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1. Burrow MF, Tagami J, Negishi T. Early tensile bone strengths of several enamel and dentin bonding systems. J Dent Res 1994; 74: 522-8.

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2. McKeage K. Tobramycin inhalation powder: a review of its use in the treatment of chronic pseudomonas aeruginosa infection in patients with cystic fibrosis. Drugs 2013; [Epub ahead of print] Available from: http://link.springer.com/article/10.1007%2 Fs40265-013-0141-0

Book

3. Mueller HJ, Freeman D. FT-IR spectrometry in materiolography. 2nd Ed., Ohio: American Society for Metal 1994, p.51-56.

Chapter in a book

4. Alexander RG. Considerations in creating a beautiful smile. In: Romano R, editor. The art of the smile. London: Quintessence Publishing, 2005, p.187-210.
5. Hudson FB, Hawcroft J. Duration of treatment in phenylketonuria. In: Seakins J, Saunders R, editors. Treatment of inborn errors of metabolism. London: Churchill Livingstone, 1973, p.51-56.

Thesis

6. Maden I. Effect Of Nd:YAG Laser Treatment In Addition To Scaling And Root Planning. Doctoral Dissertation, Istanbul University Institute of Health Sciences Periodontology Department, 2009.

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Figure 1. Panoramic radiograph of the patient taken 6 months after surgery, note irregular borders of the lesion.

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Table 1. *Concise explanation of the table contents (SD: standard deviation, CTA: cartilage tissue area, NBA: new bone area).*

	Control group (Mean % ± SD %)	First group (Mean % ± SD %)	Second group (Mean % ± SD %)
CTA	21.41 ± 4.2	2.5 ± 2.4	11.42 ± 4.2
NBA	11.48 ± 0.2	21.41 ± 14.22	11.41 ± 4.2

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Original research

Bolton's analysis using a photogrammetric method on occlusal photographs

Ashwin Prakash[1], Prasad Chitra[2]

Purpose
The aim of the study is to present a photogrammetric technique using standardized occlusal photographs to perform Bolton's analysis and assess reliability of this new method with plaster study casts.

Materials and Methods
The study was conducted on 16 subjects (8 males, 8 females), aged 18-25 years. Standardized occlusal photographs and plaster study casts were obtained. The occlusal photographs were calibrated in Nemoceph® software. Mesio-distal dimensions of all teeth up to first molars were calculated and Bolton's analysis was performed. Similarly, a digital calliper with 0.1 mm sensitivity was used to measure mesio-distal dimensions of all teeth on plaster study casts to perform Bolton's analysis. 28 parameters were measured on study models and corresponding occlusal photographs. Paired t test and intraclass correlation tests were carried out to test validity and reliability of the photogrammetric method. An intraclass correlation test was calculated for 4 derived parameters to test reliability of Bolton's analysis measurements obtained from occlusal photographs as compared to study models.

Results
All 28 parameters showed a statistically significant and excellent correlation (r>.80) in the Intra Class Correlation test. 4 variables used to calculate Bolton's analysis showed statistically significant correlation (r>.96) in the intraclass correlation test.

Conclusion
Photogrammetry is a reliable tool to measure mesio-distal tooth size. Bolton's analysis from standardized occlusal photographs using the described photogrammetric technique can be used as an effective clinical tool.

Keywords: *Photogrammetry, Bolton's analysis, Ophotograph, Nemoceph, Tooth dimensions*

Introduction

Effective and practical diagnostic aids that help in seamless and easy acquisition of data are useful in orthodontics. Digitization has been making an impact in the way we practise dentistry and holds a lot of promise in the future. However, when it comes to 3 - dimensional information, particularly in the pre treatment stage, plaster study models remain the most commonly used diagnostic aid.

Digital scanning technologies have been available from the mid 1990's (1) and digital study models were introduced in 1999 by Orthocad™ (2). Digital study models hold a lot of advantages over plaster study models, obviating the need for physical storage (3), allowing instant accessibility to information, quick referral and virtual treatment planning. Moreover Cone Beam Computed Tomography (CBCT) technology also allow the creation of virtual study models which give 3D visualization of dental crown and root morphology.

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Several studies have been conducted comparing plaster study models with digital study models (4,5). A systematic review concluded that digital models offered a higher degree of validity (2). In spite of evidence showing the diagnostic advantages of digital study models, their widespread clinical use has not permeated in developing countries. This could be attributed to the high cost of scanning technology and dependency on software involved in acquiring digital data. Moreover, both plaster study models and digital study models do not serve as a cost effective or time saving method for acquiring data on tooth dimensions in epidemiological studies.

The technological improvements in digital cameras over the recent years combined with their reduced costs makes digital photography a viable alternative. However, there have been very few studies comparing measurements obtained from occlusal photographs with plaster study casts. The present study describes a photogrammetric method to perform Bolton's analysis on occlusal photographs and assess the reliability of this new method with plaster study casts.

Materials and Methods

The study was approved by Ethics Committee of Army College of Dental Sciences, Secunderabad, India (ACDS/IEC/21/Jan 2018). The sample size estimation was carried out using GPower software version 3.1.9.2. Considering the effect size to be measured at 55%, power of the study at 80% and error margin at 5%, the total sample size required was 16.

The study was conducted on 16 subjects (8 males, 8 females), aged 18-25 years, with a mean age of 21 years and 5 months with a SD of 1.4.

Inclusion criteria

- All permanent teeth till first molars should be present.
- No restorations or crowns on any teeth.

Exclusion criteria

- Previous history of orthodontic or orthognathic treatment.
- Craniofacial trauma.
- Congenital anomalies.
- Neurologic disturbances

Bolton's analysis on study models

At the outset, upper and lower alginate impressions of the study sample (n=16) were taken and plaster study casts were prepared (Fig 1A). A digital calliper with .01 mm sensitivity was used to measure mesio-distal dimensions of all teeth up to the 1st molars in both arches (Fig 1B). The total arch length (mesio-distal dimensions of all teeth from 1st molar to the contra lateral molar in the same arch) and total anterior arch length (mesio-distal dimensions of all teeth from canine to the contra lateral canine in the same arch) were calculated for both arches. Subsequently, Bolton's analysis was performed using formulae as shown in Fig 2.

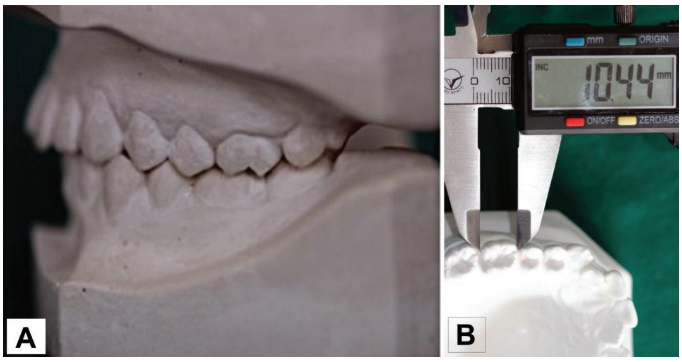


Figure 1. A) *Plaster study casts were prepared for each subject.* ***B)*** *A digital vernier calliper with sensitivity of .01mm was used to measure the mesio-distal tooth measurement of each tooth up to first molars.*

$$overall\ ratio = \frac{sum\ of\ mandibular\ 12}{sum\ of\ maxillary\ 12}\ x\ 100 \qquad anterior\ ratio = \frac{sum\ of\ mandibular\ 6}{sum\ of\ maxillary\ 6}\ x\ 100$$

If ratio < 91.3%: maxillary TM excess. If ratio > 91.3%: overall mandibular TM excess

$$Overall\ maxillary\ TM\ excess\ = sum\ of\ maxillary\ 12 - \frac{sum\ of\ mandibular\ 12}{91.3}\ x\ 100$$

$$Overall\ mandibular\ TM\ excess\ = sum\ of\ mandibular\ 12 - \frac{sum\ of\ maxillary\ 12}{100}\ x\ 91.3$$

If ratio < 77.2%: maxillary anterior TM excess. If ratio > 77.2%: mandibular anterior TM excess

$$Anterior\ maxillary\ TM\ excess\ = sum\ of\ maxillary\ 6 - \frac{sum\ of\ mandibular\ 6}{77.2}\ x\ 100$$

$$Anterior\ mandibular\ TM\ excess\ = sum\ of\ mandibular\ 6 - \frac{sum\ of\ maxillary\ 6}{100}\ x\ 77.2$$

Figure 2. *Formulas required for calculating Bolton's analysis. TM = Tooth material.*

Bolton's analysis on standardized occlusal photographs

A digital camera (Canon EOS 600D) mounted with a macro portrait lens (EF 105 mm f/2.8, 1:1 OS, Sigma) was used for obtaining photographic records. A single combination intraoral mirror designed by Ashwin, Pulgaonkar, Chitra(6) and a lip retractor with a 35 mm metal ruler bonded onto its front surface were used to obtain standardized upper and lower occlusal photographs (Figs 3A,3B) of the sample (n-16).

Method of photography: The photographs were taken in a standardized manner to achieve consistent and reproducible images. For maxillary and mandibular photographs, the subjects were seated in a dental chair in a slightly reclined position with the height of the dental chair in its lowest position. All photographs were taken by the same examiner who held the camera in his hand in a stable position and an assistant who retracted the lips and placed the intra oral mirror. For maxillary occlusal photographs, the mirror was kept parallel to the occlusal plane and placed as low as possible until it touched the lower incisors. The entire maxillary arch was reflected in the mirror. For mandibular photographs, the subjects were asked to raise their tongue to the palate and breathe through their nose. The mirror rested on the gingiva distal to the last molars, so as to include all teeth. The mirror was turned upwards with the mouth wide open until it touched the incisal edges of the upper incisors. For both occlusal photographs, the mirror and camera were positioned such that the optical axis was exactly vertical to the mirror image of the occlusal plane of the maxillary or mandibular arch. The distance from mirror to camera is kept constant by pre selecting the magnification ratio to 1:2. All photographs were taken with the camera settings in manual mode. The shutter speed was set at 1/250th of a second to ensure calibration with the ring flash, aperture at f25 and ISO at 100.

The photographs obtained were uploaded into Nemoceph 10.4.2 (Nemotec Dental Systems, Madrid, Spain) software program for Windows in which the mesio-distal tooth widths of all teeth up to the first molars were calculated. The 35 mm scale in the image was used for the purpose of calibration (Fig 3C). The total arch length and anterior arch length were calculated from the photogrammetric measurements obtained and Bolton's analysis was performed.

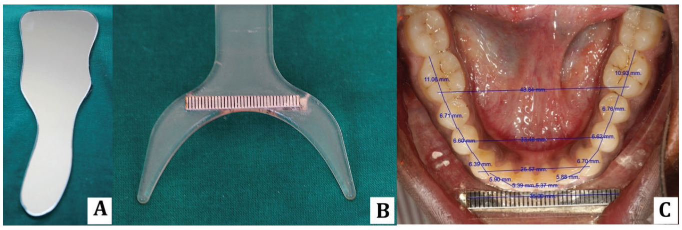


Figure 3. A) *Modified single combination intraoral mirror which is used to take the occlusal maxillary and mandibular photographs.* ***B)*** *35 mm trimmed metal scale bonded on both the surfaces of cheek retractor.* ***C)*** *The scale is used for the purpose of calibrating the images in Nemotec® software. Individual mesio-distal dimensions of all teeth up to first molars are measured in the software.*

Statistical analysis

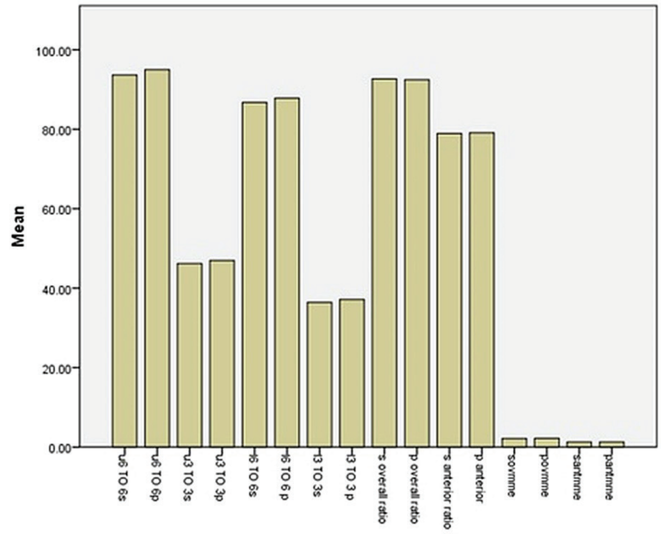
28 parameters were measured on study models and corresponding occlusal photographs of the same subjects. The parameters measured included the mesio-distal dimensions of each upper and lower tooth up to the first molars and the upper and lower total arch length and anterior arch length. The variables were paired and subsequently, paired t test and intraclass correlation coefficient (ICC) tests were performed (n=16) (Table1).

Overall ratio and anterior ratio for the study models and occlusal photographs were calculated for each subject using the measurements from Table 1. The overall and anterior maxillary/mandibular tooth material excess was calculated for study models and photographs of each subject (Table 2).

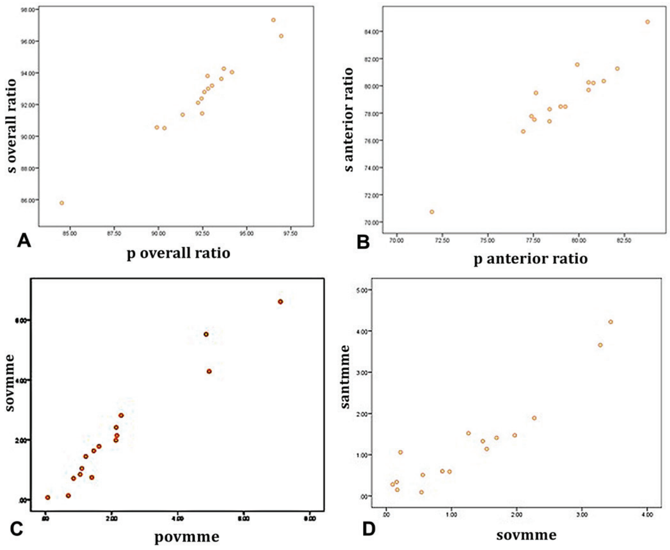
An intraclass correlation test was calculated for the 4 derived parameters to test reliability of Bolton's analysis obtained from occlusal photographs as compared to study models (n=16) (Table 3).

Results

The mean difference calculated between the 28 variables show that, in general, photographic measurement values are greater than the corresponding study model measurements, except for UR5, LL6 and LR6 (Graph 1). Moreover, all the individual mesio-distal tooth measurements show a difference less than 0.20 mm, except UR4 (-0.22 mm). All the 28 variables showed a statistically significant and excellent correlation in the intraclass correlation coefficient test (r >.75) (Graph 2). The highest correlation was obtained for LL2, LR2 and the lower anterior arch length (r = .97) and the lowest correlation was obtained for LL6 (r = 0.8) (Table 1).



Graph 1. *Bar diagram showing mean values of parameters from study model (s) with corresponding parameters from photographs (p) required to calculate Bolton's analysis. Parameters shown are: U6 to 6 = upper total arch length, U3 to 3 = upper anterior arch length, L6 to 6 = lower total arch length, L3 to 3 = lower anterior arch length, ovmme = overall maxillary tooth material excess/mandibular tooth material excess, antmme = anterior maxillary tooth material excess/mandibular tooth material excess.*



Graph 2. *Scatterplots of 4 parameters measured show excellent correlation.* ***A)*** *correlation between overall ratio in study models (s overall ratio) and photograph (p overall ratio);* ***B)*** *correlation between anterior ratio in study models (s anterior ratio) and photograph (p anterior ratio);* ***C)*** *correlation between overall maxillary/mandibular tooth material excess in study model (sovmme) and photograph (povmme);* ***D)*** *correlation between anterior maxillary/mandibular tooth material excess in study model (santmme) and photograph (pantmme).*

13 subjects showed an overall ratio greater than 91.3% in study model and photographic Bolton's analysis, indicating an overall mandibular tooth material excess. 14 subjects showed an anterior ratio greater than 77.2%, in study model and photographic Bolton's analysis, thus indicating an overall anterior mandibular tooth material excess (Table 2).

Table 1. *Mean, SD* (Standard deviation) and SE** (standard error) of the 28 parameters (n=16)*

Sample No	Variable	Study model			Photograph			SM-P (diff)	t value	Sig (2 tailed)	ICC	
		Mean	SD	SE	Mean	SD	SE				R	Sig†
1.	UR1	08.78	0.43	.10	08.90	0.42	.10	- .12	- 2.65	.018	.95	†††
2.	UR2	06.70	0.44	.11	06.89	0.39	.09	- .18	- 3.70	.002	.93	†††
3.	UR3	07.75	0.49	.12	07.85	0.47	.11	- .10	- 1.84	.085*	.94	†††
4.	UR4	07.16	0.50	.12	07.38	0.41	.10	- .22	- 3.20	.006	.90	†††
5.	UR5	06.55	0.31	.07	06.51	0.40	.10	.04	.99	.334*	.94	†††
6.	UR6	10.05	0.70	.17	10.15	0.53	.13	- .09	- .86	.401*	.87	†††
7.	UL1	08.60	0.51	.12	08.73	0.39	.09	- .12	- 2.49	.025	.94	†††
8.	UL2	06.65	0.44	.11	06.79	0.41	.10	- .13	- 2.28	.038	.91	†††
9.	UL3	07.67	0.40	.10	07.76	0.35	.08	- .09	- 1.99	.065*	.93	†††
10.	UL4	07.15	0.36	.09	07.32	0.37	.09	- .17	- 4.73	.000	.96	†††
11.	UL5	06.50	0.36	.09	06.54	0.39	.09	- .04	- .70	.493*	.89	†††
12.	UL6	10.01	0.46	.11	10.06	0.42	.10	- .05	- .52	.609*	.76	††
13.	LL1	05.29	0.28	.07	05.44	0.31	.07	- .15	- 3.00	.009	.86	†††
14.	LL2	06.05	0.41	.10	06.21	0.36	.09	- .16	- 4.89	.000	.97	†††
15.	LL3	06.92	0.40	.10	06.97	0.40	.10	- .04	- 1.07	.302*	.94	†††
16.	LL4	07.11	0.41	.10	07.25	0.42	.10	- .14	- 2.35	.033	.91	†††
17.	LL5	06.87	0.40	.10	07.03	0.36	.09	- .16	- 4.11	.001	.95	†††
18.	LL6	11.18	0.69	.17	11.13	0.68	.17	.05	.35	.726*	.80	††
19.	LR1	05.51	0.27	.06	05.59	0.26	.06	- .07	- 1.38	.188*	.81	†††
20.	LR2	05.99	0.47	.11	06.13	0.42	.10	- .13	- 3.59	.003	.97	†††
21.	LR3	06.66	0.44	.11	06.76	0.42	.10	- .10	- 2.68	.017	.96	†††
22.	LR4	07.11	0.37	.09	07.26	0.30	.07	- .15	- 3.13	.007	.91	†††
23.	LR5	06.93	0.41	.10	07.07	0.40	.10	- .14	- 2.80	.013	.93	†††
24.	LR6	11.07	0.68	.17	10.91	0.77	.19	.16	1.35	.194*	.87	†††
25.	UTAL	93.63	3.53	.88	95.00	3.04	.76	- 1.36	- 4.02	.001	.95	†††
26.	UAAL	46.18	1.77	.44	46.95	1.55	.38	- .76	- 5.00	.000	.96	†††
27.	LTAL	86.75	3.69	.92	87.81	3.18	.79	- 1.06	- 2.69	.017	.94	†††
28.	LAAL	36.44	1.69	.42	37.13	1.48	.37	- .68	- 5.83	.000	.97	†††

UR= Upper right, UL= Upper left, LL= Lower left, LR= Lower right, UTAL= Upper total arch length (sum of UR6 to LL6), UAAL= Upper Anterior arch length (sum of UR3 to UL3), LTAL= Lower total arch length (sum of LL6 to LR6), LAAL= Lower Anterior arch length (sum of LL3 to LR3) SM-P (difference in means between Study model (SM)and photograph(P), Sig= Significance, ICC= Intraclass Correlation coefficient, r= r value, Sig= Singificance † p<.05, †† p<.01, ††† p<.001

The mean difference between the 4 variables derived from Bolton analysis on study models and photographs showed that in general, photographic measurements are greater than corresponding study model measurements. The only exception to this was the mean value of overall ratio. However, all variables showed a mean difference less than 0.20 mm and a statistically significant and excellent correlation in the intraclass correlation (ICC) coefficient test (r >.75) (Table 3).

Discussion

There have been numerous studies comparing Bolton tooth size analysis between digital models and plaster study models, (7,8,9,10) all of which have shown acceptable agreement between the two methods. However, to our knowledge, no study has been conducted comparing Bolton ratio obtained from occlusal photographs to plaster study models. Moreover, only two previous studies have compared measurements obtained from occlusal photographs to plaster study models.

In 1984, Gholston (11) concluded in his study that measurements obtained from intra oral photographs were reliable. However, the Orthoscan camera the author used is no longer in production. In 2011, Normando et al. (12) presented a photogrammetric method where dental arch dimensions and tooth size widths were calculated on standardized occlusal photographs and compared with plaster study models. The authors concluded that the photogrammetric method was a reliable tool for clinical and scientific application to measure tooth size and dental arch widths, except for calculating the mesio-distal width of the upper first molar.

Table 2. *Bolton's analysis calculated on study models and photographs (n=16)*

Sample no	OR (SM)	OR (P)	AR (SM)	AR (P)	OTM (SM) Excess	OTM (P) Excess	ATM (SM) Excess	ATM (P) Excess
1.	96.32	96.95	84.7	83.77	4.28	4.95	3.66	3.28
2.	85.79	84.53	70.74	71.92	6.61*	7.11*	4.22*	3.44*
3.	94.04	94.16	80.35	81.36	1.98	2.13	1.47	1.97
4.	97.33	96.51	81.56	79.91	5.52	4.86	1.52	1.26
5.	90.51	90.34	79.48	77.64	0.84*	1.05*	1.06	0.22
6.	92.11	92.24	80.25	80.53	0.71	0.85	1.33	1.48
7.	91.44	92.47	77.39	78.38	0.13	0.69	0.09	0.54
8.	92.79	92.60	77.52	77.55	1.44	1.22	0.15	0.17
9.	92.38	92.44	78.48	78.99	1.04	1.10	0.60	0.86
10.	90.56	89.91	78.28	78.39	0.74*	1.40*	0.51	0.56
11.	91.36	91.37	77.77	77.39	0.07	0.07	0.28	0.10
12.	93.62	93.56	79.7	80.53	2.14	2.16	1.14	1.54
13.	94.26	93.70	78.47	79.25	2.81	2.29	0.59	0.97
14.	93.80	92.78	80.21	80.79	2.41	2.14	1.41	1.69
15.	93.19	93.03	76.65	76.94	1.78	1.62	0.34*	0.16*
16.	93.00	92.81	81.27	82.11	1.63	1.46	1.89	2.27

OR= Overall ratio, SM= Study model, P= Photograph, AR= Anterior ratio, OTM (SM)= Overall tooth material excess in Study model, OTM (P)= Overall tooth material excess in Photograph, ATM (SM)= Anterior tooth material excess in Study model, ATM (P)= Anterior tooth material excess in Photograph. *Maxillary excess , readings not highlighted denote mandibular excess

Table 3. *Mean, SD* (Standard deviation) and SE** (standard error) of the 4 parameters obtained from Bolton's analysis (n=16)*

Variable	Study model			Photograph			SM-P (diff)	ICC	
	Mean	SD	SE	Mean	SD	SE		r	Sig
Overall ratio	92.65	2.60	.65	92.46	2.80	.70	.19	.98	†††
Anterior ratio	78.92	2.95	.73	79.09	2.68	.67	- .16	.97	†††
Overall maxillary/mandibular excess	2.13	1.87	.46	02.19	1.86	.46	- .06	.98	†††
Anterior maxillary/mandibular excess	1.26	1.18	.29	01.28	1.05	.26	- .01	.96	†††

SM-P (difference in means between Study model (SM) and photograph (P)), Sig= Significance, ICC= Intraclass Correlation coefficient, r= r value, Sig= Sigificance † p<.05, †† p<.01, ††† p<.001

However, it should be kept in mind that correlations could be influenced negatively if the following precautions are not taken during the photographic procedure. The mirror has to be positioned correctly to ensure parallelism with the camera lens set to the correct magnification ratio of 1:2 and the optical axis of the camera perpendicular to the maxillary or mandibular occlusal plane. Occlusal photographs should be taken consistently and must reproduce the intraoral structures exactly to be of use for measurements. Moreover, if the need arises to crop the images, it must be cropped by maintaining the original ratio of the image so as to negate magnification errors.

In the current study, we evaluated the reliability of Bolton's analysis, which is an application of the photogrammetric method and requires accurate measurement of mesio-distal tooth dimensions from standardized occlusal photographs. The photogrammetric technique which we have used differs from the one used by Normando et al. in two aspects, viz., equipment and software used for calculation. Firstly, we have used a macro lens and a ring flash, which, we believe is essential for capturing standardized occlusal photographs in the correct magnification ratio. Secondly, Nemoceph® software, which we have used, allows for calibration of occlusal photographs with the help of the 35 mm scale visible in each photograph. Also, Nemoceph® software calculates the distance between any two marked points immediately in millimetres and has the distinct advantage of saving time and effort when compared to other imaging softwares such as Imagetool® which give readings in pixels and require calculations and conversions of unit. Moreover, we could not find an Imagetool® release supporting Windows 7® or higher versions. Nevertheless, further studies must be done comparing the available imaging software to ascertain which software gives the highest accuracy and reliability for calculating photogrammetric readings.

