal. As such, in the current case, internal resorption did not progress.

One hypothesis is that osteocytes reduce osteoclastogenesis activity by decreasing actin ring production (active resorbing cells) and playing an important role in bone homeostasis.[26] However, if osteocytes die via apoptosis, osteoclastogenic cytokines become active in bone resorption again.[27] At the time of tooth development, dental pulp cells and odontoblasts behave as osteocytes and choose apoptotic pathways when they encounter injury.[28] Considering that dens invaginatus is a developmental dental anomaly, it may be that there is a situation in which odontoblasts may be damaged in the process, and dens invagination may be a source of chronic infection; therefore, a tendency for internal resorption may increase. Since no findings in this case led to internal resorption, it can be considered that the pulp inflammation caused by the deep invagination of the lateral incisor caused internal resorption. As a matter of fact, in a recent study, dens invaginatus and its relationship with internal resorption drew attention.[29]

Blood in the pulp tissue at the apical region of the resorptive lesion is essential for providing clastic cells nutrients to form internal resorption, though these cells can be stimulated by the infected necrotic coronal pulp tissue as well.[9] In addition, in the current case, the occurrence of apical closure showed that vital tissues were present in the apical pulp but absent in periapical pathology. Bleeding was achieved from the lateral perforation area without touching the apical area so as not to damage the apical vital tissues.

The colonization of bacteria on the canal walls and their insufficient debridement were the main reason for this case's unsuccessful initial treatment. A recently published report also noted this knowledge, informing that histo-bacteriologic analysis underlines failed revascularization and

revitalization therapy.[30] The main reason for the first treatment's failure in that case were intra-canal microorganisms in a root canal that remained empty after the completion of possible single-stage disinfection protocols.

Alternative treatments may not have been successful in the current case, as both of the tooth's lateral walls were very thin, with even the root surface having openings in two places, so REP treatment was repeated. Optimum disinfection of the root canal system using intracanal medicaments is considered an important step in REP.[31] The used intracanal medicaments in REP are triple (ciprofloxacin, metronidazole, and minocycline) or double (ciprofloxacin and metronidazole) antibiotic combinations[32] and $Ca(OH)_2$.[33,34] In the present case report, $Ca(OH)_2$ was preferred as the intracanal medicament to remove the necrotic residual pulp tissues, eliminate osteoclastic activity, and control bleeding. When the first treatment failed, TAP was preferred in the second treatment to more effectively disinfect the area. Nevertheless, $Ca(OH)_2$ shows low solubility and inactivation in dentin, tissue fluids, and organic matter, so it is not sufficient to prevent bacterial colonization.[35] Indeed, intracanal antibiotic medicaments showed more benefits when compared to traditional $Ca(OH)_2$ in recent in vitro studies.[36] However, it has also been reported to produce cytotoxic effects in apical papillae stem cells[37], pulp stem cells[38], and pulp fibroblasts.[39] Therefore, research has found that $Ca(OH)_2$ or a double or triple antibiotic paste of a low concentration (0.1–1 mg/mL) sufficiently provides an effective antimicrobial drug without damaging the pluripotent stem cells within the root canal system.[40] For this reason, a TAP of 1 mg/mL was preferred in this study.

In a comparison study by Patel *et al.*[41], intraoral periapical radiography proved more effective than CBCT in diagnosing and maintaining root resorption episodes. Therefore, in the present case, the initial use of periapical radiographic imaging may have been a limitation to determining the resorption area and dentine wall thickening. However, CBCT was used after the failure for detailed examination of root canal, as three-dimensional examination allows clinicians to evaluate the internal tooth for a more complete diagnosis and treatment plan.[42]

The association of IRR with dens invaginatus is an extremely rare finding, and its incidence may be underestimated, adding to the difficulty of endodontic treatment. Root canal treatment was used in two published case reports without bone resorption.[43,44] This study can contribute to the literature as the first case report in which REP was used as an alternative treatment option in these cases. Still, more research is necessary to find a relation between IRR and dens invaginatus.

ÖZ

İnternal kök rezorpsiyonunun (IKR) dens invaginatus ile ilişkisi son derece nadir bir bulgudur ve endodontik tedavinin zorluğuna ek olarak insidansı hafife alınabilir. Dokuz yaşında erkek çocuk hasta, şişlik ve ağrı şikayetleri ile kliniğimize başvurdu. Radyolojik incelemede, kapalı apeks, Oehlers tip II dens invaginatus ve şiddetli IKR, ayrıca apikal üçlüde iki lateral kök perforasyonu ve apikal lezyon görüldü. Rejeneratif endodontik protokolde (REP) kanal içi medikament olarak kalsiyum hidroksit uygulandı. Farklı olarak lateral perforasyon alanından kanama sağlandı. Ancak, 2 ay sonra ağrı ve perküsyona duyarlılık nedeni ile REP tekrar edildi. Bu uygulamada kanal içi medikament olarak üçlü antibiyotik pat kullanıldı. 2 yıl sonra diş asemptomatikti ve radyografik incelemede rezorpsiyon alanında küçülme ve lateral kök yüzeyindeki açıklıklardan birinin kapandığı görüldü.

Anahtar kelimeler: *Dens invaginatus, internal kök rezorpsiyonu, konik ışınlı bilgisayarlı tomografi, rejeneratif endodontik tedavi, üçlü antibiyotik pat.*

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Cumhuriyet Dental Journal: 2021; 24(3)
e-ISSN 2146-2852
Doi:10.7126/cumudj.922980

Case Report

TEMPOROMANDIBULAR JOINT SYNOVIAL CHONDROMATOSIS: A RARE CASE

ABSTRACT

Synovial chondromatosis (osteochondromatosis) is a rare lesion originating from the synovial membrane. It develops as a result of metaplasia of the tendon sheath or synovial membrane of the bursa in the joint and causes the formation of chondral or osteochondral foci in the synovium. These cartilaginous lesions can cause secondary degenerative changes as free bodies within the joint. Between the ages of 30 and 50, the knee joint is less commonly seen in the hip, ankle, shoulder and elbow joints. Clinically, joint pain and progressive limitation of motion, effusion and recurrent locking in some cases are seen. In this case, we aimed to present SC radiologic (CBCT and MR) and clinical findings in TME of a 53 years old female patient who applied to our clinic for synovial chondromatosis.

Keywords: Synovial chondromatosis, Temporomandibular joint, CBCT, MR, Direct graphy.

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Received : 20.04.2021
Accepted : 15.06.2021

How to Cite Durna D, Çakur B, Bayındır N. Temporomandibular Joint Synovial Chondromatosis: A Rare Case. Cumhuriyet Dent J 2021;24:3:281-285.
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INTRODUCTION

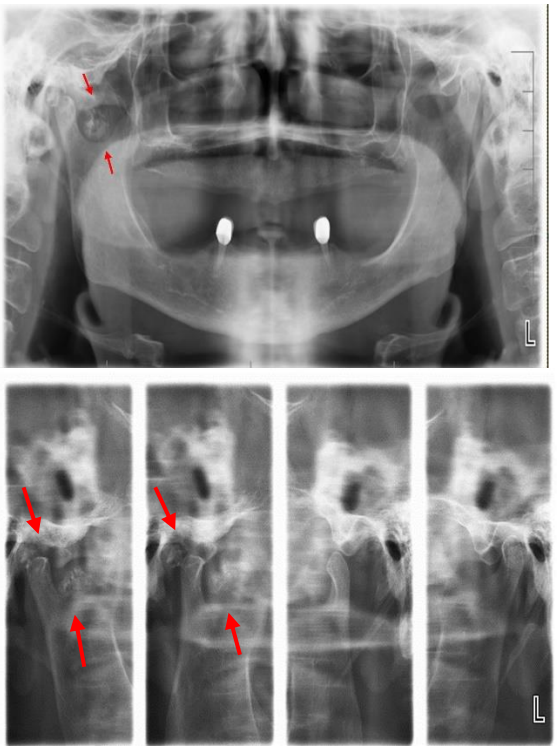
Synovial chondromatosis is a rare proliferative disease in the synovial joints characterized by the formation of metaplastic cartilage foci in the intima layer of the synovial membrane.[1] Sinovyal osteokondromatozis is benign charactered and synovial membrane, bursas, and tendon sheath are characterized by well-differentiated hyaline cartilage neoformation affecting the tendon sheath.[1] It is seen in two forms as primary and secondary. Idiopathic synovial osteochondromatosis (ISO) is the primary form.[2] The secondary form is relatively common.[3] These nodules in the affected areas break off and become liberated and numerous ossified nodules appear in the synovium. The etiology of the disease is unknown, but it is thought that it may develop after the causes that may cause irritation in the synovium.[4] Patients may complain with pain, swelling, limitation of movement, effusion or recurrent locking.[5] Clinical symptoms of patients with synovial chondromatosis characterized by pain and preauricular swelling around TMJ, limitation of mouth opening and joint sounds; there are serious cases of abnormal occlusion with crossbite and facial asymmetry.[6] Synovial chondromatosis of TMJ, first described by Auhausen in 1933, is rare with about 40 cases reported in the literature.[7]

Nonspecific clinical findings may delay diagnosis, and may also cause unnecessary medical treatments. In addition, malignant transformation has been reported, although rare, and treatment and follow-up are important.[8] Conservative or surgical methods are used for treatment. Arthroscopic or open surgical procedures are generally preferred.[9]

In this study, we aimed to present a case of synovial osteochondromatosis which is a rare disease radiologically and clinically and discuss it in the light of current literature.

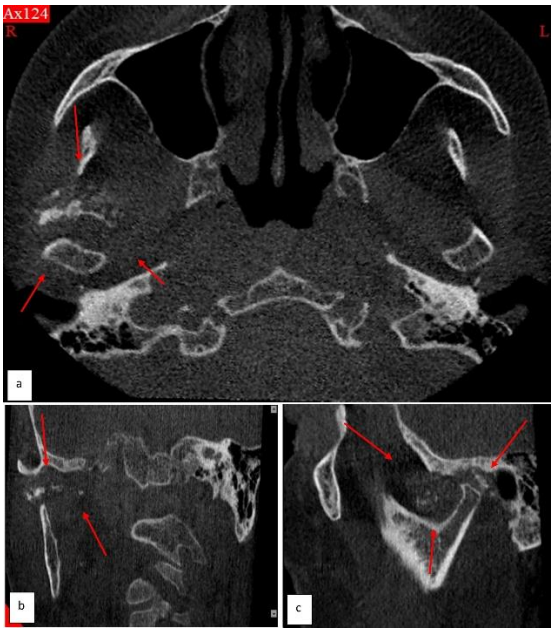
Case Report

A 53-year-old female patient applied to our clinic with the complaint of pain and limitation of movement in her right TMJ for a long time. The patient had no history of trauma. Laboratory results were within normal limits. Clinical examination revealed painful limitations in TMJ extension and flexion. Panoramic and TMJ graphs (PLANMECA ProMax -2019 / Panoramic exposure parameters; 64 kV-6.3mA, TMJ exposure parameters; 66kV-6.3mA) showed multiple, calcified bodies around the condyle in the area from the right articular fossa to the incisura mandibularis. (Figures 1a and 1b).



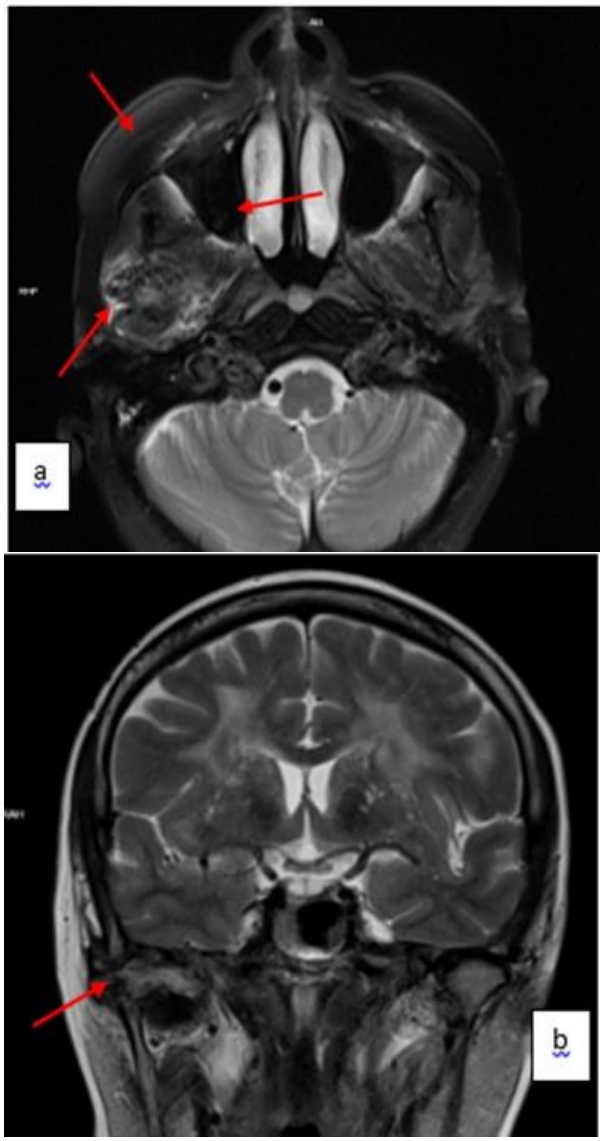
Figures 1a and 1b. Panoramic X-ray showed multiple, calcified bodies (arrows) of different sizes around the condyle in the area from the right articular fossa to the incisura mandibularis.

