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BOND STRENGTH OF THE BULK FILL COMPOSITE MATERIALS

ЈАЧИНА НА ВРСКАТА КАЈ BULK FILL КОМПОЗИТНИ МАТЕРИЈАЛИ

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Abstract

INTRODUCTION. The Bulk Fill composites possess specific features, including increased flow rates in order to achieve an appropriate cavity adaptation. An improved depth of at least 4 mm eliminates the need for layered application techniques, thereby reducing working hours. **AIM.** The aim of this study is to compare the shear bond strength (SBS) of the recently introduced three different brands of Bulk Fill composites, with one conventional micro hybrid and one nano hybrid composite. **MATERIALS AND METHODS.** In order to realize the set goals in our in vitro study, we included 35 extracted, non-carious molars in male and female patients of different ages as research samples. Three commercial Bulk Fill composites (TetricEvoCeram Bulk Fill, TetricEvoCeram Bulk Flow, SureFil® SDR® Flow) have been tested and used to control two conventional composites with a layered application technique of 2 mm (Filtek Z250, Gradia posterior). Randomly selected teeth were divided into 5 groups, with 7 teeth each. **RESULTS.** The average values and standard deviations in all groups are presented in Table 2 and Figure 1. The highest average SBS (6.24 ± 1.65MP) has been noted for the Filtek nano hybrid composite (Group IV) bound to a dentine sample, while the lowest average SBS (4.52 ± 0.65 MP) has been registered for full filling SDR composite (Group I). **CONCLUSION**. The results indicate that the application of bulk fill composite material results in an acceptable SBS comparable to that achieved through conventional RBC. As such, bulk fill composites can provide reliable alternatives to conventional composites. **KEY WORDS:** composit, tehnique, strength.

Апстракт

ВОВЕД. Bulk Fill композити поседуваат специфични карактеристики, вклучувајќи зголемена проточност за да се постигне соодветна адаптација на кавитетит. Подобрената длабочина од најмалку 4 мм ја елиминира потребата за слоевита техника на нанесување, а со тоа се намалува работното време. **ЦЕЛ.** Целта на оваа студија е да се спореди јачината на силата на смолкнување (shear bond strength - SBS) на неодамна воведените три различни брендови на Bulk fill композити, со еден конвенционален микро хибриден и еден нано хибриден композит. **МАТЕРИЈАЛ И МЕТОД**. За да ги реализираме зададените цели во нашата ин витро студија како истражувачки примерок вклучивме 35 екстрахирани, некариозни, молари кај пациенти од машки и женски род со различна возраст. Беа тестирани три комерцијални Bulk fil (TetricEvoCeram Bulk Fill, TetricEvoCeram Bulk Flow, SureFil® SDR® Flow) композити и беа користени како контрола два конвенционални композити со слоевита техника на нанесување од 2 mm (Filtek Z250, Gradia posterior). Забите по случаен избор беа поделени на 5 групи, со по 7 заби. **РЕЗУЛТАТИ.** Највисоката средна SBS (6,24± 1,65MPa) е забележана за Filtek нанохибриден композит (група IV) врзан за примерок од дентин, додека најнискиот просечен SBS (4,52 ± 0,65 MPa) е регистриран за SDR композитот со целосно пополнување (група I). **ЗАКЛУЧОК.** Резултатите покажуваат дека примената на bulk fill композитниот материјал резултира со прифатлив SBS кој е споредлив со оној постигнат преку конвенционалните RBC. Како такви, bulk fill композитите може да претставуваат сигурни алтернативи на конвенционалните композити. **КЛУЧНИ ЗБОРОВИ:** композити, техника, јачина.

Introduction

The quest for restorative material having the optimum properties of fusion with solid dental substances leads to numerous dental materials, adhesive systems and restorative techniques. The main disadvantage of resin-based materials is the contraction while bonding. The polymerization contraction of the composite breaks the bond between the dentine adhesive and the tooth tissue, especially in the insufficiently well-conditioned surfaces, and this leads to oblique micro cracks. The prevention of these micro cracks is in the creation of a stronger bond between the dentine adhesive and the composite with the tooth tissues. The strength of the polymerization contraction depends on:

1. The formula of the resin matrix and the amount of the filler into the composite;
2. The appearance of the cavity;
3. The type of the base;
4. The size, shape and location of the composite layer;
5. Light or chemical polymerization;
6. Module of elasticity (factor C);
7. The intensity of the connecting light, and
8. Degree of inbuilt porosity[1, 2, 3, 4, 5].

The Bulk Fill composites possess specific features, including increased flow rates in order to achieve an appropriate cavity adaptation. Elasticity and low polymerization lead to stress reduction and micro-flow, and thus to a reduction in postoperative sensitivity and the emergence of secondary caries. An improved depth of at least 4 mm eliminates the need for layered application techniques, thereby reducing working hours[6]. Today, traditional techniques of placing composite resins include this technique[7]. Most physicians recommend layered placement of composites with a layer thickness of 2 mm. However, every dentist will prefer composite material that can be used with Bulk Fill technique especially for posterior restorations. Therefore, the purpose of this study is to compare the shear bond strength (SBS) of the recently introduced three different brands of Bulk Fill composites, with one conventional micro hybrid and one nano hybrid composite.

Material and methods

In order to realize the set goals in our in vitro study, we included 35 extracted, non-carious molars in male and female patients of different ages as research samples, which after extraction have been stored at 4°C in a 0.5% solution of sodium hypo chloride for 24 hours before being washed with a saline solution and stored in distilled water at room temperature during the test period.

The teeth used for this study were extracted for a period not longer than 6 months. Teeth extracted more than 6 months before use may be subject to degenerative changes in dentinal protein.

We used the occlusion surface of the extracted molars. Each tooth was placed in a 25 mm internal diameter and 25 mm height mold, made of PVC (poly - vinyl - chloride). The mold was filled with mixed self-binding acrylic - ORTOpoli-PoliDent, Slovenia. When the molds were filled, they were placed in a cold water bath during the polymerization of the acrylic because the heat from the polymerization could adversely affect the samples. Immediately after hardening of the acrylic we opened the hoods and removed the prepared samples from the mold.

The coating enamel was cut by IsoMet 1000 (precision cutting machine with diamond discs of Buehler Ltd, Lake Blef, IL, USA) with water cooling, ensuring to reach only the surface dentin.

We used surface dentine as close as possible to the enamel, in order to reduce the test variations. We created a standard, repetitive, flat surface. We kept the surfaces of the teeth wet at any time, because dentine is particularly sensitive to dehydration. Exposing the surface of the teeth in the air for several minutes can cause irreversible changes in the adhesive bond.

Three commercial Bulk Fill composites were tested and used to control two conventional composites with a layered application technique of 2 mm. The materials used in the study are presented in Table 1.

Randomly selected teeth were divided into 5 groups, with 7 teeth each.

- **Group I:** After the adhesive application of each sample with G Bond-GS, using a technique of self-etching, we set a pier with SDR Flow (Dentsply. Konstanz. Germany) composite resin with a height of 3.0 mm and a diameter of 3.0 mm. Each composite excess was carefully removed and then polymerized for 20 seconds using a diode for transmitting polymerizing light (LED) with intensity of 1000 mW/cm^2 (Elipar S10, 3M ESPE, Seefeld, Germany).
- **Group II:** After the adhesive application of each sample with G Bond-GS, a pier with a composite resin TetricEvoCeram Bulk Flow (Ivoclar. Vivadent. AG. Liechtenstein) with a technique of self-etching was placed with a height of 3.0 mm and a diameter of 3.0 mm. Each composite excess was carefully removed and then polymerized for 20 seconds using a light emitting polymerizing light (LED) with a power of

Table 1. Materials used in the study.

Type of material	Name	Manufacturer	Volume of filler (%) (wt)
Nanohybride composite	Filtek Z250	3 M ESPE	73,5%
Nanohybride composite	TetricEvoCeram Bulk Fill	Ivoclar. Vivadent. AG. Liechtenstein	79% - 81%
Flow composite	TetricEvoCeram Bulk Flow	Ivoclar. Vivadent. AG. Liechtenstein	
Flow composite	SureFil® SDR® Flow Dentsply	Konstanz. Germany	68%
Microhybrid composite	Gradia posterior	GC Corp. Tokyo. Japan	81%

1000 mW/cm^2 (Elipar S10, 3M ESPE, Seefeld, Germany).

- **Group III:** After the adhesive application of each sample with G Bond-GS, a pier with composite resin TetricEvoCeram Bulk Fill (Ivoclar. Vivadent. AG Liechtenstein) with a height of 3.0 mm and a diameter of 3.0 mm was set with a self-etching technique. Each composite excess was carefully removed and then polymerized for 20 seconds using a light emitting polymerizing light (LED) with a power of 1000 mW/cm^2 (Elipar S10,3M ESPE, Seefeld, Germany).
- **Group IV:** After the application of the adhesive asset, G Bond-GS, a column of conventional nano hybrid composite resin Filtek Z250 (3M ESPE) with a height of 3.0 mm and a 3.0 mm diameter was placed. Each layer was two millimeters thick and polymerized with LED polymerizing light with intensity of 1000 mW/cm^2 for 20 seconds (Elipar S10, 3M ESPE, Germany).
- **Group V:** Following the application of the adhesive asset, G Bond-GS, a column of conventional monohybrid composite resin Gradia posterior GC (Corp. Tokyo. Japan) was installed with a height of 3.0 mm and a 3.0 mm diameter. Each layer was two millimeters thick and polymerized with LED polymerizing light with a intensity of 1000 mW/cm^2 for 20 seconds (Elipar S10, 3M ESPE, Germany).

Once prepared, the samples were stored in a 37 °C incubator with a 100% humidity over 24 hours before testing the shear bond strength (SBS) of the resin bond using a universal test machine (INSTRON 4301) at a speed of 0.5 mm/min. SBS of the dentin composite resin was recorded in Newton and calculated in MP, and the cross-sectional area of the composite composition was taken into account.

Statistical analysis was carried out with SPSS Statistics v. 22 software (IBM, NY, USA). One-way ANOVA was used for group comparison of the relation strength. Shapiro-Wilk and Levene tests were carried out in order to assess the assumptions on the data normality and the difference homogeneity. Post hoc testing for multiple comparisons among groups was carried out by the Tukey's HSD test. P-values were adjusted by the Bonferron correction method for multiple comparisons. The confidence level was set at 95% ($p<0.05$).