In the current study, even though all variables showed a statistically significant and excellent correlation in the intraclass correlation coefficient test (r >.75), only 18 out of 28 variables showed a non significant result in the paired t test (Table 1). The parameters that showed a significant difference (p< .05) include the mesio-distal widths of upper and lower first molars, upper second premolars, upper canines and LL3 and LR1. This is in agreement with the findings obtained by Normando et al. who also found that despite the high reliability between the two methods, the paired t test revealed statistical differences in the validity of the two methods. The non significant result in the paired t test of the first molars and upper premolars could be due to the posterior location of the tooth and the difficulty in obtaining standardized images, which could have been influenced by the variations in the angle formed between the mirror and arch. The differences in UR3, LL3 and LR1 could be attributed to the increased occurrence of rotations with respect to these teeth. However, the mean differences between the mesio-distal dimensions of the measured teeth were less than 0.20 mm (except UR4 = -0.22 mm), which is close to the human eye resolution of 0.2mm. (13) Hence, these minor differences are not of clinical significance.

Similarly, the intraclass correlation test of the 4 derived parameters obtained from Bolton's analysis, show excellent correlation between the two methods (r>0.96, for all variables) (Table 3). The mean differences between the measurements obtained for the two methods for the 4 parameters are below 0.2mm, showing that Bolton's analysis measurements from occlusal photographs are clinically useful. This could be used as an advantageous measuring tool in epidemiological and research studies, for assessing Bolton's discrepancy during treatment progress and in conditions were procuring a dental arch impression proves difficult. Moreover, with advances in technology it is expected that digital cameras would further improve their accuracy and be low cost imaging tools for clinicians. Taking standardized occlusal images is not time consuming and negates the need for making study models at various stages of treatment. Measurements can me made directly on occlusal photographs without the need to remove archwires as required prior to making alginate impressions which is a time saver in busy practices. We also believe the photogrammetric method can be used as an effective clinical control for self assessment and to assess changes that occur in the dental arch in between appointments (e.g. assessing midline discrepancies, Bolton discrepancy, changes in arch width due to expansion devices etc).

Conclusion

The present study shows that photogrammetry is a reliable tool to measure mesio-distal tooth size and that quantitative data obtained from photogrammetric measurement of standardized occlusal photographs can provide clinicians with useful and accurate information negating the need for plaster study models. However, taking standardized photographs is a technique sensitive procedure and so the clinician must train himself in taking repeatable photographs with minimal errors. Also there is a need for development of free software that allows for calibration and measurement of distances between two or more points in a photograph so that more clinicians can apply photogrammetry in their clinical practice.

Türkçe Öz: *Oklüzal Fotoğraflarda Fotogrametrik Bir Yöntem Kullanılarak Bolton Analizi. Amaç: Bu çalışmanın amacı, standardize oklüzal fotoğraflar kullanılarak Bolton Analizi yapılmasını sağlayan yeni bir fotogrametrik tekniği sunmak ve alçı çalışma modelleri ile bu yeni yöntemin güvenilirliğini değerlendirmektir. Gereç ve Yöntem: Bu çalışma yaşları 18-25 arasında olan 16 birey üzerinde (8 erkek, 8 kadın) gerçekleştirilmiştir. Standart oklüzal fotoğraflar ve alçı çalışma modelleri elde edilmiştir. Oklüzal fotoğraflar Nemoceph® yazılımıyla kalibre edilmiştir. 1. büyük azı dişlerine kadar olan tüm dişlerin mezio-distal boyutları hesaplanmış ve Bolton analizi yapılmıştır. Benzer şekilde, alçı çalışma modellerindeki tüm dişlerin mezio-distal boyutları 0,1 mm hassasiyete sahip bir dijital kompas kullanılarak Bolton analizi yapmak için ölçülmüştür. Çalışma modellerinde ve ilgili oklüzal fotoğraflarda 28 parametre ölçülmüştür. Fotogrametrik yöntemin geçerliliğini ve güvenilirliğini test etmek için eleştirilmiş t testi ve sınıf içi korelasyon testleri yapılmıştır. Okluzal fotoğraflardan elde edilen Bolton analiz ölçümlerinin çalışma modellerine kıyasla güvenilirliğini değerlendirmek için 4 parametreye sınıf içi korelasyon testi yapılmıştır. Bulgular: 28 parametrenin tamamı, sınıf içi korelasyon testinde istatistiksel olarak anlamlı ve mükemmel bir korelasyon (r> .80) göstermiştir. Bolton analizini hesaplamak için kullanılan 4 değişken, sınıf içi korelasyon testinde istatistiksel olarak anlamlı korelasyon (r> .96) göstermiştir. Sonuç: Fotogrametri mezio-distal diş boyutunu ölçmek için güvenilir bir araçtır. Standart oklüzal fotoğraflardan fotogrametrik teknik kullanılarak yapılan Bolton analizi etkili bir klinik araç olarak kullanılabilir. Anahtar Kelimeler: Fotogrametri, Bolton analizi, oklüzal fotoğraf, Nemoceph, diş boyutları*

Ethics Committee Approval: The study was approved by Ethics Committee of Army College of Dental Sciences, Secunderabad, India (ACDS/IEC/21/Jan 2018).

Informed Consent: The informed consents were provided by the participants.

Peer-review: Externally peer-reviewed.

Author contributions: AP and PC designed the study. AP participated in generating the data for the study. AP participated in gathering the data for the study. AP and PC participated in the analysis of the data. AP wrote the majority of the original draft of the paper. AP and PC participated in writing the paper. All authors approved the final version of this paper.

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Original research

Effects of surface coating on the flexural strength of fluoride-releasing restorative materials after water aging for one year

Muhittin Uğurlu[1]

Purpose
To evaluate the effects of surface coating and one-year water storage on the flexural strength of fluoride-releasing restorative materials.

Materials and Methods
Forty specimens were prepared from each material; GCP Glass Fill (GCP), Amalgomer CR (AHL), Zirconomer (Shofu), Fuji IX GP Capsule (GC), Beautifil II (Shofu), Estelite Σ Quick (Tokuyama) and reliaFIL LC (AHL). The specimens were randomly divided into two groups; surface coated with G-Coat Plus (GC) and uncoated. Each group was subdivided into two groups stored in distilled water at 37°C for 24 h and 1 year before testing (n=10). The flexural strength was evaluated using three-point bending test according to the ISO 4049:2009 standard using a universal testing machine. After flexural strength test, a cross-section of the coated specimens was evaluated with scanning electron microscopy (SEM).

Results
A significant increase was observed on the flexural strength of Amalgomer CR, Zirconomer and Fuji IX GP after 24 h when G-Coat Plus was applied ($p<0.05$). This significant increase was observed on the flexural strength of only Amalgomer CR and Zirconomer after 1 year ($p<0.05$). The highest flexural strength was obtained with Beautifil II, Estelite Σ Quick and reliaFIL LC after 24 h and 1 year ($p<0.05$). After 1 year, there was decrease on the flexural strength of the other materials except Beautifil II, Estelite Σ Quick and reliaFIL LC.

Conclusion
The resin coating improved the flexural strength of some glass ionomer-based materials but the water aging decreased the same physical properties.

Keywords: *Flexural strength, Glass ionomer cement, Scanning electron microscopy, Surface coating, Water aging*

Introduction

The glass-ionomer cements (GICs) have been widely used in dentistry due to their beneficial properties, such as biological compatibility, chemical adhesion to tooth structure, and especially fluoride release which contribute to caries preventive character (1,2). However, some characteristics of the GICs can limit their indications for clinical use (3). The long setting reaction time and the water sensitivity during setting reactions may cause low mechanical properties of the GICs (4,5). During the setting process, water has an important role for proper maturation of GICs (5). The initial stage, which is the clinical setting reaction, occurs within the first 10 minute after mixing. The second stage, involving the release of the calcium and aluminum ions within the matrix, is a slower continuation of the acid-base reaction that lasts 24 h (4). The material is very sensitive to water uptake at the first reaction, while the material is very susceptible to dehydration during the second step. Both water contamination and

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dehydration result in incomplete or inadequate maturation of GICs and thus to inferior mechanical properties (4).

When selecting a material to restore teeth, one of the main considerations is mechanical properties of the material (6). The mechanical properties of a direct restorative material need to be strong enough to withstand the forces associated with mastication and other possible loading (7). The materials must also maintain mechanical properties for a long term (8,9). The GICs have been introduced in dental practice by Wilson and Kent in the early 1970s (1). Since then, several researches have been done to enhance their mechanical properties and to expand their clinical applications. Consequently, fluoride-releasing and glass ionomer-based materials have been recently developed. Some of these materials are the high viscosity GIC, the ceramic reinforced GIC, the zirconia reinforced GIC and the GIC containing calcium fluorapatite nanocrystals (10). One of the recent developments in the fluoride-releasing restorative materials has been introduction of the giomer materials. The giomer is a hybridization material of GIC and composite resin, containing surface pre-reacted glass ionomer (S-PRG) filler particles within a resin matrix (3).

In previous studies, the resin coating has been recommended for increasing the clinical performance of glass-ionomer restoration (11) and the mechanical properties of GICs by preventing the water contamination and dehydration (12-16). The coating agent acts as barriers to water so the hardening and maturation processes of GIC can take place unaffected by water uptake and water loss (13,16). It has been reported that the self-adhesive resin coating agent provided a seal of the GIC's surface through high hydrophilicity and low viscosity (17). It has been additionally stated that the coating agent could improve the mechanical properties by filling the surface micro porosities of the materials (14). Reviewing the literature, there is little data on the mechanical properties of the recently developed fluoride-releasing materials and, no information is available regarding the effect of resin coating and water aging on the mechanical properties of these materials (3,10,12-16).

Therefore, the objective of this study was to evaluate the effect of resin coating and one-year water aging on the flexural strength of the fluoride-releasing materials. The null hypothesis tested was the resin coating and water aging would not affect the flexural strength of the materials.

Materials and Methods

Restoratives

Five different fluoride-releasing restorative materials were tested in the present study. The restorative materials were a glass carbomer (GCP Glass Fill; GCP, Vianen, Netherlands), a ceramic reinforced GIC (Amalgomer CR; Advanced Healthcare Ltd, Tonbridge, UK), a zirconia reinforced GIC (Zirconomer; Shofu, Kyoto, Japan), a high viscosity GIC (Fuji IX GP Capsule; GC, Tokyo, Japan) and a giomer (Beautifil II; Shofu, Kyoto, Japan). As control, a nano-filled composite resin (Estelite Σ Quick; Tokuyama, Tokyo, Japan) and a nano-hybrid composite resin (reliaFIL LC; Advanced Healthcare Ltd, Tonbridge, UK,) were used. The materials are listed in Table 1 with the composition, manufacturer and lot number. A nano-filled surface sealant agent (G-Coat Plus; GC, Tokyo, Japan, Lot:1710031) was also tested.

Specimen preparation

The 25x2x2 mm bar-shaped forty specimens were prepared from each material. After the materials were inserted into the teflon mould, the polyester strips (Mylar strip; SS White Co., Philadelphia, PA, USA) were pressed onto the mould surfaces with glass plates to extrude excess material and obtain a flat surface. The giomer and composite resins were polymerized through the glass plate using a LED light-curing unit (Smartlite Focus; Dentsply, Milford, DE, USA) according to the manufacturer's instructions (Table 2). The intensity of the curing light (Smartlite Focus; Dentsply, Milford, DE, USA) was measured before and after application and the light output was never below 1000 mW/cm^2. For GCP Glass Fill and Fuji IX GP, a capsule mixer (Silver Mix; Stomamed, Bratislava, Slovakia) was used for 10 seconds of mixing before application of the material. Amalgomer CR and Zirconomer were mixed within a total of 30 seconds according to the manufacturer's instructions (Table 2). After the light curing and setting cycle, the specimens were removed from the mould. In order to obtain flat surface, both side of the specimens were gently polished manually with a circular motion with 1000-grit and 1500-grit wet silicon carbide papers. Each specimen was brief rinsed in tap water between each grit. After the polishing procedure, the specimens were randomly divided into two groups according to coated with G-Coat Plus and uncoated. G-Coat Plus was applied using a micro-tip applicator, then gently air thinned for 5 seconds and light cured for 20 seconds with the LED light curing unit (Smartlite Focus; Dentsply, Milford, DE, USA, 1000 mW/cm^2) according to manufacturer's instructions. Only one surface of the specimens was coated as in a clinical application. All the specimens were prepared at room temperature (21±1°C) in 55% relative humidity. The temperature and humidity were measured with a digital thermometer. Each group was subdivided into the two groups stored in distilled water at 37°C for 24 h and 1 year before testing. The ten specimens were tested in each subgroup (n=10).

Flexural strength

The flexural strength was evaluated using three-point bending test according to the ISO 4049:2009 standard with a 20-mm span at a crosshead speed of 1 mm/min on a universal testing machine (Autograph AGS-X; Shimadzu, Kyoto, Japan). Before testing, the specimen dimensions were measured using a digital caliper (Digimatic Caliper, Mitutoyo, Tokyo, Japan). The flexural strength (FS) of the material was calculated by $FS = 3P_{max}L/(2bh^2)$, where P_{max} is the maximum load (N) on the load-displacement curve, L is the span length (mm), b is the width of the specimen (mm) and h is the thickness of the specimen (mm).

SEM analysis

After flexural strength test, a cross-section of a specimen was randomly selected in each coated group for SEM analysis. All specimens were adhered with conductive carbon tape to aluminum stubs and observed under SEM (Quanta Feg 250, FEI, Netherlands) with secondary electrons at ×500, ×1000 and ×2000 magnification by 20 kV.

Table 1. *The composition of the materials according to the manufacturers' data*

Materials	Type	Composition	Manufacturer	Lot
GCP Glass Fill	Glass carbomer	Fluoroaluminosilicate glass, nano fluoro/ hydroxyapatite, polyacids	GCP, Vianen, Netherlands	71702144
Amalgomer CR	Ceramic reinforced GIC	*Powder:* Fluoroaluminosilicate glass, polyacrylic acid powder, tartaric acid powder, ceramic reinforcing powder. *Liquid:* Polyacrylic acid, distilled water	Advanced Healthcare Ltd, Tonbridge, UK	011804-81
Zirconomer	Zirconia reinforced GIC	*Powder:* Fluoroaluminosilicate glass, zirconium oxide, pigments *Liquid:* Polyacrylic acid solution, tartaric acid	Shofu, Kyoto, Japan	02160281
Fujı IX GP	High viscosity GIC	Polyacrylic acid, fluoroaluminosilicate glass, polybasic carboxylic acid	GC, Tokyo, Japan	180110A
Beautifil II	Giomer	BISGMA, TEGDMA, inorganic glass filler, aluminium oxide, silica, prereacted glass ionomer filler, Camphoroquinone	Shofu, Kyoto, Japan	111787
Estelite Σ Quick	Nano-filled composite resin	Bis-GMA, TEGDMA, silica zirconia fillers, silica-titania fillers, photoinitiators	Tokuyama, Tokyo, Japan	E699
reliaFIL LC	Nano-hybrid composite resin	Bis-GMA,TEGDMA, fluoroboroaluminosilicate glass fillers, photoinitiators	Advanced Healthcare Ltd, Tonbridge, UK	021722-8

Bis-GMA: Bisphenol A diglycidyl methacrylate; TEGDMA: Triethylene glycole dimethacrylate

Table 2. *The application procedures of the materials according to manufacturer instructions*

Materials	Application procedure
GCP Glass Fill	Before activation shake the capsule or tap its side on a hard surface to loosen the powder. For activation push the plunger on a plane surface to the end of the capsule. Insert the capsule into a universal capsule gun and click once to standardize. Insert the capsule into a mixer and mix the capsule for 10-15 seconds with high frequency mixers. Remove the pin from the nozzle after mixing. Insert the capsule into the capsule gun and pull the lever 2 times (2 clicks) to prime. Within 15 seconds maximum after mixing, start to extrude the mixture directly into the preparation.
Amalgomer CR	Powder to liquid ratio 3.6g /1.0g (3.6:1.0 m/m) Use a glass block for best results and a stainless steel "Silicate" spatula. Incorporate half the powder into the liquid as quickly as possible (5-10 seconds) and then add the remainder and spatulate to a thick putty-like consistency. Total mixing time 30 seconds. Do not add powder in small increments.
Zirconomer	Powder to liquid ratio 3.6g /1.0g (3.6:1.0 m/m) Dispense two level scoops of powder with the measuring scoop provided onto a mixing pad. Then, dispense one drop of liquid separately. Divide the dispensed powder into 2 equal portions; introduce the first half to the dispensed liquid and mix for 5-10 second with the plastic spatula Then, add the remaining half and mix until it reaches a thick putty-like consistency. Mixing must be completed within a total of 30 second.
Fujı IX GP	Before activation, shake the capsule or tap its side on a hard surface to loosen the powder. To activate the capsule, push the plunger until it is flush with the main body and hold it down for 2 seconds. Immediately set it into a mixer (or an amalgamator) and mix for 10 seconds (~ 4,000 RPM) Immediately remove the mixed capsule from the mixer and load it into the GC Capsule Applier. Make two clicks to prime the capsule then syringe. The working time is 2 minutes from start of mixing. Within 10 seconds maximum after mixing, start to extrude the mixture directly into the preparation.
Beautifil II	Dispense the necessary amount of material from the syringe. Light cure for 20 seconds (halogen lamp) or 10 seconds (high power LED light).
Estelite Σ Quick	Dispense the necessary amount of material from the syringe. Light cure for 20 seconds (halogen lamp) or 10 seconds (high power LED light).
reliaFIL LC	Dispense the necessary amount of material from the syringe. Light cure for 30 seconds (halogen lamp) or 10 seconds (high power LED light).

Statistical analysis

Statistical analyses were performed with the SPSS Program, version 20.0 (Statistical Package for the Social Sciences; SPSS, Chicago, IL, USA). The Kolmogorov-Smirnov test was applied to verify if the data were normally distributed. The mean flexural strength values of the material groups were compared using one-way ANOVA and Duncan post-hoc tests. An independent t test analyzed the differences in flexural strength values of the materials, evaluating the effect of coating and aging. The p-value less than 0.05 was considered statistically significant for all statistical analyses.

Results

The flexural strength values were shown in Table 3 and graphically presented in Figure 1. The higher flexural strength values were obtained with Beautifil II, Estelite Σ Quick and reliaFIL LC than other materials after 24 h and 1 year regardless of coating (p<0.05). A significant increase was observed on the flexural strength of Amalgomer CR, Zirconomer and Fuji IX GP after 24 h when G-Coat Plus was applied (p<0.05). After 1 year, the coating significantly increased the flexural strength of Amalgomer CR and Zirconomer (p<0.05). The water aging significantly decreased the flexural strength of GCP Glass Fill, GCP Glass Fill Coated, Amalgomer CR Coated, Zirconomer, Fuji IX GP Coated groups (p<0.05).

Table 3. *The mean flexural strength values (MPa) and standard deviations of the materials (n=10 for each subgroups)*

	Flexural Strength		
	24 hours	***1 year***	$p^{\ddagger}$
GCP Glass Fill	31.27 ± 4.18^{a}	25.60 ± 3.94^{a}	0.002
GCP Glass Fill Coated	30.54 ± 4.06^{a}	26.46 ± 3.91^{a}	0.013
$p^{\dagger}$	0.698	0.630	
Amalgomer CR	35.74 ± 5.29^{ab}	31.86 ± 4.65^{ab}	0.161
Amalgomer CR Coated	45.06 ± 4.41^{cd}	41.95 ± 5.09^{c}	0.008
$p^{\dagger}$	0.000	0.000	
Zirconomer	35.58 ± 3.94^{ab}	33.96 ± 3.81^{b}	0.008
Zirconomer Coated	44.12 ± 4.81^{c}	41.90 ± 5.33^{c}	0.321
$p^{\dagger}$	0.000	0.001	
Fujı IX GP	41.29 ± 4.95^{bc}	45.27 ± 4.46^{c}	0.122
Fujı IX GP Coated	51.82 ± 5.48^{d}	48.27 ± 3.46^{c}	0.014
$p^{\dagger}$	0.000	0.110	
Beautifil II	114.75 ± 10.64^{e}	111.34 ± 10.16^{d}	0.232
Beautifil II Coated	115.51 ± 12.08^{e}	114.17 ± 11.38^{de}	0.729
$p^{\dagger}$	0.884	0.564	
Estelite Σ Quick	121.04 ± 11.34^{e}	119.10 ± 10.00^{e}	0.185
Estelite Σ Quick Coated	122.58 ± 11.44^{e}	120.23 ± 10.64^{e}	0.437
$p^{\dagger}$	0.766	0.810	
reliaFIL LC	117.95 ± 11.17^{e}	116.62 ± 11.42^{de}	0.673
reliaFIL LC Coated	117.40 ± 11.68^{e}	114.94 ± 11.03^{de}	0.362
$p^{\dagger}$	0.916	0.742	

Same small superscript letter indicates no statistical difference in the column; **p**†: Significance levels of the uncoated and coated groups of each material; **p**‡: Significance levels of the 24 hours and 1-year groups.

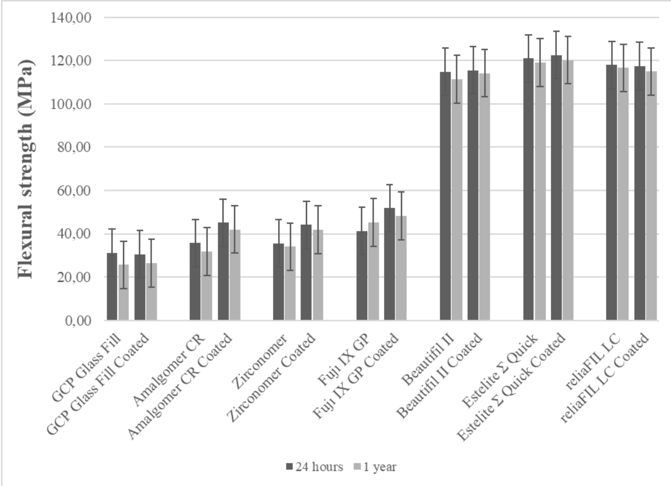

Figure 1. *The mean flexural strength values of the materials after 24 hours and 1 year.*

The SEM micrographs were presented in Figure 2. The SEM micrographs showed that there was a micro-mechanical interlocking between the materials and the coating agent after 24 h and 1 year.

Discussion

This study evaluated the flexural strength of the fluoride-releasing restorative materials and the composite resins which were commonly used as restorative materials. The effects of surface coating and one-year water aging on the flexural strength of the materials were investigated in the present study. The flexural strength test is commonly used to evaluate and compare the mechanical properties of dental materials in laboratory conditions (18-20). The flexural strength has been defined as the maximum stress that a material subjected to a bending load can resist before failure (20). It is regarded as the most important measure of strength for dental materials because considerable flexural stresses occur during the complex mastication process (18,20). The restorative materials must have high flexural strength to enhance the longevity of the restorations. (13,18). The minimum requirement of flexural strength for occlusal restorations is 80 MPa according to ISO 4049 (18). In the present study, GCP Glass Fill, Amalgomer CR, Zirconomer and Fuji IX GP did not meet the minimum requirement of ISO 4049 for occlusal restorations. The resin coating and water aging influenced the flexural strength of some fluoride-releasing materials. Therefore, the null hypothesis, that the resin coating and water aging would not affect the flexural strength of the materials, was partially rejected.

The setting process of GICs generally is characterized by interaction between a polyacid liquid and a glass powder in form of acid-base reaction. This reaction continues by a stepwise rather long-lasting setting (21). The changes in mechanical properties of GICs occur within the first 24 h and, the changes can be observed over several weeks or months (5). The coating is recommended during the initial setting stage of conventional GICs for a proper maturation (5,12). The setting process of GCP Glass Fill, Amalgomer CR, Zirconomer and Fuji IX GP occur in form of acid-base reac-

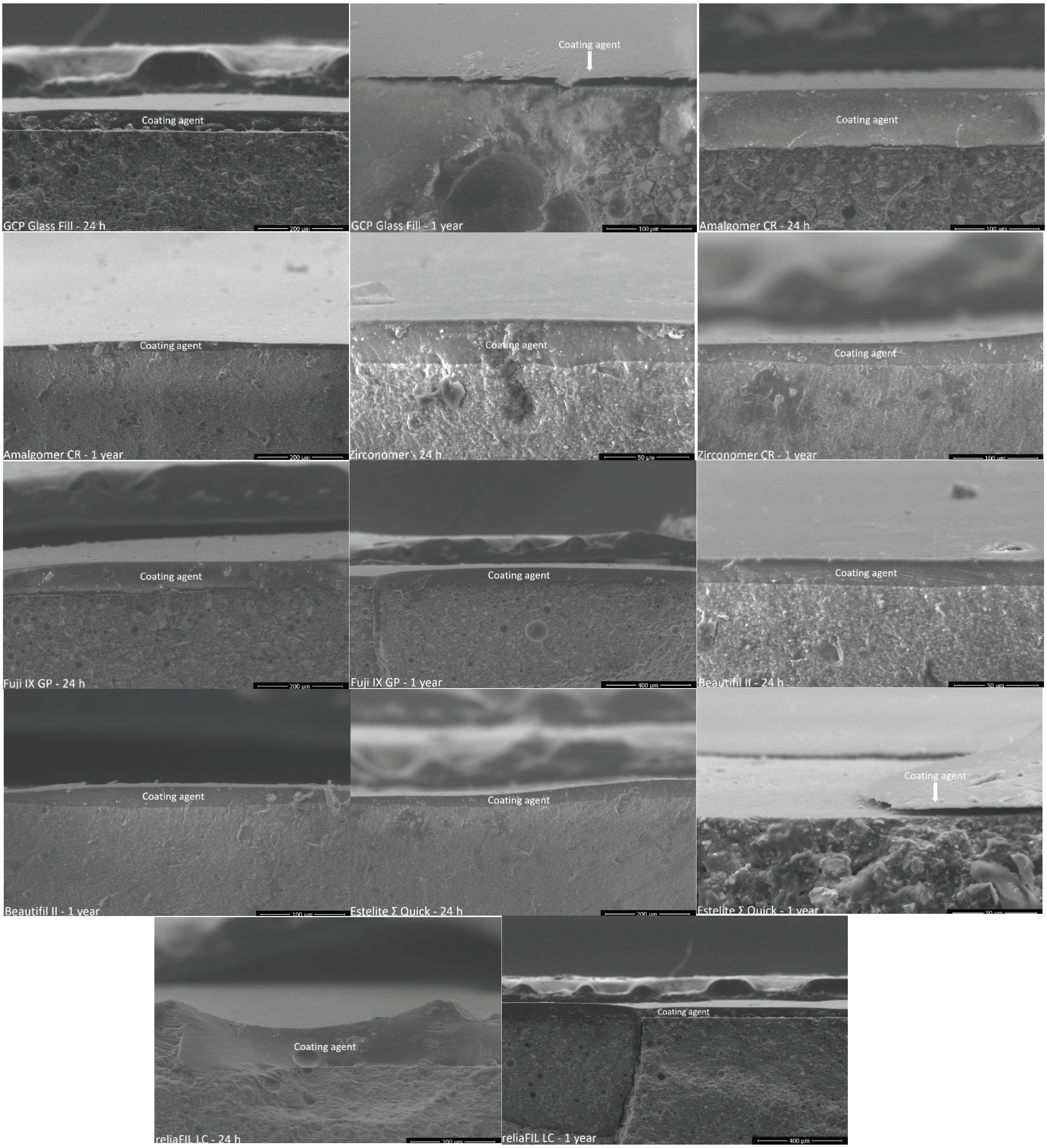

Figure 2. *SEM photomicrograph of the cross-section of the coated specimens after 24 h and 1 year. The SEM micrographs of all the materials showed that there was a micro-mechanical interlocking between the materials and the coating agent after 24 h and 1 year.*

tion like a conventional GIC. In the present study, the surface coating significantly increased the flexural strength of Amalgomer CR, Zirconomer and Fuji IX GP after 24 h. As reported in previous studies, the increase could be due to that the coating agent exerted control on the setting process of the materials within 24 h (12-16).