Cone Beam Computed Tomography (CBCT–device name) revealed multiple nodular, hyperdense calcified / ossified bodies (Figures 2a, 2b and 2c).



Figures 2a, 2b and 2c. Starting from the right joint space to the incisura mandibularis, 0.5 mm axial and 1 mm crossectional sections of the multiple calcified areas (red arrows) around the right mandibular condyle were created and tomographic axial (a), coronal (b) and sagittal (c) images.

Magnetic resonance imaging (MRI-Siemens Aera 2016) revealed ossified free fragments of similar size. No pathology was observed in the bone structure and soft tissue formation of the joint. In all sequences of MRI, the periphery of the sebest fragments was hypointense like the cortical bone and the middle was bone marrow intensities (Figures 3a and 3b).



Figures 3a and 3b. Axial (a) and coronal (b) MR images of the sections (red arrows) hypotensively observed in T2-weighted sequences and around the condyle in T2-weighted sequences.

Sagittal oblique T1 and T1 weighted sequences for TMJ were used when the mouth was open and closed. Section thickness 3mm. T1 and T2-weighted sequences in the TMJ interval and around the condyle were consistent with calcification, synovial thickening, and minimal fluid. These described findings were characteristic for osteochondromatosis. Since there was no pathology that could explain the formation of intraarticular free bodies in the bone cortex and medulla on direct X-ray, CT and MRI, the disease was found to be SC and the diagnosis was supported by biopsy.

DISCUSSION

There are two types of synovial osteochondromatosis: primary and secondary. The primary type shows monoarticular involvement, mostly involving large joints such as knees, elbows, shoulders and hips. It is more common in the third and fourth decades of life and in men.[3] The secondary type is encountered at a later age and is secondary to trauma, osteoarthritis and osteochondritis dissecans. It affects knees, hips and shoulders more often. Nodules are smaller in number, larger and may not be similar in size.[3,10] No history of trauma, normal joint spacing and joint-forming structures distinguish the primary type from secondary synovial ostecondromatosis.[11] In our case, the patient's advanced age, osteoarthritis in the right joint, radiographic The presence of a small number of nodules in the study and the large and dissimilarity of the nodules strengthened the diagnosis of secondary type synovial osteochondromatosis. Synovial osteochondromatosis usually presents with nonspecific complaints such as pain, swelling, tenderness, limitation of movement and locking in the involved joint. Physical examination may show effusion, crepitation, instability, and limited range of motion.[2] Murphey *et al.*[10] reported pain in 85-100%, swelling in 42-58%, and restriction in joint range of motion in 38-55%. It is; On physical examination, they found diffuse swelling, tenderness, crepitation, locking, and nodule or mass on the joint. Trias *et al.* Reported that at least 21 patients had muscle atrophy.[12] The disease was insidious and the duration of symptoms before diagnosis was long. Our case was a female patient and she had involvement in TMJ joint where it was rarely localized. Our patient had no history of trauma and had right TMJ pain. Physical examination revealed pain, crepitation and instability during joint movement. Diagnosis of this disease is difficult and delayed due to nonspecific complaints and clinical findings. They may be followed up as chronic joint problems with

chronic and progressive joint pain for a long time. Laboratory tests are not useful in the diagnosis, but may be useful in the differential diagnosis of inflammatory diseases.[13] Therefore, imaging methods are needed to make the diagnosis. The first option is a direct x-ray. In 70% of patients, abnormal findings such as numerous calcified nodules on the joint, bursa or tendon sheaths are seen on radiographs. Periarticular erosion may be observed in 30% of the patients due to the pressure effect on the small joints of the hip, elbow and hand-foot. However, erosions in the knee and shoulder joints are rare. Periarticular erosions may also occur in the absence of calcified nodules. In addition, secondary osteoarthritis findings caused by intraarticular bodies can be observed in untreated disease.[14] Iyengar *et al.*[15] found that physical examination findings were generally non-specific and challenging for differential diagnosis; that the diagnosis can be made mostly by the presence of radiopaque bodies on direct radiographs; reported that synovial biopsy materials should be examined histopathologically for differential diagnosis of other proliferative synovial diseases such as synovial chondromatosis and pigmented villonodular synovitis. Fujita *et al.*[16] mentioned the importance of the presence of calcification or ossification in the nodules observed in direct radiographs in the definitive diagnosis of synovial osteochondromatosis. In addition, it should be kept in mind that the nodule may not be seen on direct radiography because it is non-calcified in 5-30%. Therefore, localization, nature and changes in the neighboring structures can also be detected by computed tomography (CT) and MRI examinations, especially in the stages before ossification.[16,17] CT can show better calcified nodules and bone erosions than direct radiographs. Because of the superior soft tissue contrast and multi-planar imaging features, the typical image detected on MRI is joint articular effusion and intraarticular, intrabursal and tenosynovial soft masses with nodules. These soft masses are hypo / isointense relative to muscle in T1-weighted images and hyperintense in T2-weighted images.[10] MRI is considered the most useful method in early diagnosis.[18] Multiple, calcified and ossified nodules in the popliteal fossa on direct X-ray, Figure 2: calcified nodules on CT scan is an excellent method for the evaluation of synovial pathologies such as osteochondromatosis. MRI imaging features; It depends on the pulse sequence used, the presence and degree of calcifications and / or ossifications.[10] In our case, multiple, different sizes of calcified bodies were observed on the tmj direct radiographs (Figure 1). Conical beam computed tomography (CBCT– device name) examination showed multiple nodular, hyperdense calcified / ossified bodies (Figure 2). Magnetic resonance imaging (MRI-DEVICE NAME) examination revealed large and dissimilar, close to each other, ossified free fragments. Osteophyte and degenerative changes were observed in the bone structure and soft tissue formation of the right joint. In all sequences of MRI, the free fragments were hypointense like the cortical bone and the middle was bone marrow intensities (Figures 3a, 3b and 3c). T1 and T2 weighted sequences in the TMJ interval and around the condyle were hypotensively observed with calcification, synovial thickening and minimal fluid. The described findings are characteristic for osteochondromatosis.

CONCLUSIONS

In conclusion, synovial osteochondromatosis may cause joint pain alone or secondary to many causes. It should be kept in mind that it often involves large joints, usually monoarticular, and should be considered in such cases. CBCT and MRI should be the first choice after direct radiography in patients with a diagnosis of synovial osteochondromatosis. In addition to assisting the diagnosis, CBCT and MRI will be a good guide before surgery in the patient group planned for surgery. Treatment should be planned considering the risk of malignancy and especially the risk of early degeneration of the burdened joints. Conservative treatment may be effective in non-load-bearing upper extremity joints; however, early degeneration risk should be considered and surgical treatment should be recommended.[19]

ACKNOWLEDGEMENTS

None

CONFLICT OF INTEREST STATEMENT

The authors deny any conflicts of interest related to this study

ÖZ

Sinoviyal kondromatozis (osteokondromatozis) sinoviyal membrandan kaynaklanan nadir bir lezyondur. Eklemde bursanın tendon kılıfının veya sinovyal zarının metaplazisi sonucu gelişir ve sinovyumda kondral veya osteokondral odakların oluşmasına neden olur. Bu kıkırdak lezyonlar, eklem içinde serbest cisimler olarak ikincil dejeneratif değişikliklere neden olabilir. 30-50 yaşları arasında diz eklemi daha az sıklıkla kalça, ayak bileği, omuz ve dirsek eklemlerinde görülür. Klinik olarak eklem ağrısı ve bazı vakalarda ilerleyen hareket kısıtlılığı, efüzyon ve tekrarlayan kilitlenme görülür. Bu olguda sinovyal kondromatozis nedeniyle kliniğimize başvuran 53 yaşındaki kadın hastanın SC radyolojik (CBCT ve MR) ve TME klinik bulgularını sunmayı amaçladık.

Anahtar Kelimeler: *Sinoviyal kondromatozis, Temporomandibular eklem, CBCT, MR, Direkt grafi.*

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Cumhuriyet Dental Journal: 2021; 24(3)
e-ISSN 2146-2852
Doi:10.7126/cumudj.871091

Reviews

ANALGESIC EFFECT OF PRE-EMPTIVE ORAL NSAIDS ON POST-ENDODONTIC PAIN LEVELS IN SINGLE VISIT ENDODONTICS -A SYSTEMATIC REVIEW

ABSTRACT

Pre-emptive analgesia is an anti-nociceptive treatment that reduces postoperative pain by preventing altered afferent input. As most of the patients present with pain preoperatively have higher levels of released local inflammatory mediators. Thus, pretreatment analgesia decreases the establishment of central sensitization, a mechanism by which spinal neurons increase their response to the peripheral nociceptive impulse. This systematic review aims to compare and evaluate the postoperative pain levels and analgesic intake on NSAIDs' preoperative oral administration in single visit root canal treatment. According to PRISMA guidelines, the present review was done and was registered in the PROSPERO (Centre for Reviews and Dissemination University of York.). Registration number-CRD42020195775. The research question was formulated based on the PICO strategy. A comprehensive electronic literature search was conducted across PubMed/Medline, Scopus and Cochrane Database independently by two reviewers. Articles published from January 1990 to May 2019 that focused on pre-emptive strategies in single visit root canal treatment were included in the present review. Based on specified inclusion and exclusion criteria's, the selected articles were subjected to quality assessment, and the risk of bias was evaluated. A total of 6 articles were included, out of which three were hand searched. The overall risk of bias of included studies was moderate, and the study limitations were high. Among the studies included, Ibuprofen was considered as the best drug of choice in single visit endodontics.

Keywords: Diagnosis, Permanent Tooth, Root Canal.

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Received : 30.01.2021
Accepted : 17.05.2021

How to Cite: Teja KV, Ramesh S, Apoorva Vasundhara K. Analgesic Effect of Pre-Emptive Oral NSAIDs on Post-Endodontic Pain Levels in Single Visit Endodontics -A Systematic Review. Cumhuriyet Dent J 2021;24:3:.
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INTRODUCTION

The pain is the primary reason for which most of the patients seek endodontic treatment frequently.[1] Endodontic procedures help to restore a tooth affected irreversibly by bacterial invasion and infection.[2] The therapy aims at complete debridement of the inflamed and necrotic pulp tissue from the root canal space and sealing it from the external environment.[2,3] There is a significant relief in pain, preceding an appropriate and flawless endodontic procedure.[1,4,5,6] However, the pain relief may not be immediate and absolute.[1] Although it is diminished to some extent, immediately following the root canal treatment in most patients, 30% of them still experience moderate to severe postoperative pain due to the residual effects of inflammation.[4] So, an endodontic procedure should consider both primary concerns of the patient and long-term complications such as postoperative pain. Postoperative pain is usually at its worst in the first 24 hours after an endodontic procedure. Gradually the levels decrease in the subsequent 3 to 7 days.[4]

Mechanical, thermal, chemical and immunological stimulation of afferent nociceptors, which synapse in the dorsal horn of spinal cord lead to direct sensation of thalamus and presents as pulpal and periapical pain.[5] Nocicieptors are stimulated by the endogenous inflammatory mediators such as bradykinin, calcitonin-gene related peptide, substance p, growth factors, cytokines, chemokines and arachidonic acid.[6] These substances are generated as part of the innate immune response following a tissue injury or destruction. The arachidonic acid metabolite is converted to prostaglandin by enzymes such as cyclooxygenase enzyme 1 and 2 (COX-1, COX-2). The converted prostaglandins in turn cause the sensitization of afferent nociceptors leading to the decreased pain threshold.[6] Therefore, pharmacologic interventions mainly aim to target cyclooxygenases, limiting prostaglandin production and limiting nerve fibres' sensitization.[7]

Perception of pain is a highly subjective and variable experience. It is not only influenced by the performed treatment procedure, but multiple physical and psychological factors also play a role in perceiving pain.[8] Conventional dental procedures are associated with pain postoperatively, but the incidence and severity are highest with root canal therapy.[9] The post endodontic pain is mainly due to the extrusion of canal contents, debris and microbes from root canal space to the periapical area. These conditions lead to an intense inflammatory response and infection.[10] Various non-narcotic analgesics like Non-Steroidal Anti-inflammatory Drugs NSAID'S are used in treating endodontic pain. The mechanism of action is mainly by inhibiting prostaglandin synthesis at the sites of inflammation.[11] Several clinical studies have evaluated NSAID'S use to treat postoperative pain in endodontics with conflicting results. Some studies state conclude the beneficial effect in administering non-narcotic analgesics, whereas others claim no efficient results.[12,13] Various other drugs that are used along with NSAID'S include opioids and combination of medications for treating endodontic pain.[14]

Previous studies showed conflicting results when the drugs were prescribed preoperatively and postoperatively to reduce the endodontic pain.[13,15] Pre-emptive analgesia is an anti-nociceptive treatment that reduces postoperative pain by preventing altered afferent input processing.[16] As most of the patients present with pain preoperatively have higher levels of released local inflammatory mediators. Thus, pretreatment analgesia decreases the establishment of central sensitization, a mechanism by which spinal neurons increase their response to the peripheral nociceptive impulse.[13]

METHODS

The present review aims at evaluating the postoperative pain levels and analgesic intake on preoperative oral administration of Non-Steroidal Anti-inflammatory Drugs in single visit root canal treatment.