Results

The average values and standard deviations in all groups are presented in Table 2 and Figure 1. The highest average SBS (6.24 ± 1.65MP) was noted for the Filtek nano hybrid composite (Group IV) bound to a dentine sample, while the lowest average SBS (4.52 ± 0.65 MP) was registered for full filling SDR composite (Group I). From Table 3 we can see that this difference between Group IV and I is statistically significant ($p=0.031$). Although Group III showed higher bonding strength than group I, group II, and group V, these differences were not considered statistically significant (p values between group III and I, group III and II, group III and V, were found as $p=0.196$, $p=0.272$ and $p=1.000$

Table 2. Average Values and Standard Deviations respectively

Descriptive Statistics

Dependent Variable: SBS

Composite	Mean	Std. Deviation	N
SDR	4.5223	0.65986	7
Tetric EvoCeram flow	4.8716	1.03139	7
Tetric EvoCeram bulk fill	5.8462	0.72230	7
Filtek	6.2481	1.65103	7
Gradia	5.0344	0.54211	7
Total	5.3045	1.14579	35

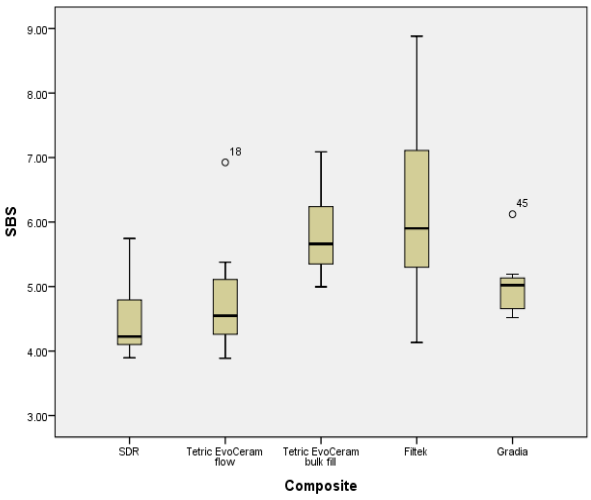

Figure 1. Average Values and Standard Deviations

Table 3. Pairwise Comparisons

Dependent Variable: SBS

(I) Composite	(J) Composite	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference [b]	
					Lower Bound	Upper Bound
SDR	Tetric EvoCeram flow	- 0.349	0.537	1.000	-1.975	1.277
	Tetric EvoCeram bulk fill	-1.324	0.537	0.196	-2.950	0.302
	Filtek	-1.726*	0.537	0.031	-3.352	-0.100
	Gradia	-0.512	0.537	1.000	-2.138	1.114
Tetric EvoCeram flow	SDR	0.349	0.537	1.000	-1.277	1.975
	Tetric EvoCeram bulk fill	-0.975	0.537	0.794	-2.601	0.651
	Filtek	-1.377	0.537	0.156	-3.003	0.250
	Gradia	-0.163	0.537	1.000	-1.789	1.463
Tetric EvoCeram bulk fill	SDR	1.324	0.537	0.196	-0.302	2.950
	Tetric EvoCeram flow	0.975	0.537	0.794	-0.651	2.601
	Filtek	-0.402	0.537	1.000	-2.028	1.224
	Gradia	0.812	0.537	1.000	-0.814	2.438
Filtek	SDR	1.726*	0.537	0.031	0.100	3.352
	Tetric EvoCeram flow	1.377	0.537	0.156	-0.250	3.003
	Tetric EvoCeram bulk fill	0.402	0.537	1.000	-1.224	2.028
	Gradia	1.214	0.537	0.311	-0.412	2.840
Gradia	SDR	0.512	0.537	1.000	-1.114	2.138
	Tetric EvoCeram flow	0.163	0.537	1.000	-1.463	1.789
	Tetric EvoCeram bulk fill	-0.812	0.537	1.000	-2.438	0.814
	Filtek	-1.214	0.537	0.311	-2.840	0.412

Based on estimated marginal means

*. The mean difference is significant at the 0.05 level. b. Adjustment for multiple comparisons: Bonferroni.

Univariate Tests

Dependent Variable: SBS

	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power [a]
Contrast	14.392	4	30.244	3.569	0.017	0.322	14.276	0.810
Error	30.244	30	1.008					

The F tests the effect of Composite. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Computed using alpha = 0.05

Table 4. Sapiro-Wilk test of normality

	Composite	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
SBS	**SDR**	0.245	7	0.200*	0.864	7	0.165
	Tetric EvoCeram flow	0.226	7	0.200*	0.863	7	0.161
	Tetric EvoCeram bulk fill	0.172	7	0.200*	0.949	7	0.719
	Filtek	0.208	7	0.200*	0.950	7	0.727
	Gradia	0.243	7	0.200*	0.853	7	0.131

*. This is a lower bound of the true significance. a. Lilliefors Significance Correction

Although the binding strength of Tetric evo ceram bulk fill composites was the highest (5.84 ± 0.72) compared with the other two bulk fill composites, SDR (4.52 ± 0.65) and Tetric evo ceram flow (4.87 ± 1.03), no statistically significant differences between bulk fill composites were registered.

Table 5. Leven test of homogeneity

		Levene Statistic	df1	df2	Sig.
SBS	Based on Mean	2.291	4	30	0.083
	Based on Median	1.498	4	30	0.228
	Based on Median and with adjusted df	1.498	4	18.465	0.244
	Based on trimmed mean	2.198	4	30	0.093

Table 6. Post Hoc Tests

(I) Composite	(J) Composite	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
SDR	**Tetric EvoCeram flow**	-0.3493	0.53669	0.965	-1.9060	1.2074
	Tetric EvoCeram bulk fill	-1.3239	0.53669	0.126	-2.8807	0.2328
	Filtek	-1.7258*	0.53669	0.024	-3.2825	-0.1691
	Gradia	-0.5121	0.53669	0.873	-2.0688	1.0447
Tetric EvoCeram flow	**SDR**	0.3493	0.53669	0.965	-1.2074	1.9060
	Tetric EvoCeram bulk fill	-0.9746	0.53669	0.384	-2.5314	0.5821
	Filtek	-1.3765	0.53669	0.103	-2.9332	0.1802
	Gradia	-0.1628	0.53669	0.998	-1.7195	1.3940
Tetric EvoCeram bulk fill	**SDR**	1.3239	0.53669	0.126	-0.2328	2.8807
	Tetric EvoCeram flow	0.9746	0.53669	0.384	-0.5821	2.5314
	Filtek	-0.4019	0.53669	0.943	-1.9586	1.1549
	Gradia	0.8118	0.53669	0.563	-0.7449	2.3686
Filtek	**SDR**	1.7258*	0.53669	0.024	0.1691	3.2825
	Tetric EvoCeram flow	1.3765	0.53669	0.103	-0.1802	2.9332
	Tetric EvoCeram bulk fill	0.4019	0.53669	0.943	-1.1549	1.9586
	Gradia	1.2137	0.53669	0.186	-0.3430	2.7705
Gradia	**SDR**	0.5121	0.53669	0.873	-1.0447	2.0688
	Tetric EvoCeram flow	0.1628	0.53669	0.998	-1.3940	1.7195
	Tetric EvoCeram bulk fill	-0.8118	0.53669	0.563	-2.3686	0.7449
	Filtek	-1.2137	0.53669	0.186	-2.7705	0.3430

Based on observed means.
The error term is Mean Square(Error) = 1.008. *. The mean difference is significant at the 0.05 level.

Table 7. Tukey HSD[a,b] test
SBS

Tukey HSD[a,b]

Composite	N	Subset	
		1	2
SDR	7	4.5223	
Tetric EvoCeram flow	7	4.8716	4.8716
Gradia	7	5.0344	5.0344
Tetric EvoCeram bulk fill	7	5.8462	5.8462
Filtek	7		6.2481
Sig.		0.126	0.103

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 1.008.
a. Uses Harmonic Mean Sample Size = 7.000.
b. Alpha =0.05.

Discussion

The purpose of this study was to do research and compare SBS of the composite resins with the bulk application technique and the conventional composite resins with a layered material application technique. Thus, although Bulk Fill composites showed lower SBS values than the conventional composite, a significant difference between Filtek and SDR was registered. Thus, the first null-hypothesis was partially rejected.

Agarwal et al.[8] evaluated the cervical marginal and internal adaptation of composite resins with posterior full charge with different viscosities, before and after thermo cycling. They found that liquid bulk fill composites demonstrated significantly better marginal adaptation. However, researchers found no difference between conventional composites and bulk fill composites[8].

Previous studies have shown that samples tested with composite resins with bulk fill application technique show better polymerization depth than those treated with conventional composite resins[9]. However, in this study, there was no significant difference between the three bulk fill composites, despite the fact that tetric evo ceram bulk fill demonstrated higher SBS than the SDR and Tetric evo ceram flow, which is in line with a study by Hakan Colak et al. Thus, the second null-hypothesis was accepted.

In his in vitro study, Miroslaw Orlowski et al. comparing the marginal integrity between the four types of Bulk Fill composite materials came to the conclusion that bulk fill liquid and sonic activated liquid composites have better marginal adaptation than bulk fill composites in paste form[10].

Peutzfeldt and Asmussen showed that fluidity level in the application of the composite material affects the marginal adaptation; the increased fluidity of the composite makes it better to adhere to the walls of the cavity[11]. In our study, although there was a difference between the strength of the relationship in the three bulk fill composites, the results obtained are not extremely significant, which is correlated with the study of Alrahlah et al.[12].

In this study, bulk fill composite filling showed statistically similar SBS values. This may be because they have shown very similar mechanical properties and consistency[13, 14]. As confirmed in various in vitro studies, resinous full-fill composite materials can be polymerized into a thicker layer of material, since the degree of polymerization and micromechanical properties can be maintained within the 4-millimeter layers at a radiation time of up to 20 seconds[10].

Conclusion

Although this study has a number of limitations, the results indicate that the application of bulk fill composite material results in an acceptable SBS comparable to that achieved through conventional RBC. As such, bulk fill composites can provide reliable alternatives to conventional composites. This can be of potential benefit to dentists because the bulk fill composite materials are simpler than the conventional composites and can be more efficiently applied. However, further research in this area is required to better understand how the relation forces of these adhesion systems relate to clinically acceptable conditions.

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THE ROLE OF ROTARY SYSTEMS IN ENDODONTIC TREATMENT- REVIEW ARTICLE

УЛОГАТА НА МАШИНСКИТЕ СИСТЕМИ ВО ЕНДОДОНТСКИ ТРЕТМАН-РЕВИЈАЛЕН ТРУД

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Abstract

The instrumentation of the root canal system involves the enlargement and shaping of the complex endodontic space and its disinfection. Various instruments and techniques have been developed and described for this critical phase of treatment of the root canal system. Although many data on the cleaning and shaping of the root canal system can be found in the literature, the definitive scientific evidence of quality and the clinical proper use of various instruments and techniques remain incomplete. The reasons for this outcome are the existence of methodological problems, due to which it is difficult to make a comparison between the various studies that relate to the different endodontic systems and their implementation in endodontic treatment-retreatment. Therefore, the main purpose of this study is to summarize the data from previous research related to the examination and comparison of various endodontic systems and to consider the role of individual endodontic systems in the final success of the endodontic treatment-retreatment. Key words: efficiency, rotary systems for retreatment, root canals, evaluation, debris extrusion, instrument design.