The protective effect of the coating from extrinsic water may allow complete maturation of the GIC reaction with delayed water exposure, thus possibly creating a stronger material while it may not reinforce the surface of the material (16). Previous studies concluded that significant improvement of wear resistance (13), shear punch strength (16), and flexural strength (13-15) of Fuji IX GP after coating with G Coat Plus before water contamination. It has been also reported that the strength increases in coated GIC resulted from that the protective coating contributes to the GIC strength by improving the maturation process and not by the inherent strength of the coating layer (12). In this study, the surface coating did not affect the flexural strength of GCP Glass Fill after 24 h. It could result from different moisture sensitivity of GCP Glass

Fill. According to the manufacturer, heat application is recommended for GCP Glass Fill during the setting reaction to increase its mechanical properties. But it has been concluded that the gloss and heat application with LED curing unit did not influence the flexural strength of GCP Glass Fill (22). This result has been attributed to different chemical composition and moisture sensitivity of the material (22). After 1 year, the coating increased the flexural strength of Amalgomer CR and Zirconomer. As reported in a previous study, it could be due to that the coating agent reduced the surface porosity and crack propagation on the GICs (16).

In this study, the glass ionomer-based materials GCP Glass Fill, Amalgomer CR, Zirconomer and Fuji IX GP showed lower flexural strength than Beautifil II and the composite resins regardless of coating and water aging. It has been previously reported that the giomer and composite resins had higher mechanical properties than GICs (14,23-25). In the present study, the coating did not influence the flexural strength of Beautifil II and the composite resins regardless of water storage. This result can be due to the high flexural strength of the materials. It has been stated that during the three-point bending test, the crack starts from within the specimen not from the surface, therefore the coating does not play a role on materials which are more resistant to flexural stresses (13).

The water aging is one of the most widely used procedures in experimental studies to evaluate the performance of materials and simulate the physiological aging of materials (8). It has been stated that the storage agent had a low effect on the mechanical properties, furthermore the storage time was more important factor (4,15). The water aging can cause detrimental effect on GICs, as it erodes the surface of the material and induces hydrolysis and dissolution of GICs' components (26,27). The water uptake in conventional GIC is rapid due to the hydrogel structure and large micropores on the surface, therefore a substantial decrease in strength and elasticity of the material may occur (28). The water aging can also cause plasticization of the resin component in the composite resins due to water sorption. Therefore, the long-term storage in water can influence mechanical properties of the composite resins (29). Furthermore, it has been also reported that the effects of water aging could be related to the composition of composite resins and GICs (7,29).

A previous study has concluded that the flexural strength of Fuji IX GP showed an increase up to 3 months and then, decreased after 6 months water aging (14). The improvement in the strength up to 3 months has been attributed to the acid-base reaction that proceeds slowly until final maturation completion which may take a few months (30). It has been stated that the storage time was an effective factor in the flexural strength of either uncoated and coated GICs (14). In the present study, the 1-year water aging did not affect the flexural strength of Beautifil II and the composite resins regardless of coating; however, it decreased the flexural strength values on GCP Glass Fill, Amalgomer CR, Zirconomer and Fuji IX GP. As stated in a previous study, the decrease could attribute to water uptake of the materials (7). The decrease of flexural strength was not observed on Zirconomer coated group. It could be due to that the coating can reduce water uptake. It has been reported that the coating with G Coat Plus could be beneficial for reducing water absorption of GIC (31). But, in this study, the coating did not show the same effect for each glass ionomer-based material. The differences could result from different chemical composition and water uptake of the materials. Unfortunately, in this study, the water uptake was not evaluated.

In the present study, the SEM micrographs showed that there was still a micro-mechanical interlocking between the materials and the coating agent after 1 year, but it was stated that the masticatory forces could cause debonding the coating agent over time in oral environment (12). The *in vitro* researches cannot exactly reflect the actual status of the oral cavity since oral environment is dynamic and different from laboratory conditions. But the laboratory studies simulating most clinical conditions are very useful to assess behavior of biomaterials (14,16). The longevity is one of the most important considerations of restorations (32). Therefore, the restorative materials are evaluated with *in vitro* studies to determine if they are susceptible to degradation during long-term using. Besides the *in vitro* studies, further clinical studies are also needed to investigate the performance of the fluoride-releasing materials and the effects of resin coating.

Conclusion

Within the limitations of this study, the resin coating provided a valuable support for some of the glass ionomer-based materials, since it led to significant improvements in flexural strength of the materials. The giomer and composite resins had higher mechanical properties than the glass ionomer-based materials regardless of coating and water aging. The one-year water aging decreased the flexural strength of the glass ionomer-based materials while it did not affect the flexural strength of the giomer and composite resins.

Türkçe Özet: *Yüzey örtülemenin bir yıl suda yaşlandırmadan sonra florid salan restoratif materyallerin eğilme dayanımına etkisi. Amaç: Yüzey örtüleme ve bir yıl suda yaşlandırmanın florid salan restoratif materyallerin eğilme dayanımı üzerindeki etkilerini değerlendirmek. Gereç ve Yöntem: Her materyalden kırk örnek hazırlandı; GCP Glass Fill (GCP), Amalgomer CR (AHL), Zirconomer (Shofu), Fuji IX GP Kapsül (GC), Beautifil II (Shofu), Estelite Σ Quick (Tokuyama) ve reliaFIL LC (AHL). Örnekler, G-Coat Plus (GC) ile yüzey örtülenmiş ve örtülenmemiş olarak rastgele iki gruba ayrıldı. Her grup testten önce 37 °C'de distile su içinde 24 saat ve 1 yıl saklanan iki alt gruba ayrıldı (n = 10). Eğilme dayanımı, universal test cihazında ISO 4049:2009 standardına göre üç nokta eğilme testi kullanılarak değerlendirildi. Eğilme dayanımı testinden sonra, yüzeyi örtülenmiş örneklerin bir kesiti taramalı elektron mikroskobu ile değerlendirildi. Bulgular: Amalgomer CR, Zirconomer ve Fuji IX GP'nin 24 saat sonundaki eğilme dayanımında, G-Coat Plus uygulandığında önemli bir artış gözlendi (p <0.05). Bu artış, 1 yıl sonunda sadece Amalgomer CR ve Zirconomer 'in eğilme dayanımında gözlenmiştir (p <0.05). En yüksek eğilme dayanımı değerleri 24 saat ve 1 yıl sonunda Beautifil II, Estelite Σ Quick ve reliaFIL LC ile elde edildi (p <0.05). Beautifil II, Estelite Σ Quick ve reliaFIL LC hariç diğer materyallerin eğilme dayanımı 1 yıl sonunda azalmıştır (p> 0.05). Sonuç: Rezin örtüleme, bazı cam iyonomer bazlı materyallerin eğilme dayanımında artışlar sağlamıştır. Suda yaşlandırma, cam iyonomer bazlı materyallerin eğilme dayanımını azaltmıştır. Anahtar kelimeler: Cam iyonomer siman, Eğilme dayanımı, Suda yaşlandırma, Taramalı elektron mikroskobu, Yüzey örtüleme*

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Original research

Influence of exposure to phosphoric and polyacrylic acids on selected microscopic and physical/chemical properties of calcium hydroxide cements

Isabela Dantas Torres de Araújo[1], Renato Barbosa Soares[1], Camila Pessoa Lopes[1], Isana Álvares Ferreira[1], Boniek Castillo Dutra Borges[1]

Purpose
This study aimed to evaluate if the contact of calcium hydroxide cements with polyacrylic and phosphoric acids would alter selected microscopic and physical and chemical properties.

Materials and Methods
Chemically activated (Hydro C and Dycal Advanced Formula II) and resin-modified photoactivated (Ultra-blend Plus) calcium hydroxide cements were examined after exposure to the following different strategies: contact with no substance (control group); rinsing with water and drying; contact with polyacrylic acid, rinsing with water, and drying; and contact with phosphoric acid, rinsing with water, and drying. Surface morphology, determined by scanning electron microscopy (SEM), water sorption and solubility, and the release of hydroxyl ions were evaluated.

Results
SEM showed a greater impact of the conditioning acids on the surface of the chemically activated cements. Ultra-blend Plus obtained the highest value of sorption (516.8 $\mu g/mm^3$) and solubility (381.1 $\mu g/mm^3$) and Hydro C had the lowest values 251.9 $\mu g/mm^3$ and 206.3 $\mu g/mm^3$ respectively. Considering the release of hydroxyl ions in comparison with time, Hydro C and Ultra-blend Plus presented significant statistical difference for polyacrylic and phosphoric acid subgroups.

Conclusion
Hydro C and Dycal presented intensification of surface irregularities after contact with conditioning acids. The chemically activated materials suffered a decrease in sorption and solubility. The action of the conditioning acids promotes greater increase of the release of hydroxyl ions for Hydro C and Dycal.

Keywords: *Calcium hydroxide, Hydrogen ion concentration, Solubility, Surface morphology, Water absorption*

Introduction

Protection of the dentin/pulp complex is characterized by the use of one or more protective agents in the dental cavity in order to stimulate dentin neoformation, maintain pulp vitality, and neutralize/eliminate the action of the remaining microorganisms (1). Among the protective materials used, calcium hydroxide (CH) cements are widely used as the protective agent in deep cavities and with restricted application to the bottom wall of the dental cavity due to the potential of antibacterial odontoblast stimulation (1–4).

Calcium hydroxide cements' use is based on high alkalinity, biocompatibility, antibacterial action, thermal insulating action, and stimulating action in the process of dentin neoformation (1,5–7). However, it presents disadvantages, such as low mechanical resistance, solubility in the buccal medium, no adhesiveness, limited working time required, and under acidic conditions it

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dissolves, causing marginal infiltration (7). Thus, resin-modified photoactivated CH materials were designed and produced by manufacturers as a way to overcome these deficiencies and make restorative steps easier to professionals (8).

As CHs have poor mechanical properties and high solubility, the dissolution of the material and/or sorption in water, when aggravated by exposure to the conditioning acids or exposure to aqueous medium, favor the inability of the material to remain stable under restoration. This can leave the restoration unsupported, as well as the cavity without the necessary protection (8,9). Still, there are no studies that verify whether contact of cements with acids affects their fundamental properties.

Based on this, this study aimed to evaluate if the contact of CH cements with polyacrylic and phosphoric acids would alter surface micromorphology, water sorption, water solubility, and hydroxyl ion release. The null hypotheses tested in this study was that the contact of CH cements with the conditioning acids would not alter the surface morphology, water sorption, water solubility, or the release of hydroxyl ions.

Materials and Methods

Experimental design

In the present study, surface micromorphology (SM), water sorption (WSp), water solubility (WSol), and release of hydroxyl ions (ROH) were the response variables analyzed.

For SM, WSp, and WSol, the factors under study were CH cement (Hydro C, Dycal, and Ultra-blend Plus) and surface treatment (contact with no substance; rinsing with water and air-drying; exposure to polyacrylic acid, rinsing with water, and air-drying; and exposition to phosphoric acid, rinsing with water, and air-drying).

For ROH, the surface treatment and timepoint factors (0 h, 3 h, 12 h, 24 h, and 48 h) were under study for each CH cement individually (Figure 1).

Descriptions of the materials used in this investigation and their compositions are presented in Table 1.

Preparation of specimens

A total of 156 specimens (n=52) were produced using a pre-fabricated silicon mold (1 mm thick × 5 mm diameter) following the directions from each manufacturer.

Ultra-blend Plus (Ultradent, South Jordan, UT, USA) specimens were made after filling the mold with the material. Subsequently, a Mylar strip and a glass plate (1 mm thick)

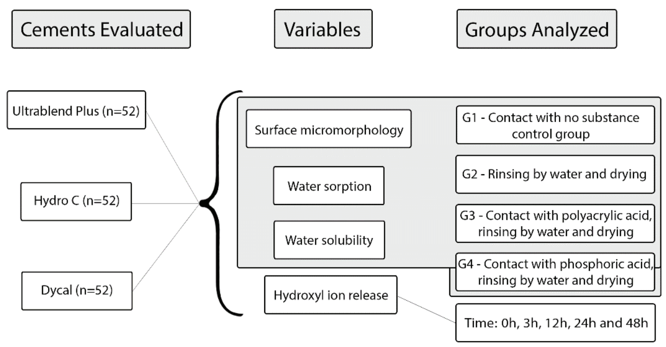

Figure 1. *Experimental design diagram.*

were placed on the cement, with slight digital pressure to obtain regularity and surface smoothness. Finally, there was photoactivation of the material with a Coltolux LED 1200 mW/cm^2 (Coltène, Altstätten, Switzerland) following the manufacturer's directions.

The specimens made with Hydro C (Dentsply, Petrópolis, RJ, Brazil) and Dycal (Dentsply, Petrópolis, RJ, Brazil), which are chemically activated CH-based cements, were made using two insulin syringes for their standardization. About 0.3 IU of the catalyst and base pastes were placed on a glass plate. The portions were mixed with the aid of a spatula until homogeneity of the cement was achieved. Then, the mixture was inserted into the mold until it was filled. Subsequently, a Mylar strip and a glass plate (1 mm thick) were placed on the cement and it was held with digital pressure until the material was secured. After production, specimens were randomly subjected to one of the following strategies:

- Control: specimens did not come in contact with any substance.
- Rinsing: after material cure/photoactivation, the specimen surface was washed with distilled water via a triple syringe for 15 s and then dried with air from the same syringe for the same time.
- Polyacrylic acid: after material cure/photoactivation, 25–30% Riva Conditioner (SDI, Bayswater, Victoria, Australia) was applied (0,1 UI) to the surface of the specimen for 15 s, followed by washing with distilled water from a triple syringe for 15 s and drying with air from the same syringe for the same time.
- Phosphoric acid: after material cure/photoactivation, 37% Super etch (SDI, Bayswater, Victoria, Australia) phosphoric acid was applied (0,1 UI) to the specimen surface for 15 s, followed by washing with distilled water from a triple syringe for 15 s and drying with air from the same syringe for the same time.

Then, SM (n=3), WSp, WSol, and ROH (n=10) were analyzed.

Surface morphology

Surface morphology was analyzed qualitatively with a low vacuum scanning electron microscope (HITACHI, model TM 3000, Hitachi Ltd., Tokyo, Japan) which doesn't require a prior sample preparation. The magnification of 400x was used for surface impact assessment. Three samples from each subgroup were analyzed immediately after receiving treatments.

WSp, WSol, and ROH

WSp and WSol tests were based on the standard ISO 4049:2019 method (10), except for the dimensions of the specimens. Samples were stored in a desiccator, in an incubator at 37°C and weighed daily with a Sartorius CC 1201 precision balance (Sartorius, Goettingen, Germany) until mass stabilization, that is, a mass in which the variation amounted to less than 0.2 mg within any 24-h period, resulting in M1. Subsequently, the thickness and diameter of the specimens were measured at three different points using a digital caliper (Mitutoyo Corporation, Tokyo, Japan). These measurements were used to calculate the volume of each specimen.

The samples were then placed in Eppendorf pots filled with distilled water (pH 6.34) and stored in an oven at 37ºC

Table 1. *Analyzed materials, it's compositions and batches*

Materials	Composition	Manufacturer	Batch	Local Manufacturing
Chemically activated calcium hydroxide cement (Hydro)	Catalyst: Calcium hydroxide, zinc oxide, ethyltoluene sulfonamide, zinc stearate, and mineral dyes Base: Ester glycol salicylate, barium sulfate, titanium dioxide, silica, and mineral dyes	Dentsply	125671H	Petrópolis, RJ, Brazil
Chemically activated calcium hydroxide cement (Dycal Advanced Formula II)	Catalyst: Ethyltoluene sulfonamide, calcium hydroxide, zinc oxide, titanium dioxide, zinc stearate, and mineral dyes Base: Ester glycol salicylate, calcium phosphate, calcium tungstate, zinc oxide, and mineral dyes	Dentsply	116185H	Petrópolis, RJ, Brazil
Resin-modified calcium hydroxide cement (Ultra-blend Plus)	Calcium hydroxide, Urethane dimethacrylate, and Tricalcium salt Triethylene glycol dimethacrylate	Ultradent	D017X	South Jordan, UT, United States of America
Polyacrylic acid 25–30% (Riva conditioner)	Polyacrylic acid, balance ingredients	SDI	140355	Bayswater, Victoria, Australia
Phosphoric acid 37% (Super etch)	Phosphoric acid, balance ingredients	SDI	130694	Bayswater, Victoria, Australia

for seven days, then dried on absorbent paper and weighed to obtain M2. To measure ROH, the pH of the distilled water was analyzed with a digital pH meter (Lucadema, LUCA-210. Serial No. 25553/1607) in periods of 0 h, 3 h, 12 h, 24 h, and 48 h, with no water exchange during periods. To obtain M3, the samples were stored inside a dissector and weighed daily until a constant mass was obtained. The obtained results were added to the following formulas to obtain the sorption and solubility of the tested materials (10):

- WSp=(M2–M3)/Volume
- WSol=(M1–M3)/Volume

Statistical analysis

Data from WSp and WSol tests were statistically analyzed by two-way ANOVA and the Tukey's test ($p<0.05$). ROH data was analyzed by two-way ANOVA for repeated measurements and the Tukey's test ($p<0.05$). SM was descriptively analyzed. ASSISTAT Beta (Federal University of Campina Grande, Campina Grande, PB, Brazil) software was utilized to perform statistical tests.

Results

SM

Hydro C presented a surface regularity in the control subgroup compared to the others which presented an increase of dark precipitate, surface porosity and fissures, as well as pore size for the polyacrylic acid subgroup and exposure of darker granules with larger diameters for the phosphoric acid subgroup (Figure 2).

Ultra-blend Plus presented a smoother surface morphology independent of the surface treatment (Figure 3). For Dycal Advanced Formula II, the presence of surface regularity for control and rinsing subgroups was evident and the presence of fissures could be determined by the specimens' exposure to the vacuum generated by the analysis apparatus (Figure 4). In the polyacrylic acid subgroup, the presence of zones of precipitate and dissolution of the material was observed, associated with less exposure of white granules. For the phosphoric acid subgroup, surface layer dissolution was noted, associated with the exposure of black granules and increased exposure of white granules.

WSp and WSol

The sorption and solubility values of the cements/groups are described in Table 2 and Table 3, respectively. In relation to group A (Hydro C), the samples referring to the rinsing subgroup presented higher sorption and solubility averages (400.6 µg/mm^3 and 242.8 µg/mm^3, respectively). For both properties the decrease in values of the polyacrylic acid and phosphoric acid subgroups is highlighted. For sorption values, there was statistically significant difference between

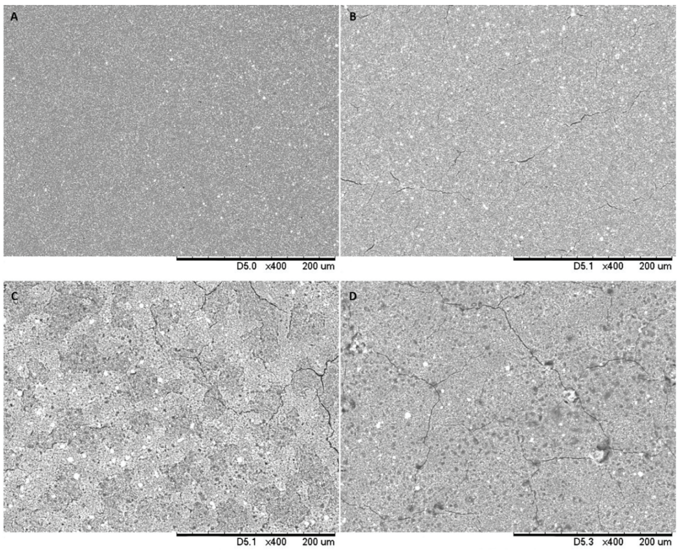

Figure 2. *Scanning electron microscopy images of Hydro C corresponding to;* ***A)*** *Control subgroup,* ***B)*** *Rinsing subgroup,* ***C)*** *Polyacrylic acid subgroup and* ***D)*** *Phosphoric acid subgroup.*

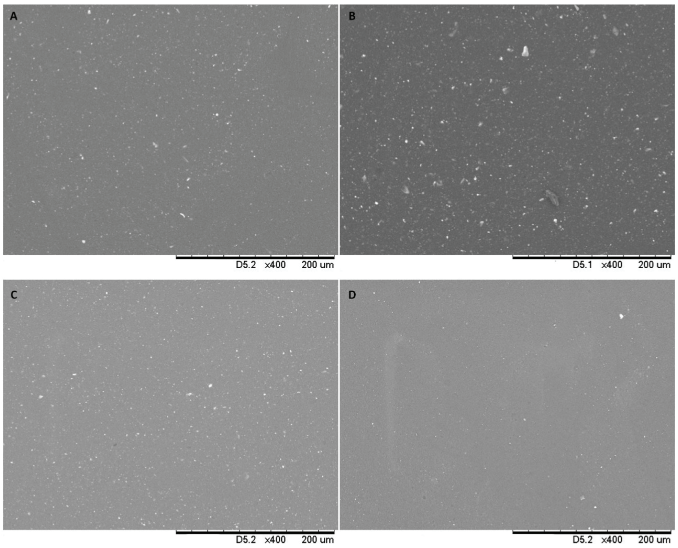

Figure 3. *Scanning electron microscopy images of Ultra-blend Plus corresponding to;* ***A)*** *Control subgroup,* ***B)*** *Rinsing subgroup,* ***C)*** *Polyacrylic acid subgroup and* ***D)*** *Phosphoric acid subgroup.*

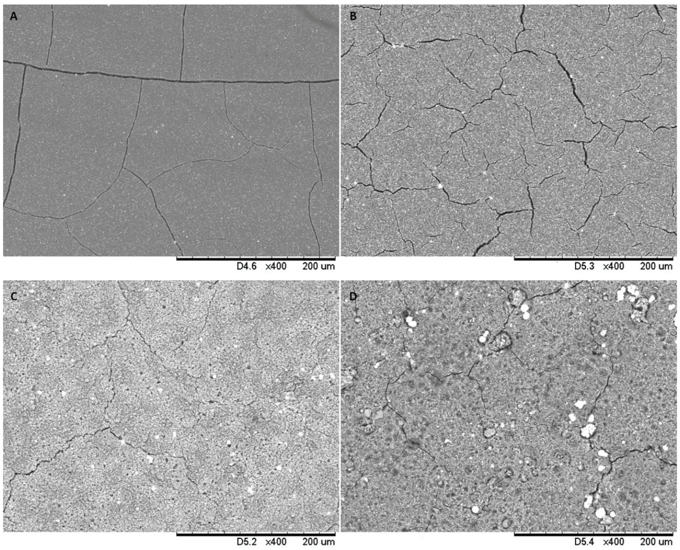

Figure 4. *Scanning electron microscopy images of Dycal Advanced Formula II corresponding to;* ***A)*** *Control subgroup,* ***B)*** *Rinsing subgroup,* ***C)*** *Polyacrylic acid subgroup and* ***D)*** *Phosphoric acid subgroup.*

Table 2. *Mean (standard deviation) of sorption in water (μg/mm³) according to calcium hydroxide cement and surface treatment performed*

	Treatment Performed			
Materials	**Control**	**Rinsing**	**Polyacrylic Acid**	**Phosphoric Acid**
Hydro C	342.4 (69.3) aAB	400.6 (83.4) aA	272.1 (30.1) bB	251.9 (32.4) cB
Ultra-blend Plus	373.1 (66.4) aB	401.2 (61.2) aB	444.8 (57.3) aAB	516.8 (47.9) aA
Dycal Advanced Formula II	427.4 (72.5) aA	433.5 (106.7) aA	288.2 (50.7) bB	367.2 (45.2) bAB

Different upper-case letters indicate significant statistical differences ($p<0.05$) between the treatments performed for the same calcium hydroxide cement. Different lowercase letters indicate significant statistical differences ($p<0.05$) between calcium hydroxide cements for the same surface treatment.