Objectives:

The systematic review mainly concentrated on the pretreatment analgesia on post endodontic pain levels in patients undergoing single visit root canal treatment. The present review gives a comprehensive idea on analgesics used and the

regimen preferred in single visit root canal treatment.

PICO Question:
Is there any variation in the postoperative pain levels and postoperative analgesic intake on preoperative administration of NSAIDs in single visit root canal treatment?

PICO analysis:
Population: Teeth undergoing single visit root canal treatment.

Intervention: Oral preoperative administration of NSAIDs.

Comparison: Experimental group compared to placebo, a different NSAID, an extra dose of the experimental NSAID.

Outcome:
Primary outcome: Postoperative pain levels.

Secondary outcome: Postoperative analgesic intake.

Protocol for registration:
According to PRISMA guidelines, the present review was done and was registered in the PROSPERO (Centre for Reviews and Dissemination University of York; http://www.crd.york.ac.uk/PROSPERO). Registration number - CRD42020195775.

Source
To identify studies included or considered for this review, detailed search strategies were developed for the database searched. The MEDLINE search used the combination of controlled vocabulary and free text terms.

Searched Data Bases
- PubMed (January 1990 to May 2019)
- PubMed Advanced Search
- MEDLINE
- Cochrane Database of Systematic Reviews

Full-text articles in English were only applied during the electronic search to include all the possible clinical trials in the systematic review's potentially relevant article search phase. No time restriction was applied. Reference list of the reviews and identified randomized controlled trials were also checked for possible additional studies.

Hand Searching:
The following journals were hand-searched:

- Journal of Endodontics
- International Endodontic Journal
- Journal of Conservative Dentistry
- Indian Journal of Dental Research
- Journal of International and Oral Health
- Journal of Dental School
- Caspian Journal of Dental Research

Language

Full-text articles in English were only selected.

Inclusion Criteria

Criteria for considering studies for this review

Types of studies

- Randomized controlled trials
- Clinical trials
- Prospective Invivo studies

Types of Participants

- Patients under the age group of 18 years to 65 years undergoing single visit root canal treatment.

Types of Interventions

- Patients prescribed preoperatively with oral NSAIDs before a single visit root canal treatment.

Types of Comparisons
- Compared to placebo, the experimental group, a different NSAID, or a different experimental NSAID dose.

Types of Outcome Measures
1. Primary Outcome: Postoperative pain levels
2. Secondary Outcome: Postoperative analgesic intake

Exclusion Criteria
The following studies were excluded

1. Case reports/Case series
2. Invitro Studies

3. Animal studies
4. Review articles
5. Absence of baseline pain
6. Invivo studies comparing NSAIDs in multiple visit root canal treatment
7. Invivo studies comparing corticosteroids and opioids in single visit root canal treatment
8. Studies not mentioned in the inclusion criteria

Search

A search was carried out in an electronic database (i.e.,) PubMed Central, Cochrane, Lilac, Science Direct, Google Scholar) using the following search terms alone employing PUBMED search builder from January 1990 up to May 2018. Various "search terms" or "keywords" were used to execute the PubMed search. The searched terms were: "Teeth", "permanent teeth", "Pain", "Endodontic pain", "Pre-emptive", "Preoperative", "Analgesics", "NSAIDS", "Analgesic intake", "Post endodontic pain", "Single visit root canal treatment".

Description of Studies

Duplicates were excluded, and 392 articles were identified. Out of those 373 articles were excluded looking title and abstract. The remaining 19 articles were assessed fully by two independent examiners according to the inclusion criteria. Out of which 16 articles were excluded with reason (Table 1).

Table 1. Table Depicting the List of studies excluded based on the criteria

S.No	Author	Year	Reason for Exclusion
1.	Menhinick *et al.*[17]	2004	Discussed on pain intensity of the patient following Pulpectomy on using ibuprofen or a combination of ibuprofen
2.	Rogers *et al.*[19]	1999	The study discussed on efficacy of dexamethasone and ketorolac tromethamine when used as an intracanal medication, with oral ibuprofen and placebo on post-treatment endodontic pain
3.	Mehrvarzfar *et al.*[15]	2011	The study compared the efficacy of tramadol, novafen and in pain reduction after root canal preparation
4.	Modaresi *et al.*[19]	2006	The study compared the efficacy of ibuprofen, acetaminophen-codeine and placebo premedication therapy on the depth of anesthesia during the treatment of inflamed teeth
5.	D.R. Mehlish *et al.*[20]	2010	The study compared the analgesic efficacy of concurrent ibuprofen and paracetamol with ibuprofen and paracetamol alone in the management of moderate to severe acute postoperative pain after removal of impacted teeth
6.	Wells *et al.*[21]	2011	The study compared the efficacy of ibuprofen and ibuprofen combined with acetaminophen on post-treatment pain after emergency debridement of teeth in symptomatic patients with a pulpal diagnosis of necrosis
7.	Elazaki *et al.*[22]	2016	The study the compared efficacy of non-steroidal anti-inflammatory drugs in controlling post-endodontic pain after root canal preparation
8.	Metri M *et al.*[23]	2016	The study results were reported as a change in the intensity of the pain rather than absolute pain on pretreatment with diclofenac sodium on post endodontic pain in single visit root canal treatment
9.	S. Jenarthanan *et al.*[24]	2018	The study compared the efficacy of diclofenac sodium administered using different delivery routes in the management of endodontic pain
10.	Taggar *et al.*[25]	2017	The study compared two ibuprofen formulations in the reduction of spontaneous pain and mechanical allodynia measured using a bite force transducer
11.	Nabi *et al.*[26]	2018	The study compared the analgesic effect of preoperative ibuprofen in controlling the post endodontic pain with or without low-level laser therapy in single visit root canal treatment
12.	Joshi *et al.*[27]	2016	The study compared the efficacy of oral and intraligamentary Piroxicam for the management of post endodontic pain after a single visit root canal treatment
13.	Ramazani *et al.*[28]	2013	The study compared the prophylactic effects of oral zintoma and ibuprofen on post endodontic pain after a single visit root canal treatment
14.	Praveen *et al.*[29]	2017	The study evaluated the premedication of ketorolac and prednisolone on post endodontic pain in single visit root canal treatment
15	Torabinajed *et al.*[12]	1994	The study evaluated the effectiveness of various medications on post endodontic pain after instrumentation
16.	Sethi *et al.*[30]	2014	The study compared the effect of single-dose pretreatment analgesia with different analgesics on postoperative endodontic pain after root canal preparation

Finally, six studies obtained from hand and electronic search matching the inclusion criteria were assessed for systematic review (Figure 1), (Figure 2).

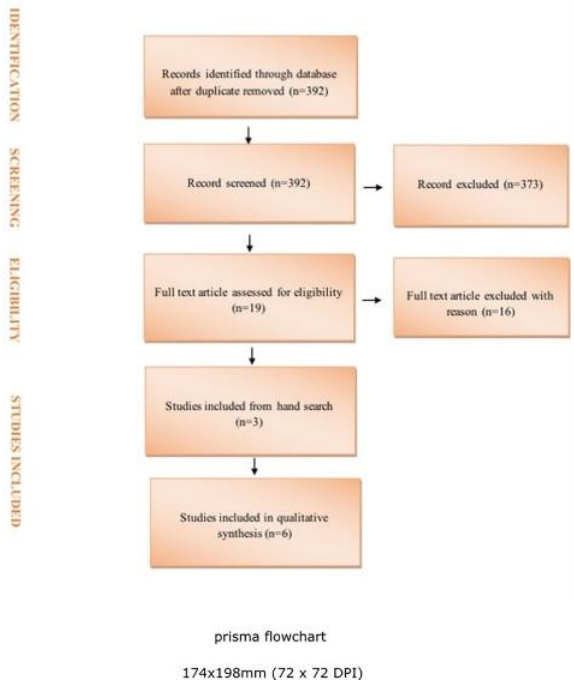

Figure 1. PRISMA Flow Chart Depicting the Search Stratergy

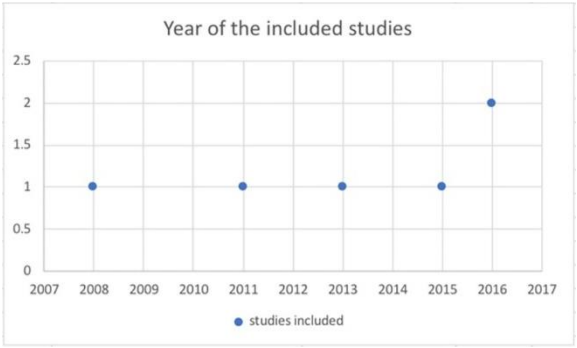

Figure 2. Graph Depicting the Year of the Included Studies

Two independent reviewers did the above analysis, and in case of disagreement, a consensus was reached after discussion with a third reviewer.

The checklist's methodology was followed based on the Quality Assessment of Diagnostic Accuracy Studies Tool (QUADAS). Only clinical trials were included for this systematic review.

Data extraction and statistical analysis:

The extracted included the type of treatment, protocol followed, drugs and controls used in each study, the time drugs administered, the time points at which the pain was measured, and the outcome variables used to measure pain, and postoperative analgesic intake. Information on the pre and postoperative pain at measured time points were gathered from each of the treatment arms of the included articles were derived, and the graphs were plotted. The included six eligible studies used various pain assessment scales, including Heft-Parker visual analogue scale, modified visual analogue scale (VAS) and categorical pain scales. All data was converted to standardize 170mm Heft-Parker VAS scoring for interpreting the results. A meta-analysis can yield a more precise overall estimate of the treatment effect. However, it was not possible in the present study due to the heterogeneity among the included studies such as differences in the sample sizes and follow up periods. Hence, only a descriptive evaluation of data has been performed.

RESULTS

Summary of included studies

The electronic and manual search identified 392 studies after de-duplication. Three other records were identified through hand searching and were included in the present study. Three hundred ninety-two records were screened out of which 373 studies were excluded, and 19 full-text articles were assessed for eligibility. Sixteen studies were excluded with reason. Finally, after assessing full texts, six articles from manual and electronic search on postoperative endodontic pain management with NSAIDs in single visit endodontics were identified and included in the study.[13,31-35] The drugs that were studied included ibuprofen 200mg tablet, tenoxicam 20mg table, ketorolac 10mg tablet, celecoxib 400mg capsule, gelofen 400mg capsule (ibuprofen 400mg capsule), novafen 200mg capsule (ibuprofen 200mg capsule), tenoxicam 20mg capsule, ibuprofen liquigel capsule, ibuprofen 600mg tablet, ibuprofen 600mg liquigel capsule, ibuprofen 400mg tablet and indomethacin 25mg tablet. All the included studies provided baseline demographic data. Ethnicity was not reported in any of the included studies.[13,31-35] The mean baseline pain for the selected studies was 73.217 on a 170mm Heft parker VAS, ranging from 52 to 150. The study by Mokhtari *et al.*[35] Categorized the treated tooth and arch type and reported the tooth's specific diagnosis. Bali *et al.*[31] specified the diagnosis, but the treated tooth and arch type was not specified. Ashraf *et al.*, specified the arch and the tooth type, but the diagnosis was unclear. The time of evaluating the postoperative pain levels was different, and the disparity was observed in all

the included studies.[13.31-35] Characteristics of included studies are found in (Table 2).