Апстракт

Подготовката на коренскиот канален систем вклучува проширување и обликување на комплексниот ендодонтски простор и негова дезинфекција. Различни инструменти и техники се развиени и опишани за оваа критична фаза на третман на коренскиот канален систем. Иако многу податоци за подготовка на коренскиот канален систем можат да се најдат во литературата, дефинитивните научни докази за квалитетот и клиничкото соодветно користење на различни инструменти и техники остануваат нецелосни. Причините за ваквиот исход се постоење на методолошките проблеми, поради кои е тешко да се направи споредување меѓу различните истражувања, кои се однесуваат на различните ендодонтски системи и нивната имплементација во ендодонтскиот третман-ретретман. Поради тоа, главна цел на оваа студија е да се сублимираат податоците од претходните истражувања кои се однесуваат на испитување и споредба на различните ендодонтски системи и да се согледа улогата на поединиот ендодонтски системи во конечниот успех на ендодонтскиот третман-ретретман. Клучни зборови: ефикасност, ротациони системи за ретретман, коренски канали, евалуација, екструзија на дебрис, дизајн на инструментот.

Introduction

The ultimate goal of endodontic therapy is a fully filled canal system with adequate fillers and preserving a healthy apical periodontium. The success of the endodontic therapy is correlated with the efficiency of the treatment system to be used in this procedure. Technological advances and its implementation in dentistry offer a variety of modern endodontic systems. In the past, endodontic treatment was realized only with the help of manual instruments.

Mechanical instrumentation of the root canal system is a very important stage in endodontic therapy, because it enables the shaping and enlargement of the root canal system and thus allows the irrigation and application of medicaments for more effective results in order to eliminate bacteria and their products. This remains to be one of the most difficult tasks in endodontic therapy. The main objectives of the instrumentation of the root canal system are prevention of periradicular infections and/or realization of endodontic treatment in cases where the infection already exists through:

- Removal of vital and necrotic tissue from the main root canal
- Creation of sufficient space for irrigation and medication
- Preservation of the integrity and location of the apical canal anatomy

- Avoiding iatrogenic damage to the canal system and root structure
- Making the root canal filling easier
- Avoiding further irritation and / or infection of periradicular tissues

The techniques for processing the root canal include: manual instrumentation, root canal instrumentation, ultrasonic instrumentation, use of laser systems and NIT systems (separately or in combination). Ingle[1] formalized a technique for the instrumentation of the root canal system, which was then accepted as a "standardized technique", which compares all other techniques that follow it. In this technique, each subsequent instrument is actively introduced to the working length, after which the canal shape is achieved, which corresponds to the shape and size of the final instrument. Shilder[2] pointed out to the need of removing all organic contents from the entire root canal space using instruments and abundant irrigation and he considers that the final shape of the canal system does not depend solely on the individual and unique anatomy of each root canal, but also on the technique of work and the material for final filling.

Shilder described five design goals for the root channel system:

1. Continuous enlargement of the canal to the funnel form
2. The diameter of the cross section of the canal should be narrower at each point, descending apical
3. The instrumentation of the root canal should follow the shape of the original canal
4. Apical anatomy should remain in its original position
5. The apical opening should be preserved in its original size.

He also described four biological principles:

1. The configuration of the instruments should be appropriate to the root canals
2. To prevent the extrusion of necrotic debris beyond foramen apicale
3. Removal of all tissue from the root canal space
4. Creating a sufficient space for intra-canal medicaments.

Starting from these design goals and biological principles, which are necessary for successful endodontic treatment, the complete endodontic treatment or retreatment is a real challenge. The complexity of the anatomy of the root canal system, including a wide range of variations in number, length, curvature, and root canal diameter; the complexity of the apical anatomy with the accessory canals; the communications between the canal space and the lateral periodontium as well as the furcation area are the factors that affect the final result of the endodontic therapy.

Fauchard, one of the founders of modern dentistry, describes instruments for endodontic instrumentation of root canals and the removal of the pulp in his book "Le chirurgien dentist", giving a systematic description of the instrumentation of the root canal system for the first time at that time. Edvard Maynard is responsible for the development of the first endodontic manual instruments[4, 5]. In 1852, Arthur used thin instruments for processing the root canals[5-9]. In 1885, Gates Glidden instruments were introduced, and in 1915 K-file instruments, and until today, they are part of the standard endodontic instruments. Although standardization of instruments was proposed by Trebitsch in 1929 and again by Ingle in 1958, ISO specifications for endodontic instruments were only accepted in 1974[10].

The first description of the use of machine rotary devices (dental hand pieces) is by Oltramare[11]. He used endodontic rectangular instruments that he introduced passively into the root canal up to the foramen apicale, and then started their rotation. He insists that in curved channels only thin instruments should be used to avoid fractures of the instrument. In 1889, William H. Rollins was the first to use canal instruments with a different design of the working part developed for machine root canal preparation with a 360° rotation. In order to avoid instrument fractures, the rotational speed was limited to 100 r.p.m.[12]. In the following years, several different endodontic rotary systems were introduced, but they all used the same principle of operation (rotation of 360° with rotational speed of 100 r.p.m). In 1928, W & H (Burmoos, Austria) created a system that used a combination of rotational and vertical motion of the instrument. Due to well-managed marketing, Europe's most popular endodontic hand pieces were Hander-handpiece (W & H) in 1958 and Giromatic (MicroMega, Besancon, France) in 1964. The root canal instruments in both systems were made of stainless steel, and their work was limited to only one type of motion; either rotational or vertical motion of the instrument up and down. Racer instruments used vertical motion and Giromatic reciprocal with rotation of 90˚. The dentist could only affect the rotational speed of the hand-piece and the vertical amplitude of the endodontic instrument by moving the dental hand-piece[10, 13].

Then a period of modified endodontic hand-pieces followed, with a main goal to achieve a flexible movement in order to follow the anatomy of the root canal. Excalibur (W & H), which enables the lateral oscillation of the instrument and Endoplaner (Microna,

Spreitenbach, Switzerland) are examples of the development of endodontic hand-pieces using flexible motion[10, 13]. The endodontic hand-pieces made of nickel-titanium (NiTi) were first described by Walia et al.[14]. NiTi rotary instruments were introduced later and they used a 360° rotation at low speed. Contemporary endodontics continued to use manual instruments, but NiTi rotary instruments and new techniques for their use (a variety of endodontic motor-rotary systems) offer new perspectives for the instrumentation of the root canal system with the potential to overcome some of the major disadvantages of traditional endodontic instruments.

Discussion

Methodological aspects in the evaluation of the quality of the root canal preparation

During the past decades, a number of studies have been conducted and published on the instrumentation of the root canal system. Unfortunately, the results are partly contradictory and do not result in definitive conclusions that would point to the advantage of certain endodontic systems (manual or motor systems). There is also a significant deficit of studies in terms of the quality of the root canal instrumentation. The fact is that despite the use of endodontic instruments for almost a century, there is no defined mode of operation that would represent a gold standard for their usage[15-17]. In most experimental studies published in the literature, there are a small number of rotary systems or rotational techniques that are examined and compared. Only a few studies included the comparison of four[18, 16, 19-23], five[24] or six and more[11, 25, 26, 27-32] devices and techniques. In most of these published studies, the survey involves a limited number of parameters, which yield results of a limited character. Most of the studies are still focusing primarily on, or only on, the shape of the root canal system and much smaller is the number of studies that analyze the ability of endodontic systems for root canal cleaning. Data on the working time with a certain endodontic system, as well as safety at work (in terms of instrument fractures and periapical debris extrusion as prevention of additional infection) are not usually the target of specific experiments, but are accompanying observations of research designed for other purposes. The wide range of experimental designs of endodontic instruments and different working methods, as well as the various evaluation criteria, do not allow the comparison of results from different studies, even when performed with the same endodontic system and technique.

Many publications do not contain sufficient data on the composition of the examined sample, the experience of the operator, and in particular there has been criticism that many study protocols have been modified by the researchers instead as proposed by the manufacturer of the endodontic system, which may result in inadequate usage of the instruments and lead to false results and conclusions.

Evaluation criteria for endodontic treatment

The first criterion is the cleanliness of the root canal space after endodontic treatment. Several different protocols have been described. Some of these studies are only of descriptive nature[20, 21, 33, 34], whereas others use predefined results. These scoring systems include those with three results[35-36], four results[21, 37], and even seven results according to examined endodontic systems[(38)]. In most of the studies, the cleanliness of the root canal space has been shown to be superior in the coronary section of the root canal compared to the apical third[13]. Because of this, it seems that the evaluation procedure that specifies the results for different parts of the root canal is more adequate.

The second criterion is the evaluation of the postoperative form of the root canal system. The purpose of these type of studies is to evaluate the preservation of the original shape of the canal[39, 40]. It was ascertained that working with samples of extracted human teeth provides better reproduction of the clinical conditions, in contrast to studies using acrylate blockages[41]. On the other hand, the wide spectrum of variations in the three-dimensional morphology of the root canal system makes standardization of the procedure difficult to achieve[42].

There are variations in the length and width of the root canal, the density of the dentin, the irregular calcification of the pulp, the size and location of the apical constriction, and in particular the angle, radius, length and location of the curvatures of the root canal, including the three-dimensional nature of the curvatures[43, 44]. Several techniques have been described to determine the characteristics of the curvature and most commonly used is the Schneider technique[45].

The third criterion is to determine the quantity of debris extrusion through the apical constriction, carried out by collecting and measuring this material during the preparation of the extracted teeth[11, 46-50]. The methods of evaluating the apical debris extrusion are different in various studies, but some can be compared. A significantly higher amount of apical-extruded debris was found in endodontic retreatment with manual instruments compared to motor dental hand-pieces, which is consistent with the results of other studies[51, 52, 53]. Bharathi et al.[52] measured a significantly lower amount of apical extruded debris in endodontic retreatment with ProFile

instruments, compared to Hedström's manual instruments. In addition, machine endodontic systems (Mtwo and Reciproc) have proved to be more successful than Hedström's manual instruments in terms of the quantity of apical extrusion (residues in the root canal preparation)[53]. In a study by Topçuoğlu et al.[54], all evaluated techniques for endodontic retreatment caused apical debris extrusion. The researchers concluded that the hand instruments produced significantly more apical extruded material than the ProTaper, D-RaCe and R-Endo rotary systems, while there was no statistical difference between the rotating systems, which is consistent with the results of Pešić et al.[55]. Findings from previous studies have shown that machine endodontic systems tend to direct debris in root canal preparation up to a maximum percent coronary, rather than apically[51, 52, 53, 56]. These studies indicate that the Crown-down technique reduces the possibility of debris extrusion to apical, that is, allows the evacuation of the debris of the root canal treatment in the coronary direction[57].