Table 3. *Mean (standard deviation) of solubility in water (μg/mm³) according to calcium hydroxide cement and surface treatment performed*

	Treatment Performed			
Materials	**Control**	**Rinsing**	**Polyacrylic Acid**	**Phosphoric Acid**
Hydro C	228.8 (42.2) aA	242.8 (37.4) aA	206.3 (28.8) aA	208.9 (24.1) aA
Ultra-blend Plus	249.8 (11.4) aA	238.9 (62) aA	307.3 (47.8) bAB	381.1 (21.5) bB
Dycal Advanced Formula II	229.4 (31.7) aA	290.7 (110) aA	226.6 (31.5) abA	225.1 (21.3) aA

Different upper-case letters indicate significant statistical differences ($p<0.05$) between the treatments performed for the same calcium hydroxide cement. Different lowercase letters indicate significant statistical differences ($p<0.05$) between calcium hydroxide cements for the same surface treatment.

treatments for the rinsing subgroup (WSp 400.6 μg/mm³). For both properties, lower WSp and WSol with a statistically significant difference between cements was observed for phosphoric and polyacrylic acid subgroups (WSp 251.9 μg/mm³ and WSol 206.3 μg/mm³).

Group B (Ultra-blend Plus) presented increasing rates of sorption and solubility values in each subgroup, presenting the lowest mean value for sorption in the control subgroup (373.1 μg/mm³) and highest in the phosphoric acid subgroup (516.8 μg/mm³). For solubility the lowest mean value was in the rinsing subgroup (238.9 μg/mm³) and highest in the phosphoric acid subgroup (381.1 μg/mm3). It is evident, for both properties, a statistically significant difference between treatments for the phosphoric acid subgroup (WSp 516.8 μg/mm³ and WSol 381.1 μg/mm³). Considering the difference between cements, a statistically significant difference was observed for polyacrylic (WSp 444.8 μg/mm³ and WSol 307.3 μg/mm³) and phosphoric acid (WSp 516.8 μg/mm³ and WSol 381.1 μg/mm³) subgroups.

Group C (Dycal Advanced Formula II) had the highest mean values of sorption and solubility for the samples of the rinsing subgroup (433.5 μg/mm³ and 290.7 μg/mm³, respectively). It was observed an important decrease in sorption values for polyacrylic acid subgroup and a decrease for phosphoric acid subgroup in comparison with control and rinsing subgroups. Otherwise for solubility values, a slight increase for rinsing subgroup and a slight decrease for acids subgroups were observed. For sorption values, there was statistically significant difference between treatments for the polyacrylic acid subgroup (WSp 288.2 μg/mm³). For both properties, average WSp and WSol with a statistically significant difference between cements was observed for phosphoric and polyacrylic acid subgroups (WSp 367.2 μg/mm³ and WSol 226.6 μg/mm³).

ROH

Considering the release of hydroxyl ions for Hydro C and Ultra-blend Plus, a statistically significant difference between treatments and control was observed from 3 h for the polyacrylic and phosphoric acid subgroups and from 12 h for the rinsing subgroup of Hydro C. On the other hand, Dycal Advanced Formula II presented a statistically significant difference between treatments and control only in the 3rd hour for the rinsing, polyacrylic and phosphoric acid subgroups (Tables 4, 5, 6).

Concerning the difference between time for the same treatment, Hydro C presented statistically significant difference between 0 h to 24 h for acids subgroups and between 0 h to 48 h for control and rinsing subgroups. Ultra-blend Plus presented statistically significant difference between 0 h to 12 h for acids subgroups, between 0 h and 12 h and 48 h for rinsing subgroup and finally, between 0 h and 12 h and 24 h for control subgroup. Dycal Advanced Formula II presented statistically significant difference between 0h and 3 h for acids subgroups, between 0 h to 12 h for rinsing subgroup and finally, between 0 h and 12 h for control subgroup (Tables 4, 5, 6).

When analyzing the pH values obtained in each measurement period, similar results for Hydro C and Dycal Advanced Formula II was noticed considering the period of higher rates of alkalinization. Concerning the first cement, it was observed the start of a higher rate of alkalinization in the period of 3 hours for the polyacrylic acid (pH 8.1) and phosphoric acid (pH 8.3) subgroups (Tables 4, 5, 6).

Regarding the second cited cement, the beginning of a higher rate of alkalinization was also set in the period of 3 hours for polyacrylic (pH 8.5) and phosphoric acid (pH 8.6) subgroups. In addition, rinsing subgroup was identified (pH 8.3) as well. Finally, for Ultra-blend, the beginning of a higher rate of alkalinization was in the period of 12 hours for rinsing (pH 8.0) and control (pH 8.1) subgroups (Tables 4, 5, 6).

Table 4. *Mean (standard deviation) of the Hydro C pH according to surface treatment and time*

	Treatment Performed			
Time	Control	Rinsing	Polyacrylic Acid	Phosphoric Acid
pH (0 h)	6.04 (0.00) Aa	6.04 (0.00) Aa	6.04 (0.00) Aa	6.04 (0.00) Aa
pH (3 h)	7.9 (0.05) Ab	7.8 (0.03) Ab	8.1 (0.04) Bb	8.3 (0.04) Cb
pH (12 h)	9.7 (0.15) Ac	9.2 (0.07) Bc	9.0 (0.04) Cc	9.1 (0.03) BCc
pH (24 h)	10.0 (0.08) Ad	9.5 (0.03) Bd	9.2 (0.08) Cd	9.3 (0.09) Cd
pH (48 h)	10.2 (0.10) Ae	9.6 (0.05) Be	9.3 (0.05) Cd	9.4 (0.09) Cd

Different upper-case letters indicate significant statistical differences ($p<0.05$) between the treatments performed for the same time. Different lowercase letters indicate significant statistical differences ($p<0.05$) between time for the same surface treatment.

Table 5. *Mean (standard deviation) of the Ultra-blend Plus pH according to surface treatment and time*

	Treatment Performed			
Tempo	Control	Rinsing	Polyacrylic Acid	Phosphoric Acid
pH (0 h)	6.04 (0.00) Aa	6.04 (0.00) Aa	6.04 (0.00) Aa	6.04 (0.00) Aa
pH (3 h)	6.0 (0.01) Aa	6.1 (0.04) Aa	6.2 (0.06) Bb	6.3 (0.04) Bb
pH (12 h)	8.1 (0.03) Ab	8.0 (0.05) ABb	7.9 (0.02) Bc	7.9 (0.02) Bc
pH (24 h)	8.4 (0.16) Ac	8.0 (0.07) Bb	7.9 (0.02) Cc	7.9 (0.02) Cc
pH (48 h)	8.5 (0.15) Ac	8.2 (0.12) Bc	8.0 (0.03) Cc	7.9 (0.03) Cc

Different upper-case letters indicate significant statistical differences ($p<0.05$) between the treatments performed for the same time. Different lowercase letters indicate significant statistical differences ($p<0.05$) between time for the same surface treatment.

Table 6. *Mean (standard deviation) of the Dycal Advanced Formula II pH according to surface treatment and time*

	Treatment Performed			
Time	Control	Rinsing	Polyacrylic Acid	Phosphoric Acid
pH (0 h)	6.04 (0.00) Aa	6.04 (0.00) Aa	6.04 (0.00) Aa	6.04 (0.00) Aa
pH (3 h)	7.0 (0.03) Aa	8.3 (0.03) Bb	8.5 (0.06) Bb	8.6 (0.09) Bb
pH (12 h)	9.2 (0.18) Ab	9.4 (0.10) Ac	9.2 (0.06) Ab	9.1 (0.07) Ab
pH (24 h)	9.3 (0.06) Ab	9.4 (0.07) Ac	9.4 (0.08) Ab	9.3 (0.08) Ab
pH (48 h)	9.4 (0.08) Ab	9.4 (0.08) Ac	9.4 (0.07) Ab	9.4 (0.07) Ab

Different upper-case letters indicate significant statistical differences ($p<0.05$) between the treatments performed for the same time. Different lowercase letters indicate significant statistical differences ($p<0.05$) between time for the same surface treatment.

Discussion

In this study, the null hypothesis that the application of phosphoric and polyacrylic acids, in addition to rinsing/drying procedures, would have no influence on the sorption and solubility properties, release of hydroxyl ions, and surface morphology of calcium hydroxide cements was rejected since it was evident that the contact with acids impacted such properties.

Usually, chemically activated CH cements are used in indirect pulp capping, which are widely studied with an emphasis on their low physical properties and high solubility in water. It is noteworthy that their stability in relation to the fundamental physical and chemical properties is of paramount importance to maintain their protective action, and they must be stable to dissolution in aqueous medium, organic solvents, and conditioning acids (4,8).

The analysis of the surface morphology indicated a greater impact of the conditioning acids on the surface of the chemically activated cements. Hydro C and Dycal Advanced Formula II presented fissures which were aggravated throughout the treatments analyzed and that may be the result of loss of some minerals (10,11) as well as the SEM's vacuum. The resin-modified material was inert to the action of the acids when under analysis at 400x magnification. The increase in porosity in the chemically activated CH can determine the behavior of the pH obtained in this study.

In the study of de Souza et al, SEM analysis of the rinsing group for both cements tested showed similar surface characteristics as the present study's control and rinsing subgroup for Hydro C and Dycal Advanced Formula II confirming that the action of conditioning acids is responsible for surface alterations in those cements (10).

The increase in the contact surface between the surface of the material and the storage medium (distilled water), represented by the increase in porosity, influences the higher capacity of release of hydroxyl ions in the initial period (3 h) for the polyacrylic acid and phosphoric acid subgroups, highlighting the need for new studies so that the behavior presented can be clearly defined. Also, the differences in the chemical composition of the cements must be taken into account, in addition to the action selectivity of the acids used.

Regarding sorption and solubility, it is known that the solubilization of these protective materials is beneficial and desirable for therapeutic action to be obtained, however, it must be controlled (4). In the present study, sorption showed a significant statistical difference between treatments in Hydro C (rinsing subgroup), Dycal Advanced Formula II (polyacrylic acid subgroup) and Ultra-blend Plus (phosphoric acid subgroup). Considering sorption and solubility, Hydro C and Dycal were affected by the acids in regards to a decrease in those values. Only Ultra-blend Plus showed an increase in sorption and solubility values among acids subgroups.

However, in a recent study, Hydro C presented higher mean of sorption compared to a resin-containing self-curing CH cement (Life - Kerr, Karlsruhe, Germany) when in contact with polyacrylic acid which was explained by the association between the type of conditioning agent and the basic composition of each cement used in the experiment (10).

Also, differently from the present study's results, Francisconi et al observed a lower sorption in water of the resin-modified CH cement (Biocal), with a percentage of 2.5% when compared to chemically activated CH cements (Dycal and Hydro C), with a percentage of 5.49% and 8.27%, respectively. A lower solubility of the resin modified cement was also found (Biocal, 0.72%) when compared to Dycal (4,21%) and Hydro C (7,25%) (8).

Biocal and Ultra-blend contain the UDMA monomer in their compositions, but Ultra-blend also has TEGDMA monomer (12). It is suggested in the literature that the copolymerization of UDMA with TEGDMA can result in a three-dimensional network configuration with more heterogeneity. When a network of polymers presents high heterogeneity, the spaces created between high and low density areas of the network are large and can accommodate a large amount of water (13).

Thus, based on the difference in the values of sorption and solubility between the cements of the present work with the study of Francisconi et al. (8), it is possible to indicate that Ultra-blend, which presents a significant amount of hydrophilic groups, would promote an absorption of water that would be retained in its network of polymers (13).

When the Ultra-blend Plus was exposed to phosphoric acid, higher sorption and solubility was observed in relation to the control group. This can be explained by the fact that the presence of the urethane monomer favors the consolidation of the hydrogen bonds, therefore impacting the sorption in water (14,15). Thus, the elevation of the sorption mean in water in these subgroups may be related to an increase in the amount of hydrogen bonding caused by the action of the acids on the surface of the material (11). However, it should be emphasized that in performing the test the cements' immersion medium exerts an influence on the solubility of these materials. It is proven that CH cements have lower solubility when immersed in dentin fluid, differently when immersed in distilled water (16).

The findings related to the release of hydroxyl ions suggest that the stability of Dycal is related to the fact that some components of the Dycal base paste, such as calcium phosphate and calcium tungstate, that are not present in Hydro C, are not influenced by the action of the acids, and therefore do not alter the hydroxyl ion (OH^-) release capacity when subjected to these conditioning agents (6). Dycal presented a high release of hydroxyl ions, with a mean hydroxyl ion release (pH) of approximately 9.4 in the 24-h period. However, this value was slightly lower when compared to the previous studies, with values around 10 and 10.90. This difference can be attributed to the different storage temperature, which was 37°C for the study in question and 25–30°C (ambient temperature) for the present study (11,17).

One study analyzed the release of hydroxyl ions (pH) from various CH-based cements, including Hydro C and Ultra-blend, at 3 h, 24 h, 72 h, and 168 h. It was observed that the Ultra-blend did not promote the release of hydroxyl ions at any moment during evaluation, obtaining the lowest pH values among the analyzed materials (3). Another study compared the release of hydroxyl ions of Hydro C and Life and all samples from both cements were able to alkalize distilled water, except for those exposed to phosphoric acid ($p<0.05$). Both cements demonstrated a low release of hydroxyl ions ability, with an average pH 7.2 (10).

In the present study, Ultra-blend, independent of the subgroups, was able to release hydroxyl ions, obtaining a higher pH value than distilled water. However, this value was low when compared to other cements. Analyzing the Ultra-blend Plus alone, it was observed that the worst release of hydroxyl ions was when this material was exposed to acids. These facts suggest the existence of some substance in its composition that prevents the release of hydroxyl ions, especially after contact with the conditioning acids.

These findings are worrying since the determinant factor for the protective action of CH cements originates from their ionic dissociation when in aqueous medium, promoting the release of hydroxyl (OH^-) and calcium (Ca^{2+}) ions. The OH ions determine the alkalinity characteristic of these materials, in addition to acting as bacterial enzymatic inhibitors and causing damage to the bacterial cytoplasmic membrane, factors that motivate its antimicrobial action (18).

Although it has a protective character, it should be emphasized that CH-based materials do not act as biostimulants. The cells in contact with the CH undergo necrosis due to their high pH, forming a layer called a zone of cauterization. The pulp tissue adjacent to this layer is responsible for pulp healing and is associated with the formation of a hard tissue barrier (7,19). Thus, regardless of the treatment applied, the low release of hydroxyl ions of Ultra-blend Plus, when exposed to acids can generate a lower necrotic layer, casting doubt on the biological and therapeutic effects of these materials under the mentioned conditions, and thus making it essential to conduct further studies to confirm these findings (20).

Despite this is an *in vitro* study that evaluated the effect of the exposure of conditioning acids to chemically activated and resin-modified calcium hydroxide cements, there are no studies in the literature that makes the same comparison. Therefore, and also considering this study's limited design, it is important to carry out new laboratory studies to evaluate the action of these agents on the properties of these cements and clinical trials that evaluate the in vivo repercussion on the protective capacity of calcium hydroxide-based materials after exposure to the conditioning acids.

Conclusion

The analysis of the surface morphology, Ultra-blend Plus was stable to the action of the conditioning acids. On the other hand, Hydro C and Dycal presented intensification of surface irregularities after contact with phosphoric acid. The chemically activated materials suffered a decrease in sorption and solubility properties presenting an inverse behavior compared to the resin-modified material. The analysis of the release of hydroxyl ions has shown that the action of the conditioning acids promotes greater increase of the release of hydroxyl ions for the chemically activated cements but lower increase for Ultra-blend Plus. The action of the conditioning acids intensified the alkalization from 3 hours onwards for Hydro C and Dycal Advanced Formula II in all subgroups. However, Ultra-blend Plus had intensified its alkalinization from 12 hours onwards.

Türkçe Özet: *Kalsiyum hidroksit simanların poliakrilik ve fosforik asitlerle temasının mikroskopik ve fiziksel/kimyasal özellikleri üzerine etkileri. Amaç: Bu çalışma kalsiyum hidroksit simanların, poliakrilik ve fosforik asitlerle teması halinde, mikroskobik, fiziksel ve kimyasal özelliklerini değiştirip değiştirmeyeceğini değerlendirmeyi amaçlamaktadır. Gereç ve Yöntem: Kimyasal aktivasyonlu (Hydro C ve Dycal Advanced Formula II) ve rezin modifiye ışık aktivasyonlu (Ultra-blend Plus) kalsiyum hidroksit simanlar farklı stratejilere maruz bırakıldıktan sonra değerlendirilmiştir. Bu stratejiler şu şekilde belirlenmiştir: Hiçbir madde ile temas halinde olmama (kontrol grubu); su ile durulama ve kurutma; poliakrilik asit ile temasın ardından su ile durulama ve kurutma; fosforik asit ile temasın ardından su ile durulama ve kurutma. Taramalı Elekron Mikroskopisi (SEM) ile görüntülenen yüzey morfolojisi, su emilimi ve çözünürlük, hidroksil iyon salınımı değerlendirilmiştir. Bulgular: SEM sonuçları, yüzey düzenleyici asitlerin kimyasal aktivasyonlu simanların yüzeyinde daha büyük bir etki yarattığını göstermiştir. Ultra-blend Plus en yüksek değerdeki emilimi (516.8 $\mu g/mm^3$) ve çözünürlüğü (381.1 $\mu g/mm^3$) sağlarken Hydro C ise en düşük değerleri, 251.9 $\mu g/mm^3$ ve 206.3 $\mu g/mm^3$, sergilemiştir. Hidroksil iyonlarının zamana kıyasla salınımı göz önüne alındığında Hydro C ve Ultra-blend Plus, poliakrilik ve fosforik asit alt gruplarına göre önemli istatistiksel farklılıklar göstermiştir. Sonuç: Hydro C ve Dycal materyallerinin yüzey düzenleyici asitler ile temaslarından sonra yüzey düzensizliklerinin arttığı belirlenmiştir. Kimyasal aktivasyonlu bu materyallerin emilim ve çözünürlük değerlerinde düşüş saptanmıştır. Yüzey düzenleyici asitler, Hydro C ve Dycal'ın hidroksil iyon salınımı değerlerinin daha fazla yükselmesine katkıda bulunmuştur. Anahtar Kelimeler: Kalsiyum hidroksit, hidrojen iyon konsantrasyonu, çözünürlük, yüzey morfolojisi; su emilimi*

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Author contributions: RBS,CPL, IÁF, and BCDB designed the study. IDTA, RBS and CPL participated in generating the data for the study. RBS and CPL participated in gathering the data for the study. IDTA, RBS,CPL, IÁF, and BCDB participated in the analysis of the data. RBS and CPL wrote the majority of the original draft of the paper. IDTA, RBS,CPL, IÁF, and BCDB participated in writing the paper. All authors approved the final version of this paper.

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Original research

The level of two trace elements in carious, non-carious, primary, and permanent teeth*

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Purpose

The boron and fluoride mainly accumulate in the bones and teeth of the human body. The purpose of this study is to determine boron or fluoride levels in the whole tooth, to evaluate the correlation between their levels and to compare these levels in primary/permanent, carious, and non-carious groups.

Materials and Methods

The boron and fluoride levels of thirty-six teeth, separated such as primary carious (n=9) and non-carious (n=9), permanent carious (n=9) and non-carious (n=9), were determined by ICP-MS and ion-selective electrode, respectively.

Results

While boron levels were between 0.001 and 5.88 ppm, the fluoride levels were between 21.24 and 449.22 ppm. The boron level of non-carious teeth was higher than those of carious teeth in primary and permanent tooth groups. However, this difference was not statistically significant ($p>0.05$). The fluoride level of non-carious teeth was higher than those of carious teeth in primary ($p=0.062$) and permanent teeth groups ($p=0.046$). Negative correlation, found between boron and fluoride in all groups, was significant only in non-carious teeth group ($r=-0.488$, $p=0.040$).

Conclusion

The results of our study proved the importance of fluoride as a protective factor for dental caries once more. The boron levels in non-carious teeth were also higher than carious teeth. However, it was not significant. Moreover, there was negative correlation between teeth boron and fluoride levels. Therefore, it is necessary to conduct more detailed studies on the tooth boron level and its relation with caries formation and with fluoride levels.

Keywords: *Boron, Fluoride, Teeth, Trace elements, Dental health*

Introduction

Dental caries, the most common oral disease and the major cause of tooth loss, are also considered as the third among non-communicable disease that endangers human health (1). Studies have shown that some of the trace elements such as F, Al, Fe, Se, Sr, Mn, Cu, and Cd are closely related to dental caries; some prevent dental caries while others accelerate dental caries (2). Amount of trace elements in teeth can provide information on environmental factors, eating habits, and oral health (3).

Boron is a trace element found in a daily diet and it is as a potentially essential element for humans (4-8). It is known that distributed throughout the human body with the highest concentration in the bones and teeth (9,10). It has been shown in various studies that boron plays important roles, especially in mineral metabolism and bone development (11,12). It has been shown that boric acid reduced alveolar bone loss in

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rats with experimental periodontitis and osteoporosis (13). In a study performed with pre-osteoblastic cells, boron has been found to affect mRNA expression of collagen-I, bone sialoproteins, osteocalcin, osteopontin, and extracellular matrix proteins (14). Recent studies on the development of boron-containing dental composites due to their antibacterial properties are being carried out in order to prevent secondary caries formation (15). However, in the literature, it is unclear whether the boron has a cariogenic, anticariogenic or cariostatic effect on the teeth. Moreover, studies on the tooth boron level are very limited (3,16).

Fluoride is found mostly in bones and teeth because of its affinity to calcium. It prevents caries in adults and children by making the external surface of teeth more durable to the acid attacks (17). However, it is also associated with dental fluorosis and if consumed in excessively, it has potential health risks such as bone fragility. Fluoride can be incorporated into the structure of the teeth by means of nutrients and drinking water as well as by the use of agents such as toothpaste, mouthwashes (18).

This study is important in terms of being the first study that determines boron levels of human teeth in Turkey. The purpose of this study is to determine boron or fluoride levels in the whole tooth, to evaluate the correlation between their levels and to compare these levels in primary/permanent, carious, and non-carious groups.

Materials and Methods

Samples

Thirty-six teeth of thirty-six patients, who admitted to the Marmara University Faculty of Dentistry Department of Oral and Maxillofacial Surgery between May 2017 and July 2017, were included in this study. The non-carious permanent teeth were extracted for orthodontic purposes or because of periodontal diseases, the non-carious primary teeth were extracted due to delayed physiologic root resorption. The carious primary and permanent teeth were extracted because of excessive caries. The collected thirty-six teeth were divided mainly into two groups such as primary (n=18) and permanent (n=18). Each group has two sub-groups as carious (n=9) and non-carious (n=9) teeth. Enamel, dentin, and other parts of teeth were not separated and the decayed tissue of carious teeth was not removed. The whole tooth was used for boron and fluoride determinations. This study has been reviewed and approved by the Ethical Committee of Marmara University Health Sciences Institute (03.04.2017-108). Informed consent was obtained from all individual participants included in this study.

ICP-MS method for the boron assay

Each whole tooth was first washed and cleaned in the saline solution (0.9% NaCl). Then they were ground and weighed. Five mL of concentrated HCl (Merck, Darmstadt, Germany) per gram of tooth was used to dissolve them and they were filtered later by using the syringe filter (0.2 µm). One mL of them was taken into the falcon tube. Then, it was diluted to 5 mL with ultrapure water. Boron levels were determined in all diluted samples by using an inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Scientific X Series 2, nebulizer gas, 1.2/min; cooling gas, 13/min; power, 1051 W; auxiliary gas, 0.9/min). Calibration solutions (Chem Lab, Zedelgem, Belgium) at different boron concentrations as 0.002, 0.02, 0.2, 2, 20, 200, 2000 and 20000 ppb were used in the experiment.

Ion-selective electrode method for the assay of fluoride levels

Sodium acetate solution (15 %, 900 µL) was added to increase the pH of each 100 µL of dissolved tooth samples in concentrated HCl. Then, to adjust the total ionic strength, 900 µL of this was taken and 100 µL of TISAB-III buffer solution (Orion 940911) was added. The fluoride level of these tooth solutions was then determined using an ion-selective electrode (Orion-96-09). The electrode was calibrated with six standard fluoride solutions ranging from 1 to 10^6 µM. Electrode potentials of standard solutions are measured and plotted on the linear axis against their concentrations on the log axis. The fluoride levels of the tooth solutions were calculated using the slope of the calibration curve.

Statistical analysis

Statistical Package for the Social Sciences for Windows software, version 24.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. The Shapiro-Wilk test was used to evaluate the normal distribution of continuous variables. Student t-test was used for two independent group comparisons for normally distributed variables which were given as mean ± standard deviation. Mann-Whitney U test was used for two independent group comparisons for non-normally distributed variables which were given as median values. Correlation of normally and non-normally distributed variables were used Pearson and Spearman correlation, respectively. The confidence interval was set to 95% and $p < 0.05$ was considered statistically significant.

Results

According to the results of boron analysis, the average boron level of all teeth (n=36) was 0.63 ± 1.19 ppm. The boron level of non-carious teeth was higher than those of carious teeth in primary and permanent tooth groups. However, these differences were not statistically significant (p>0.5) (Fig. 1). As seen in Figure 1, boron levels of primary teeth were higher than those of permanent teeth (p<0.001).