Table 2. Table Depicting the Data and Characteristics of the Included Studies

Author and year	**Treatment Groups (n)**	**Time of Delivery**	**Time of Pain Evaluation**	**Outcome Variable**	**Inclusion Criteria**	**Anesthetic agent used, Irrigation Protocol, the file system used, Sealer Used and Obturation Technique Followed**	**Drugs Prescribed Postoperatively**	**Postoperative pain Scores and Analgesic intake**
Bali et al 2016.[31]	Group 1: 200mg of ibuprofen (30) Group 2: 20mg of tenoxicam (30) Group 3: 10mg of ketorolac (30)	Single dose orally half an hour before the procedure	Baseline,0,6, 12,24	Postoperative pain: 10point visual analogue scale	Permanent teeth diagnosed with symptomatic irreversible pulpitis	2ml of xylocaine 2% adrenaline 1:200,000, 5.25% NaOCl and EDTA gel (volume and time of irrigant used was not specified), instrumentation (not specified), Calcium hydroxide based sealer(seal apex), lateral condensation technique	Not mentioned	Pain scores: Group 1: Baseline: 5.32; 0hrs: .72; 6hrs: .85; 12hrs: 0.87; 18 hrs: 0.73; 24hrs: 0.66 Group 2: Baseline: 5.20; 0hrs: .78; 6hrs: .68; 12hrs: 0.56; 18hrs: 0.66; 24hrs: 0.44 Group 3: Baseline : 6.02; 0hrs: .75; 6hrs: .69; 12hrs: 0.55; 18hrs: 0.40; 24hrs: 0.42 Analgesic intake: Not specified.
Ashraf et al 2013.[32]	Group 1: 400mg of Celecoxib capsules (15) Group 2: Placebo capsules (15)	Group 1 received single dose orally half an hour before the procedure. Group 2 received two placebo capsules half an hour before the procedure.	Baseline,4,8, 12,24	Postoperative pain: 170mm Heft-Parker VAS	Patients complaining of pain in mandibular first and second molars	2% lidocaine with1/80,000 epinephrine, 2.5% NaOCl(volume and time of irrigant used is not specified), instrumentation (not specified), resin based sealer (AH26 sealer, Dentsply, Konstanz, Germany), lateral condensation technique	Three 325mg acetaminophen tablets prescribed to be taken if required.	Pain scores: Group 1: Baseline: 148.9; 4hrs: 55.2; 8hrs: 55.2; 12hrs: 70.0; 24hrs: 31.4; 48hrs: 15.7 Group 2: Baseline: 150.0; 4hrs: 92.8; 8hrs: 92.8; 12hrs: 100.7; 24hrs: 48.5; 48hrs: 21.07 Analgesic intake: Not specified.
Dejkam et al 2015.[33]	Group 1: 400mg of Gelofen capsule (20) Group 2: 200mg of Novafen capsule (20) Group 3: 500mg of flour and starch placebo capsules (20)	Each participant received two capsules 60 minutes before the treatment.	Baseline,4,8, 12,24,48 hours postoperatively	Postoperative pain: 10point Visual Analogue Scale	Permanent teeth with pulpal diagnosis not specified	2%Lidocaine with 1/80,000 epinephrine, irrigation protocol and instrumentation not specified, resin based sealer(AH26 sealer), lateral condensation technique.	Acetaminophen codeine as and when required.	Pain scores: Group 1: Baseline: 5.55; 4hrs: 2.7; 8hrs: 1.75; 12hrs: 2.55; 24 hrs: 1.75; 48hrs: 1.0 Group 2: Baseline: 5.95; 4hrs: 3.25; 8hrs: 2.45; 12hrs: 2.8; 24hrs: 1.65; 48hrs: 0.7 Group 3: Baseline: 5.15; 4hrs: 2.35; 8hrs: 2.5; 12hrs: 3.4; 24hrs: 1.8; 48hrs: 1.0 Analgesic intake: Placebo: 7 patients: 3 tablets 3 patients: 2 tablets Celecoxib group: 3 patients: 3 tablets 2 patients: 2 tablets 1 patient: 1 tablet
Arslan et al 2011[34]	Group 1: 20mg of tenoxicam capsule (16) Group 2: 200mg of liquigel ibuprofen capsule (16) Group 3: Sugar placebo (16)	The Single-dose prescribed orally before root canal treatment	Baseline,6,12,24,48,72 hours postoperatively	Postoperative pain: 100mm Visual Analogue Scale	Pain originating from a tooth >=50mm of VAS included pulpal and periapical diagnosis not specified	4% Articaine HCl with 1:100,000 epinephrine(ultracaine D-S forte), 5.25% NaOCl, EDTA gel (volume and time of irrigant used was not specified), final irrigation with 1ml of 15% liquid EDTA for 1 minute, followed by 3ml of 5.25% NaOCl, instrumentation (not specified), calcium hydroxide based sealer(seal apex), lateral compaction technique.	Instructed to take extra dosage of test medication(dosage not specified)	Pain scores: Group 1: Baseline: 82.6; 6hrs: 7.92; 12hrs: 9.62; 24hrs: 4.09; 48hrs: 4.80; 72hrs: 1.8 Group 2: Baseline: 83.2; 6hrs: 2.83; 12hrs: 15.3; 24hrs: 3.49; 48hrs: 2.34; 72hrs: 0.0 Group 3: Baseline: 83.2; 6hrs: 35.1; 12hrs: 19.8; 24hrs: 16.4; 48hrs: 13.6; 72hrs: 8.86 Analgesic intake: Not specified.

Attar et al 2008[13]	Placebo (12) 600mg Ibuprofen tablets (14) 600mg Ibuprofen liquigel (13)	Single dose orally before the treatment	Baseline, during treatment, 6,12,18,24 hours postoperatively	100mm VAS, 170mm HP-Categorial VAS, Categorial	Pain >=30 on VAS included pulpal and periapical diagnosis not specified.	Long acting anesthetic was administred (agent not specified), 3% or 6% NaOCl (volume and time of irrigant used was not specified), Flexofiles (Dentsply, Mallifier, Tulsa), Size 2 to 4 gates gidden burs (Dentsply, Mallifier), GT nickel-titanium rotary files (Dentsply, Mallifier), Roth's 801 or ZOE or AH Plus resin seler was used. Obturation by down packing with system-B (SybronEndo Corp, Orange, CA) and backfilling using obtura (Obtura Spartan, Fenton, MO)	Tylenol ES (500mg) was prescribed as escape medication	Pain scores: Group 1: Baseline: 65.6; 6hrs: 17.9; 12hrs: 20.4; 24hrs: 11.9 Group 2: Baseline: 64.7; 6hrs: 26.2; 12hrs: 24; 24hrs: 23.5 Group 3: Baseline: 65.9; 6hrs: 28.1; 12hrs: 31.8; 24hrs: 21.6 Analgesic intake: Not specified.
Mokhtari et al 2016[35]	Group A: 400mg of ibuprofen tablet (22) Group B: 25mg of indomethacin tablet (22) Group C: Placebo (22)	Single-dose orally one hour before the procedure	Baseline, during treatment ,8,12 and 24 hours postoperatively	Postoperative pain: 100mm VAS	First and second mandibular molars with irreversible pulpitis	1.8ml of 2% lidocaine with 1:80000 epinephrine, 2.5% NaOCl and saline (volume and the time of irrigant used was not specified), instrumentation (not specified), AH-26 sealer (Dentsply,Tulsa), lateral condensation technique.	Postoperative medication not specified	Pain scores: Group 1: Baseline: 54.6; 8hrs: 4.2; 12hrs: 17.2; 24hrs: 8.4 Group 2: Baseline: 57.4; 8hrs: 8.6; 12hrs: 17.6; 24hrs: 14.4 Group 3: Baseline: 57.4; 8hrs: 24.2; 12hrs: 18.6; 24hrs: 9.2 Analgesic intake: patients were asked to record the analgesic intake, but the number of drugs taken were not specified.

Qualitative review

Significant differences between the studies selected included the treatment drugs, the dose of the medication, sample size and population type, time of the outcome variable measurement. The quality of evidence was average as most of the studies included had a moderate risk of bias as measured using a quality assessment tool. Sequence generation was specified only in the study reported by Arslan *et al.*[32] Allocation concealment was set in Ashraf *et al.*[32], and Mokthari *et al.*[35], study. The information about the blinding of patients and the care provider and assessor, the information about the other sources of bias were clearly specified by Ashraf *et al.*, Arslan *et al.*, and Mokhtari *et al.* studies.[32,34,35] The quality assessment and risk of bias is found in (Table 3).

Table 3. Table Depicting the Quality Analysis and Risk of Bias Assessment of Included Studies using Cochrane Quality Assessment Tool

Author, year	**Sequence generation adequate?**	**Allocation adequately concealed?**	**Blinding of participants?**	**Blinding of care providers?**	**Blinding of outcome assessors?**	**Incomplete outcome data adequately addressed? Was the overall attrition sufficiently low/differential attrition sufficiently low?**	**Was the study free of selective outcome reporting?**	**Were there other sources of bias? Were there important baseline differences in prognostic factors?**	**Risk of bias**
	Acceptable methods must be truly random; randomized table, computer-generated random numbers, etc. patients should be accepted sequentially.	**The group selection must be hidden from patients, personel and assesors until treatment is rendered; otherwise randomization may be tampered with bias increased.**				**How many people dropped out of the study? <20% is good, <15% is better for smaller studies. This avoids the risk of bias in the population sample.**	**Did they not report the data you know they observed? This is not especially relevant to these papers.**	**Were the groups similar at baseline? For any of the baseline groups, a difference of <10% is acceptable**	
Bali, 2016.[31]	Unclear-methods of randomisation not described	Unclear	Unclear	Yes	Unclear	Unclear	Yes	Yes	High

Ashraf, 2013.[32]	Unclear-methods of randomisation not described	Yes-sequentially numbered containers	Yes	Yes	No	Yes/Yes- 6.6% patients lost	Yes	Yes	Moderate
Dejkam, 2015.[33]	Unclear-methods of randomisation not described	Unclear	Yes	Yes	No	Unclear	Yes	Yes	Moderate
Arslan, 2011.[34]	Yes- block randomisation	Unclear	Yes	Yes	Yes	Yes/Yes	Yes	Yes	Moderate
Attar, 2008.[13]	Unclear-methods of randomisation not described	Unclear	Unclear	Unclear	Unclear	Yes/No-13% patients lost to follow up	Yes	Yes-differences in gender and diagnosis distribution	High
Mokhtari, 2016.[35]	Unclear-methods of randomisation not described	Yes- table of random numbers	Yes	Yes	No	Unclear	Yes	Yes	Moderate

The data obtained was reorganized into bar graphs (Graph 1), which illustrates the VAS scores at 4, 6, 8, 12, 24, 48 and 72 hours. At 4 hours, only two studies compared the pain reduction out of which gelofen 400mg capsule (Ibuprofen 400mg capsule) proved better than celecoxib 400mg capsule and Ibuprofen 200mg capsule.[32,33] At 6 hours' time interval, three studies analyzed the outcome of all the drugs compared. Ibuprofen 200mg liquigel capsule seemed to have the highest VAS score reduction, followed by tenoxicam 20mg tablet and ketorolac 10mg tablets.[31,35] Three studies compared postoperative pain reduction at 8 hours' time interval. Ibuprofen 400mg tablet had least VAS scores followed by indomethacin 25mg tablet compared with celecoxib 500mg and 200mg and 400mg novafen and gelofen capsules.[32.33,35]

All the six studies[13,31-35], compared the postoperative pain scores at 12 and 24 hours' time interval, there were varied reduction scores obtained. However, Bali *et al.*[32] seemed to have least VAS scores among the drugs compared; ketorolac 10mg tablet had least scores followed by tenoxicam 20mg tablet and Ibuprofen 200mg tablet. At 24 hours' time interval, VAS score reduction was evident in Arslan *et al.*, study. Ibuprofen 200mg liquigel capsule had least VAS scores of 3.49, followed by tenoxicam 20mg capsule and ketorolac 10mg tablet.[33]

Three studies[32,33,34], compared the outcome at 48 hours out of which, the VAS point reduction at 48 and 72 hours was observed in Arslan *et al.*[33], study, where the least VAS scores were reported with Ibuprofen 200mg liquigel capsule both at 24 and 72 hours, as compared to Indomethacin 25mg and placebo. At 48hour interval, the VAS point reduction with Ibuprofen liquigel capsule was 2.[36] Whereas at 72 hours it was reported to be nil or 0. General observations of the included studies showed a gradual trend of increased pain scores from 8 to 12 hours in all the groups, representing the peak pain levels experienced by the patient postoperatively. The pain scores tapered gradually at 48hour interval, and the reported pain levels were almost nil at 72hour intervals. Thereby it can be justified that the pain after a single visit root canal treatment may last for 24 to 72hour intervals after which the pain experienced by the patient is almost nil.

The study by Attar *et al.* had varied results in which placebo proved beneficial compared to the other tested drugs at all-time intervals. The study was determined to be dissimilar compared to the other included studies. The reason could be the smaller sample size, different timing of the treatment dose, and the placebo group's behaviour. The study was categorized into two sessions where partial treatments were temporized and completed treatments were obturated in single visit. Because the treatments were not stated to be identical, the placebo group may have perceived their treatment to be more effective, and the patients may be more suggestible to placebo.[13]

However, these comparisons' major disadvantage is the difference in the study designs and a significant difference in the baseline VAS scores. The study by Ashraf *et al.* reported that the baseline VAS score was 148.9 for celecoxib group, which was reduced to 15.7 at 48 hours. The mean

VAS score reduction for celecoxib was 133.3.[32] Whereas, the Ibuprofen 200mg liquigel capsule, which has the least VAS score of 2.34 at 48 hours, showed a mean VAS reduction of only 80.86.[34] Hence, the baseline pain scores should also be taken into consideration before concluding. Future trails have to concentrate more on other groups of NSAIDs. COX-2 and NSAID therapy combination have to be explored more in patients with moderate to severe baseline VAS scores.