The fourth criterion is about safety work, and with regard to instrument fractures, apical blockades, loss of working length, perforation. Most of these questions have not been systematically examined in specially designed research for this purpose[58-59]. The instrument fractures may be related to the type, design and quality of the instruments, the material from which they are produced, the rotational speed and torque, the pressure during preparation, the angle and radius of curvature of the root canal, the frequency of use, the technique of sterilization, as well as the level of expertise of the operators.

The fifth criterion is the evaluation of working time in order to obtain conclusions about the efficiency of the system or technique. Data on working time show great differences in identical instruments and techniques, which is due to different methodological approaches, but also due to individual factors (operator)[60, 61].

Conventional rotary systems

Gottingen and coworkers[11] have realized a series of experiments comparing the ability to prepare the root canal system, the ability to completely clean it without residues and the work reliability of various conventional machine endodontic instruments[11]. The study includes a total of 15 groups with 15 teeth. The following endodontic systems were examined: Giromatic, Endolift, Endocursor, Canal-Leader, Canal-Finder, Intra-Endo 3-LDSY, manual preparation, Excalibur, Endoplaner, Ultrasound and Rotofile NiTi Instruments (known as MiTy-Roto-Files).The average curvature of the root canal of the various groups in this study was between 17.81˚ and 25.11˚, and all the root canals were prepared to size # 35 of the instrument. Further studies were performed on Excalibur[62] and Endoplaner. All of these studies have shown that the instrumentation of curved canals using conventional stainless steel instruments in many cases resulted in a serious change in the anatomy of the root canal system, as well as large depositions of debris and smear layers along the canals[20, 29, 63-65]. In addition, in some of the machine endodontic systems there were identified major deficiencies in terms of work safety (apical blockages, loss of working length, perforations and fractures of the instrument[11, 21, 27, 28, 32, 49, 63].

NiTi systems

Metallurgical aspects

The metallurgical aspects of the NiTi instruments refer to the two main characteristics of this alloy composed of approximately 55% nickel and 45% titanium, namely: the memory shape of the metal and the superior elasticity. The elastic limit of bending and torsion is two to three times higher than that of steel instruments. The modulus of elasticity is considerably lower for NiTi alloy than for steel and therefore forces applied to the radicular dentine in the instrumentation of the canal are much smaller than when working with steel instruments.

These unique properties are related to the fact that NiTi is the so-called "alloy with memory of shape", which exists in two different crystalline forms: austenite and martensite. The austenitic phase is transformed into a martensitic phase during stress at a constant temperature and in this form a small force for working with the instrument is required. After the release of the metal from the stress moment, it returns to the austenitic phase and the instrument returns to its original form. Because of these properties of the NiTi alloy, it became a reality to produce endodontic instruments with a larger cone of 2%, which is a standard for steel instruments[66].

Design of NiTi systems

Over the years, several different NiTi systems have been designed and introduced to dentists. The characteristics of the design of the endodontic instrument such as: cutting angle, number of blades, top design, cone and intersection will affect its flexibility, cutting efficiency and torsion resistance.

NiTi Rotary Systems

Initially, NiTi instruments were used to work with conventional motor dental hand-pieces resulting in clinically unacceptable number of instrument fractures. For this reason, manufacturers have created controlled torque motors with individual adjustment of the torque

limits for each individual instrument, which aims to allow the instrument to operate under the limit of its elasticity, which reduces the risk of fracture[67].

Root instrumentation studies using various NiTi systems in recent years have focused on analyzing the ability to maintain the curvature of the root canal and maintaining its original form as well as the safety of operations with these new motor systems, while relatively little information is available for their cleaning ability in terms of the quantity of the extrusion material and the absence of debris and smear layer of the walls of the root canal. The results for the Quantec instruments were clearly superior to the hand instruments for the middle and apical third of the roots canals and with best results for the coronary third of the root canal[37]. In a further study, the differences between Quantec SC and Lightspeed[68] were analyzed, with both systems showing almost complete removal of debris. In most samples in both groups, the cleanliness was better in the coronary than in the apical part of the root canals. Studies that analyzed the endodontic systems: FlexMaster, ProTaper and Hyflex showed almost complete removal of the residues of the canal instrumentation[69, 70].

All studies together point to the fact that the various endodontic rotary NiTi systems vary in their efficiency, which is probably due to the different design and technological processing of the NiTi alloy. Regarding the safety aspects, there is a significant incidence of instrument fractures during the instrumentation of the root canal[71]. There may be two types of fractures: torsion and flexural fractures[72]. Flexural fractures may arise from defects in the surface of the instrument and occur after cyclical fatigue[73]. Anatomical conditions such as radius and curvature of the root canal, frequency of use, different torque as well as operator experience are among the main factors for fractures[74-78].

Additional aspects of occupational safety, such as the frequency of apical blockades, perforations, loss of working length or apical residue extrusion, have not been systematically evaluated so far. From the studies presented so far it can be concluded that loss of working length and apical blockages actually occur in some cases, while the perforation is considered to be insignificant. The quantity of apical extruded material was analyzed in a minimal number of studies, and it was found that the difference between manual, conventional machine and rotary NiTi endodontic systems was not significant[13, 49].

Most of the comparative studies present some evidence of shorter working hours with rotary NiTi systems compared to manual instrumentation. The reason for this is considered to be the smaller number of instruments in NiTi systems (ProTaper), which are required for complete endodontic instrumentation.

Conclusion

All the results obtained so far have shown that the use of NiTi endodontic systems results in less tension and better preservation of the form in the instrumentation of the root canals, especially those with curvature; the use of NiTi instruments alone does not provide completely clean walls of the root canal (the purity decreases from the coronal to the apical part of the root canal); the use of EDTA during instrumentation does not completely remove the smear layer; the use of NiTi instruments with the active blade is superior to the instruments with radial surfaces in terms of the purity of the channel walls; the use of NiTi instruments in accordance with the manufacturer's recommendations shows that they are safe to use; it is necessary to use a special engine (endodmotor) at a constant speed that is low and with torque control. All of these conclusions indicate that the application of NiTi instruments facilitates the preparation, especially of curved root canals.

Modern technological developments allow those benefits to be implemented by the manufacturers of endodontic systems in their product range. Therefore, it is necessary to carry out new research that will examine the benefits and characteristics of the new endodontic systems and whose results will give new practical knowledge of the efficiency of these systems in the realization of their goal: endodontic instrumentation of the root canal system with preserved anatomy, maximum possible cleanliness of radicular walls, minimal debris extrusion, shorter working time and greater safety.

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DMFT EVALUATION OF FIRST PERMANENT MOLARS AMONG 12 YEAR OLD CHILDREN IN THE REPUBLIC OF MACEDONIA

ЕВАЛУАЦИЈА НА КЕП ИНДЕКС НА ПРВ ТРАЕН МОЛАР КАЈ 12 ГОДИШНИ ДЕЦА ВО РЕПУБЛИКА МАКЕДОНИЈА

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Abstract

Introduction: The first permanent molar plays an important role in mastication and it preserves the vertical dimension of the jaws. Because of the particular anatomical form, the early eruption and the carelessness of the parents, it is usually prone to caries. The presence of caries on this tooth is often an indicator of the condition of other teeth. **Aim:** To evaluate the DMFT and its components of first permanent molars among 12 year old children in all regions of the Republic of Macedonia. Material and method: 19355 children at the age of 12 were examined by 147 calibrated dentists in dental offices. The data were entered in forms recommended by the WHO and statistically processed. **Results:** The DMFT was 1.59 (1.52 in males, 1.66 in females). The lowest DMFT value was recorded in the Northeast region - 0.92, and the highest in the Polog region - 2.96. The F component was the major contributor to DMFT Index (51.24%) followed by the D component (43.78%) and the M component (4.97%). Conclusion: Analysis has shown that consistent application of preventive measures is needed to reduce each of the DMFT components. **Key words:** DMFT index, first permanent molar, 12 year old children, preventive measures.

Апстракт

Вовед: Првиот траен молар има важна улога при мастикацијата и ја чува вертикалната димензија на вилиците. Заради специфичната анатомска форма, раната ерупција и невнимание на родителите, најчесто е подложен на кариес.Присуството на кариес на овој заб често е индикатор за состојбата на останатите заби. **Цел:** Да се направи евалуација на КЕП индексот кај 12 годишни деца во сите региони во Република Македонија. Материјал и метод: Беа прегледани 19355 деца на 12 годишна возраст од 147 калибрирани стоматолзи во стоматолошки ординации.Податоците беа внесени во формулари препорачани од СЗО и статистички обработени. **Резултати:** КЕП вредноста изнесуваше 1.59 (момчиња-1.52, девојчиња-1.66). Најниска КЕП вредност беше забележана во Североисточен регион 0.92, а највисока во Полошки регион 2.96. П компонентата беше главен носител на КЕП индексот (51.24%) следена од компонента К (43.78%) и компонента Е (4.97%). Заклучок: Анализата на КЕП6 покажа дека е потребна доследна примена на превентивни мерки за намалување на секоја компонента на КЕП. **Клучни зборови:** КЕП индекс, прв траен молар, 12-годишни деца, превентивни мерки

Introduction

Good oral health is essential for the optimal general health and the overall quality of life. Furthermore, oral health incorporates the integrity and good condition of the specific parts of the oral cavity - teeth, oral mucosa, masticatory muscles, tongue, TMJ, and salivary glands - which participate in the performance of the vital functions such as chewing, speech and swallowing[1].

Having this in mind, an important aspect to be mentioned when talking about oral health is the dental caries. In theory, it is a multifactorial disease that occurs for several reasons, such as irregular nutrition, the presence of pathogens, the morphology of teeth, inadequate oral hygiene, genetic predisposition and time[2]. Furthermore, caries and periodontal disease are historically considered as the most important global burden of oral health. The research points out that dental caries is still a major health problem in most industrialized countries since it occurs in 60-90% of school children and the vast majority of adults. In 2004, WHO updated the epidemiological information available in the databases[3, 4].