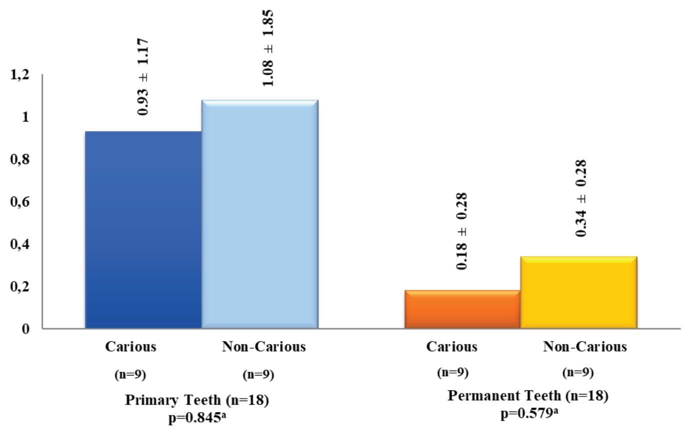

Figure 1. *Boron levels (ppm) of teeth.*
Values are given as Mean ± SD. SD: Standard Deviation. a: t-Test

The fluoride level of all teeth was 93.05 ± 82.00 ppm (n=36) and it was higher than that of the boron level. The fluoride level of non-carious teeth was higher than those of carious teeth in primary (p=0.062) and permanent teeth groups (p= 0.046) (Fig.2). As seen in Figure 2, fluoride levels of primary teeth were lower than those of permanent teeth in contrast to boron results.

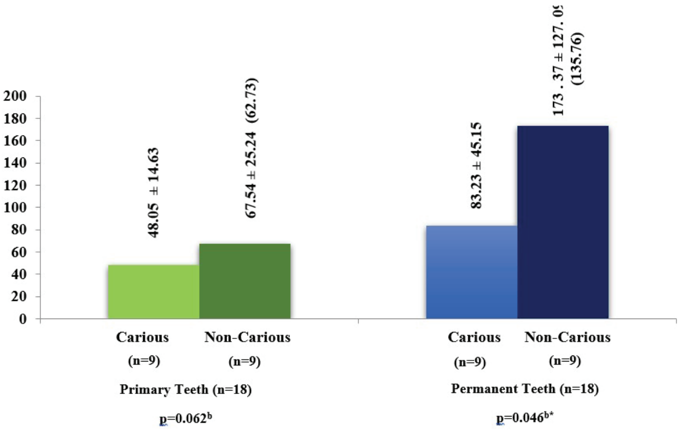

Figure 2. *Fluoride levels (ppm) of teeth.*
Values are given as Mean ± SD, SD: Standard Deviation. Median values are given in parentheses. b: Mann-Whitney U Test

Negative correlation, found between boron and fluoride in all groups, also in all teeth, was significant only in non-carious teeth group (r = -0.488, p = 0.040).

Discussion

The effects of trace elements on the protection of oral health are very controversial issue. Trace elements of teeth have been investigated for various reasons, for example, there are some dental health studies where trace element concentrations have been correlated with dental caries such as Si, Se, Cd, and Pt (19). As mentioned above, studies on the evaluation of teeth boron levels and their relationship with caries are very limited in the literature. Its reason is the difficulty in standardizing environmental factors with other elements. In various studies, the levels of some trace elements in the whole tooth were determined (20-22). However, to our knowledge, there is one study to determine the levels of boron and fluoride in the whole tooth (23). Therefore, it is very difficult to compare our studies with the literature.

Similar to the present study, Riat and Sharma (23) determined boron levels in the whole teeth of 15 healthy and 15 carious permanent teeth. The reason for using the whole tooth instead of tooth parts such as enamel or dentine was explained by that tooth cutting procedure causes positive contamination with trace elements. In contrast to our study, which ICP-MS was used, the ICP-Atomic Emission Spectroscopy has been used and found that boron levels measured in carious teeth (49.85 ± 18.15 ppm) were significantly higher than those of non-carious teeth (39.05 ± 8.19 ppm). Moreover, teeth boron levels have been found higher than ours. The reason of this may be due to the differences in methods used for boron determination; sample size differences; the differences in the causes of caries formation; differences due to individuals and environmental; and geographical differences.

In another study, dental enamel boron concentration has been determined by the ICP-Atomic Absorption Spectroscopy method and found that the amount of boron in the healthy permanent teeth was higher than that of the healthy primary teeth (24). Kumagai *et al*. (25) determined the boron level in the dentin of 121 healthy permanent teeth as about 1.63 ppm by the ICP-MS method.

In a healthy adult, dentin is composed by approximately 45% mineral, 33% organic matrix, and %10 water by volume. Enamel is composed by about 87% mineral by volume. The most mineralized part of the tooth is enamel, this is followed by dentin (26). Therefore, when the whole tooth is examined, it may be correct that we find it lower.

It has been suggested that fluoride could prevent caries in permanent and primary teeth (24,27.28). Our results support this suggestion. Because the fluoride level in non-carious teeth was found to be higher than that of carious teeth in primary or permanent groups. Although it was non-significant, boron levels in non-carious teeth were also higher than those of carious teeth. This may show their cariostatic effect while boron and fluoride are together. However, negative correlation was found between fluoride and boron levels in all teeth and groups. The significant negative correlation was present only in non-carious teeth group. In Lius' study (29), when boron and fluoride are added together to drinking water in rats fed a cariogenic diet, boron has been shown to reduce the caries protection effect of fluoride. It has been suggested that boron may inhibit the fluoride absorption from the gastrointestinal system.

Conclusion

The results of our study also proved the importance of fluoride as a protective factor for dental caries once more. Although not statistically significant, our results showed that non-carious teeth have contained more boron than carious teeth. Moreover, there was negative correlation between teeth boron and fluoride levels. The further detailed studies about tooth boron level and its relation with caries formation and with fluoride levels should be done using large samples, comparing different geographic areas. It is also necessary to identify the boron and fluoride level in water or food samples and their cariogenic effects.

Türkçe Özet: *Çürüklü, çürüksüz süt ve daimi dişlerde iki eser elementin düzeyleri. Amaç: Bor ve florür, insan vücudunda başlıca kemik ve dişlerde birikir. Bu çalışmanın amacı, dişin tamamında bor ve florür düzeylerini belirlemek, bu düzeyler arasındaki korelasyonu değerlendirmek ve çürüklü, çürüksüz süt ve daimi dişlerde karşılaştırmaktı. Gereç ve Yöntem: Çürüklü süt dişler (n = 9) ve çürüksüz süt dişler (n = 9) ile çürüklü daimi dişler (n =9) ve çürüksüz daimi dişler (n = 9) olarak ayrılan otuz altı dişin bor ve florür seviyeleri sırasıyla ICP-MS ve iyon seçici elektrot ile belirlendi. Bulgular: Bor seviyeleri 0.001 ile 5.88 ppm arasında iken, florür seviyeleri 21.24 ile 449.22 ppm arasındaydı. Süt ve daimi dişlerde, çürüksüz dişlerin bor düzeyleri çürüklü dişlerden daha yüksekti. Ancak bu fark istatistiksel olarak anlamlı değil (p>0.05). Süt dişlerinde (p = 0.062) ve daimi dişlerde (p = 0.046), çürüksüz dişlerin florür düzeyleri çürüklü dişlerden daha yüksekti. Tüm gruplarda bor ve florür düzeyleri arasında bulunan negatif korelasyon sadece çürüksüz olmayan diş grubunda anlamlıydı (r = - 0.488, p = 0.040). Sonuç: Çalışmamızın sonuçları, diş çürüğü için koruyucu faktör olan florürün önemini bir kez daha kanıtlamıştır. Çürüksüz dişlerdeki bor seviyeleri çürüklü dişlerden daha yüksekti. Ancak, anlamlı değildi. Hatta diş bor ve florür düzeyleri arasında negatif korelasyon vardı. Bu nedenle, diş bor düzeyi, çürük oluşumu ve florür düzeyi ile ilişkisi hakkında daha ayrıntılı çalışmalar yapmak gerekmektedir. Anahtar Kelimeler: Bor; florür; diş; eser elementler; diş sağlığı*

Ethics Committee Approval: This study has been reviewed and approved by the Ethical Committee of Marmara University Health Sciences Institute (03.04.2017-108).

Informed Consent: Informed consent was obtained from all individual participants included in this study.

Peer-review: Externally peer-reviewed.

Author contributions: SA, AY and FS designed the study. RK, GB, SY and PNT participated in generating the data for the study. RK, GB, SY and PNT participated in gathering the data for the study. SA, AY and FS participated in the analysis of the data. RK and AY wrote the majority of the original draft of the paper. RK and AY participated in writing the paper. All authors approved the final version of this paper.

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Original research

Use of a new skin colour measurement method for the investigation of relationship between skin and tooth colour

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Purpose
There is conflicting information about the relationship between tooth color and skin color in the literature. The aim of the present study was evaluation of the correlation between L, a, b values of skin and tooth shade using a new skin color measurement method.

Materials and Methods
CIELab values of teeth were obtained through measurements from the middle third of the labial surfaces on central incisors of individuals using a clinical spectrophotometer. CIELab values of the skin were measured through facial images using a software which was manufactured for present study. A statistical analysis program (SAS 9.4) was used for the analysis of the data. Kolmogorov-Smirnov test, t-test, and multivariate regression analysis were used to evaluate the data (α=0.05).

Results
Correlations between variables revealed that while the lowest and statistically insignificant correlations were observed with a* values of the skin, significant correlations did not exceed moderate level (p<.05). When considering regression analysis results, b* values of the skin had a statistically significant effect in describing b* values of the tooth, while L* and a* values of the skin were observed to be insufficient in describing L* and a* values of the teeth.

Conclusion
Results of this study suggest that different significant correlations were observed between the skin and tooth color for different L*, a*, and b parameters (p<0.05 and p<0.01). The results indicated that skin color can be used for tooth color selection in case of loss of natural teeth or when discoloration is present on existing teeth.

Keywords: *Shade selection, Tooth shade, Skin colour, CIELab, Esthetics*

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Introduction

In prosthetic dentistry, esthetics of final restoration is one of the most important factors influencing the success of therapy. Dental treatment should be in harmony with the rest of the face, and a life-like appearance which is acceptable by the patient should be aimed.[1,2] Selection of the correct tooth shade is one of the leading factors for an acceptable esthetics. Presence of teeth mostly provides convenience in selecting tooth shade. However, internal and external factors may influence the tooth shade; teeth may not have a natural appearance, and therefore, it may be hard to identify color using present teeth.[3,4] Selection of tooth shade may become even more difficult for the clinicians when there is no tooth to be used as reference. In these situations, selection of tooth shade may more subjective, and accordingly, some methods to overcome this issue were studied in the past and published.[5,6] Other characteristics of the patients

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can be utilized for tooth shade selection in these situations. Some previous studies have reported that individual's eye, skin, hair color, and factors like sex and age can be used when selecting the tooth shade.[5-7]

Some studies has indicated that tooth shade of women is lighter than men [8-10]. Some others reported that teeth with darker shade should be preferred for elderly individuals.[11,12] A correlation was determined between hair color and tooth shade even if it was poor.[7] While a significant correlation was observed in some studies investigating the correlation between the skin color and tooth shade, some studies reported no correlation.[5,7,10]

Different methods are used to determine the skin or tooth shade. While some researchers preferred using shade guides and various classifications providing more subjective data, and some others used computer programs and digital instruments to determine the color.[11,13-16] The methods used in studies on a delicate issue like colorimetry are critical in terms of obtaining true and precise results. Digital methods were used to obtain the values that belong to both skin and teeth, however, a well-accepted method for skin color measurement has not been reported in the literature previously.

The aim of this study was to investigate any correlation between L*, a*, b* values of skin and tooth shade considering the gender using a novel color measurement technique. The null hypothesis was that the tooth and skin color would not be in a correlation.

Materials and Methods

This study was conducted on 149 dental students (67 male, 82 female, age range 18-23 and mean age of 20.8 ± 2). Ethics committee approval and informed consent of all volunteers were received for the study (Decision Number: 70904504/335).

Individuals who had completely erupted maxillary right central incisors without decay and restoration, no skin disorder, and no postoperative facial cicatrix and individuals who did not undergo any color changing procedure on the face or skin were included in the study. Individuals who had restored teeth, teeth undergoing root canal therapy, teeth with internal or external discoloration, and who were smokers were excluded from the study.

Recommendations from previous studies were followed to design the image capturing technique for teeth and face in this study.[14,17,18] Two weeks before the shade measurements of teeth, the test teeth were cleaned with polishing brush and polishing paste attached to a low-speed hand piece. Measurements were made from the middle third of labial surfaces of right maxillary central incisors of individuals using a clinical spectrophotometer (VITA Easyshade Advance, VITA Zahnfabrik) and according to the instructions of the manufacturer. CIE L*a*b* values were recorded.

Facial images were taken[14,18] A digital camera (Nikon D5200) with 24.2 megapixel resolution was used in the present study. The participants were asked to wash their faces gently and remove their make up before their photographs were taken. They were kept in an environment with normal room temperature for 20 minutes before the photographs were taken. Camera adjustments included a macrolens with focal length ranging from 90 to 120 mm, shutter speed of 1/50, and lens aperture of f/5. Soft box light sources were used for illumination and an opaque white background was used. External light sources and ambient conditions were kept constant for all individuals. Before photographs were taken, eyeglasses, jewellery, and hat were removed. Images of frontal appearances were taken by ensuring individuals to pose when Frankfurt horizontal plane was parallel to the ground and midsagittal plane was perpendicular to the ground.

CIELab values of the skin were measured through facial images by using a software which was specially manufactured for present study. The software automatically selects three zones on the face including frontal, right, and left malar areas, and the measurement of excessive changes of color (rashes, moles, etc.) on the skin is also automatically excluded through a button in the interface of the software (Figure 1). Average of CIE L*,a*, and b* values were taken automatically from approximately 100.000 separate points in total in selected zones, excluding blotchy areas. Using this method, it was aimed to prevent zonal skin color changes to deviate the results of the measurements. SkinL, Skina, Skinb and ToothL, Tootha and Toothb were the codes to represent the L*, a* and b* values of skin and teeth.

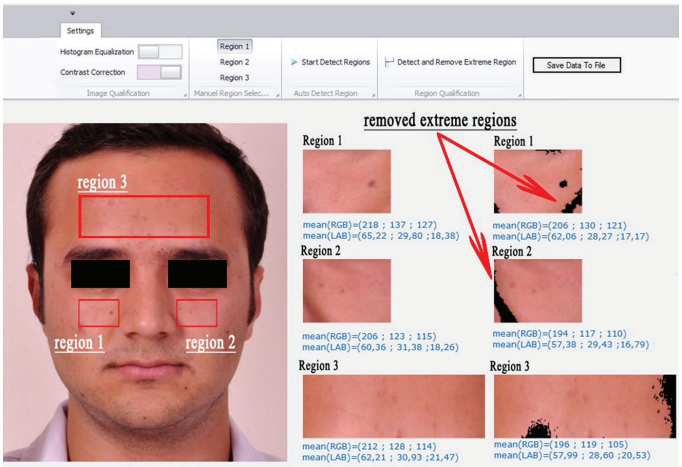

Figure 1. *Use of the computer software to obtain CIELab values of skin color.*

Statictical analysis

Descriptive statistics and t-tests were used for L*, a*, and, b* parameters of the skin and tooth color, and regression and correlation analysis were performed to reveal any correlation between them. Regression analysis was performed between the components (coordinates) which define the same properties of the color of the skin and the teeth. Regression analysis was also performed when values which define different chromatic properties were highly correlated A statistical software was used for all analyzes (SAS 9.4).

Results

Table 1 displays overall data of dependent and independent variables of individuals, descriptive statistics for male and females, and the results of t-test. According to these results, significant differences were observed with respect to gender between mean values of skinL, skinA, skinB, and toothA ($p<0,01$).

Table 2 displays the correlation between dependent (toothL, toothA, and toothB) and independent (skinL, skinA, and skinB) variables for males and females together, males, and females. There was high, moderate, no correlation between values of varying components. While the toothL component does not correlate with any other component, the toothA component was moderately correlated with the skinB component in for only males and for both genders together situation. ($p<.05$). In addition, the toothA component had a correlation with the skinB component for males ($p<.05$).

A high correlation was observed between the toothB component and the skinL component in all three cases of gender ($p<.01$). While the toothb component for females correlated moderately with the skinb component ($p<.05$), a high correlation was observed between these components for males and for both genders situation ($p<0.01$).

Table 3 displays the regression analysis results between each of the dependent variables of tooth (toothL, toothA, and toothB) and corresponding independent variables of skin. Considering these results, skinB had a statistically significant effect in describing toothB ($p<0.01$) , while skinL and skinA were observed to be insufficient in describing toothL and toothA ($p>.05$). This was associated with the fact that there was scarcely any correlation between skinL and toothL and skinA and toothA. The highest regression value was observed between the toothB and skinL parameters.

Discussion

Tooth shade is a complex phenomenon directly associated with the esthetics of the individual and involving subjective and objective factors. There is no sufficient scientific information about the correlation between tooth and skin color, information or reports in agreement. Some researchers stated that there was a reverse correlation between tooth and skin color; whereas, some others indicated a linear correlation. In the present study, it was observed that while a linear correlation was observed between some parameters of color, reverse correlation was observed in other parameters. There was a linear and significant correlation between b* values of the skin and the tooth. This result is similar to the result by Haralur et al.[14] In contrast with this result, a powerful reverse correlation was observed between L* values of skin and b* values of tooth. This is in line with the results by Jahangiri et al.[19] and N'Guessan et al.,[20] who found a reverse correlation between the tooth and skin color. Some researchers reported that there was no correlation between skin color and tooth color.[8,10,21] The difference in

Table 1. *Descriptive statistics and t-test results of variables of skin and tooth (*p<0.05, **p<0.01)*

	Male					Female					Male and Female					
	n	Mean	Ss.	Min.	Max	n	Mean	Ss.	Min.	Max	n	Mean	Ss.	Min.	Max	P
skinL	67	56.103	3.762	46.293	67.493	82	59.447	4.790	48.163	73.867	149	57.943	4.653	46.293	73.867	0.000**
skinA	67	22.250	2.202	17.143	26.963	82	21.291	1.985	16.213	25.287	149	21.722	2.133	16.213	26.963	0.006**
skinB	67	15.327	2.617	8.820	21.817	82	14.220	2.662	5.647	20.511	149	14.718	2.691	5.647	21.817	0.012*
toothL	67	84.369	3.387	71.667	90.200	82	85.124	2.490	80.100	91.133	149	84.785	2.941	71.667	91.133	0.119
toothA	67	-0.562	0.709	-1.867	1.500	82	-0.869	0.679	-2.300	0.767	149	-0.731	0.708	-2.300	1.500	0.008**
toothB	67	19.503	3.525	10.700	27.100	82	18.344	4.196	5.233	26.833	149	18.866	3.938	5.233	27.100	0.074

Table 2. *Correlations between dependent (toothL, toothA, and toothB) and independent (skinL, skinA, and skinB) variables (*p<0.05, **p<0.01)*

	Male and Female		
	skinL	skinA	skinB
toothL	0.00515	-0.06765	-0.04012
toothA	-0.17089*	0.04513	0.08258
toothB	-0.28683**	-0.06146	0.25561**
	Male		
toothL	-0.10784	-0.05953	0.05026
toothA	-0.26698*	-0.07481	0.16683*
toothB	-0.31778**	-0.09817	0.29397**
	Female		
toothL	0.00829	-0.01698	-0.08488
toothA	0.00594	0.06360	-0.06620
toothB	-0.21927**	-0.09944	0.19292*

Table 3. *Results of regression analysis between each of dependent variables of tooth (toothL, toothA, and toothB) and corresponding independent variables*

	Parameter Estimation	Standard error	t value	p
Dependent toothL				
Intercept	84.5963	3.03048	27.92	0.0001
skinL	0.0032	0.05213	0.06	0.9503
Dependent toothA				
Intercept	-1.05606	0.59664	-1.77000	0.07880
skinA	0.01497	0.02734	0.55000	0.58470
Dependent toothB				
Intercept	13.3588	1.74611	7.65000	0.000
skinB	0.3742	0.11672	3.21000	0.00170
Dependent toothL				
Intercept	32,9317	3,8871	8,4718	0,000
SkinL	-0,2427	0,0668	-3,6301	0,000

those results might be associated with varying methods used in different studies. In several previous studies, color records were visually made using shade guides or various classifications for skin and tooth color selection.[11,15,16] In visual method, skin color or tooth shade is generally categorized. Color selection depends on subjective assessment of researcher and ambient light may directly influence the type of color selection. These limitations in visual color selection might have affected the these study results. In the present study, digital methods were preferred when measuring both tooth and skin color, and the effect of light was minimized by eliminating the subjective factors. Haralur et al.[14] used a spectrophotometer, which is not affected from external lights when measuring tooth color and facial images when measuring the color skin. Seck et al.,[13] on the other hand, used facial images in both processes. Researchers compared the results by taking color value from a few points determined on the face in photographs. When human skin was examined closely, excessive color changes were observed on small areas. Therefore, it is possible to obtain different CIE Lab values on every pixel where color sample is taken. In order to avoid this problem, a new software was used when obtaining facial color on the images in the present study. The software gives the average of L*, a* and b* values taken from approximately 100.000 points by excluding severe color changes detected on the face from the measurement.

While the t-test results obtained in our study revealed a significant difference for gender in terms of mean values, no highly significant differences were found in the correlation analysis between teeth and skin color. When the results of tooth-skin correlation of male, female, male and female were examined separately, it was observed that the significances were similar (Table 2). There was no effect of gender found in describing tooth color and skin color correlation.

When the results from the regression analysis were examined, b* values of skin had a statistically significant effect in explaining the b* values of tooth. The highest correlation was observed between the b* values of the tooth and the L* values of the skin, which define the different characteristics of the color (Table 2). This was confirmed by the results of the regression analysis (Table 3). These findings indicate that skin color data which were digitally obtained are likely to be used for estimating tooth shade through various mathematical equations.

In the present study, skin color measurements were performed on digital images obtained by standardizing the lighting conditions. The use of standard light is one of the main limitations of the current and similar previous studies.[13,14] However, in this study, color determination from approximately 100,000 different points and an average CIELab value is remarkable for skin color standardization. Further studies should investigate whether different lighting conditions have an impact on the relationship between skin color and tooth color.

This study focused on potential correlations between the color components of skin and teeth. The accuracy of the system will be evaluated and reported in future studies. The results obtained in the present study may help researchers to have a better understanding about the correlation between tooth and skin color. Xiao et al. described a new software process for measuring skin color.[22] In this respect, the results of this study should be interpreted considering that a new, specifically manufactured software was used and different software may lead to different results. Further studies are needed to evaluate the effect of age on the correlation between tooth and skin color.

Conclusion

Within the limitations of the present study, different significant correlations were observed between the skin and tooth color for different CIELab parameters. When the color components of the skin and teeth are evaluated separately, the b* values of the teeth and the L* and b* values of the skin are highly correlated. The results indicated that some CIELab parameters of the skin color may be used for tooth color selection in case of loss of natural teeth or when existing teeth are discolored.

Türkçe Özet: *Cilt ve diş rengi arasındaki ilişkinin araştırılmasında yeni bir cilt rengi ölçüm yönteminin kullanılması. Amaç: Literatürde diş rengi ile cilt rengi arasındaki ilişki hakkında çelişkili bilgiler vardır. Bu çalışmanın amacı, cilt ve dişlere ait L, a, b değerleri arasındaki ilişkinin yeni bir cilt rengi ölçüm yöntemi kullanılarak değerlendirilmesidir. Gereç ve Yöntem: Dişlere ait CIELab değerleri, klinik spektrofotometre kullanılarak bireylerin orta keser dişlerinin labial orta üçlüsünden yapılan ölçümlerle elde edildi. Cilde ait CIELab değerleri, bu çalışma için özel olarak üretilmiş bir yazılım kullanılarak bireylerin fotoğrafları üzerinden ölçüldü. Verilerin analizi için bir istatistiksel analiz programı (SAS 9.4) kullanıldı. Verilerin değerlendirilmesinde Kolmogorov-Smirnow testi, t-testi ve çok değişkenli regresyon analizi kullanıldı (α = 0.05). Bulgular: Değişkenler arası korelasyonlar deri rengine ait a* değerleri için düşük ve istatistiksel olarak anlamlı değilken, diğer değişkenler arası görülen anlamlı olan korelasyonların ise orta düzeyi geçmediği gözlendi (p<.05). Regresyon analizi sonuçları dikkate alındığında, cilde ait b* değerleri ile dişlere ait b* değerleri istatistiksel olarak anlamlı düzeyde tanımlarken, cilde ait L* ve a * değerlerinin dişlere ait L* ve a * değerlerinin tanımlanmasında yetersiz olduğu gözlendi. Sonuç: Bu çalışmanın sonuçları, L*, a* ve b* parametreleri için cilt ve diş rengi arasında farklı korelasyonların bulunduğunu göstermiştir (p<0.05 ve p<0.01). Sonuçlar, doğal dişlerin tamamen kaybedilmesi veya mevcut dişlerde renk değişikliği olması durumunda, cilt renginin diş rengi seçimi için kullanılabileceğini göstermektedir. Anahtar Kelimeler: renk seçimi, diş rengi, ten rengi, CIELab, estetik*

Ethics Committee Approval: Ethics committee approval and informed consent of all volunteers were received for the study (Decision Number: 70904504/335).

Informed Consent: The informed consents were provided by the participants.

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Author contributions: NT, USB, IK, and BY designed the study. NT and USB participated in generating the data for the study. NT participated in gathering the data for the study. NT, USB, IK, and BY participated in the analysis of the data. NT and USB wrote the majority of the original draft of the paper. NT, USB, IK, and BY participated in writing the paper. All authors approved the final version of this paper.

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Knowledge, attitude and practice of dentists in Coronavirus disease 2019 pandemic in Turkey

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Purpose
The aim was to assess the knowledge, attitudes and anxiety of the dentists in relation to COVID-19.

Materials and Methods
This cross-sectional survey was conducted amongst 590 dentists employed in Turkey from March to April 2020. The electronic survey consisted of 22 questions related to demographic profile, knowledge, clinical practice and anxiety level of the participants towards dental management of COVID-19. The survey was sent to participants by email and mobile phone messages to be filled electronically.