As far as the analgesic intake is concerned, (Table 4) study by Ashraf *et al.*, has specified the amount of postoperative analgesic consumption where the amount of analgesic intake in the placebo group was higher celecoxib group.[32]

Table 4. Table Depicting the Data of Analgesic Intake, Rescue Medication and Incidence of adverse events

Author, year	Treatment groups (n)	Rescue medication	# Patients withdrawn	Adverse events
Bali, 2016.[31]	Ibuprofen 200mg (30) Tenoxicam 20mg (30) Ketorolac 10mg (30)	Not specified.	No patients took extra medication for pain control.	The patients reported no additional side effects.
Ashraf, 2013.[32]	Celecoxib 400mg capsules (15) Placebo capsules (15)	Three 325mg acetaminophen tablets prescribed to be taken if required.	Two patients were excluded from the study as one underwent third molar surgery, and the other was not able to answer the questions. Rescue medication: Placebo: 7 patients: 3 tablets 3 patients: 2 tablets Celecoxib group: 3 patients: 3 tablets 2 patients: 2 tablets 1 patient: 1 tablet	The patients reported no side effects.
Dejkam, 2015.[33]	Gelofen capsule 400mg (20) Novafen 200mg capsule (20) Placebo 20mg capsule (20)	Acetaminophen codeine as and when required.	Not specified.	The patients reported no side effects.
Arslan, 2011.[34]	Placebo (16) Tenoxicam 20mg (16) Ibuprofen 200mg (16)	Extra dose of treatment medication	No patients took the rescue medication.	The patients reported no side effects.
Attar, 2008.[13]	Placebo (12) Ibuprofen 600mg tablet (14) Ibuprofen 600mg liquigel (13)	Tylenol ES (500mg)	6 patients lost the follow-up by not returning questionnaires. No patients took the escape medication.	The patients reported no side effects.
Mokhtari, 2016.[35]	Ibuprofen 400mg tablet (22) Indomethacin 25mg tablet (22) Placebo (22)	Additional analgesics not specified.	Not specified.	The patients reported no side effects.

Although the patients were prescribed with rescue medication, the intake was not specified in Mokhtari and Dejkam *et al.*[33,35], studies. None of the included studies reported any adverse events or side effects noted on the intake of a placebo, or any experimental NSAID prescribed[13,31-35] except in the Bali *et al.*[31], study all the other studies specified on the type of rescue medication prescribed.[13,32-35] None of the included studies reported any adverse events or side effects[31,32-35] except for Attar *et al.*[11], study, where the data was not reported. Only 2 included studies[13,32] reported on patients withdrawal, which is specified in (Table 3).

Quantitative Review

All the studies included a measure of variance in the form of data tables, like graphs and p-value.[13,31-35] However, these studies cannot be considered for meta-analysis due to the heterogenicity among the included studies such as differences in the sample sizes and follow up periods. Hence, only a descriptive evaluation of data has been performed.

Strength of Evidence

The strength of evidence of the included studies was graded based on the AHRQ EPC methodology. The study limitations were high. Although the study designs were randomized controlled trials, the risk of bias was higher. The studies were direct; the interventions and comparisons were similar as specified in the review question. The studies seem to be unrestrained by using VAS to measure outcomes, which aligns with the review question's outcome. The studies were inconsistent in that they had varying

effect sizes and in different directions. The studies were imprecise, as they have inadequate power given the amount of difference predicted between the placebo and experimental groups (Table 5).

Table 5. Table Showing the Strength of evidence of Included Studies

Outcome: post-operative pain reduction						
Domains						
Study set:	**Number of studies and participants**	**Study limitations**	**Directness**	**Consistency**	**Precision**	**Grade for strength of evidence**
Overall data set	6 RCT's N= 333	High	Direct	Inconsistent	Imprecise	Insufficient

DISCUSSION

Findings and conceptual text

The study's goal is to give comprehensive information on the preemptive analgesic efficacy of oral NSAIDs in patients undergoing single visit root canal treatment. The present review aimed to evaluate the effective oral NSAID for reducing postoperative pain levels in single visit root canal treatment. The review question was, Is there any variation in the postoperative pain levels and postoperative analgesic intake on preoperative administration of NSAIDs in single visit root canal treatment? The literature search has shown that Ibuprofen 200mg liquigel capsules and tablets, Tenoxicam 20mg tablets, and capsules and ketorolac 10mg tablets were the most effective drugs in reducing the postoperative pain levels in single visit root canal treatment at 12 and 24 hour time intervals. However, the results cannot be generalized as there was insufficient evidence on outcomes of this present review, based on the Agency for Healthcare Research and Quality (AHRQ) Evidence-based Practice Centers (EPC) strength of evidence methodology.[36]

The present review showed that Ibuprofen 200mg liquigel capsules and tablets, Tenoxicam 20mg capsules and tablets and ketorolac 10mg tablets effectively relieving post-endodontic pain levels in single visit root canal treatment at 12 and 24 hour time intervals. Previously the literature reviews on pain were obtained from an oral surgery or medical pain perspective and applied to the field of endodontics, which may not be an appropriate way to categorize the pain of endodontic origin. The medical research is mainly concerned with acute surgical pain, which may not be relevant to the endodontic pain. A patient undergoing endodontic treatment may have pre-existing pain for an extended time; the pain may have undergone central sensitization and progressed from an acute to chronic stage.[37,38] Analgesic regimens are useful in chronic pain reduction, may not be effective in acute cases. In addition to the quality of presented pain, the studies concerned with oral surgery tend to have different baseline population presenting to the operatory for the extraction of their wisdom teeth and likely to be young and healthy, so they might have mild or no preoperative pain.

In the present review, no studies mentioned the combination therapies for significant postoperative pain reduction and none of the included studies compared or described the combination NSAIDs in postoperative pain reduction.[13,31-35] As far as dosage is concerned, the single prophylactic dose was administered before the procedure with different doses and dosage schedules. No conclusion can be drawn from the studies, about the time of initial dosage that would lead to the most significant reduction in pain for the patient or how long they should maintain the analgesic regimen. Compared to higher dosages of tablets, capsules with lower dosages have proven beneficial in postoperative pain reduction.

Strength and Limitations of the Review

The systematic review's strengths are the sum of research pretreatment analgesia on post endodontic pain levels in patients undergoing single visit root canal treatment. The present review gave a comprehensive idea on analgesics used and the regimen preferred in single visit root canal treatment.

This review's limitations are the small number of included studies and the sample size of the included studies. Other limitations include the significant heterogeneity in the included studies; the trails differed in the drug administration's timing, the dose and the time after the administration when the effect was measured.

Future Research

More research is indicated to elaborate on Ibuprofen's impact on post endodontic pain. Various trials have to be performed using the different combination of NSAID's and the research has to stress on COX-2 inhibitors in single visit root canal treatment. Future trails have to concentrate on studies comparing the fast-acting formulations of various NSAID's which seems to have the highest action in terms of both speeds of pain relief and efficacy of pain relief.

Summary

This systematic review aimed to compare and evaluate the postoperative pain levels and analgesic intake on NSAIDs' preoperative oral administration in single visit root canal treatment. An electronic search was carried out on the PUBMED database for the articles that could be used to evaluate preemptive oral NSAIDs on postoperative pain levels in patients undergoing single visit root canal treatment.

Article search was narrowed down based upon the pre-stated inclusion and exclusion criteria. A total of six articles were included in this systematic review for detailed evaluation (Figure2).

Assessment of postoperative pain levels and postoperative analgesic intake were the variables of interest.

Based on the result of this systematic review, Ibuprofen 200mg liquigel capsules and tablets, Tenoxicam 20mg capsules and tablets, ketorolac 10mg tablets were effective in relieving post endodontic pain levels in single visit root canal treatment at 12 and 24 hour time intervals. Among all the NSAIDs compared in this systematic review, Ibuprofen was the most widely studied and used almost in all the included studies except for Ashraf *et al.*[32], study. Taking its more comprehensive account of comparison, within the limitations of the current systematic review, Ibuprofen was considered the best drug of choice in single visit endodontics.

CONCLUSIONS

Taking into a more comprehensive account of comparison among the studies included in the present systematic review, Ibuprofen was considered as the best drug of choice in single visit endodontics. It can also be justified that the pain after a single visit root canal treatment may last for 24 to 72hour intervals after which the pain experienced by a patient is nil.

ACKNOWLEDGEMENTS

None

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Cumhuriyet Dental Journal: 2021; 24(3)
e-ISSN 2146-2852
Doi:10.7126/cumudj.915422

Reviews

SOLID ORGAN TRANSPLANT CANDIDATES AND RECIPIENTS: DENTISTS' PERSPECTIVE

ABSTRACT

In recent years, dental treatment need in organ transplant candidates and recipients increased in proportion to the increased number of organ transplantations due to advances in immunosuppressive drugs and medical surgical technology.

For the transplant candidates and recipients, dental treatment usually requires standard practice procedures that apply to the management of the severely medically compromised patient. However, there are no guidelines, clinical trials or outcome assessments on appropriate dental treatment for these patient groups. Moreover, medicine often forgets dentistry, as there is no consensus among medical transplant specialists as to whether dental infections pose a risk to organ transplant candidates and recipients.

The aim of this article is to briefly review the most common oral manifestations in solid organ transplant candidates and recipients, and to suggest a specific dental management protocol to guide medical and dental professionals for general dental management before and after solid organ transplantation.

Keywords: Solid organ transplantation, oral manifestations, dental management.

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Received : 13.04.2021
Accepted : 15.06.2021

How to Cite: Gümrü B, Tarçin B. Solid Organ Transplant Candidates and Recipients: Dentists' Perspective. Cumhuriyet Dent J 2021;24:3:299-309.
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INTRODUCTION

Transplantation, the replacement of a failing organ, tissue or cellular element with another from a donor for therapeutic purposes, is considered one of the most important achievements in medicine and science in the late 20th century.[1,2] This has been made possible by gaining further insight on the immune response, the improvement of immunosuppressive drugs so as to prevent or delay the rejection of the transplanted organ, and the advances in medical surgical technologies and techniques.

Currently in Turkey, all organ transplantation centres and operations are under the control of the National Tissue and Organ Transplantation and Dialysis Coordination Centre affiliated to the Ministry of Health. According to the data on the number of transplantations, donors, and centres in the last 10 years available on the website of the Ministry of Health, mostly solid organ transplantation (SOT) (kidney and liver) is performed in the majority of organ transplantation centres across Turkey.

As our scientific knowledge continues to increase and transplantation techniques improve, the transplantation procedures will have more successful outcomes, will be performed more frequently, will become economically feasible, and the life expectancy of transplant recipients will increase significantly. As a result, contacts between the dental practitioners and SOT patients are likely to become more often.[1-3] Therefore, the dentist should have knowledge on the specific dental management of these patients.

This article briefly reviews the oral manifestations in SOT candidates and recipients, and suggests a specific dental management protocol by reviewing the literature on general dental management before and after SOT.