Additionally, the first permanent molar is the most important unit in the masticatory system and for the overall oral health. Early loss of this tooth can significantly affect the efficiency of mastication, decrease the vertical dimension, cause premature eruption of the second and third molar and dislocate the neighboring teeth[5].

Due to its specific anatomical form, early eruption and ignorance about oral health by the side of the parents is very likely to cause initial caries to this tooth. The presence of caries of the first permanent molar is often an individual indicator of the oral health condition[6].

Maintaining healthy lasting first molars is important because they erupt at an early age and are the first erupted teeth of the series of permanent dentition. Therefore, epidemiological studies with molars can be a powerful tool in planning an appropriate health system and health strategies[7].

The age of 12 years is universally accepted as the most appropriate age for monitoring dental caries, since all permanent teeth, other than the third molars, are usually erupted[3].

The World Health Organization (WHO) considers children of 12 years of age as one of the most important target groups, since in most countries children at this age attend a school that facilitates their mobilization in epidemiological research[8].

The most important demographic indicator for evaluation and measurement of dental caries is the DMFT Index. This index shows the number of permanent teeth with cavities, the number of extracted teeth and the number of sealed teeth. The DMFT Index is used as an important criterion for assessing the status of oral health. But this index does not illustrate the true prevalence of teeth caries in a community. Therefore, the percentage of people without caries is used as a complementary index of the prevalence of caries in epidemiological studies[9].

Aim

To evaluate the value and structure of the DMFT index in 12 year old children in Republic of Macedonia

Material and methods

The epidemiological study was conducted during September and October 2014 in all primary schools in eight regions in the Republic of Macedonia. All students from 6th grade, who were present at school on this day, were examined. The total number of children at the age of 12 (born in 2002), was 20.602. Precisely 19.355 (93.94%) were examined, of which 9.938 (51.34%) were boys, and 9.417 (48.65%) were girls. The dental examinations were performed by 147 dentists calibrated according to WHO standards for equalizing diagnostic criteria[10].

The examinations were performed in a dental office with a probe and a mirror. However, X-ray methods to confirm the diagnosis were not used. The data were entered into unified translated and adapted forms recommended by the WHO, by dental assistants and then they were statistically processed.

Also, the data was categorized according to the WHO criteria for ranking the DMFT values for 12 year old individuals, including 0-none; 0-1.1 very low; 1.2-2.6 low; 2.7-4.4 medium; 4.5-6.5 tall; more than 6,6 very high caries rank[11].

Additionally, all components of the DMFT Index were analyzed, with emphasis on component K as an indicator for the necessary dental treatment of the examined category of participants.

The obtained data was also evaluated by sex and according to the region where the respondents come from, regardless of whether they come from an urban or rural area of the region and regardless of their socio-economic status.

Because the Ministry of Education is an official collaborator of the National Strategy for Prevention of Oral Diseases in Children 0-14 Year in the Republic of Macedonia, and the dental examinations are carried out every year according to the program frameworks and evaluation of the Strategy, no special permission was required for the implementation of this epidemiological study.

Results

The results of the epidemiological study showed that the DMFT index of the first permanent molar in 12 year old children in Republic of Macedonia is 1.59. Separately in the regions, the results are as follows: 0.79 in the Northeast region, 1.27 in the Pelagonia region, 1.50 in the Skopje region, 1.58 in the Southwest region, 1.70 in the Southeast region, 1.77 in the East region, 2.03 in the Vardar region and 2.12 in the Polog region (Table 1).

When taking the DMFT structure's aspect, the F (filled first permanent molars) was dominant in all regions except in the Polog and the Northeast region. Additionally, the results showed that in the Skopje region 56.14% of the first permanent molars were filled and 39.9% were decayed; 61% of the first permanent molars in the Pelagonia region were filled, and 33.94%

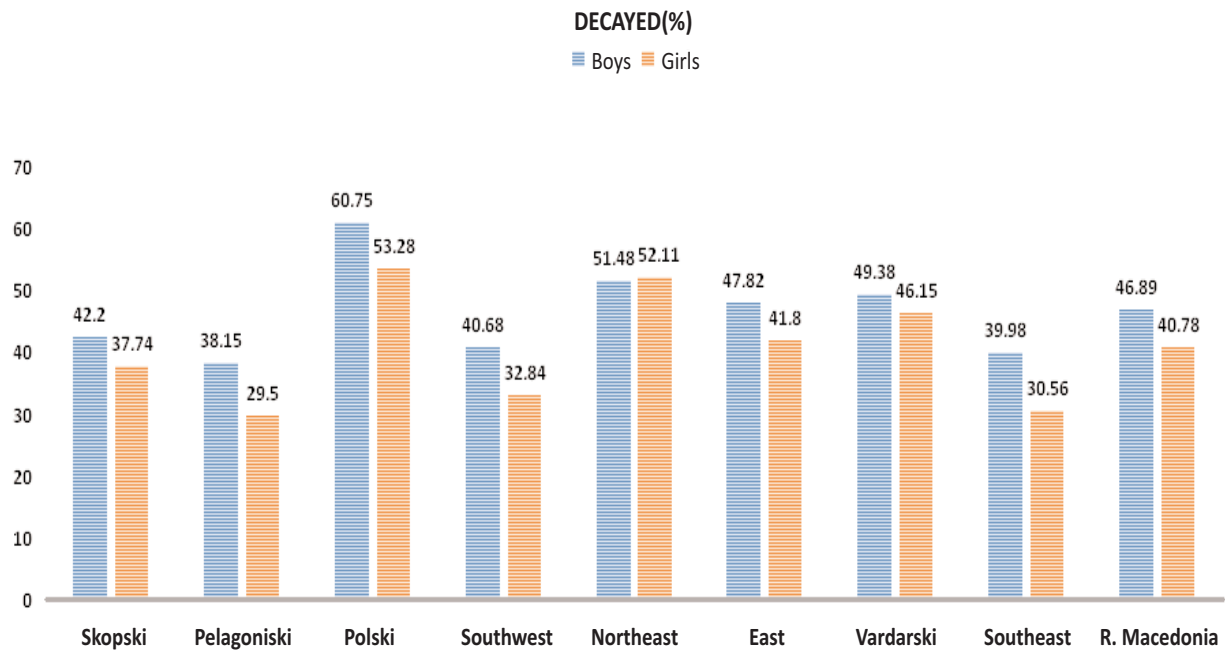

Figure 1. *Decayed first permanent molar among children at the age of 12 across regions in RM*

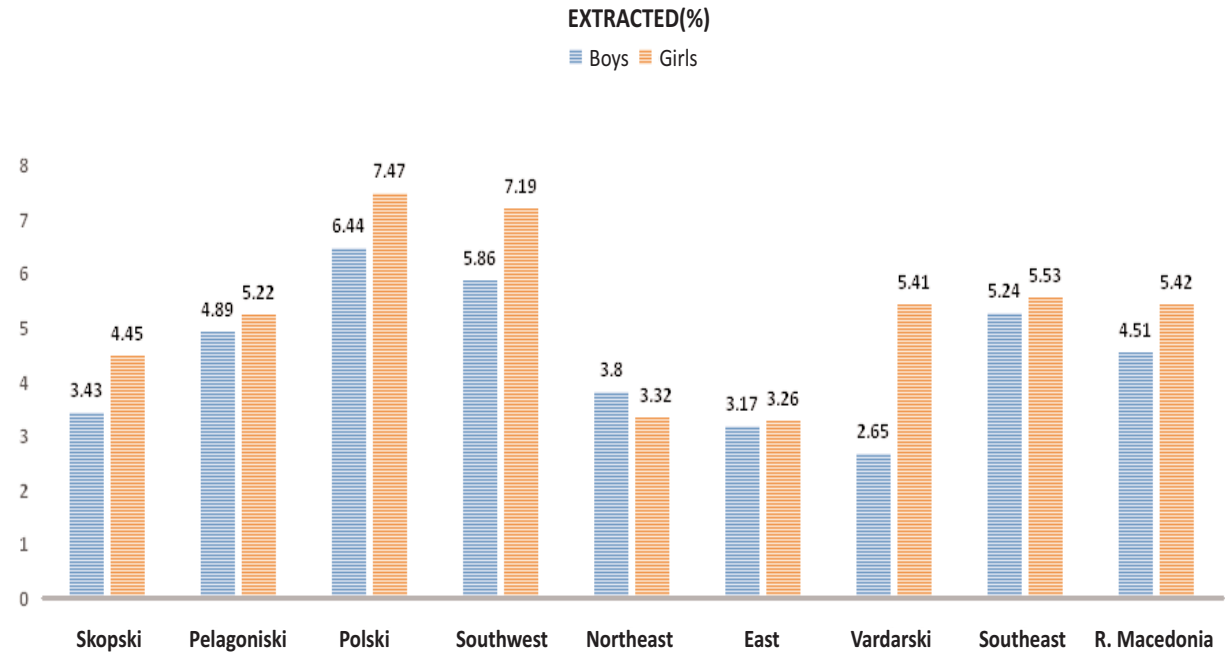

Figure 2. *Extracted first permanent molar among children at the age of 12 across regions in RM*

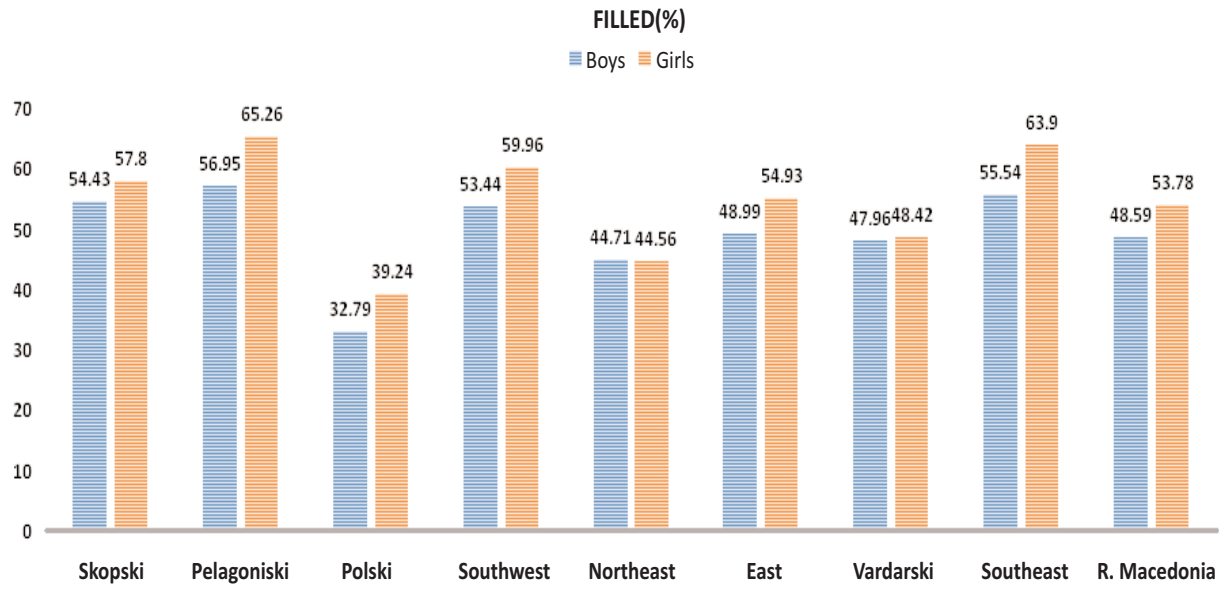

Figure 3. *Filled first permanent molar among children at the age of 12 across regions in RM*