Results
Majority of respondents pointed to aerosol (98.5%), saliva (90.6%) and mucosal contact (71.4%) as the transmission routes of COVID-19. Amongst all clinical symptoms of the disease questioned, fever (99.8%), dry cough (99.1%), fatigue (90.0%) were associated with COVID-19 by vast majority of population. 96.4% of respondents agreed the usage of gloves as personal protective equipment and it was followed by face shield (88.1%), disposable gown (83.4%), protective goggles (73.9%). Increased usage of FFP2/N95 (33.9%) and FFP3/N99 (10.9%) respirators, contrary to reduction of surgical mask usage (50.7%), were observed in aerosol-generating procedures comparing to dental procedures without producing aerosol and these differences were highly significant ($p<0.001$, $p=0.004$, $p<0.001$). The mean anxiety level of respondents was 3.35 ± 1.18 (ranging from 0 to 5).

Conclusion
These results demonstrated an adequate knowledge, especially in clinical symptoms of COVID-19 amongst dentists. However, the respirator usage rate in aerosol-generating procedures was not satisfactory which suggesting to necessity of dentists' education about pandemic.

Keywords: *COVID-19, Dentistry, Mask, Respirator, Attitude*

Introduction

Several cases with unidentified viral pneumonia was reported in early December in Wuhan, China and was confirmed by the World Health Organization (WHO) on 31 December (1). After analysis of respiratory samples, the causative agent was identified as novel coronavirus (2). On 11 February 2020, WHO and International Committee on Taxonomy of Viruses (ICTV) officially named for the virus as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease as Coronavirus Disease 2019 (COVID-19) respectively (3). According to WHO situation report on 28 April 2020 of COVID-19, it has been documented 2,954,222 cases and 202,597 deaths worldwide and the cases and deaths continue to increase daily (4).

Coronavirus which includes alpha, beta, delta, and gamma subtypes is a single strand RNA virus (5). Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) and Middle East Respiratory Syndrome coronavirus (MERS-

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CoV) are among the identified types of coronavirus that cause disease in humans. They belong to beta coronavirus subtype and cause respiratory infection similar to SARS-CoV-2 (6).

According to report of WHO-China Joint Mission on COVID-19, the most common symptoms of the disease were fever, cough, fatigue, expectoration, dyspnea and sore throat while nausea, diarrhea and hemoptysis were also reported (7). In addition, Ai *et al.* (8) claimed most patients (%90) had bilateral lung involvement in COVID-19 pneumonia and Shi *et al.* (9) reported the involvement as ground glass opacity, consolidations, air bronchogram and thickening of interlobular septa and adjacent pleura in computed tomography (CT) imaging. Furthermore, patients may experience wide range of complications, including respiratory system and heart injury, impaired renal and liver function and even death (10).

SARS CoV-2 invades the cell through the Angiotensin-converting enzyme 2 (ACE2) cell receptor which were found copiously in respiratory tract (11). Although, its transmission routes are not still completely clear, human to human transmission with contact transmission including oral, nasal and eye mucous membranes and direct transmission via droplets related to respiratory activities was proved (12). Moreover, there are reports about the detection of SARS-CoV-2 in saliva, blood, gastrointestinal tract and urine in the literature (13,14).

Dentists are exposed to pathogenic microorganisms when performing aerosol-generating procedures. The risk of transmission of infectious diseases increases with their close contact with their patients and handling of sharp instruments. Dentists are one of the most vulnerable profession group against COVID-19 because of the inability to take definitive preventive measures during the performing of aerosol-generating procedures and the disease threatens the health of the dental health care workers and patients as well as the community. Thus, dentists should have information about the disease in order to protect their own and patients' health. The objective of this study was to evaluate the knowledge, attitudes and anxiety level of dentists in relation to COVID-19.

Materials and Methods

This cross-sectional study was conducted from March 2020 to April 2020. The study was approved by Research Ethics Committee of Kocaeli University (Registration number: GO-KAEK-2020/5.09 2020/93). The electronic questionnaire which contained about knowledge, attitude and anxiety level of dentists relation to COVID-19 was evolved in Goggle Forms and were sent to participants by e-mail and mobile phone messages.

To be proper for inclusion into the study, the participants had to be dentists who were working in Turkey actively. The questionnaire was developed based on guidelines of American Dental Association (ADA), United Kingdom National Health Service (NHS) and Turkish Dental Association (TDA) about COVID-19.

The questionnaire, containing 22 questions, includes four parts. In the first part, participants were asked about demographic data (age, sex, year of experience, profession and comorbidities related to COVID-19); in the second, on knowledge about COVID-19 (dental emergency procedures and transmission routes and symptoms of COVID-19); in the third, on attitudes (usage of protective equipments and selection of masks or respirators); in the fifth, on anxiety level related to COVID-19.

Statistical analysis

Data were analyzed statistically with a statistical program (SPSS Statics 26, IBM Inc., Armonk, NY, USA) in the presented study. The standard descriptive methods such as the mean, standard deviation and frequency were applied to determine the characteristics of the sample. Chi-square test and Fischer's exact test were performed to identify the relationship and difference between the mask types used by the participants for the procedures with and without aerosol respectively. For all tests, P-value <0.05 was considered statistically significant.

Results

Demographic data and risk factors

A total number of 590 participants included in this study. Out of them, 325 were females (55.0%) and 265 were males (44.9%). 284 of the participants (48.1%) were in the age range of 20-30, 166 of them were in the range of 31-40, 87 of them were in the range of 41-50, 49 of them were in the range of 51-60 and 4 of them were over 60 years. 240 of the participants (40.6%) had professional experience range of 0-5 years, 120 of them were range of 6-10 years, 74 of them were range of 11-15 years, 55 of them were range of 16-20 years and 101 of them were range of 21+.

360 of all participants (61.0%) were general dentists, whereas 230 of them (38.9%) were dental specialists. According to distribution of specialization, 59 of them (10.0%) were oral and maxillofacial surgeons, 46 of them (7.7%) were pedodontists, 30 of them (5.0%) were prosthodontists, 25 of them (4.2%) were orthodontists, 23 of them (3.8%) were endodontists, 22 of them (3.7%) were periodontists, 22 of them (3.7%) were restorative dentistry specialists and 3 of them (0.5%) were oral and maxillofacial radiologists. According to types of clinics, 158 of them (26.7%) were private practitioners, 151 of them (25.5%) worked at dental clinics, 141 of them (23.8%) worked at university hospitals and 140 of them (23.7%) worked at the state dental health centers.

Most dentists (89.2%; n=521) stated that they are not at high risk about COVID-19 (age >60 and/or comorbidities including, immunosuppression, respiratory disease, diabetes mellitus, hypertension, cardiovascular disease), while 63 of them (10.7%) are. The majority of participants (86.9%; n=508) delayed to treat their patients (asymptomatic patients) due to the concerns about COVID-19. Surprisingly, this rate was higher in participants at low risk than participants at high risk (87.5%; n=456, 82.5%; n=52).

COVID-19 knowledge level

Participants responded that COVID-19 transmitted by aerosol (98.5%; n=579) , saliva (90.6%; n=533), mucosal contact (71.4%; n=420), blood (36.2%; n=213), fecal-oral (32.8%; n=193) and vertically (6.6%; n=39).

For the question about clinical symptoms of COVID-19, the most given answer was fever by 587 participants (99.8%)

and it was followed by dry cough (99.1%) fatigue (90.0%), sore throat (84.9%), diarrhea (64.5%), nausea and vomiting (56.6%), headache (54.3%), dyspnea (46.1%), myalgia (32%), sputum formation (29.9%), arthralgia (21.3%).

251 participants (42.9%) responded the question about the limit for the body temperature defined for COVID-19 to be considered high as 38.5 °C. Other answers were 37.5 °C by 136 participants (23.2%), 37.7 °C by 89 participants (15.2%), 37.3 °C by 86 participants (14.7%) and 37.1 °C by 23 participants (3.9%).

Regarding dental emergency procedures, the majority of population defined acute pulpal pain (94.7%), severe intraoral bleeding (90.9%), tooth fracture with pain (87.3%), maxillofacial fracture (80.8%), alveolar osteitis (74.0%), severe pericoronitis (73.7%), abscess (67.9%), tooth avulsion (65.6%) and breakage of orthodontic wires with soft tissue trauma (63.8%) as emergency dental situations. However, cementation of fixed dentures (12.6%), modification of removable prothesis (8.2%) , cementation of implant retained prothesis (6.2%), asymptomatic tooth fracture (5.8%), periodic examination and asymptomatic tooth extraction (1.2%) was also described as emergency dental procedures by respondents.

Attitudes

Majority of respondents was able to use gloves (96.4%; n=558), face shield (88.1%; n=510), disposable gown (83.4%; n=483), protective goggles (73.9%, n=428) and disposable coat (20.9%; n=121) after the WHO declares pandemic.

In procedures which did not generate aerosol, mostly surgical mask with single use (53.7%; n=311) were worn by participants and FFP3/N99 mask with single use (1.0%; n=6) were the least used amongst all mask types. In aerosol-generating procedures, the most used mask type was also surgical mask with single use (36.0%; n=200) and FF1 mask with multiple use (1.6%; n=9) were the least. In aerosol-generating procedures, FFP2/N95 (33.9%; n=189) and FFP3/N99 (10.9%; n=61) mask usage were higher comparing procedures that did not generate aerosol (FFP2/N95: 12.2%; n=71, FFP3/N99: 5.8%; n=34) However, surgical mask usage was lower in aerosol-generating procedures (50.7%; n=282) comparing other procedures (77.5%; n=448), the differences were highly significant (p<0.001, p=0.004, p<0.001). FFP1 mask usage for both procedures were similar and statistically insignificant (p=1.00). The current values related to mask usage of the participants are presented in Table and Figure 2.

Majority of respondents (87.0%; n=508) stated that they would refer the patients, who presented COVID-19 symptoms, to specials clinics for COVID-19 instead of performing the dental treatment while only 6 respondents (1.0%) would refer them to university hospitals. 66 respondents (11.3%) would prefer to perform only dental emergency procedures, whereas only 4 respondents (0.7%) chose to do routine dental treatment. Moreover, regarding asymptomatic patients, more than half of respondents (53.9%) stated that they would perform only dental emergency procedures.

Anxiety level

Regarding to evaluation of anxiety of participants, every participants were asked to mark their anxiety level (ranging

Table 1. *Mask/respirator usage of the participants in different dental procedures*

Mask/ respirator type	Surgical mask	FFP1	FFP2/N95	FFP3/N99
Aerosol-generating procedures	282	23	189	61
Other procedures	448	23	71	34
p value *	p<0.001	1.00	p<0.001	0.004

* Fischer's exact test

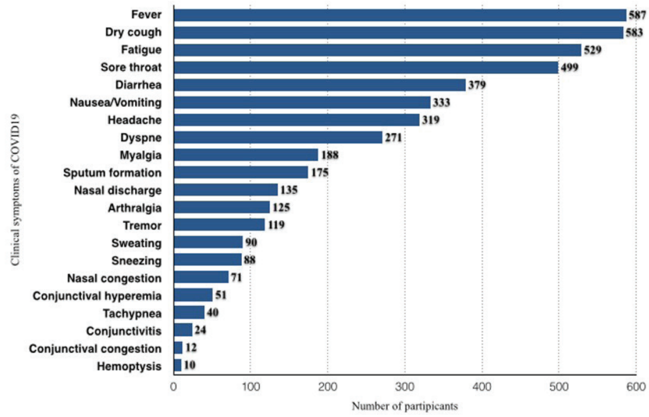

Figure 1. *Distribution of responses to 'What are the clinical symptoms of COVID 19?' according to number of participants.*

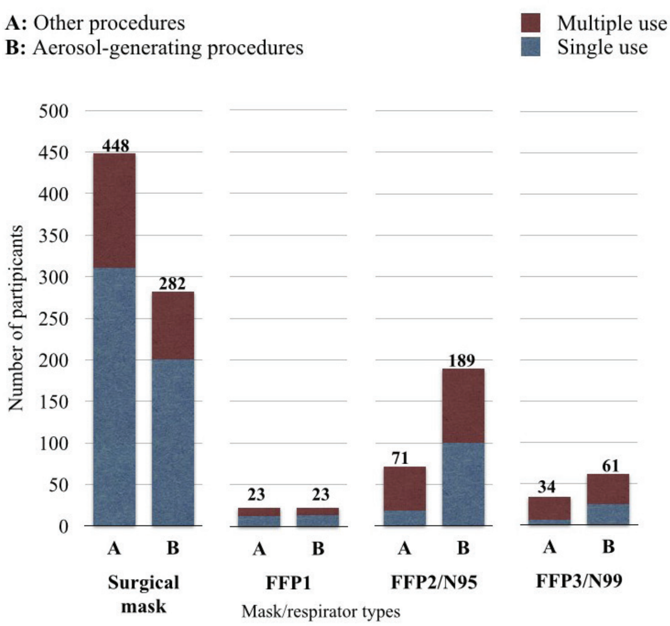

Figure 2. *Distribution of responses to 'Which type of mask/respirator do you use in procedures without aerosol (A) and with aerosol (B)?' according to number of participants.*

from 0 to 5) related to pandemic and the mean anxiety level was reported as 3.35 ± 1.18. Furthermore, majority of the participants (83.1%) though that the disease could be transmitted to their selves even by protective equipment and/or preventions while treating a patient with COVID-19. 16% of all, stated that protective equipment and/or preventions protects from the transmission of disease, but 0.9% of the population determined that protective equipment and/or preventions is not required to prevent the disease.

Discussion

The major outbreak has spread to 210 countries and territories globally and the total number of cases has increased to 2,954,222 at the time of writing (4). This situation revealed that dentists are at high risk for the transmission of the disease, as it affects all healthcare workers. Furthermore, dental procedures may increase the spread of the disease and it can also be mentioned about the negative effects of public health. In present situation, procedures that dentists should and should not perform became more crucial. Thus, the purpose of this study was to evaluate the knowledge attitudes and anxiety level of dentists about COVID-19.

Age is an important demographic parameter to predict to prognosis of the disease. Arshad *et al.* (15) study demonstrated that younger people had slower disease progression. These findings coincide with Chinese Center for Disease Control and Prevention (CCDC) which claimed more than 80% of deaths were seen in people over 60 years old (16). Moreover, comorbidities associated to COVID-19 is also influence the progression of the disease, such as cardiovascular disease, diabetes, hypertension, chronic respiratory disease and cancer (7). In our study, 10.7% of all participants were stated that they are in high risk group and the majority of this group (%82.5) postponed to performing treatment because of their concerns about the disease. Although the rates of those in the high risk group seem low in our study, we estimate that the rate among dentists is actually higher. The probable reason that it appeals to younger groups in electronic survey studies, due to the more usage of the internet in these groups.

The possible transmission routes of COVID-19 were previously mentioned in this paper (11-14). The majority of participants responded the way of transmission as microorganism containing aerosol (98.5%), saliva (90.6%) and mucosa contact (71.4%), respectively. On the other hand, our results also showed poor knowledge of our population about the contamination with blood (36.2%), fecal-oral (32.8%) and vertical contamination (6.6%) routes. The probable reason for the missing information may be that the disease is a newly defined and the mechanism of transmission has not yet been fully described.

As a part of dental procedures, microorganism containing aerosols are generated with high-speed handpieces or ultrasonic devices and these, which are contaminated with saliva and blood, spread and cause long distance contamination (17). Therefore, many authors suggested in order to reduce the transmission potential of this disease, only emergency dental treatments in patients without COVID-19 symptoms are performed in dental clinics (18). Therefore, dentists' knowledge about the protocol and clinical symptoms of the COVID-19 is critical. Most of the participants answered the question about the clinical symptoms of COVID-19 correctly in the presented study. Moreover, the majority of participants (87%) responded that a patient who showed symptoms of COVID-19 applied to their clinic, they would refer her/him to special clinics for COVID-19 instead of doing dental treatment.

Rothe *et al.* (19) reported an asymptomatic case who infected many patients in his incubation period. In addition, Guan *et al.* (20) have obtained results that the incubation period may be extended up to 19 days which increase the risk of transmission. Besides, atypical symptoms were reported in patients who are highly contagious in another study (21) and they claimed that more than 90% of patients are not diagnosed correctly. In addition, some authors (22) reported that nucleic acid amplification tests (NAAT) and real-time fluorescence polymerase chain reaction (RT-PCR) tests used in the diagnosis of the disease may even lead to false-negative results in the literature. Hence, every patient should be evaluated as a carrier of the disease and preventive measures should be taken urgently.

The temperature of individuals including staff, patients and their companions should be recorded (23). United States Center for Disease Control and Prevention (US CDC) recommended to defer the treatment when the body temperature is over 38 °C (24). In contrast, a recent report suggested that the highest limit is 37.3 °C. In our study, only 14.7% of all participants were answered the highest limit as 37.3 °C while half of them chose 38.5 °C (25).

There is no consensus on the selection of masks and respirators (FFP2/N95 or FFP3/N99) in the dental procedures. European Center for Disease Control and Prevention (ECDC) and US CDC recommended respirators for health workers in all procedures, whereas the WHO suggested respirators only in aerosol-generating procedures. Moreover, which respirator will be used in different procedures is also not clear in the literature. ECDC recommended using FFP3 respirators in aerosol-generating procedures, while Public Health England (UK) recommended them for all procedures, for instance (24,26). In the presented study, respirator using was higher in aerosol-generating procedures [FFP2/N95 (33.9%), FFP3/N99 (10.9%)] comparing other procedures [FFP2/N95 (12.2%), FFP3/N99 (5.8%)]. Unfortunately, the usage of respirators in dental procedures was not enough for proper protection in our study. Some authors (27) recommended using FFP2/N95 or higher respirators for health care workers in all conditions, due to the unclear transmission mechanism of COVID-19. It was confirmed by Wang *et al.* (28) have also shown a significant decrease in number of infected medical staff with N95 respirators, although 8.33 times more exposure, compared to no-mask group.

The rapid spread of the disease, the difficulty of its control and the gradually increase in the number of deaths affect the mental health of the health care workers as well as their physical burden. We reported the majority of our respondents think that the protective equipments are not enough to prevent the disease's transmission and the mean anxiety level of the population was 3.35 because of COVID-19. *Lai et al.* (29) also found similar results which 44.6% and 71.5% of 1257 health care workers are showed symptoms of anxiety and depression respectively.

Conclusion

The findings of the presented study indicated that variable levels of understanding of COVID-19 and prevention methods among dentists in Turkey. Participants have good knowledge about the emergency dental procedures and disease's transmission routes and symptoms. However, some gaps were observed in respirator usage. Although most of the dental the clinics are closed, dentists continue to perform emergency dental treatments. Hence, dentists should improve their knowledge and attitudes to protect community's and their own health during these challenging times.

Türkçe Özet: *COVID-19'da diş hekimlerinin bilgi seviyesi ve davranışı. Amaç: Çalışmanın amacı, diş hekimlerinin COVID-19 ile ilgili olarak bilgi seviyesini, davranışlarını ve kaygı düzeyini değerlendirmektir. Gereç ve Yöntem: Bu kesitsel anket çalışması Türkiye'de görev yapan 590 diş hekimi ile Mart 2020 ile Nisan 2020 tarihleri arasında yapılmıştır. Elektronik anket, katılımcıların demografik bilgileri, COVID-19'un dental yönetimine ilişkin bilgi seviyeleri, klinik uygulamaları ve kaygı düzeyleri ile ilgili 22 soru içermektedir. Anket, katılımcılara elektronik olarak uygulanması amacı ile e-posta ve cep telefonu mesajı ile iletilmiştir. Bulgular: Katılımcıların çoğunluğu aerosol (% 98,5), tükürük (% 90,6) ve mukozal teması (% 71,4) COVID-19'un bulaşma yolları olarak tanımlamıştır. Katılımcıların büyük çoğunluğu ise hastalığın tüm klinik semptomları arasında ateş (%99,8), kuru öksürük (%99,1) ve halsizliği (%90,0) COVID-19 ile ilişkilendirmiştir. Eldiven, katılımcıların % 96,4'ü tarafından kişisel koruyucu ekipman olarak kullanılmakta ve bunu siperlik (% 88,1), tek kullanımlık tulum (% 83,4) ve koruyucu gözlük (% 73,9) takip etmektedir. Aerosol üretilen işlemlerde FFP2/N95 (% 33,9) ve FFP3/N99 (% 10,9) respiratuarların kullanımı aerosol üretilmeyen işlemler ile karşılaştırıldığında daha fazla iken, cerrahi maske kullanımı (% 50,7) daha azdır ve bu farklılık istatistiksel olarak anlamlıdır (p<0.001, p=0.004, p<0.001). Katılımcıların ortalama kaygı düzeyi 3.35 ± 1.18 (0 ile 5 arasında) olarak tespit edilmiştir. Sonuç: Bu sonuçlar, özellikle COVID-19'un klinik semptomlarında olmak üzere diş hekimlerinin yeterli bilgi düzeyine sahip olduğu göstermektedir. Ancak, aerosol üretilen işlemlerde respiratuar kullanım oranının yetersiz olması diş hekimlerinin pandemi ile ilgili olarak eğitilmesi gerekliliğini göstermektedir. Anahtar Kelimeler: COVID-19, diş hekimliği, maske, respiratuar, davranış*

Ethics Committee Approval: The study was approved by Research Ethics Committee of Kocaeli University (Registration number: GOKAEK-2020/5.09 2020/93).

Informed Consent: The informed consents were provided by the participants.

Peer-review: Externally peer-reviewed.

Author contributions: BT and FMC designed the study. BT and FMC participated in generating the data for the study. BT participated in gathering the data for the study. BT and FMC participated in the analysis of the data. FMC wrote the majority of the original draft of the paper. BT and FMC participated in writing the paper. All authors approved the final version of this paper.

Conflict of Interest: The authors had no conflict of interest to declare.

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Original research

The dilemma of COVID-19 in dental practice concerning the role of saliva in transmission: a brief review of current evidence

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From December 12, 2019, a pandemic of acute respiratory syndrome, the novel human coronavirus disease (COVID-19), caused by a novel β-coronavirus (2019-nCoV) began to grow globally by person-to-person transmission. The production of airborne material during aerosol generating dental procedures would expose dental team and patients to remarkable risk of transmission concerning the face-to-face communication and splattered saliva, blood, and other body fluids. Dental professionals can be a substantial help in preventing the transmission of COVID-19. This study has reviewed relevant current evidences in literature that has addressed the role of saliva and the threats that may be inherent in transmission of the disease during dental procedures. The study also offers feasible proactive and preventive measures for dental practice during the outbreak to block possible person-to-person or indirect transmission in dental settings.

Keywords: *SARS-CoV-2, COVID-19, Saliva, Transmission, Infection Control*

Introduction

The novel coronavirus 2019 (2019-nCoV) or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is an enveloped positive-sense RNA virus which belongs to the Coronaviridae family. This RNA virus seems to be correlated but different from the other coronaviruses causing Severe Acute Respiratory Syndrome (SARS-CoV) and Middle East Respiratory Syndrome (MERS-CoV) (1,2). From December 12, 2019, a pandemic of acute respiratory syndrome, the novel human coronavirus disease (COVID-19), began to grow among humans by person-to-person transmission (3,4). It is believed that Rhinolophus affinis bat and pangolins are natural and intermediate hosts of the virus, respectively (4). The common clinical symptoms of this infection include fever, cough, fatigue, acute respiratory disease with abnormal chest CT (5); despite these symptoms, COVID-19 mostly presents with mild severity. On the other hand, compared to COVID-19, higher mortality rates were reported for either SARS-CoV (10%) or MERS-CoV (37%) (3); however, COVID-19 can be more contagious and spread faster than the other two mentioned respiratory syndromes (6). During this pandemic, dentistry is considered as one of the most high-risk jobs, since dentists are in close contact with patients' oral cavity especially saliva (7). Oral cavity is a main source of spreading respiratory droplets, which contains millions of virus in infected individual. These droplets may involve oral, nasal and ocular mucosa of dental health workers directly or indirectly through contact or contaminated surfaces (8). In spite of some uncertainties about the definite characteristics of SARS-CoV-2, it has recently been reported that this novel virus could be detected in saliva of infected patients (9). It is still not clear if SARS-CoV-2 viruses survive in human saliva and if yes, how long this endurance would be.

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Likewise, it is not verified whether SARS-CoV-2 is detectable in human saliva during the early incubation period or not. Evidently, many biomarkers in saliva help early diagnosis of oral cancer. However, it is still unclear if any salivary biomarker could be feasible for early diagnosis of COVID-19. Moreover, there are many established antiviral proteins in the saliva that can be regarded in protection against COVID-19 (9–12). Because of the presence of the virus in the saliva, it might be transmitted directly or indirectly from individuals without apparent respiratory symptoms (9). Since many of dental procedures are spatter- and aerosol-generating, the risk of airborne contamination through droplet nuclei in dental clinics is very high (6,13). Considering all these disputes, health care professionals specially dentists must protect themselves and their patients from the spread of the virus, and subsequently adopt extra-protective measures in order to prevent the transmission of SARS-CoV-2 (5,6). Therefore, concerning the role of saliva in transmission, this brief and rational overview was conducted to find, discuss, and summarize the current relevant evidences to highlight the empirical role of dental practitioners and their staff on prevention and protection of themselves, their patients, and public from this world-spread infection.

Search strategy

A search was made through electronic databases (Web of Science, MEDLINE, Scopus and Google Scholar) with particular focus on the latest information on World Health Organization (WHO), American Dental Association (ADA), Centers for Disease Control and Prevention (CDC) and United Kingdome National Health Service (UK NHS) websites. The keywords; 'SARS-CoV-2' or 'COVID-19' or '2019-nCoV' and 'Dentistry' or 'Infection Control, Dental' or 'Saliva' were searched in title/abstract of publications; limited to 2019 to 2020. The inclusion criteria were all articles relevant to dentistry and dental practice published in English. Moreover, the reference lists of the selected papers were reviewed for possible inclusion. Titles and abstracts of all selected papers were reviewed and complete text of the articles has been carefully checked upon confirmation for potential inclusion. To sum up, 39 articles, which demanded qualities to meet our criteria for this review were recited and retrieved.