Oral Manifestations in Solid Organ Transplant Candidates

The likelihood of untreated dental disease and poor dental health is increased in SOT candidates due to a number of factors. Disability and loss of employment as a result of organ failure and accompanying complications may result in patients not having regular access to dental care. In addition, stress, anxiety, fatigue, depression, cognitive impairments, substance abuse, and other incapacitation sources may further compromise the maintenance of optimal dental health.[1,4,5] Preoccupation of the patients with their primary disease leads to underestimation or ignorance of the dental symptoms and postponement of the dental examinations.[1,6]

Renal dialysis patients have been reported to show deficits in oral health behaviour, evidence of periodontal disease, and high decayed, missing, and filled teeth (DMFT) index for various reasons.[7] Haemodialysis, reduction of oral fluid intake, and multiple medication usage for the management of complications accompanying renal failure and dialysis cause xerostomia, which may contribute to and promote dental caries, periodontal disease, fungal and viral infections in the renal transplant candidate.[1,8]

Alcoholic liver disease is one of the most frequent indications for liver transplantation. Heavy alcohol use is often associated with dental neglect as well as heavy smoking which is a risk factor that can contribute to and exacerbate periodontal disease.[1,6] Another indication of liver transplantation is the treatment of Hepatitis C, which is usually in association with alcohol and drug abuse, possibly concomitant smoking, dental neglect, and untreated dental disease.[1,6,9] The use of diuretic agents for the management of the frequently encountered cirrhosis, ascites, and/or oedema complications may reduce salivary secretion, which may promote dental plaque accumulation.[5,6,10] Mood modifiers with anticholinergic activity may be required in liver transplant candidates and may cause a decrease in salivary secretion.[6] The possibility of xerostomia is reported to increase with the number of medications taken. Chronic active hepatitis, autoimmune hepatitis, and primary biliary cirrhosis have also been associated with Sjögren's syndrome which is another cause of xerostomia.[11,12]

High prevalence of deficient dental hygiene, poor oral health, xerostomia, periodontal disease, dental caries, and periapical lesions in the liver

transplant candidates has been reported.[9,13] Oral health status, oral health behaviours, and oral mucosal pathologies of liver transplant candidates were evaluated in two studies by Guggenheimer *et al*.[5,6] The most important determinants of dental disease were detected to be intervals longer than 1 year since the last dental visit, smoking, and diuretic therapy.[6] The most common oral pathologies were reported as fissured tongue, atrophy of tongue papillae, angular cheilitis, and oral candidiasis associated with xerostomia due to diuretic use.[5]

Oral Manifestations in Solid Organ Transplant Recipients

Although the survival rate of transplant recipients has increased due to the inclusion of cyclosporine A (CsA) and tacrolimus to the post-transplantation immunosuppressive protocol, SOT recipients become more prone to fungal, viral, and bacterial infections as a consequence of long-term immunosuppressive therapy.[2,3,10,14-16] Oral Candida, Cytomegalovirus (CMV), Herpes Simplex Virus (HSV) and Varicella Zoster Virus (VZV) infections, and Epstein-Barr Virus (EBV) associated hairy leukoplakia were observed in these patients.[1,3,10,17]

Oral ulcerations and stomatitis have been considered a possible post-transplantation complication due to immunosuppressives such as tacrolimus, everolimus, and mycophenolate mofetil (MMF), however the reports on this topic are controversial.[18-21]

An additional side effect of CsA is gingival enlargement, which usually appears within the first 3 months, affects the interdental papillae in the anterior region, while does not appear to affect edentulous areas. Gingival enlargement prevents the maintenance of the oral hygiene causing an increased susceptibility to caries, periodontal diseases, and infections. The frequency of gingival enlargement has been reported to be 7-74.1% in renal, and 22% in liver transplant recipients.[15,22-25] The severity of gingival enlargement has been shown to vary depending on factors such as age (more severe in young patients), gender (more frequent in male patients), genetic predisposition, oral hygiene status, type of drug used, and concomitant drug use (calcium channel blockers).[15,22,24] Other immunosuppressive agents such as tacrolimus, sirolimus, and azathioprine may also cause gingival enlargement.[22,23]

It has been shown that the risk of developing oral malignancy increases following SOT, and a considerable amount of squamous/basal cell carcinoma, and Kaposi's sarcoma cases have been reported.[26-30] The multifactorial development of these malignancies may involve suppression of the inherent immune mechanisms against malignant cells, smoking, and activation of human papilloma virus (HPV) and other oncogenic viruses such as EBV, CMV, HSV8, Hepatitis B and C Virus.[31-33]

The prevalence of tongue pathologies such as fissured, saburral, atrophic, hairy, and geographic tongue have been reported to be high in liver transplant recipients.[2,5,15]

Collecting data on oral health of patients before and after SOT, including dental, periodontal, and oral hygiene findings, Schmalz *et al*.[7] and Ziebolz *et al*.[34] reported that SOT candidates and recipients showed similar dental findings (DMFT index), but worse in comparison to the general population. Additionally, in both groups most of the patients were reported to have pronounced periodontal treatment need.[7,34] Oral hygiene findings were significantly worse in SOT recipients compared to the patients on the waiting list.[34]

Do the Dental Infections Pose Risk for the Transplant Candidates/Recipients?

Although the combined effects of increased infection susceptibility, untreated dental disease, and poor dental health theoretically imply that dental infections may pose a considerable risk for the physically debilitated transplant candidates and for immunosuppressed transplant recipients, current literature examining the complication of infection in transplant recipients rarely refers to dental infections.[35-39] The absence or scarcity of similar references indicates that dental infections are not observed, or those observed are not reported.[4,40]

In retrospective studies, significantly higher incidence of post-operative complications, such as infection and transplant rejection, were reported in transplant candidates who did not undergo dental examination and treatment in comparison to those who did.[41,42]

Questionnaire survey studies conducted by Guggenheimer *et al*.[4] in the United States (US) organ transplant centres and by Ziebolz *et al*.[34] in the German organ transplant centres provided information on dental screening and dental infections prior to transplantation procedures. The results of these studies showed that most organ transplant centres (80% and 89%, respectively) routinely requested pre-transplant dental evaluation, although the majority indicated this specifically for certain organs, especially the heart and kidney. The emergence of a dental infection that caused postponement or cancellation of the scheduled transplantation procedure was reported in 38%, and post-transplantation sepsis from a possible dental source in 27% of the US questionnaires.

The increasing number of organ transplantations, in combination with the increasing age of transplant recipients and their longer survival, makes it possible for dental disease to become a more common complication source in the transplant population.[4,16] Standardized protocols regarding pre- and post-transplant dental care based on continuous observation and documentation from patient experiences should be established and implemented to minimize this possibility until further prospective and controlled clinical studies are conducted in the closely supervised and monitored transplant population.[1,4]

General Dental Management Before and After Solid Organ Transplantation

The dental practitioner may encounter a SOT candidate for the first time during the pre-transplantation evaluation phase, because protocols of some transplant centres require dental examination and treatment of existing dental disease as a part of the pre-transplant evaluation process.

As the extensive transplantation literature does not document that dental disease or dental infections have a critical impact on neither SOT candidates nor recipients, no data or outcome assessments are available regarding the optimal dental management of these patients.[1,6] However, a number of pragmatic recommendations that agree on several guidelines have been published.[1-3,16,43-45] In this context, we strongly recommend the pre- and post-transplantation dental protocols outlined in Tables 1 and 2.

Table 1. General dental management BEFORE solid organ transplantation

1. Consultation with the patient's physician • Concomitant medical conditions that lead to or develop as a result of organ failure (Diabetes mellitus, cardiovascular disease, metabolic disorders, anaemia, anticoagulant use, coagulation disorders)
2. Patient education and motivation to maintain proper oral hygiene • Education and motivation for proper oral hygiene maintenance • Information about the risks and problems that may be encountered in the oral cavity after transplantation • Starting the use of fluoride compounds and antiseptic mouthwashes
3. Identification of active dental diseases and potential infection foci • Detailed clinical examination of the dentition, periodontium, oral mucosa, lymph nodes and salivary glands • Pulp vitality testing of all teeth • Radiographic examination including a complete series of periapical radiographs (including edentulous areas) or panoramic radiography supplemented with periapical radiographs

4. Elimination of all active dental diseases and removal of all potential acute or chronic infection sources

- Supra/subgingival plaque removal by scaling, root planning, and curettage
- Restoration of carious teeth with favourable prognosis
- Endodontic treatments
- Extraction of teeth having very deep or extensive caries, more than 5-6 mm pocket depths, furcation defects, endoperiodontal lesions, periapical lesions and teeth requiring root-canal treatment which is technically difficult or with uncertain prognosis
- Removal of residual root fragments, and partially impacted teeth
- Necessary adjustments in existing dentures

5. Preparation of a detailed written report that the patient has been treated

Table 2. General dental management AFTER solid organ transplantation

1. Immediate post-transplantation period	**1. Consultation with the patient's physician** Medical conditions resulting from the use of immunosuppressives and corticosteroids after transplantation (metabolic disorders, electrolyte imbalances, diabetes mellitus, hypertension, coagulation disorders, drug interactions) **2. Emergency dental treatment performed in a hospital setting with antibiotic prophylaxis** **3. Palliative and local dental treatment** • Prevention of xerostomia • Education and motivation of the patient to maintain proper oral hygiene • Elimination of the risk factors (smoking, alcohol) • Improvement of the diet • Removal of existing dentures and orthodontic appliances • Dental examination for the risk of malignant lesion development • Prevention from infections
2. Stable post-transplantation period	**1. Consultation with the patient's physician** **2. Palliative and local dental treatment** • Prevention of xerostomia • Education and motivation of the patient to maintain proper oral hygiene • Elimination of the risk factors (smoking, alcohol) • Improvement of the diet • Removal of existing dentures and orthodontic appliances • Control of gingival enlargement • Dental examination for the risk of malignant lesion development • Prevention from infections **3. Elective dental treatment** **4. Invasive dental treatment performed with antibiotic prophylaxis**
3. Post-transplantation rejection period	**1. Consultation with the patient's physician** **2. Emergency dental treatment performed in a hospital setting with antibiotic prophylaxis**

Pre-transplantation dental care

1. Consultation with the patient's physician

There are a number of concomitant medical conditions that lead to or develop as a result of organ failure in the transplant candidate (e.g. poorly controlled diabetes and cardiovascular disease often accompanying end-stage renal disease, multiple metabolic and coagulation disorders resulting from end-stage liver or kidney disease). Before any dental procedure, consultation with the physician to assess the patient's current medical condition, the required laboratory tests, and also the need for introducing antibiotic prophylaxis or prescription of certain drugs (antibiotics or anti-inflammatory analgesics), as well as their doses, is recommended.[1,2,43-46]

2. Patient education and motivation to maintain proper oral hygiene

Education and motivation for proper oral hygiene maintenance and information about the risks and problems that may be encountered in the oral cavity after transplantation should be given to the patient. Instructions for oral hygiene should be

given and the use of fluoride compounds and antiseptic mouthwashes (e.g. chlorhexidine) should be recommended and started.[1,2,44]

3. Identification of active dental diseases and potential infection foci

The exacerbation of an infection prior to the transplantation procedure may result in the postponement or cancellation of the surgery and obtaining another compatible and suitable donor organ may cause extra delays. In addition, if any pre-existing infection is transferred to the immediate after transplantation period, it may have devastating consequences, as a more intense regimen called "induction immunosuppression" is used over a period of several weeks to prevent acute graft rejection. Therefore, dental evaluation prior to transplantation should be focused on the identification and elimination of potential infection sources.[1,4,6,10,42]

For dental treatment planning, a detailed clinical examination of the dentition, periodontium, and oral mucosa along with the head and neck region, including the lymph nodes and salivary glands, is extremely important.[3] Pulp vitality testing of all teeth should be performed. A radiographic examination including a complete series of periapical radiographs or panoramic radiography supplemented with periapical radiographs should be conducted.[2,16,43]

4. Elimination of all active dental diseases and removal of all potential infection sources

All active dental diseases should be treated, all potential acute or chronic infection sources should be eliminated, but elective treatment should be delayed.[2,44]

Adequate periodontal health should be maintained by removal of supra/subgingival plaque by scaling, root planning, and curettage. Carious teeth with favourable prognosis should be restored, and endodontic treatments should be performed. Teeth having very deep or extensive caries, more than 5-6 mm pocket depths, furcation defects, endoperiodontal lesions, periapical lesions and teeth requiring root-canal treatment which is technically difficult or with uncertain prognosis should be extracted. Residual root fragments and partially impacted teeth should be removed. If the laboratory tests are abnormal, the use of anti-fibrinolytic agents, local haemostatic measures, plasma or platelet transfusion, and vitamin K should be considered before any dental surgical procedure.[46,47] In haemodialysis patients, the dental treatments are recommended to be performed one day after dialysis.[44] And finally, necessary adjustments should be performed in the existing dentures.

Implant surgery should be postponed until the stable post-transplantation period. If implant treatment is carried out prior to transplantation, care should be taken to allow sufficient time to assess the biological response and osseointegration.[2]

During dental treatment procedures, the prescription of non-steroidal anti-inflammatory drugs (NSAIDs) should be limited or avoided. Pain management should preferably be performed with adjusted doses of acetaminophen.[44,46] The use of antibiotics such as tetracyclines and cephalosporins should be usually avoided or the time period between the doses should be increased.[14] In studies concerning the use of certain anaesthetics such as Lidocaine/Xylocaine, Bennett *et al*.[48] asserted that their use should be avoided if possible or they should be used only by increasing the interval between doses, while Byrne[49] stated that they may be used both in end-stage renal and hepatic disease without changing the doses.

There is increasing evidence on the antibiotic resistance resulting from unnecessary use of beta-lactams and on the significant correlation between antibiotic use and increased risk of fungal infection.[50] Cocero *et al*.[51] concluded that extractions can be performed without antibiotic prophylaxis in liver transplant candidates, and satisfactory healing can be achieved using atraumatic techniques. Similar results were also reported by Pereira *et al*.[47], Helenius-Hietala *et al*.[52], and Perdigão *et al*.[53] Adherence to the American Heart Association (AHA) regimen is recommended if prophylactic antibiotic use is necessary.