Table 1. DMFT of first permanent molar among children at the age of 12 across regions in the Republic of Macedonia

Generation 2002 – first permanent molar Analyses 2014		REGIONS								
		Skopski	Pelagoniski	Poloski	Southwest	Northeast	East	Vardarski	Southeast	R.Macedonia
DMFT	Total	1.50	1.27	2.12	1.58	0.79	1.77	2.03	1.70	1.59
	Boys	1.43	1.25	2.04	1.47	0.81	1.61	1.98	1.61	1.52
	Girls	1.58	1.30	2.20	1.69	0.78	1.94	2.09	1.79	1.66

Table 2. DMFT components of first permanent molar among children at the age of 12 across regions in the Republic of Macedonia

Generation 2002 – first permanent molar Analyses 2014			REGIONS								
			Skopski	Pelagoniski	Poloski	Southwest	Northeast	East	Vardarski	Southeast	R.Macedonia
DECAYED	total	Number	3867	947	3723	1048	710	1219	1087	885	13486
		Percentage	**39.9**	**33.94**	**56.98**	**36.57**	**51.78**	**44.65**	**47.75**	**34.76**	**43.78**
EXTRACTED	total	Number	383	141	455	188	49	88	92	137	1533
		Percentage	**3.95**	**5.05**	**6.96**	**6.56**	**3.57**	**3.22**	**4.04**	**5.38**	**4.97**
FILLED	total	Number	5441	1702	2355	1629	612	1423	1097	1524	15783
		Percentage	**56.14**	**61.0**	**36.04**	**56.85**	**44.63**	**52.12**	**48.19**	**59.85**	**51.24**

were decayed; 56.85% of the first permanent molars in the Southwest region were filled, and 36.57% were decayed. 52.12% of the first permanent molars in the East region were filled, and 44.65% were decayed; 48.19% of the first permanent molars in the Vardar region were filled, and 47.75% were decayed; 59.85% of

Table 3. MFT components of first permanent molar among children at the age of 12 across regions in the Republic of Macedonia in relation to gender

Generation 2002 – first permanent molar Analyses 2014			REGIONS								
			Skopski	Pelagoniski	Poloski	Southwest	Northeast	East	Vardarski	Southeast	R.Macedonia
DECAYED	Boys	Number	2012	546	1969	555	365	617	559	465	7088
		Percentage	42.12	38.15	60.75	40.68	51.48	47.82	49.38	39.98	46.89
	Girls	Number	1855	401	1754	493	345	602	528	420	6398
		Percentage	37.74	29.50	53.28	32.84	52.11	41.80	46.15	30.56	40.78
EXTRACTED	Boys	Number	164	70	209	80	27	41	30	61	682
		Percentage	3.43	4.89	6.44	5.86	3.80	3.17	2.65	5.24	4.51
	Girls	Number	219	71	246	108	22	47	62	76	851
		Percentage	4.45	5.22	7.47	7.19	3.32	3.26	5.41	5.53	5.42
FILLED	Boys	Number	2600	815	1063	729	317	632	543	646	7345
		Percentage	54.43	56.95	32.79	53.44	44.71	48.99	47.96	55.54	48.59
	Girls	Number	2841	887	1292	900	295	791	554	878	8438
		Percentage	57.80	65.26	39.24	59.96	44.56	54.93	48.42	63.90	53.78

the first permanent molars in the South-East region were filled, and 34.76% were decayed. From this, the overall results showed that at the level of the Republic of Macedonia 51.24% of the first permanent molars were filled, and 43.78% were decayed.

Alongside with this, the component M (missing first permanent molars) participated with the lowest percentage in the structure of the DMFT index. In the Republic of Macedonia 4.97% of the first permanent molars were extracted. Region wise, the results pointed out that 3.95% of the first permanent molars were extracted in the Skopje region, 5.05% in the Pelagonia region, 6.96% in the Polog region, 6.56% in the Southwest region, 3.57% in the Northeast region, 3.22% in the East region, 4.04% in the Vardar region and 5.38% in the South-East region (Table 2).

Taking the aspect of gender distribution into consideration, girls in the Republic of Macedonia have higher values of the DMFT index, 1.66, compared to the boys that accounted for 1.52. The F component is dominant in both sexes, but higher in girls compared to boys, except for the Northeast region where the value of the DMFT index as well as all DMFT components are approximately present in both girls and boys. The M component is also with higher values among girls in all regions, but the D component is higher among boys in each region and in the country level of the Republic of Macedonia (Table 3).

Discussion

Many authors put emphasis on the aspect that teeth with deep fissure have a higher caries risk than teeth with smooth surfaces[12].

To start with, *Fleger and all.* predicate that in dental morbidity, first permanent molars play a big role and one third of this group of teeth is devitalized until the of age 18. Only 0.7% of adolescents have four healthy first permanent molars[13].

Furthermore, the study of *MC Donald* shows a high frequency of cavities on the occlusal surfaces of the first permanent molars for all age groups and that the first permanent molar remains the most common location of caries for a very short period after its eruption[14].

Serban and all, according to analyzes from their study, claim that the presence of the carious lesions of the first permanent molar reach their peak at about 12 years of age and this condition is in direct correlation

with the hygiene-dietary habits and with the health concept of the state[15].

Additionally, *Wyne AH* believes that caries prevalence data are the basis for building health policies and preparing preventive programs and points out the need for treatment among the population[16].

All of these claims as well as our personal experiences were a motive for the preparation of this study, even more when taking into consideration that, although caries is a widespread oral disease, and this is a well-documented condition among different population groups in many areas around the world, it is not the case in the Republic of Macedonia. In particular, the condition of the first permanent molar with the structure of all components of the DMFT index, especially among the 12 year old children participating in our study, has not been investigated and documented.

Also, our goal was to evaluate the condition of the same group of teeth that were sealed immediately after eruption (when participants were 6-year-old children and included in the national preventive program) because the same children at the age of 12 are no longer subject for intensive monitoring by the preventive teams.

According to the results of the study, the DMFT index of the first permanent molar in the 12 year old children in the Republic of Macedonia was 1.59, which is in the frame of the values that we found in our research. In the Republic of Macedonia, the value of the DMFT index for girls was 1.66 and for boys 1.52, which also coincided with the situation in other countries where this data has been researched.

Speaking of the other studies that have researched the same data, *Sadeghi* estimated that the DMFT value of the first permanent molar is 1.9 ± 1.6 (boys 1.83, girls 1.98)[17];

A HaerianArdakani and all. estimate that the DMFT value of the first permanent molar is 1.17 ± 1.26 (boys 0.88, girls 1.33)[6];

Parnian Poureslami and all. predicate that the DMFT value of the first permanent molar is 1.97-2.60 (boys 2.13, girls 2.43)[18];

Massom T and all. concluded that the DMFT value of the first permanent molar is 2.17 ± 1.39 (boys 2.19, girls 2.22)[19].

Wei Yin and all. estimated that the DMFT value of the first permanent molar is 0.61 (boys 0.47, girls 0.75)[20];

Gorgi Z. and all. claim that the DMFT value of the first permanent molar is 1.00 ± 1.36 (boys 0.93 ± 1.33, girls 1.07 ± 1.39)[21].

Regarding the structure of the DMFT components, research has shown that in the Republic of Macedonia the F component is the leader component in this index with 56.14% as in many countries in Eastern and Central Europe, but this is not the case around the world where the D component is the dominant one[22, 17, 23].

Considering the fact that the oral health condition of the first permanent molar is the main carrier of the DMFT value, then the current condition of the first permanent molar among our participants clearly indicates improvement of the oral health of these children who were included in the National Preventive Program since their age of 6.

This fact is confirmed by *Alvarez-Arenal and all.* noting that the prevalence of caries in 12-year-olds was 71%, and the first permanent molar accounted for 64.1% of this percentage[24].

Conclusion

The results of this study show that oral health in 12-year-olds in the Republic of Macedonia needs to be improved by the consistent implementation of preventive measures at an earlier age since it reduces the value of the DMFT Index and all its components in permanent molar and the overall dentition in both sexes.

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SALIVARY FLUORIDE CONCENTRATION DURING ORTHODONTIC TREATMENT USING TWO TYPES OF ADHESIVES

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Abstract

The importance of fluoride concentration in saliva is well established, based on the main role of the fluoride ions, that is decreasing the demineralization and enhancing the remineralization of enamel, even in patients with carious risk. The **aim** of the present study is to measure the fluoride concentration in saliva in patients under fixed orthodontic treatment with metal braces by using two different types of orthodontic adhesives – composite and resin-reinforced glass ionomer cements. The subjects for this study were 60 patients scheduled for orthodontic therapy in the Department of Orthodontics, University Clinic of Dentistry "St. Pantelejmon" – Skopje. Patients were divided in two groups according to the adhesive type used for bonding: - The first group comprosed of 30 patients whose braces were bonded with composite adhesive, - The second group comprised of 30 patients whose braces were bonded with resin-reinforced glass ionomer adhesive (RRGICs). The **results** showed that resin-reinforced glass ionomer adhesive (RRGICs) releases fluoride one day after bonding the braces and there was rapid decrease of fluoride concentration in saliva one month later. The outcome was different in the patients with composite adhesive where we had a slow decrease of fluoride concentration in T1 period. **Key words:** Demineralization; Composite; Glass ionomer cement.