Transmission

The transmission of COVID-19 most probably occurs by contact, droplet, and conceivably airborne under particular conditions concerning the previous evidences on SARS-CoV outbreaks.(14) SARS-CoV-2 is transmitted through droplets both directly and indirectly (8). The direct transmission occurs via coughing, sneezing, and droplet inhalation. Whereas, the indirect transmission occurs by touching eye, nose or mouth after contacting a contaminated surface (8).

Mechanism of transmission

Previous study demonstrated that SARS-CoV-2 show 79% similarity in nucleotide sequencing to SARS-CoV.(2) In addition, both viruses use the same host receptor named Angiotensin Converting Enzyme 2 (ACE2) receptor to enter cells (6). Xu et al. (15), in a study showed that ACE2 receptors are being expressed in oral mucosa, especially in tongue tissue which makes the oral cavity more susceptible for SARS-CoV-2 infection. In a study on Chinese rhesus macaques, which mimics the human situation, it was demonstrated that their $ACE2^{+}$ epithelial cells lining salivary gland ducts were early targets of SARS-CoV infection (16). Although no study has been done so far, due to similarities in the mechanism of cell entry, it seems SARS-CoV-2 have the potential to infect salivary gland ducts cells (14). Besides, these infected cells may constitute an important source of virus in saliva. Herein, we elucidate various possible transmission routes of SARS-CoV-2 in dental practice.

Routes of transmission

Droplet and aerosol transmission

As announced by WHO, transmission primarily occurs by means of respiratory droplets (>5-10 µm) within 1 m. Moreover, during aerosol-generating procedures, airborne transmission may be possible through droplet nuclei (≤5µm) which can spread over distances exceeding 1 m (13). Droplets and aerosols of individual's respiratory system spreads around by talking, laughing, singing, coughing and sneezing, by which infected individual can transmit disease (17). Geralton et al. (17) have demonstrated that in an individual with symptomatic respiratory viral infection both droplets and aerosols, which contain viral RNA, are produced by coughing or breathing. However, some studies have reported that asymptomatic individuals might spread the SARS-CoV-2 (18,19). It is recently documented that SARS-CoV-2 could be viable and infectious for hours in aerosols and for days on surfaces which make it feasible to be transmitted through aerosol and fomite (20). Previous study showed that in dental clinics large amounts of droplets and aerosols are produced by particular dental procedures in patients' oral cavity, which are mixed by patients' saliva or even blood. These particles are small enough to stay in the air for an extended period and be transmitted to clinicians directly or settle down on surfaces and be transmitted indirectly (6). Furthermore, To et al. (9) have detected SARS-CoV-2 in saliva of infected individuals by viral culture. Therefore, it seems that during dental procedures, SARS-CoV-2 has the potential to be transmitted via droplets and aerosols of saliva, from symptomatic, presymptomatic, or probably asymptomatic individuals.

Contact and contaminated surfaces transmission

WHO declared that SARS-CoV-2 could be transmitted through droplets, large particles that settle down on surfaces in short periods of time (8). Previous studies have shown that other Coronaviridae members that share similarities with SARS-CoV-2, like SARS-CoV, MERS-CoV and endemic human coronaviruses (HCoV) can stay afloat on plastic, metal and glass surfaces for up to 9 days (21). Moreover, the results of a very recent study have shown that SARS-CoV-2 can be viable up to 72 hours on plastic and stainless steel surfaces (20). Another recent study (22) has evaluated the stability of SARS-CoV-2 considering various factors such as temperature, different surface materials, and verified virucidal effects of common disinfectants. Their results have shown that the virus remains highly stable at 4°C, but as the temperature is increased to 70°C, the viability time decreases to 5 minutes. They have also figured out that SARS-CoV-2 can be stable on different surfaces such as printing and tissue papers (3 hours) wood and cloth (2 days), but more stable on smooth surfaces such as glass (4 days), stainless steel and plastic (7 days). The most concerning point was that the virus has been detected after 7 days on outer layer of laboratory infected surgical masks. They also examined the virucidal effects of various common disinfectants such as household bleach (1:49,1:99), hand soap solution (1:49), ethanol (70%), povidone-iodine (7.5%), chloroxylenol (0.05%), chlorhexidine (0.05%), and benzalkonium chloride (0.1%) and determined that the infectious virus could not be detected after a 5-minute incubation at room temperature (22ºC) except for hand soap solution. In addition, they depicted that SARS-CoV-2 is particularly stable in broad-spectrum pH values (pH: 3-10) at room temperature. They concluded that, although highly stable in a favorable environment, SARS-CoV-2 is susceptible to standard aforementioned disinfection methods (22). Therefore, since many surfaces involved in dental settings are made of these examined materials, if contaminated, they can be an impending source of infection in dental clinics where possible infectious droplets and aerosols are highly generated.

Another study (23) has shown that SARS-CoV and possibly MERS-CoV were found in samples taken from hospital surfaces at concentration far beyond of infective dose. So touching mouth, nose or eyes with prior contact with these surfaces could make individuals vulnerable for developing SARS or MERS (23). Thus, health care professionals especially dentists who have direct or indirect contact with patients' oral fluids, contaminated dental instruments or environmental surface are at a high risk of getting the COVID-19. In addition, close contact of patients' conjunctiva, nasal and oral mucosa with droplets and aerosols might do the same.

Oral-fecal transmission

While some published articles (24,25) have supported the idea of oral-fecal transmission, this route of transmission has not been verified by WHO, since there are no sufficient evidences (13). The study of Wu et al. (25), have shown that SARS-CoV-2 RNA can be detected in fecal samples of infected individuals 47 days after onset of first symptoms. Besides, SARS-CoV-2 could be found in fecal samples of some patients for a mean of 11.2 days even after respiratory tests became negative. Moreover, The study of Hindson (24) brought up the idea of digestive shedding of the virus which may lead to oral-fecal transmission. Nevertheless, proving this idea demands further studies.

Protection and Prevention

Since SARS-CoV-2 is abundantly present in patient's saliva, any dental procedure that has the ability to produce excessive amount of droplets and aerosols from saliva or even blood may cause airborne contamination (6). The latent period of COVID-19 is about three days , however, since infected people might be asymptomatic or be in incubation period (99%≤ 14 days) (26), it is almost impossible to make utmost prevention in dental clinics. Thus, according to different guidelines, all patients must be evaluated for possibility of infection with SARS-CoV-2; moreover, protective, preventive, and proactive measures must be adopted in order to avoid the transmission of SARS-CoV-2. Many authorities in different countries have proposed guidelines and guidance reports. Iran as a leading country fighting against corona virus has followed the guidelines of WHO and offered a dental guideline for COVID-19 pandemic available at (27) in Farsi language.

UK offered an official guidance issued mutually by the Department of Health and Social Care (DHSC), Public Health Wales (PHW), Public Health Agency (PHA) Northern Ireland, Health Protection Scotland (HPS), Public Health England (PHE) and NHS England (28). Their proposed guidance provides the infection prevention and control recommendations for health and social care workers who are involved with potential or confirmed COVID-19 patients.

ADA (29) has also proposed provisional recommendation and guidance resources for optimal protection against the risk of COVID-19 transmission as their primary infection control goal. CDC has stated that dental practice has distinctive features that necessitate additional infection control attentions. CDC's Interim Infection Prevention and Control Recommendations for patients with COVID-19 has been published with information that increments, but does not replace, the general infection prevention and control (IPC) recommendations for COVID-19 (30). In this guidance, CDC endorses a multi-step method, which concludes all preventive measures that should be regarded before and after arrival of the patient and during their presence under dental procedures (29). They have stated that dental health care personnel should adhere to standard precautions described as the minimum infection prevention practices in any health care setting that relates to patient care, regardless if the patient is suspected or confirmed to be infected (31).

Patient evaluation

This part is inevitably the first and most imperative part in preventive measures during the early stages of pandemic. Hierarchical figures of various guidelines illustrate most countries proposed different approaches to evaluate patients coming to dental clinics during the pandemic of SARS-CoV-2. Nevertheless, they all agreed on using their questionnaire to screen a patient. The recommended guidelines until the date of this report are summarized in Figures 1 to 4.

Hand hygiene

Proper hand hygiene is an important protective measure for transmission of COVID-19, as it is for other infectious diseases. Having appropriate hand hygiene before examination and dental procedures, and after touching patient's oral cavity, secretion, blood, body fluids, and contaminated dental equipment is of ultimate importance (6). WHO has recommended 11-step hand hygiene technique with soap and water for duration of 40-60 seconds in the instance that hands are noticeably contaminated with blood or other body fluids. Moreover, WHO has suggested 8-step technique with alcohol-based hand rub for duration of 20-30 seconds to be appropriate (32). If there's no alcohol-based hand rub and soap accessible, then using 0.05% chlorinated water

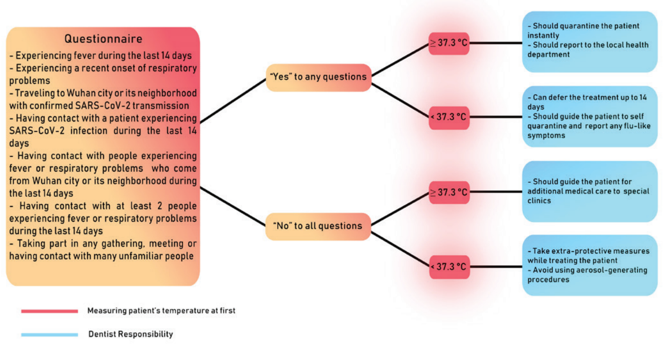

Figure 1. *The summary of patient evaluation in dental clinics according to the 5th edition of the guideline for the diagnosis and treatment of novel coronavirus pneumonia drafted by the National Health Commission of the People's Republic of China (6).*

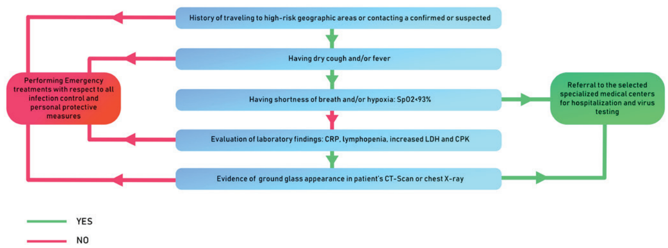

Figure 2. *The summary of patient evaluation in dental clinics according to Iran's dental guideline for COVID-19 pandemic (27).*

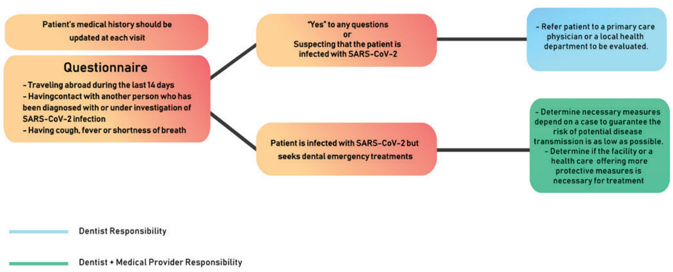

Figure 3. *The summary of ADA interim guidance for screening patients in dental clinics (46).*

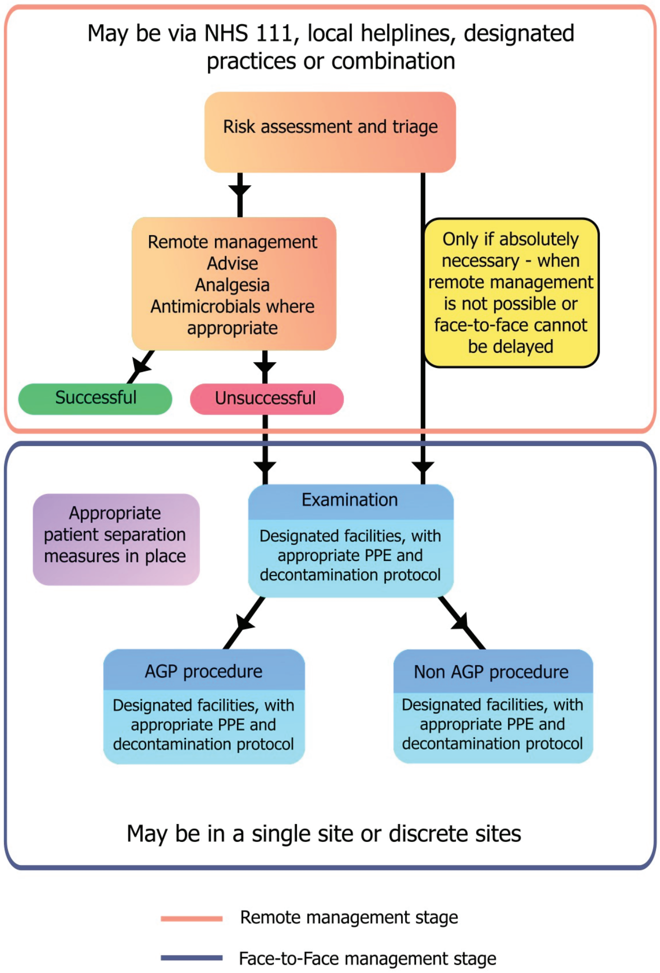

Figure 4. *The summary of COVID-19 urgent dental care pathway according to NHS guideline. From: https://www.england.nhs.uk/coronavirus/publication/covid-19-guidance-and-standard-operating-procedure-urgent-dental-care-systems-in-the-context-of-coronavirus/. Site accessed April 15, 2020.*

for hand washing is an alternative; however, repeated use can lead to asthma and dermatitis that may subsequently increase the chance of infection (33). Also, WHO has suggested using solution comprised of ethanol (80%), glycerol (1.45%) and oxygen peroxide (0.125%), or combination of isopropyl alcohol (75%), glycerol (1.45%) and oxygen peroxide (0.125%) for hand disinfection (34).

Disinfection

SARS-CoV-2 can remain on contaminated surfaces, from a few hours up to several days, depending on the environment's temperature and humidity (20). Therefore, decontamination plays a significant role in preventing viral transmission, especially in healthcare facilities. Corona viruses are one of the easiest ones to be killed with disinfectants since they are enveloped viruses (21). According to a review of literature (21) on all available data for inactivation of coronaviruses family including SARS-CoV and MERS-CoV, various surface disinfectants have shown to be efficient. Nevertheless, the authors have concluded that only 0.1% sodium hypochlorite or 62-71% ethanol for 1 min on surfaces can meaningfully decrease coronavirus infectivity and they have expected the same outcome for SARS-CoV-2 infection. The WHO suggested thorough cleaning of environmental surfaces with detergent and employment of hospital level disinfectants such as 0.5% sodium hypochlorite (5000 ppm). This solution which can be prepared by dilution of 1-part home bleach (5% sodium hypochlorite) with 9 parts water (33) ensure an effective result of disinfecting surfaces (35). Furthermore, 70% ethanol was recommended for disinfecting small surfaces (33). For surface decontamination, ADA refers to disinfectant materials approved by the Environmental Protection Agency (EPA). The list of recommended EPA-approved products against SARS-CoV-2 has been available for public (36). In addition, UK NHS has worked with PHE and assessed the available evidence and proposed the guidance for health professionals concerning UK's infection prevention and control recommendations for COVID-19 after some updates and revisions (37).

Personal protective equipment (PPE)

During the early phase of a pandemic, particularly when the vaccine is not introduced, personal protective equipment (PPE) plays a foremost role in controlling the disease (38). PPE comprises gloves, medical masks, goggles or a face shield, and gowns, and also respirators and aprons for particular instances. Respirators such as N95, Filtering Face piece Particles 2 (FFP2) or any equivalent standard), eye protection (goggles or face shield), long-sleeved gowns, gloves and also fluid-resistant aprons (if gowns are not waterproof) must be used to protect the health care workers including dentists during aerosol-generating procedures in pandemic of SARS-CoV-2 (39). Since SARS-CoV-2 is a virus with a diameter of ~0.1 µm (40) and N95 masks are able to filter ≈99.8% of particles with the same diameter size (41), these masks are capable of filtering free virions mostly (40). In addition, it seems that N95 masks are capable of protecting against large droplets (> 5 µm in diameter) and droplet nuclei (≤ 5 µm in diameter) which are formed by sneezing (~100 µm) and coughing (~1 µm).(40) However, during SARS-CoV outbreak in Toronto, using N95 masks by healthcare workers have decreased their mental performances and increased headaches among them (42). Considering all the facts, respirators like N95 mask or FFP2 or any equivalent standards are important parts of PPEs and are widely used even in short supply of PPE (39). WHO has recommended N95 usage for healthcare workers during SARS-CoV-2 pandemic especially when performing procedures that produce aerosols (39). In addition, WHO emphasizes to wear the mask appropriately; completely covering the mouth and nose and be tight to the face to minimize the gap between face and the mask. Once it is worn, it shouldn't be touched (43). Using one mask or respirator for more than 4 hours can cause discomfort and it should be avoided (39). Masks must be changed when they become damp. They must be removed from behind. Single-use masks must be discarded after each use and not be used again (43). WHO recommends cleaning utility gloves and discarding single-use gloves (nitrile or latex) after each use and forbids reusing them, moreover, hygiene should be accomplished after PPE is removed. It is suggested that leakage rates of both latex and nitrile gloves are almost similar to one another. Whereas, vinyl gloves have more defects compared to latex ones, especially after use (32). Different studies have shown that latex gloves are stronger barriers against virus penetration than vinyl or polyethylene (44,45). Even in exposure to 70% ethanol which is frequently used for disinfection, latex gloves still remain good barriers in spite of having 1% penetration (44). Figures 5-6 and Table 1 portray the PPE recommended by different authorities and guidelines.

Mouthwash

As SARS-CoV-2 is susceptible to oxidation, ADA has recommended 1% hydrogen peroxide mouthwash before appointment in order to decrease salivary load of oral microbes including SARS-CoV-2 (46). Moreover, chlorhexidine (CHX) has been shown to have antibacterial, antifungal and also antiviral activity against some lipid-enveloped viruses (47). As SARS-CoV-2 is a lipid-enveloped virus, it could be sensitive to this solution. However, in a brief review of literature by Harrel et al. (48), it was mentioned that CHX is unlikely to affect neither the blood coming directly from the operating site nor the nasopharynx-harbored viruses and bacteria. According to available literature, povidone-iodine mouthwash was verified to be the only sufficient mouthwash for pre-procedural rinsing in dental practice against Coronaviridae family. Whereas, CHX was shown to be ineffective against coronaviruses (49). The study reported by Peng et al. (6) recommends the use of oxidative agents against SARS-CoV-2. Hence, mouthwashes containing 1% hydrogen peroxide or 0.2% povidone can be regarded feasible in elimination or reducing the load of SARS-CoV-2 in saliva. Moreover, mouthwashes are intensely advocated in dental procedures where the rubber dam is not, or could not be used (50).

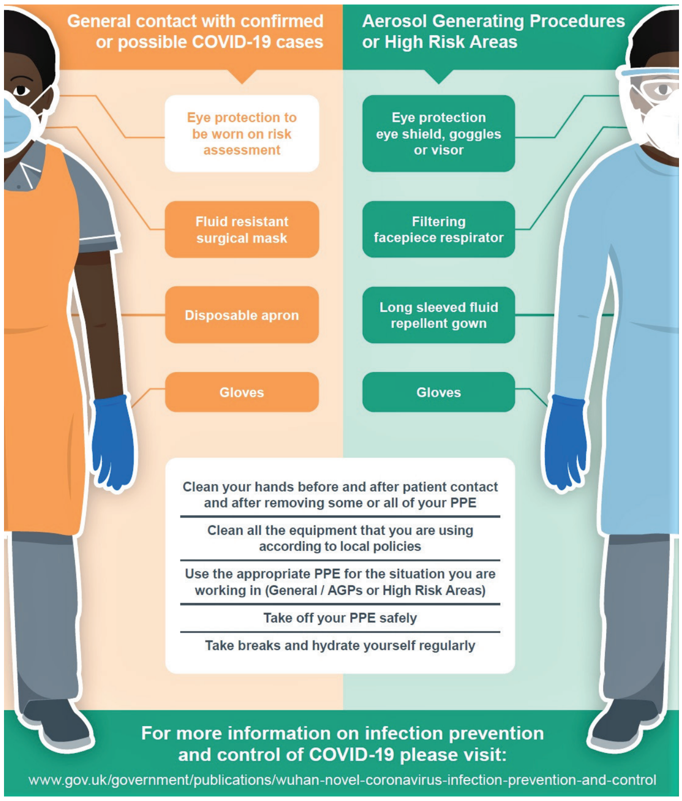

Figure 5. *Visual guide to safe PPE according to guidance on infection prevention and control for COVID-19 by PHE. From: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/878056/PHE_COVID-19_visual_guide_poster_PPE.pdf. Site accessed April 24, 2020.*

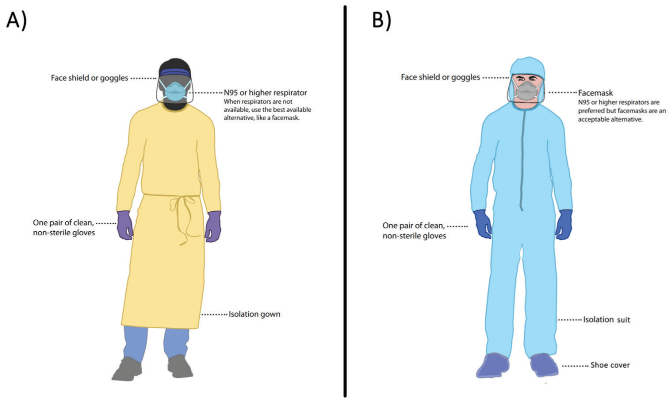

Figure 6. *A schematic figure of PPE for dental procedures during outbreak recommended by current guidelines. From: https://www.cdc.gov/coronavirus/2019-ncov/downloads/COVID-19_PPE_illustrations-p.pdf. Site accessed April 3, 2020.*

Table 1. *PPE for urgent dental procedures recommended by NHS. 1- Fluid-resistant gowns (or long-sleeved waterproof apron) must be worn during aerosol generating procedures (AGPs). If non-fluid-resistant gowns are used, a disposable plastic apron should be worn underneath. 2- If wearing an FFP3 that is not fluid-resistant, a full-face shield/visor must be worn. 3- Eye protection should be discarded after each use. Otherwise, if polycarbonate safety glasses/goggles or any equivalent are used, they should be disinfected according to manufacturers' guidance. Available at: https://www.england.nhs.uk/coronavirus/publication/covid-19-guidance-and-standard-operating-procedure-urgent-dental-care-systems-in-the-context-of-coronavirus/. Site accessed April 15, 2020*

Personal protective equipment (PPE) for COVID-19 urgent dental care settings

	Waiting room/reception No clinical treatment	**Dental surgery Non AGP treatment**	**Dental surgery Treatments involving AGPs**
Good hand hygiene	√	√	√
Disposable gloves	X	√	√
Disposable plastic aparon	X	√	X
Disposable gown (1)	X	X	√(1)
Fluid-resistant surgical mask	√	√	X
Filtering piece (FFP3) respirator (2)	X	X	√
Eye protection (3)	X	√	√

With all these debates on, further studies are required to prove which mouthwash is more efficient against SARS-CoV-2.

Protective measures in dental procedures

As SARS-CoV-2 might be transmitted via aerosol, using aerosol-generating instruments and performing such procedures including ultrasonic and sonic scalers, air polishing, air-water syringe, tooth preparation with turbine handpiece or air abrasion should be minimized as much as possible (26,48). While using PPE during aerosol-generating procedures is prerequisite, WHO has recommended to perform these dental procedures in a room with minimum ventilation rate of 160 L/s per patient or in negative-pressure rooms with minimum 12 air changes per hour and regulated air flow direction when mechanical ventilation is used. Furthermore, individuals presenting in the room should be as minimum as required for patient treatment (35).

Two methods are available to decrease airborne contamination produced from the treatment site before and after it becomes airborne. Practically, it is easiest to eliminate as much airborne contamination as possible before it outflows from the proximate of operational and treatment region (48). One method is to remove the airborne contamination before it escapes from the most closed area surrounding the operational site. The employment of a high-volume evacuator (HVE) has been proved to reduce the contamination occurring from the operative site by more than 90% (48). It has been proved that the number of colony-forming units, produced during dental procedures is decreased greatly when an assistant uses an HVE. The usual HVE used in dentistry has a large opening (usually ≥8 millimeters) and is joined to an evacuation system that removes a large air volume (100 cubic feet / minute). A typical saliva ejector in the dental units cannot be categorized as an HVE because of its small opening that does not remove sufficient large volume of air (48). The other method includes employing devices that eliminate the contaminated material from the air of the operational area after it has become airborne. The most frequently methods are the use of a high efficiency particulate air (HEPA) filter and the use of ultraviolet (UV) chambers in the ventilation system. While both of these systems are effective, they are almost expensive (48).

Rubber dams and HVEs reduce aerosol or spatter in dental procedures. Using rubber dams can greatly decrease the development of saliva- and blood-contaminated aerosols or spatters, particularly when high-speed handpieces and ultrasonic dental devices are used (6). It was reported that the use of rubber dam could significantly reduce airborne particles by 70 % in the operating field's ~3-foot diameter (6). When rubber dam is implemented, extra HVE for aerosol and spatter together with usual unit suction should be used during the operations. In this case, it is also important to adopt a full four-hand operation (6). Although in some cases isolation of the rubber dam is not feasible, manual tools such as Carisolv and hand scaler are advised for caries removal and periodontal scaling to reduce aerosol generation as much as possible (6). Moreover, Extra-oral evacuation devices and special aerosol reduction devices deliberated to use with ultrasonic scalers are also regarded to reduce droplets and aerosols generated during scaling procedures (51).

In addition to the above precautions, controlling patient's gag, cough and vomit reflex in dental clinics can minimize droplet and aerosol production (51). Several strategies for avoiding gagging are proposed in literature, including behavioural modifications as well as pharmacological and non-pharmacological management. Behavioural modification techniques include relaxing, distraction, suggestion/hypnosis, and so on. Pharmacological techniques such as using topical and local anaesthetics as well as antihistamines, sedatives, CNS depressants and so on could be used to manage gag reflex. Using salt on the anterior part of the tongue as a suitable non-pharmacological remedy could have the same effect as well (52).