5. Preparation of a detailed written report that the patient has been treated

A detailed written report should be prepared informing the medical team that the patient has been treated and that his/her dental status will not affect the medical treatment.[44]

Post-transplantation dental care

The post-transplantation period is divided into 3 periods as immediate, stable, and rejection period.[1,2]

Transplant patients are treated with immunosuppressive drugs such as CsA, azathioprine, and tacrolimus, often together with glucocorticoids, for the rest of their lives in order to prevent rejection of the transplanted organ. Among the medical problems that the dental practitioner may be co-fronted are metabolic derangements, electrolyte disturbances, diabetes mellitus, hypertension, coagulation disorders, and drug interactions. Clinically, oral manifestations that should be considered may appear in these patients due to immunosuppressive therapy.[1,2] Consultation with the patient's physician is recommended to discuss the overall condition of the patient before any dental procedure in all periods after transplantation.[1,2,44,45]

"Immediate post-transplantation period" (the first three months following surgery), is considered as the maximum life-threatening period. Therefore, only emergency dental treatments should be performed in a hospital setting and with antibiotic prophylaxis after consultation with the patient's physician.[2,44] Essentially, palliative and local dental treatment should be aimed to the prevention of xerostomia (use of mouthwashes containing 0.5% aqueous solution of sodium carboxy cellulose every two hours, synthetic saliva substitutes, sugar-free chewing gum), education and motivation of the patient to maintain proper oral hygiene (use of extra soft toothbrush, fluoride toothpaste, and antiseptic mouthwashes such as chlorhexidine), elimination of the risk factors (smoking and alcohol), improvement of the diet (soft diet, avoiding irritant, cariogenic or excessively hot foods), removal of the dentures and orthodontic appliances, examination for the risk of malignant lesion development, and prevention from infections.[2] Prevention and control of fungal and viral infections is usually carried out by prophylactic administration of nystatin and acyclovir, respectively.[1,54]

In the ***"stable period"*** (more than three months following surgery), elective dental treatment can be performed. However, the optimum time for dental treatment is six months following transplantation.[2] Since the epithelization of the graft anastomosis is not completed and the patient is highly immunosuppressed, antibiotic prophylaxis and laboratory tests are recommended when invasive dental treatment is required.[1,2,4]

Gingival enlargement should be controlled in patients using CsA.[2] Decreasing the dose of CsA or substitution of CsA with alternative new generation immunosuppressive agents such as tacrolimus, sirolimus, rifampicin, or MMF together with meticulous oral hygiene advice are reported to help reduce the frequency and severity of gingival enlargement.[1,23,55,56] However, gingival changes may also occur under tacrolimus or sirolimus therapy and azathioprine and MMF combination therapy may help to reduce the gingival hyperplasia prevalence in transplant patients.[22,23,34,56] In case where the nature of the transplanted organ does not allow for the drug to be replaced or the dose reduced, the treatment should be based on the elimination of the predisposing factors and the maintenance of proper oral hygiene.[2,56] The need for surgical treatment should be carefully evaluated because recurrence was observed in 34% of surgical cases within 18 months.[23]

During the evaluation of a transplant recipient, the dental practitioner should be careful about malignant lesions, including squamous/ basal cell carcinomas[57]. The risk of oral cancer is higher particularly in liver transplant recipients who are more likely to have a history of tobacco and alcohol use.[31,32] Since early diagnosis directly affects the prognosis, strict follow-up is important due to the high risk of malignancy development in this population.[30]

NSAIDs are not recommended to be used in transplant recipients during dental treatment procedures, as they exacerbate gastrointestinal disorders associated with corticosteroid administration, potentiate the nephrotoxic effects of CsA and tacrolimus, and increase bleeding.[1,2,14,44,58] Antibiotics (erythromycin, clarithromycin) and azole antifungals (ketoconazole, fluconazole, and itraconazole) may increase serum CsA levels, resulting in a more severe immunosuppression than desired.[1,2,44,58] Co-trimoxazole, tetracyclines, aminoglycosides, and quinolones are known to have nephrotoxic effects.[1,2] The use of macrolide antibiotics may lead to elevated serum CsA or tacrolimus levels and an increased risk of toxicity and infection.[58] Considering all these facts before prescribing or administering any drug during dental treatment, the dental practitioner should consult with the patient's physician for confirmation of the medical condition and immunosuppression level of the patient in post-transplant periods.

For SOT recipients, post-operative guidelines often recommend antibiotic prophylaxis prior to dental procedures[44], but evidence-based data from controlled clinical trials to support this recommendation or a consensus is not available.[1,4,9] Prophylactic antibiotic use raises concerns about the risk of infection by opportunistic organisms and potential adverse drug interactions.[58] Collaboration with the patient's physician and compliance to the current guidelines of AHA recommending a single-dose regimen minimizes the concerns about adverse drug reactions. Questionnaire surveys of US and German organ transplant centres provided data on antibiotic prophylaxis.[4,34] Most centres (83%) routinely recommended antibiotic prophylaxis before dental treatment for all dental procedures, whether invasive or not, after SOT.

In patients under corticosteroid treatment for a long time, the patient's physician should be consulted to ascertain the need for corticosteroid supplementation before dental treatment to prevent adrenal crisis.[2,44]

"Transplant rejection" may be acute or chronic. In case of rejection, dental treatment should be postponed and only emergency dental treatment should be carried out.[2]

CONCLUSIONS

Creating a healthy oral environment before transplantation is important as well as maintaining it after transplantation, with a special attention to eliminate oral sources of infection. The lack of consensus on the need for dental assessment before transplantation suggests that dental infections are not considered as a major concern by the healthcare specialists dealing with all aspects of organ transplantation. Based on our own experience as dental professionals, we observe that transplant candidates with dental infection foci are usually referred for dental assessment and treatment only a few days before transplantation procedure, and therefore dental treatments have to be performed under time pressure, so the opinion that "*medicine forgets dentistry*" comes true.

CONFLICTS OF INTEREST STATEMENT

The authors declare no conflict of interests.

ÖZ

Son yıllarda organ nakli adaylarında ve organ alıcılarında dental tedavi ihtiyacı, immünsüpresif ilaçlar ve medikal cerrahi teknolojideki gelişmeler sayesinde artan organ nakli sayısı ile orantılı olarak artış göstermiştir. Organ nakli adayları ve organ alıcılarının dental tedavisi genellikle ciddi sistemik hastalıklara sahip hastaların tedavisi için geçerli olan standart prosedürleri içermektedir. Bununla birlikte, bu hasta grupları için uygun dental tedavi konusunda herhangi bir kılavuz, klinik araştırma veya sonuç değerlendirmesi mevcut değildir. Bunun yanı sıra, organ nakli uzmanları arasında dental enfeksiyonların organ nakli adayları ve organ alıcıları için bir risk teşkil edip etmediği konusunda fikir birliği bulunmamakta ve tıp genellikle diş hekimliğini unutmaktadır. Bu makalenin amacı, solid organ nakli adayları ve organ alıcılarında sık görülen ağız belirtilerini kısaca gözden geçirmek ve solid organ nakli öncesi ve sonrasında genel dental tedavi için tıp ve diş hekimlerine rehberlik edecek spesifik bir dental tedavi protokolü önermektir. ***Anahtar kelimeler:*** *Solid organ transplantasyonu, ağız belirtileri, dental tedavi*

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Cumhuriyet Dental Journal: 2021; 24(3)
e-ISSN 2146-2852
Doi:10.7126/cumudj.948193

Reviews

CONTEMPORARY ENDODONTIC OBTURATION TECHNIQUES: A COMPREHENSIVE LITERATURE REVIEW

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ABSTRACT

The success of an endodontic treatment is excessively depends on obturation as well as chemomechanical shaping. Hence, achiving a homogenious, impermeable and gap-free obturation plays a crucial role in endodontic treatment success. Today, many materials and techniques have been developed for endodontic purpose such as those which applied with heat or vibration, those softened with non-toxic chemicals and those uses ultrasound energy. Some of these techniques are still preferred by proffessionals when some are abandoned. However, gutta-percha is the most used material either with or without sealer. Gutta-percha can be applied with cold or warm techniques. It's known that warm gutta-percha technique leads a more homogenious obturation than cold techniques. Nevertheless, different obturation methods are convenient for different anatomic variations of root canal system. This review article summerize the contemporary and conventional obturation techniques with evaluation of their current status in endodontic implementation.

Keywords: Endodontic Obturation Techniques, Contemporary Obturation Techniques, Cold Gutta-Percha Techniques, Softened Gutta-Percha Techniques, Endodontic Treatment.

Received : 04.06.2021
Accepted : 07.09.2021

How to Cite: Zan R, Demir AŞ. Contemporary Endodontic Obturation Techniques: A Comprehensive Literature Review. Cumhuriyet Dent J 2021;24:3:310-317.
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INTRODUCTION

A total root canal obturation is a major objective in endodontic treatment procedures.[1–3] The primary purpose of root canal obturations is preventing the microleakage and bacterial invasion between root canals and periradiculer tissues. Composing a both apically and coronally, three dimentional sealing is crucial in order to ensure maximum preservation of root canals from microorganisms and inhibit their penetration to periradicular tissues.[4,5] Unsuccessful obturations that caused by necrotic debris, bacteria, bacterial toxins and pulp residues left in root canals can cause periradicular diseases and bacterial growth.[2,6] It has been reported that insufficent endodontic obturations can cause unexpected postoperative complications in endodontic approaches with 65% rate.[2] There have been advancements in endodontic materials, equipments and techniques over the past two decades as we increased our knowledge about the nature of endodontics, endodontic diseases and the definition of endodontic success and failure.[2,4,5,7] The standard obturation materials consist sealers and a solid core material placed in the center of the root canal space.[6] Necessities of an obturation material have been explained in previous studies.[3,6] The ideal obturation material should be biocompatible, anatomically compatible, well adapted to the walls in three dimensions, cover the root canal spaces well apically and coronally, easily removed when needed and definable in radiographs. It also should have dimentional stability to prevent irregularities.[3,6] Thus, gutta-percha has been the most preferred obturation material due to its useful properties but most of the cases, the root canal spaces do not be completely filled with the obturation materials and that causes problems.[3,6,8] For many years, gutta percha was the priory option for a gap free and homogeneous fillings both in the apical, middle and coronal third of root canals.[8] However, different obturation materials and systems have been developed and used over the years for a better endodontic treatment.[3] The aim of this review is investigating the contemporary root canal obturation techniques in endodontics.

1) Cold Gutta-Percha Techniques

A. Single Gutta Percha Technique:

In this technique, the obturation made by single cone which is master cone and do not necessity of the use of accessory cones. Single gutta percha technique shows exceptional adaptation to the root canals especially when the root canals expanded with rotary nickel-titanium systems. Because master cone can match the geometry of last rotary instruments used and provide a void free, nonpermeable filling as it prevents the leakage between apical of roots and the periradicular tissues.[5] Using of single gutta percha technique has increased recently and the number one reason to prefer this method is the time advantage and simplicity of the technique.[9,10] Chybowski *et al.* reported the clinical success of single cone technique used with bioseramic sealer. The useful qualties of bioseramic sealers and its compatibility with single cone technique have influenced the success by causing an advanced sealing in the root canals of the teeth. This technique also provided a more conservative preperation rather than traditional warm lateral and vertical compaction techniques.[11] It's been also proven in a study, warm lateral condansation and vertical compaction techniques are disadvantageous due to their nonhomogenity and poor adaptation characteristics.[12] In a study that made with two groups of maxillary and mandibulary canines with single straight canals, using single cone technique with RoekoRSA sealing in one year follow-up study on leakage that demonstrated by Wu *et al.* as there was no leakage after 1 year of the treatment. Because of the RSA sealer was insoluable, it showed a promising result when used with single cone technique.[13]

Altough single cone with a sealer technique has an important advantages as it fits precisely and composes a gap free filling in root canals that has shaped with a rotary instruments, there's disadvantages of this method such as the single cone is not compatible with the irregularities in the middle and coronal 1/3 of the canal. Thus, if these areas filled only with sealer; occured gaps can damage the mass integrity in terms of the long term success of the endodontic treatment. Another fact about the single cone technique is the cytotoxicity

of solvents. Morover, gutta percha shows dimensional changes when the solvent dries. Besides, single cone technique has the risk of flooding and disjunction because of the chemical features of gutta percha.[9]

B. Lateral Compaction Technique:

Lateral condensation technique is the most frequently used technique in endodontics.[8,14,15] This technique based on a master apical cone sent to the full working length and accessory cones placed around the master gutta percha cone with sealer. Spreaders used for lateral compaction, sent to the 1-2 mm shorter than the working length until the spreader can not move more than 1-2 mm in root canal opening.[9,16] Lateral compaction technique offers a more controlled obturation but disadvantageous for treatment time. In this method, gutta perchas poorly adopts the root canal walls and comprises inhomogenity.[17] It's been reported in a study that compares sealing efficacy of 2 warm vertical compaction technique with single cone and lateral compaction technique, single cone and lateral compaction technique showed the lowest fluid permeability with a similar result.[18] Besides, this technique offers a greater dimensional stability.[1] Also, lateral compaction technique has been accepted and applied succesfully by dentists for years due to its easiness of controlling and favorable characteristic of providing patients comfort.[1] However, it has been reported that the new core carrier systems are more promising than traditional lateral condensation and single cone techniques in the manner of void-free filling in root canal systems.[19]