Апстракт

Важноста на концентрацијата на флуориди во плунката е добро утврдена врз основа на главната улога на флуоридните јони, што ја намалува деминерализацијата и ја подобрува реминерализацијата на емајлот, дури и кај пациенти со ризик од кариес. **Целта** на оваа студија е мерење на концентрацијата на флуориди во плунката кај пациенти под фиксен ортодонтски третман со метални брекети со користење на два различни ортодонски атхезиви - композитни и смолести модифицирани глас-јономер цементи. Предмети за оваа студија беа 60 пациенти закажани за ортодонтска терапија во одделот за Ортодонција, Универзитетска клиника за стоматологија "Св. Пантелејмон"- Скопје. Пациентите беа поделени во две групи според типот на атхезивот користен за врзување: - Првата група од 30 пациенти кај кои е користен композитен атхезив; - Втората група од 30 пациенти, каде е користен смолесто модифициран глас-јономерен атхезив (RRGICs). **Резултатите** покажаа дека смолесто модифициран глас-јономерен атхезив (RRGICs), го ослободува флуоридот еден ден по поврзувањето на протезите и брзото намалување на концентрацијата на флуорид во плунката еден месец подоцна. Ситуацијата е друга кај пациентите со композитен атхзив, каде што имаме бавно намалување на концентрација на флуорид во Т1 период. **Клучни зборови:** Деминерализација, Композит, Глас-јономер цемент

Introduction

Cleaning orthodontic brackets and bands represents a challenge for the patient wearing them, these attachments act as plaque-retaining structures leading to demineralization of adjacent enamel[1].

Recent studies have shown that 50% to 75% of all orthodontic patients develop demineralization on the labial surface during fixed appliance therapy[2, 3].

The importance of fluoride concentration in saliva is well established, based on the main role of the fluoride ions, which is decreasing the demineralization and enhancing the remineralization of enamel, even in patients with carious risk[4, 5, 6].

The absorption of the fluoride ions from the oral fluids in the sound enamel is low and limited at a neutral pH. If the fluoride ions are present in the mouth at the time when the pH is decreasing and the carious lesion is

starting, their effect is to inhibit the demineralization of the enamel by promoting remineralization[7, 8, 9, 10].

In Orthodontics, white spot lesions and marginal gingivitis raise much concern among professionals, who have been tackling this problem by making use of materials to decrease and prevent such damage to oral health, among which are the ionomer cements (GICs). Since their introduction in 1971, GIC have been employed for a number of applications mainly due to its chemical adhesion to enamel, dentin and other surfaces in addition to rereleasing fluoride[19].

The evolution of GIC properties has contributed to the decrease in dental caries among orthodontically treated patients due to the biological and chemical characteristics of the material[20].

Despite these favorable characteristics, the adhesion of brackets to dental enamel is not entirely adequate, often not being strong enough to resist to masticatory forces and orthodontic movements[21].

In order to overcome this problem, manufacturers have developed hybrid products by incorporating a resin matrix system to GICs, thus combining the retentive capacity of resins with the well known beneficial properties of GICs[22].

These materials were denominated as resin-reinforced glass ionomer cements (RRGICs).

RRGICs can be used in Orthodontics due to their resistance to orthodontic forces, thus becoming a useful material for bonding orthodontic accessories and preserving the dental enamel. It is accepted that the RRGICs analysed in this study release fluoride and are used for bonding brackets and attaching[23].

The aim of the present study is to measure fluoride concentration in saliva in patients under fixed orthodontic treatment with metal braces by using two different types of orthodontic adhesives - composite and resin-reinforced glass ionomer cements.

Material and methods

The subjects for this study were 60 patients scheduled for orthodontic therapy in the Department of Orthodontics, the University Clinic of Dentistry "St. Pantelejmon" - Skopje. Patients were divided in two groups according to the adhesive type used for bonding:

- The first group was composed of 30 patients whose braces were bonded with composite adhesive
- The second group comprised of 30 patients whose braces were bonded with resin-reinforced glass ionomer adhesive (RRGICs).

All experimental procedures were conducted in accordance with the Declaration of Helsinki's recommendations guiding physicians in biomedical research involving human subjects. All participants and their parents or guardians received written information about the aims and design of the study and signed a written informed consent form.

The criteria for inclusion were as follows: permanent dentition period, crowding, age 12-25 years, good general health, general dentistry completed and consent to participate. Criteria for exclusion were: diabetes mellitus, autoimmune connective tissue diseases, any syndrome and antibiotic therapy in the last 3 months. The orthodontic process in all subjects started with 0.012 NiTi. Subjects were required to establish good oral hygiene status, none used supplementary fluoride during the study and none of them received any periodontal procedure before or during the active orthodontic treatment.

Saliva collection

Samples of saliva were collected into plastic specimen containers as wholly unstimulated saliva for a period of two minutes in three periods:

T_0 – collecting saliva before bonding the braces
T_1 – collecting saliva the day after bonding
T_2 – collecting saliva one month later.

The fluoride content of the saliva samples was analysed by the Taves[24] micro diffusion method as described in detail by Zero at al.[25]. The volume of the saliva was adjusted to 3ml with double deionized water, and 0.1 ml of 1.65 mol/l NaOH was added to the central trap. One milliliter of 6 mol/l HCl, saturated with hexamethyldisiloxane, was added to the sample before the dish was sealed. The samples were rotated for 18 hours on a rotary shaker at 80 rpm. At the end of the diffusion period, the NaOH traps were removed. The samples contained in the traps were dried at 65^{0}C for 2 hours, and buffered with 1 ml of 0.34 mol/l acetic to a final pH at 5.0. Fluoride was then measured by a fluoride ion-specific electrode (Model 960900, Orion Research, Inc.).

The electrode was gauged every day by using standard solution of fluoride (0.05, 0.010, and 0.19 ppm). Fluoride release was measured at T_0, T_1 and T_2 periods.

Results

Orthodontic brackets and bands act as biofilm-retaining structures, which can cause demineralization of the adjacent enamel during orthodontic treatment.

Therefore, an effective prevention against enamel demineralization adjacent to the orthodontic attachments is necessary.

The amount of fluoride release from each material during the study period is shown in Table 1 and 2.

Table 1. Descriptive analysis (F in saliva in T0,T1 and T2) **Glas-jonomer cement**

	Glas-jonomer cement			
	T_0	T_1	T_2	p value T_2-T_1/T_2-T_0/T_1-T_0
F	0.04± 0.02	0.0418± 0.02	0.0399± 0.02	0.00008**/0.77/0.0001**

p (Friedman ANOVA; post hoc Wilcoxon-Matched pairs test)**p<0.01

Table 2. Descriptive analysis (F in saliva in T0,T1 and T2) - **Composite**

	Composite			
	T_0	T_1	T_2	p value T_2-T_1/T_2-T_0/T_1-T_0
F	0.04± 0.081	0.039± 0.018	0.04± 0.018	1.0 ns/0.53 ns/0.55ns

p (Friedman ANOVA; post hoc Wilcoxon-Matched pairs test)

For the group of patients with resin-reinforced glass ionomer adhesive (RRGICs) we obtained a significant higher fluoride release of 0.0418 mg/L in T_1 period, respectively at the first day and than a rapid decrease at T_2 period, after one month, respectively 0.0399 mg/L comparing with the fluoride concentration at T_0 period 0.04 mg/L.

The situation was different in patients with composite adhesive where we observed a slow decrease of fluoride concentration at T_1 period, 0.039 mg/L and stabilization of fluoride concentration as the situation before adhering the braces, respectively 0.04 mg/L in T_2 period.

Discussion

In order to reduce the occurrence of such demineralization processes, the orthodontic attachments should be maintained with materials which can release fluoride and provide adequate adhesion to both enamel and stainless steel[11].

Some studies have shown that part of the fluoride release from these materials is absorbed by adjacent dental tissues, making them more resistant to secondary caries in addition to reducing demineralization and increasing remineralization. Nevertheless, both the magnitude and the duration of the anticariogenic effects of fluoride depend mainly on its concentration and retention time within the oral cavity. Therefore, it is better to have fluoride released for longer periods of time rather than the initial "burst effect" of the material, since the longevity of the orthodontic appliance should be taken into account[26].

Kielbassa et al. reported that RRGICs have an anticariogenic effect compared to non-fluoridated composites. This anticariogenic effect is crucial in the orthodontic treatment[2].

Salivary fluoride levels vary from 0.01-0.10 mg/L depending on the water fluoride usage and the diet of the individual. Langerolf and Oliveby stated that saliva influences caries attack mainly by its rate of flow and by its fluoride content[1,2].

Daws at al. quote the normal concentration of fluoride in saliva as being about 0.019 mg/L and also confirmed that salivary fluoride levels were independent of flow rates, and that higher concentration of fluoride in saliva led to the formation of calcium fluoride which had a longer clearance time[13].

Many researchers now believe that continuous low concentration of fluoride in saliva, particularly at the plaque/saliva/enamel interface is necessary for caries prevention[14].

Leverett et al. showed that caries-free subjects had higher salivary fluoride than high caries subjects[15].

Shields et al. showed that subjects with no caries experience, from both fluoridated and non-fluoridated communities, had salivary fluoride levels of 0.04 mg/L or greater, whereas high caries subjects from both fluoridated and non-fluoridated communities had salivary fluoride levels of 0.02 mg/L or less[16].

Duggal et al. also showed consistent inverse relationship between salivary fluoride concentration and dental caries in 272 children[17].

Sjorgen et al. reported that a caries-active group in Sweden had lower salivary fluoride levels than a caries-inactive group[18]. Also they reported that type, shape and surface area of the cement can significantly influence the fluoride release process.

The model of the fluoride levels variation in this study is similar to other studies and is explained by the CaF_2 formation, which represent the major product of the reaction between fluoride with enamel, and which precipitates wherever the dental hard tissues are exposed to high concentrations of fluoride, inhibiting the enamel demineralization and enhancing the remineralization[8, 9].

CaF_2 is relatively stable at a neutral pH. When the pH is decreased, CaF_2 dissociates and fluoride ions are released and adsorbed in enamel. The dissolution of the CaF_2 formed on the teeth surfaces, in saliva and in dental bacterial plaque is the key of the preventive effect of fluoride in saliva.

The preventive effect of fluoride is unquestionably connected with the fluoride ions reserve in saliva in the periods when the pH is decreasing in the oral cavity.

Conclusion

The results showed that resin-reinforced glass ionomer adhesive (RRGICs) releases fluoride one day after bonding the braces and there is rapid decrease of fluoride concentration in saliva one month later. The outcome was different in patients with composite adhesive where we had a slow decrease of fluoride concentration in T_1 period.