In dental procedures, the high-speed dental handpiece without anti-retraction valves might aspire and eject the debris and fluids. Most specifically, the pathogens, including bacteria and viruses, can also contaminate the air and water tubes in the dental device, which can thus possibly cause cross-infection (6). In a review study, using anti-retraction high-speed handpieces was recommended during this pandemic period of COVID-19 in order to potentially prevent cross-contamination (6). This research has shown that the high-speed dental anti-retraction handpiece can substantially reduce the backflow of oral bacteria and HBV into the handpiece and dental unit tubes as opposed to the handpiece without anti-retraction feature (6).

On the other hand, it is reported that microbes might be present in the smoke and particulate debris (plume) produced during laser and electrosurgery treatments (51). Garden et al. (53) have shown that laser plume can contain and transmit papillomavirus DNA. Although those findings cannot be generalized to SARS-CoV-2, cautions should be taken when using such devices.

Radiological examination

Intraoral radiography remains the most popular x-ray technique used in dental imaging; however, the use of this technique should be replaced with extraoral radiographies such as panoramic radiography or cone beam computed tomography (CBCT), since the intra oral radiographies can induce saliva secretion, gag reflex and coughing (26,51). Otherwise, when intraoral radiographies are required, barriers should be used twice on sensors to avoid perforation and cross contamination (54). During dental radiology procedures, precautions taken to prevent nosocomial human-to-human transmission may play a substantial role in decreasing the spread of COVID-19. All relevant protective approaches should be adopted to reduce the risk of infection among staff and patients.

Proper management of medical and dental waste

Policies and procedures for the effective management of health-care waste should be followed, including determining authority and sufficient human and material assets for safe disposal of such waste. There is no proof that direct, unsafe human contact has contributed in the transmission of the COVID-19 virus during the management of the health care waste (33,55).

Any medical waste generated during treatment of COVID-19 patient should be carefully deposited in specified containers and appropriate bags, and then safely discarded, preferably on location. All those who handle medical waste should use PPE such as eye protection, mask, longed-sleeved gown, thick gloves, apron, and boots. After removal, hand hygiene should be performed (33,55).

COVID-19 diagnostic test for dental health care professionals

The significance of COVID-19 diagnostic test for dentists and dental team is unequivocal. This should be performed in hospitals with the same high priority as that of medical healthcare professionals. One should not underestimate the possibility of a dental practitioner being positive for COVID-19 and potentially infecting patients attending emergency dental services (38).

Conclusion

Saliva might have a critical role in direct and indirect transmission of COVID-19. Because of the infectious potential of saliva in transmission of COVID-19, it is essential to develop effectual policies for prevention and protection during aerosol-generating dental procedures especially for dental team and patients. These strategies include accurate perception of the transmission routes, training and testing dental staff for COVID-19, submitting proper protective and preventive measures, accurate patient evaluation, hand hygiene, employing adequate disinfection method of dental settings and office, using recommended personal protective equipment, and finally proper discarding wastes. Saliva can also be used for diagnostic methods, which are non-invasive, convenient, and cost-prohibited and may deliver fast and early detection of COVID-19.

Türkçe Özet: *Bulaşmada diş hekimliği uygulamalarında tükürüğün rolüne ilişkin COVID-19 ikilemi: güncel kanıtların kısa bir incelemesi. Yeni bir β-coronavirus (2019-nCoV) neden olduğu akut bir solunum sistemi sendromu salgını olan insan yeni coronavirus hastalığı (COVID-19) 12 Aralık 2019'dan itibaren kişiden kişiye bulaşma yoluyla küresel olarak yayılmaya başlamıştır. Diş hekimliği prosedürlerinin ürettiği aerosol sırasında oluşan havadaki uçuşan materyaller yüz yüze iletişim, sıçramış tükürük, kan ve diğer vücut sıvılarıyla aracılığıyla diş hekimliği ekibini ve hastalarını belirgin bulaşma riskine maruz bırakacaktır. Diş hekimleri, COVID-19 bulaşmasının önlenmesinde önemli yardımları olabilir. Bu çalışma, tükürük rolünü ve diş hekimliği prosedürleri sırasında hastalığın bulaşmasında doğabilecek tehditleri ele alan literatürdeki güncel kanıtları incelemiştir. Bu çalışma ayrıca, salgın sırasında diş hekimliği uygulamaları için olası kişiden kişiye veya diş hekimliği ortamında dolaylı bulaşmayı engellemek için uygulanabilir proaktif ve önleyici tedbirleri önermektedir. Anahtar Kelimeler: SARS-CoV-2, COVID-19, Tükürük, Bulaşma, İnfeksiyon Kontrolü*

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Review

Managing emerging challenges of Coronavirus disease 2019 (COVID-19) in dentistry

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Dental centers have been referred to as a hub or reservoir for infection, where healthcare professionals and other staff, patients and the public together may potentially spread pathogenic microorganisms. This may occur via saliva, skin or indirectly through air, water, and contaminated surfaces or instruments. Everyone should therefore be considered as potential sources of infection. During a pandemic, limiting unnecessary care has been adopted as a clinical measure for some patient's, to reduce the risk of cross-infection in the short term. However, in order to enable continuation of necessary and qualified care, dental processes need to follow specific infection control strategies in order to prevent transmission of emerging pandemic risks following COVID-19. In this article, we develop a tool with practical recommendations to mitigate infection risks before, during and following pandemics to enable ongoing dental care provision in primary and secondary care based on national and global recommendations.

Keywords: *COVID-19, Protection measures, Dentistry, Transmission routes, Cross-infection*

Introduction

In December 2019, the outbreak of Acute Respiratory Disorder Syndrome Corona Virus 2 (SARS-Cov2), or Corona Virus Disease 2019 (COVİD-19), started in China-Wuhan. The World Health Organization (WHO) officially announced the outbreak as a pandemic on March 12, 2020 (1). Since the virus was discovered, cases spread worldwide have caused one of the biggest and most complex infection outbreaks in history. COVİD-19 spreads mainly through symptomatic or asymptomatic persons and effectively through breathing (2–5). For this reason, it has been reported that healthcare professionals, family members, friends and patients who are in close contact with COVID-19 persons are at risk of getting infected or spreading the virus (6,7). Although, it is stated that the mortality rate of COVID-19 is lower than other Corona Virus diseases, the problem is the very rapid transmission of disease and that increasing numbers of infected persons complicate survival (3–5).

Dental Care Professionals are exposed to pathogenic microorganisms that infect the oral cavity and respiratory tract, both because they work in the oral area, they cannot maintain an advised global 1-meter public distance (2). In light of the current Coronavirus Pandemic (COVİD-19), people working in this area are subject to considerable risk of contamination with COVID-19 due to face-to-face interactions and contact to saliva, blood, other secretions, and use of sharp instruments. In addition, inhalation of aerosols and airborne particles, especially during applications using ultrasonic and high-speed dental handpiece, poses an additional higher risk of contamination for COVID-19 (2).

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Emerging challenges of coronavirus disease 19 (COVID-19) in dentistry

The COVID-19 outbreak throughout populations and, perhaps more specific to dentistry, intraoral secretions containing high concentrations of virus, are themselves important indicators that the working dental environment is dangerous (2). The findings of previous studies show that angiotensin converting enzyme II (ACE2) plays an important role in the cellular entry of virus (8,9). ACE2 is found in the oral mucosa, especially in the tongue intensely (10). These findings indicate that COVID-19 causative virus will bind to ACE2s located in the oral cavity and increase the susceptibility to infection (8–10). It is emphasized that virus density and duration of contact have an important role on the transmission and course of the disease (7,8,11). Despite the protection measures of healthcare professionals serving during the epidemic period, the number of deaths amongst high risk individuals high (6). High risk individuals may include, for example, the elderly over 70 years of age, the systemically medically compromised, in particular the immune-compromised and cardio-respiratory compromised, and pregnant patients, in particular those in the third trimester (2). In addition, it is known that the number of infected individuals who do not show clinical symptoms are as much as the individuals who have clinical symptoms (12,13). Therefore, there exists a hidden risk that individuals who do not show clinical symptoms and are unaware of their infection, have the potential to spread the virus (14–16).

COVİD-19, currently does not have a specific antiviral treatment or vaccine available, but Phase I clinical trials with the most advanced COVİD-19 vaccine candidates began in the spring of 2020 worldwide. Current treatment includes some of the antiviral drugs used for other viral diseases, and plasma replacement therapy which provides general care to support vital organ functions (17–19). Effective disinfectant and antiseptic products are essential to prevent the spread of infection in the absence of COVİD-19-specific treatments, and particularly in diseases with a high rate of transmission. In addition, hygienic measures such as wearing medical masks, protective glasses or face shields to reduce the passage through breathing are very important for protection against COVİD-19 contamination (20). Because COVİD-19 is a deadly threat, the use of antiseptics with antiviral activity is critical to help prevent contamination. However, although enveloped viruses like Corona virus are considered to be more sensitive to antiseptics, they may react differently than non-enveloped viruses in terms of the concentration of the active ingredient and the required duration of administration (21,22). Enveloped viruses are responsible for serious outbreaks such as acute respiratory failure syndrome (SARS), Middle East respiratory syndrome coronavirus (MERS-CoV), and ebola virus disease (EBOV). The recent Corona virus (COVİD-19) outbreak emphasizes the importance of prevention measures to help prevent infection and limit the spread of disease before, during and following a pandemic (15,17).

Employees and the public are therefore at high risk for infection in dentistry (2). Infection is defined as a clinical picture resulting from inflammatory reactions caused by entrance of a microorganism into the body with response from the body's immune system (23). This response may occur in a specific body area or more systematically. The best method of prevention is therefore to stop the microorganism at the cross-infection stage. Cross infection is the transition of pathogenic microorganisms from an infected person to a non-infected person that may potentially cause infection (24). In dental practices, cross infections occur either directly (person-to-person), indirectly (for example using a contaminated / non-sterilized instrument in another patient), or by droplet infection (inhalation of microorganisms suspended in the air) (23–25). The lack of necessary precautions can be responsible for the transmittance of the infection factor. COVID-19 infected persons may be grouped as individuals with symptoms that are easily recognized at the acute stage of infection and individuals without symptoms that can only be recognized by tests. In the case of a pandemic, to avoid delay in necessary clinical care provision, every person may be seen as a potential carrier regardless of the health history (2,17).

Infection control measures for all patients in dentistry during a pandemic

Clinical staff must protect themselves and the other patients from the source of infection in the clinical setting (7,11,14). In addition, the dentist should be alert to infectious diseases that they might encounter, and direct his patient for necessary medical treatment before dental treatment (24). Due to the risk of mortality, dental healthcare professionals who are in contact with a large number of people should have high awareness of this issue. In this section, infection control measures are described for dentistry in general. Nonetheless, it is important for dental centers to continue to follow national and regional advice from governments and clinical networks.

Hand hygiene

Hand hygiene has become even more prevalent for dental practice during the COVİD-19 pandemic. In fact, a regimen has been suggested for 5 handwashes, 2 prior to treatment and 3 following (14). Specially, clinicians should wash their hands before examining the patient, before dental treatment, after contacting to patient, after touching infected settings and instruments, and after touching the oral mucosa, open wound surface or any fluid associated with the patient. The dental professionals should be given more care to avoid contacting their mouth, nose and eyes (2,12,13). These recommendations go beyond the clinical handwashing protocol outside of a pandemic situation.

Patient triage

The general consensus is that triage should be conducted in order to examine the existing state of health and the existence of risk factors for COVID-19 progression while accepting patients (13,26). This may be conducted initially by phone by an appropriately trained member of staff in order to investigate dental and medical needs using structured questions (26,27). This may identify high risk patients or assess the vulnerability of patients and threat they may pose to other patients or staff in the practice (5). This should be handled empathetically and so as to not prejudice. Patients may be questioned whether they have had any interaction with persons who are infected or whether they have flown to high outbreak regions. If the patient has a history of contact and symptoms, treatment should not be performed and the patient should be directed to the relevant institutions for quarantine or hospitalization, dependent on national guidelines (14). It is advised that dental procedures be delayed for 14 days, which is the quarantine time for asymptomatic patients. Dental operations can be carried out in the absence of contact, symptoms, travel records by taking preventive measures. It is critical for those who accompany the patient to have the same protection measures implemented (26,27).

On presentation of the patient and other persons to the surgery, body temperature should be measured with a contactless thermometer on the forehead, and the presence/absence of abnormal symptoms such as cough, sneezing or difficulty in breathing should be evaluated (2,13). National guidelines should be followed and if the patient is likely to have viral infection, treatment delayed.

Application of only emergency and mandatory treatments

Emergency and mandatory treatments to be applied in dentistry in Turkey have been reported by the Ministry of Health. The list is in accordance with the guidance of Centers for Disease Control and Prevention (CDC) for providing Dental Care during COVID-19 (26,27). These treatments include:

a. Severe toothache caused by pulpal inflammation
b. Severe pain from pericoronitis or third molar
c. Postoperatively developing osteitis or alveolitis
d. Abscess or bacterial infection that causes localized pain and swelling
e. Tooth fracture causing pain or soft tissue trauma
f. Avulsion or luxation injuries due to dental trauma
g. Fractures of the jaw and face
h. Painful lesions / ulcerations of the oral mucosa
i. Life-threatening or uncontrolled bleeding
j. Intraoral / extraoral infections that threaten the patient's airway patency
k. Treatment of patients who are planned to receive, or are receiving, radiotherapy and chemotherapy and planned to take organ transplantation
l. Patients requiring dental consultation for medical problems
m. Suture removal
n. Treatment of temporary restoration losses/fractures and ulcerations of removable dentures so as not to form aerosols
o. Pain and / or infection due to injury in soft tissue as a result of fracture of brackets and wires of patients undergoing orthodontic treatment
p. Feeding plate applications of newborn patients with cleft lip and/or palate
q. Temporomandibular luxation
r. Biopsy (in cases of suspected malignancy)

Treatments other than the above procedures may be delayed based on national guidelines. It is recommended that emergency treatments be implemented by certain staff in isolated areas, and ensuring the temperature and health of all staff are monitored.

Instrument and surface decontamination

Contaminated areas such as common areas, door handles, chairs and tables, and instruments contaminated by patient fluids such as blood and saliva should first be cleaned and then disinfected. Disinfection should not be applied without cleaning (28,29). All areas should be dry and ventilated (30). For cleaning and disinfection, national guidelines may be followed. In the Ministry of Health Guide in Turkey, the following have been proposed (26),

1. Sodium hypochlorite 1:10 diluted standard bleach for blood and body fluids contaminated surfaces;
2. Sodium hypochlorite 1: 100 diluted standard bleach for external surfaces;
3. 0.5% hydrogen peroxide for exterior surfaces of equipment, floors and walls;
4. Quaternary ammonium compounds for floors and walls.
5. Elevators should be regularly disinfected, people using elevators should wear masks correctly and avoid direct contact with touch buttons if possible (2)

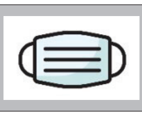
Personal protective equipment

In dental clinics and hospitals, personal protection equipment including goggles, masks, gloves, caps, face shields, and protective outerwear, is strongly recommended for all healthcare providers, especially as airborne droplet transmission is considered the main route of spread (13,14). When wearing and taking off personal protective equipment, caps, protective outwear, masks, goggles, face shields and gloves should be donned in order. While taking off, attention should be paid to the order of gloves, face shields, googles, protective outwear, caps and masks (30,31). Protective outwear should have long sleeves and made of liquid impervious fabric (30). Caps, googles, face shields and masks must be used by all staff. Goggles and face shields should be cleaned with soapy water after each patient and wiped with at least 70% alcohol-based disinfectant. If glove integrity is impaired or contamination occurs, gloves should be removed immediately, and after hand hygiene is provided new gloves should be worn (30).

In regard to masks specifically, they may be described in terms of levels according to their degree of infection prevention (32). Level 1 masks have the least fluid resistance, bacterial filtration efficiency, particulate filtration efficiency, and breathing resistance. Level 2 masks provide a moderate barrier for fluid resistance, bacterial and particulate filtration efficiencies and breathing resistance. Level 3 masks provide the maximum level of fluid resistance recognized by American Society of Testing and Materials (ASTM) and are designed for procedures with moderate or heavy amounts of blood, fluid spray or aerosol exposure (33). Furthermore, according to American Dental Association (ADA), there are 3 different types of masks (Table 1) including surgical masks, N95 and N95 equivalent masks (KN/KP95, PFF2, P2, DS/DL2, Korean Special 1st). N95 and N95 equivalent masks are recommended when treating patients during this pandemic.

Masks should fit the face and be used with other protective equipment. If the masks are not used with goggles or face shields, it is strongly indicated that there is a higher risk

Table 1. *Recommendations for masks for healthcare professionals*

Mask Types	European standard (EN 149:2001)	USA Standard Evaluated, tested, and approved by National Institute for Occupational Safety and Health (NIOSH)	Protection for healthcare professionals against COVID-19
	FFP1	-	Not recommended
	FFP2	N95	Recommended
	FFP3	N99	Recommended
	-	N100	Recommended

of infection (27,33). It should not be neglected to remove the mask lastly after leaving the patient infected zone and then to apply hand hygiene (33).

Reduce reliance on aerosol production and ensure effective Aspiration-Ventilation

During the pandemic, procedures that produce aerosols should be avoided, for example use of high-speed handpieces and ultrasonic devices. Techniques such as Atraumatic Restorative Technique (ART) with Glass Ionomers may be more useful temporary or intermediate term options for restorations than conventional composites that involve aerosol production (13,34,35). Therefore, treatment may be applied with manual instruments if possible. If handpieces are used, devices with an anti-retraction valves should be used together with high-volume aspirators and the procedure undertaken with the 4-hand technique (2,7,13).

The use of rubber-dam may also significantly reduce the formation of aerosol or droplet contaminated with saliva and blood. It has been reported that the use of rubber-dam can reduce particles in the air of the operation area with a diameter of about 90 cm by 70%. High-volume aspirators should be preferred during application and four hand technique should be used (2,7).

Additionally, it is recommended to use mouthwashes containing oxidative agents such as 1% hydrogen peroxide or 0.2% povidone, instead of routine antimicrobial mouthwashes (26). It is important to use mouthwashes especially for situations where rubber dam cannot be used.

Safe waste management

Medical wastes should be removed in accordance with the "Control of Medical Wastes" regulation of Ministry of Environment And Urbanization (36–38). In patients with no suspected infection, red colored plastic bags with black colored "International Biohazard" emblem on both sides and "ATTENTION! MEDICAL WASTE" writing should be used. In patients with suspected infection or the presence of infection, red colored plastic storage containers with black colored "International Biohazard" emblem and a black "CAUTION! PATHOLOGICAL MEDICAL WASTE" writing should be used (37).

Infection control education

Education and Training Staff and supervisors who are involved in the decontamination process should have demonstrated knowledge of national processes and infection control principles (38).

Additional infection control measures for COVID-19 patients in dentistry

Because of these features of dental treatment in which a large number of droplets and aerosols may be produced over a wide area; routine infection control precautions are not adequately efficient in regular clinical practice to control the spread of COVID-19. This problem is particularly compounded when patients are in the incubation phase, are unaware of having being exposed to or want to conceal their disease (12). As a general rule, the treatment of an infected or at-risk patient should be postponed until the patient's active symptoms disappear. The patient should proceed to his dental treatment after receiving necessary treatment for the infection. Apart from these, in exceptional circumstances where a COVID-19 patient requires care, the following rules should be observed in addition to the aforementioned infection control measures:

- COVID-19 patients should be given the last appointment of the day in order to allow aerosols to be neutralized overnight.
- Body temperature should be measured with a contactless thermometer on the forehead.
- Attention should be paid to carry out the dental treatments in a predetermined part of the clinic.
- Protective protection covers should be applied to all water and air spray guns, aerator heads of the unit and other exposed surfaces that are not used clinically

- Unit table, control buttons and reflector arms should be covered with disposable covers.
- The dental clinic and preoperative area reserved for patients with COVID-19 should be ventilated with negative pressure systems and the negative pressure level should be checked. In hospitals where there are no negative pressure systems, positive pressure systems and air conditioners should be closed.
- Going outside with any aprons worn in clinics should be prevented.
- In the case of x-ray; related apparatus should be covered with disposable plastic and taking panoramic radiography should be preferred instead of periapical radiography.
- All touched surfaces should be cleaned with effective disinfectants including flooring, walls and ceilings,
- Prior to taking the patient to the dental unit, all tools that will not be used should be removed.
- All staff in addition to patient and all healthcare professionals should wear a disposable gown and masks.
- Side protection goggles, gloves and surgical mask should be used,
- Disposable instruments should be used in risky patients as much as possible,
- If necessary, double gloves should be used according to the length of the procedure,
- The treatment should be completed as quick as possible, and unnecessary personnel should not be taken into the clinic.
- Surfaces should be disinfected after cleaning with appropriate detergent.
- Hands should be washed with antiseptic solution.
- The materials that are discarded after use should be collected by putting 'septic material' on them by placing the sharp ones in thick and safe protectors (26,30,37,38).

Recommended approaches to primary and secondary dental care during pandemics

As the pandemic continues or is brought under control in various countries, there remains possibility of second waves of COVID-19 or re-emergence of disease (39,40). Therefore, it is likely that the recommendations discussed in this article will need to continue for some time beyond the end of the pandemic phase of an infection. This will require adaptations to the way dentistry is provided in primary care and methods for provision of secondary or specialist care to support ongoing clinical care in practice in challenging situations. It will also be important to focus care towards prevention rather than operative. If possible, some less important treatment may be delayed until after the pandemic phase. In the UK, recommendations for prevention of dental disease and improving access through developing Managed Academic Clinical Networks (MACN) have been published (40).

With regard to secondary care provision, in the authors experience, appointments often necessitate a physical second dental visit and consultation with a specialist, with additional contact required between patients, staff and health care professionals. In this regard, the emerging era of digital dentistry may enable new opportunities during a pandemic for remote appointments to take place in certain clinical situations. One study assessed the viability of remote clinical consultations for the management of referrals from primary or general dental practitioners in restorative dentistry, compared to an in-person consultation with the patient also attending a secondary care environment, such as another dental practice or hospital (34). It found that remote consultations, using digital technologies, were as effective and safe as in-person consultations and had high patient acceptance. Moving on from this work, it may be possible for patients with dental care needs (who themselves are at higher risk of disease or carry an infection) to attend an appointment at a dedicated hub or site with the necessary infection control measures discussed above. Information from the examination and images from special tests may then be safely and effectively shared remotely and live with specialists at a safe distance, enabling simple and practical guidance for complex clinical care if required.

The high concern of second wave pandemic reveals the importance of the continuity of measures taken during and after the pandemic (39,40). It is clear that adding the fever measurements to routine protocol will be effective to reduce the potential transmission risk. As of today's knowledge, support should be obtained from the current technologies for patient intervention, treatment process and keeping the environment clean. In prosthetic applications, it is preferred to use digital impression, if possible, to reduce the risk of long contact with saliva or transfer to the surfaces. Applications such as ozone, ultraviolet in line with the scientific study results for the sterilization of the environment should be utilized between patient intervals. Utilization of ceilings or floor instruments providing vacuum in addition to collecting the aerosol spread in the environment would be helpful during the treatment of these patients. As the oronasal way is known to be the primary road of transmission, the use of a mask covering only the nose of the patient during treatment could also be beneficial. This measure may increase the effectiveness of the protection of clinician while working at a short distance. It could also be predicted that the use of newly developed antiviral mouthwashes by physicians, patients, healthcare professionals, individuals with high risk, and their addition to the water supply coming to the dental unit may reduce the risk of transmission. The fact that pandemic is so effective all over the world will definitely lead to an increase in some measures and the development of new technologies in dentistry. For example, the development of non-aerosol devices.

Conclusion

The recent COVID-19 outbreak shows that all people are considered potential carriers of high-risk infection. It is therefore important to ensure routine and additional implementation of infection control procedures and adaptation of methods of dental care delivery in order to reduce risks of cross-infection and enable continuation of dental care provision. The recommendations discussed in this article, provide a tool for implementation of infection control and dental care delivery by dental healthcare professionals before, during and following an infection pandemic. In addition to routine measures, it is important to include the measures implemented during the epidemic in routine protocols.

***Türkçe Özet:** Diş Hekimliğinde Coronavirüs 2019 hastalığı (COVID-19) nedeniyle ortaya çıkan zorlukları yönetmek. Diş klinikleri; sağlık profesyonelleri, yardımcı personel, hastalar ve hasta yakınlarının birarada bulunduğu ve potansiyel olarak patojenik mikroorganizmaların yayılabileceği enfeksiyon riski konusunda riskli merkezler olarak görülmektedir. Çapraz enfeksiyon, tükürük, deri veya dolaylı olarak hava, su ve kontamine yüzeyler veya aletler yoluyla meydana gelebilmektedir. Bu nedenle, bireyler, potansiyel enfeksiyon kaynakları olarak düşünülmelidir. COVID-19 pandemisi sırasında, kısa vadede, çapraz enfeksiyon riskini azaltmak için bazı tedavilerin ve klinik uygulamaların önlem olarak sınırlandırılması benimsenmiştir. Bununla birlikte, gerekli ve nitelikli bakımın sürdürülebilmesi için dental tedavi süreçlerinin COVID-19'dan sonra da enfeksiyon risklerini önlemek için spesifik enfeksiyon kontrol stratejilerini takip etmesi gerekmektedir. Bu makalede, pandemiden önce, pandemi sırasında ve sonrasında enfeksiyon risklerini azaltmak için, ulusal ve küresel önerilere dayanarak birinci ve ikinci basamakta sürekli diş hekimliği hizmetlerinin sağlanmasına olanak tanıyan pratik öneriler sunulmaktadır. Anahtar Kelimeler: COVID-19, Korunma önlemleri, Diş Hekimliği, Bulaş yolları, Çapraz enfeksiyon*

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