I. Softened Gutta-Percha Techniques

A. Heat-Softened Gutta-Percha Techniques

B. Warm Lateral Compaction Technique:

Cold lateral condansation technique is the most widely used technique in endodontics, besides the favorouble features of this technique. Its important disadvantages are homogenity of the mass of gutta percha and poor adaptation to root canal walls and irregularities of root canal system.[20,21] Thus, a better and advanced technique started to be applied.[20] Warm lateral compaction technique requires a peizoelectric ultrasonic unit, a file adaptor and a K type of file.[21] With warm lateral compaction technique, the disadvantages of cold lateral compaction technique were eliminated as well as it provides a more homogen filling with less voids in the root canal system.[20] This technique also favorable in the manner of filling accessory and lateral canals also considered as a cost effective.[21] Clinton and Himmel compared the adaptation of two different obturation techniques as lateral condansation and warm gutta percha obturation techniques on root canal walls. They conducted that warm gutta percha technique showed a better adaptation in root canal system and left less voids than the lateral condansation technique because of the flowing features of warm methods.[8] A similar another study conducted 60 extracted teeth with lateral canals, divided 6 groups and used different obturation techniques that divided into 2 subgroups for the rank of filled lateral canals, Goldberg reported that the difference between 2 subgroups was significant and the teeth obturated with Ultrafil succesfully filled the lateral canals.[22] Morover, Kersten compared several obturation techniques such as cold lateral compaction, thermoplasticized gutta percha and thermomecanical compaction. He reported that warm lateral compaction technique caused minimum leakage in the apical of the root canals.[23] Nelson *et al.*[24] compared cold and warm lateral compaction techniques and reported that warm lateral compaction method showed a significantly higher density because of the application of the heat. Furthermore, System B instrumentation were used in low heat lateral condensation technique.[24] Nevertheless, warm lateral compaction technique is more advantageous than standard lateral compaction technique in many aspects.[24]

i) Warm Vertical Compaction Technique:

This technique also know as Schilder technique as it introduced by Schilder for the first time in 1967.[21] It's also knows as wave compaction technique.[24] The purpose of this technique is to provide an enhanced plugging especially in complicated root canal systems by filling accessory and lateral canals and internal resorption defects.[9,21] Heat systems and pluggers required for this technique.[21] The technique starts with sealing the root canals with a sealer and placing the master gutta-percha cone. The heated

gutta-percha cones should be added to canals by condencing vertically with spreader until ensuring a hermetic filling.[9] According to Kececi *et al.*, contunious wave obturation technique was more favorable than lateral condansation technique in terms of treatment time. Wave obturation technique was choosen in that study because of its advantageous features such as minimal apical leakage and its duration.[16] Also, vertical compaction with warm gutta-percha presents the most frequent filling in lateral canals and foramina among other techniques.[1] Interestingly, in a study demonstrated by Aminsobhani et. al., 3 obturation techniques that are lateral compaction, warm vertical compaction and Guttaflow System were compared by their permeability to saliva and their coronal microbial leakage and resultly no significant difference was found in 3 techniques.[25] However, warm vertical compaction technique has a few disadvantages such as the high cost of the equipment and the difficulties of length control.[21] On the other hand, this technique is preferable because it decreases apical leakage and provides a dense filling in root canal systems.[9,21] Venturi conducted that, vertical compaction technique was more successfull in creating a three dimentional homogenious filling when it's applied with apical backfilling method.[26]

C. Thermomechanical Compaction Technique:

Thermomechanical compaction technique was founded by Mc Spadden and developed by Tagger *et al.* This technique is based on heating the gutta-perchas and thermoplastised them by thermomechanical compactors.[27] The heat occurs when an inverted Hedstrom file driven into the gutta-percha with slow handpieces.[21] The application of this technique should be as pressuring apically, lateraly and coronally in the root canals.[21,27] Plasticized gutta-perchas performs an enhanced homogenity and adaptation in root canals. Thus, this technique is advantageous and preferred by dentists especially difficult root canal shapes.[28] Also it's preferable because of the easiness to apply and good filling quality in canal irregularities.[29] However, thermomechanical compaction technique allows more leakage than HeroFill Soft-Core technique but less than lateral compaction method according to Boussetta *et al.*[30] It also has some disadvantages such as contraindication of narrow root canals because of the risk of the breakage of compactor and vertical root fractures.[9] According to a study, if different gutta-percha materials compared in the canals that obturated with thermomechanical obturation technique, Resilon gives better results in the percentance of lateral canal filling. Also, the rates were higher especially in the middle and apical areas.[27] It's notable that hybrid thermomechanical compaction technique causes less bond strength than lateral compaction technique.[31] Also, the higher bond strength to dentine in the root was the combination of AH Plus/GP in the canals obturated with lateral compaction technique.[31] There are many modifications of thermomechanical compaction technique such as Tagger technique. In Tagger technique, cold lateral condensation applied before inverting reverse Hedstrom file differently than the original technique. Another modification is Microseal that the flowable gutta-percha covered condensers used.[21]

D. Thermoplasticized Gutta-Percha Enjection:

In this technique, gutta-percha materials plasticized with heat and applied in the mechanically prepared root canals with pressure.[9] De-Deus *et al.*[32] reported that, thermoplastized obturation techniques caused higher results than lateral compaction technique in the percentance of gutta-percha filled area in oval shaped canals. Gulabivala *et al.*[33] reported that thermoplasticized gutta-percha techniques may be disadvantageous due to unpredictibility obturating length and they offered to fill the apical area of root with a controlled technique such as lateral condansation technique.

a. Obtura 3 Max

Obtura 3 Max system is the most recent example in thermoplasticized gutta-percha technique.[9] This sytem comprise a needle with a slicone stop and a gun that can be controllable.[9,21] It's a high working temperature device and it can heat the gutta-percha cones up to 160 degree.[9] Obtura 3 Max system is favorable due its well adaptation to root canal walls.[34]

b. Ultrafil 3D

This system consists of a low working temperature (70 ˚C) device and 3 different disposable needles for different canal shapes.[29] The device is portable and nonreactive to moist or heat. It's notable that the low expanding percentance results a better sealing in the root canal system.[35] This injection should be made slowly and under control. It's also important to not pressure to needle to prevent possible gaps in the root canals.[9]

E. Trifecta:

This technique considered as a safer technique with a gutta-percha barrier in the apical 2 mm area and filling the middle and apical areas with thermoplasticized injection. The gutta-percha barrier prevents the flowing of gutta-percha from apical of the root canals.[9] Al-Dewani *et al.* compared the seability and radiographic quality of two different obturation techniques that are lateral condansation and Trifecta system. The study was made on one hundred human teeth with single root. They reported that, Trifecta showed an enhanced seability and less apical dye penetration than lateral condensation technique but it demonstrated poorer radiographic quality. They also concluded that Trifecta is a good alternative to lateral condansation technique.[36] It's worth to say that as thermoplasticized obturation techniques gives a better adaptation on root canal walls, it's necessery to use sealers for an enhanced seal.[36]

F. Thermafill:

Thermafill is a carrier based technique includes a gutta-percha coated with carrier. A gutta-percha coated core material used as a carrier and applied in the root canals after heated in special ovens. The use of sealer are necessery in Thermafill technique. Grossman formulated AH26 and AH plus sealers are suggested for this technique but TubliSeal and Watch's sealers are not recommended.[14] The heated carrier should be sent to the sealed and biomechanically prepared root canals. It's important to remove smear layer with the preparation and before filling the root canal system.[14] This technique is favorable because of time efficiency, its easily removable and impermeable nature.[9,14,21] Gutta-percha succesfully fill the lateral and accessory canals with this technique.[14] Hovewer, the risks of this technique are the possibility of post operative pain and diffuculties of length control.[21] Also, difficult retreatment prosedure and the risk of core material to get out of gutta-percha harden the endodontic treatment process.[14] There are several carriers as Protaper, Recripoc, Waveone, GuttaCore etc.[9,21]

G. Soft-Core:

This technique is similar to Thermafill. The heated carriers sent to the working lenght in the root canals. Differently than Thermafill, Soft Core consists of 2 sections which are metal prob and plastic gutta-percha cone. The metal pin does not hold the plastic core completely, and after the soft core is placed in the groove, the plastic handle is bent and removed together with the metal pin.[6]

H. Alpha-Seal:

Although the system is basically similar to the Thermafill, the different carrier part of the system which is the alpha seal gutta percha is adapted to the K type file by the dentist. In Alpha-Seal technique a sterile file that is the same size as the master apical file is used with a warmed alpha seal syringe inserted into it. After that, when the syringe removed, the gutta percha is covered around the file and can be placed in the root canals. Also, gutta-perchas heated for decreasing the moleculer weight in this technique. Alpha-Seal technique advantageous because its avaible for trying in root canals and making the corrections during treatment.[6] In a study made by Gulabivala *et al.*, thermoplasticized gutta-percha obturation techniques was compared with cold lateral condansation technique. Alpha-Seal had more overfills than Quick Fill and more extrusion of gutta-percha than lateral condensation and Thermafill. They also concluded that the thermoplasticized gutta-percha techniques were more time-efficent than lateral condensation technique.[33]

İ. SuccessFil:

SuccessFil is also a carrier based obturation technique. Carriers are placed in syringe that measure the root canal lenght. After applying sealer to the root canals, gutta percha carrier send to the canal lenght. Morover, pluggers that chosen

according to canal morphology can be used in this technique.[14] Goldberg *et al.* compared three different obturation techniques which are Trifecte, lateral condansation and combination of lateral condansation and SuccessFil. They demonstrated that if lateral condansation technique applied with successfil showed better results as enhanced adaptation to root canal walls and condensed filling.[37]

J. System B

System B is a heating system that uses with two special technics called down pack and backfilling technique.[21] In down pack technique, a thin layer of sealer should be applied in the walls of the canals, after applying sealer, master cone should be placed and heated with a System B heat source and bind with a System B plugger gently then switch to Machtou plugger until create a hermetic, homogen filling.[9,21] In backfilling technique a selected needle used to plasticized gutta-percha and fill the canals. Before canal opening, draw back the needle and use pluggers for pressure.[21] Lea *et al.* evaluated the obturation density in two control groups, comparing cold lateral and warm vertical condansation techniques with System B, conducted that warm vertical compaction technique showed significantly higher density.[17] Inan et. al. conducted that System B caused less leakage than Thermafill technique.[38] In another study the sealing ability of two different obturation methods were compared, cold lateral compaction and system B, it has been concluded that System B showed significantly more sealing than cold lateral technique and cold lateral technique showed significantly more apical leakage.[39] Calamus and Elements are other systems similar with System B. Both techniques have a gun system.[9] In these systems, gutta-perchas are heated before sending to canals up to 200.[40]

II. Other Techniques

A. Ultrasound Technique

Ultrasound is an energy of sound with over 20 kHz frequence which is higher than sensible frequency for human. It was used for cavity preparation in dentistry under minimal invasive techniques.[41] This technique was first introduced in endodontics by Richman and developed by Howard Martin.[42] Ultrasound technique might be very benefical in terms of cleaning the debriment in the root canal system. Also it has following features such as easiness to access of root canal orifice, better preparation and enhanced extirpation of root canals. Morover, ultrasound irrigation was founded more effective than passive irrigation.[41] It was reported by Townsend that ultrasonic irrigation was more effective in removing microorganisms from root canal system in an in vitro study.[43] It's also known that ultrasonic irrigation technique is more adventageous specially in curved and wide canals. It's been concluded that, ultrasonic irrigation in a combination with conventional irrigation can improve the success of endodontic treatment.[41] Nevertheless, ultrasound technique provides a preferable irrigation, cavity cleaning, removal of posts and broken instruments in the root canals.[44]

CONCLUSIONS

In endodontic evoluation, many obturation techniques have been applied and devoloped to create a homogeneous, void-free and impermeable filling. Nevertheless, gutta-percha cones are the most used material in endodontic treatment. Therefore, the root canal obturation techniques divided mainly two categories as cold and warm gutta-percha techniques. These techniques were developed due to spesific need in the treatment depending the various anatomic variations of the root canal systems. In this context, choosing the obturation technique that is suitable for the anatomic variations of the tooth in case is essential for determining the long term endodontic success. Also, using quality sealing material also plays a crucial role in the endodontic treatment. However, lateral condansation technique is the most preferred obturation technique that is widely accepted and suggested by clinicians in endodontics. In conclusion, it's known that modified contemporary obturation techniques are more efficient than the traditional techniques because of the following reasons; enhanced filling quality, minimum leakage percentances, more homogenious fillings and better obturation results in long term. Nevertheless, more compherensive

studies and developments on current obturation techniques are needed to adjust the best quality of endodontic obturation and to obtain maximum performance with minimum disadvantages.

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