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NUTRITIONAL HABITS AMONG DENTISTRY STUDENTS AND THEIR IMPACT ON PERIODONTAL HEALTH

НАВИКИТЕ ВО ИСХРАНАТА КАЈ СТУДЕНТСКАТА ПОПУЛАЦИЈА И НИВНОТО ВЛИЈАНИЕ КАЈ ПАРОДОНТАЛНОТО ЗДРАВЈЕ

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Abstract

The impact of adequate nutrition on teeth development, alveolar bone and periodontal ligament, has been proved more than once, yet it still remains unclear if particular components of nutrition have certain influence on the occurrence or severity of periodontal diseases. Periodontal health relies on a balance between the host, environmental and bacterial factors. Although there is considerable evidence linking diminished antioxidant status to increased oxidative damage and disease severity, there have been, up to now, a few dietary studies. Therefore the **aim** of our study was to analyse dietary habits among student population and their relation to periodontal and gingival index values. In order to gain a homogenous group concerning age, life style, and oral hygiene habits the group consisted of 200 dentistry students in the last two years. For the examination we developed a questionnaire of 24 questions concerning the quality and type of dietary products. At the same time all participants were subjected to periodontal examination where periodontal index values and oral hygiene were noted. The data were statistically evaluated by a correlation test and a student t-test. From the results of our research we can conclude that patients should be advised, along with oral hygiene procedure, to consume vegetables and fruits in order to maintain periodontal health or to reduce disease severity. **Key words:** nutrition, gingival bleeding, periodontal health.

Апстракт

Влијанието на адекватната исхрана врз развојот на забите, алвеоларната коска и периодонталниот лигамент е докажано многу пати, но сепак останува нејасно дали одредени компоненти од исхраната имаат извесно влијание врз појавата или степенот на развој на пародонталната болест. Пародонталното здравје се базира на рамнотежа помеѓу фактори кои зависат од индивидуата, околината и бактериите. Иако постојат значителни докази кои го поврзуваат намалениот статус на антиоксиданси со зголеменото оксидативно оштетување и сериозноста на болеста, направени се и неколку дополнителни студии. **Целта** на нашето истражување беше да се анализираат навиките во исхраната кај студентската популација и нивната поврзаност со вредностите на пародонталните индекси. Со цел да се добие хомогена група на испитаници, во однос на возраста, начинот на живот и орално-хигиенските навики, групата се состоеше од 200 студенти по стоматологија, од последните две години. За испитувањето подготвивме прашалник од 24 прашања во врска со квалитетот и видот на исхраната. Во исто време сите учесници беа подложени на пародонтолошки преглед, каде што беа забележани вредностите на пародонталниот индекс и квалитетот на одржување на оралната хигиена. Податоците беа статистички евалуирани со корелациона анализа и Студентов t-тест. Од резултатите добиени при истражувањето, можеме да заклучиме дека пациентите треба да се советуваат, заедно со процедурата за орална хигиена, да консумираат зеленчук и овошје, со цел да се одржи здравјето на пародонтот или да се намали сериозноста на болеста. **Клучни зборови:** исхрана, гингивално крварење, пародонтолошко здравје.

Introduction

Dietary quality and nutrition are important in the promotion and maintenance of health throughout the entire life span. They occupy a prominent position in disease prevention and health promotion. When combined with other modifiable risk factors, such as tobacco or physical activity, diet and nutrition may have an additive or multiplier effect on an array of chronic diseases, including cardiovascular disease, diabetes, obesity, cancer, osteoporosis, and dental diseases.

Periodontal disease includes gingival inflammation and further development of loss of soft-tissue attachment to the tooth and resorption of the alveolar bone, ulti-

mately leading to tooth loss. The disease is found primarily among adults with increased numbers in older age but can also be found in younger individuals[1,2,3]. Although convincing scientific evidence support the fact that the pathogenesis of periodontitis involves anaerobic oral bacteria and that tissue damage occurs as a result of the complex interaction between pathogenic bacteria and the host's response to infection, several local and systemic factors are known to be associated with the risk or the severity of the periodontal disease[4,5,6].

Nutrition is known to be important for maintaining periodontal health, and different dietary aspects have been put forward as possible aggravating factors. The two potential mechanisms in the relation of diet to gingival and periodontal health are diet in relation to plaque formation and the effect of different nutritional deficiencies on periodontal tissues. Research into the local effect of diet on plaque has focused largely on the effect of an abrasive diet in reducing plaque formation. Although animal studies have shown a relationship between diet and plaque formation[7,8,9,10], the significance to humans is questionable because of differences in tooth morphology

The impact of adequate nutrition on teeth development, alveolar bone and periodontal ligament, has been proved many times, yet it still remains unclear if particular components of nutrition have certain influence on the occurrence or severity of periodontal disease.

Periodontal health relies on a balance between the host, environmental and bacterial factors.

The primary etiology of periodontal disease is bacterial, but a susceptible host is also necessary for disease initiation.

The effect of nutrition as one of the modifying factors of the host immune response has been unjustly underestimated.

Although there is considerable evidence linking diminished antioxidant status to increased oxidative damage and disease severity, there have been a few dietary studies.

Reactive oxidative species cause tissue damage by variety of different mechanisms which include:

- DNA damage,
- Lipid peroxidation,
- Protein damage including gingival Hyaluronic acid and proteoglican,
- Oxidation like alfa 1 antitripsine [11,12],
- Stimulation of proinflamatory cytokines release by monocytes by activation of nuclear factor Nf kB[13].

Since the antioxidant content of fruits and vegetables may be a key factor in their beneficial effects, and lack of wider studies, on the other hand, investigating the dietary intake of such food and periodontal health, we undertook this examination whose main objective is:

- To explore the possible influence of dietary intake of food rich with vitamins on periodontal health.

Material and methods

At the Clinic of Oral Pathology and Periodontology we conducted an investigation with 200 participants. In order to gain a homogenous group concerning age, life style, and oral hygiene habits the group consisted of dentistry students in the last two years.

For the examination we developed a questionnaire of 24 questions concerning the quality and type of dietary products. For each question participants were offered three possibilities for the frequency of consuming the food that was pointed out – I rarely eat, I eat two of three times a week and a habit of consuming such food every day. According to the chosen answer participants were divided into three groups.

At the same time all participants were subjected to periodontal examination where periodontal index values and oral hygiene were noted.

Index values were compared between groups.

The data were statistically evaluated by a correlation test and a student t-test.

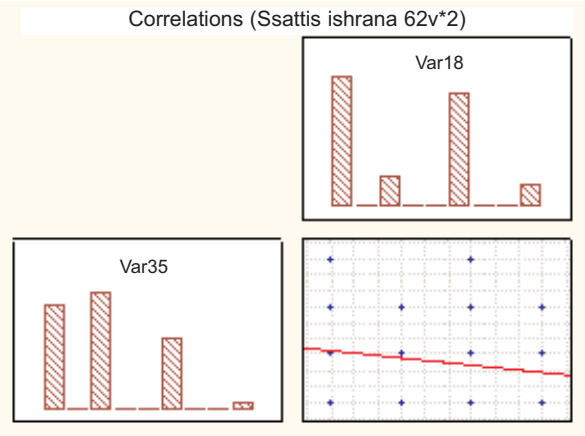
Results

Participants were divided into three groups according to their answers to the questions.

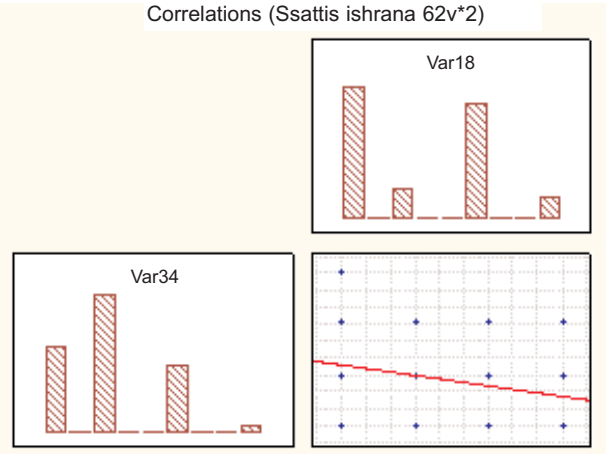
The average score for plaque index values was 0.73 according to criteria proposed by Green-Vermilion.

The highest correlation $p<0.0001$ using Spearman was determined between index values of gingival inflammation and gingival bleeding on probing (Sillnes&Loe) and question number 11 (vegetable intake) and question number 18 (citrus fruits intake). Values of the correlation coefficient are presented in Graphs 1, 2 and 3. The intake of vegetables is correlated both to gingival inflammation and gingival bleeding, with correlation coefficients of $p= -0.27$ and $p= -0.22$, accordingly. Consumption of fruits is correlated to gingival inflammation with correlation coefficients of $p = 0.17$.

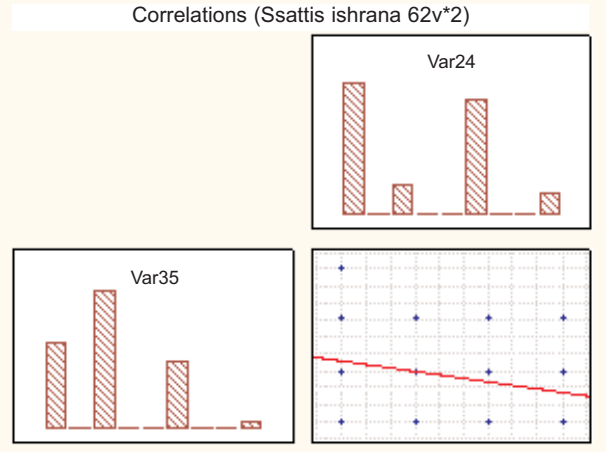
Student t-test was done for the same questions and index values of gingival bleeding and inflammation. Differences between all groups with different vegetable intake regarding gingival inflammation were found, with group A (low consumption of vegetables) differing from group B with a statistical significance of $p=0.015$ and from group C with a statistical significance of $p=0.023$.



Graph 1. *Spearman correlation matrix for Gingival inflammation and vegetable intake.*

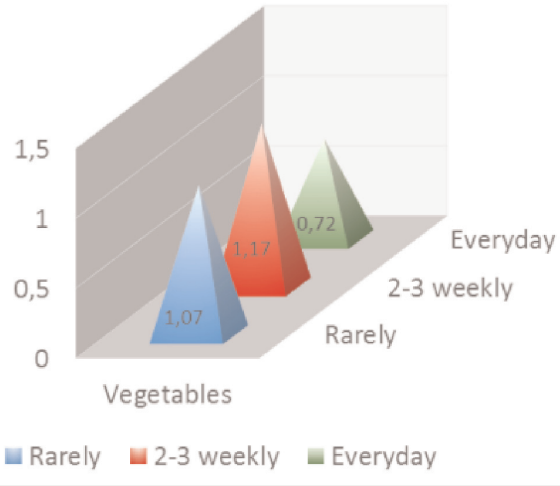


Graph 2. *Spearman correlation matrix for Gingival bleeding on probing and vegetable intake.*

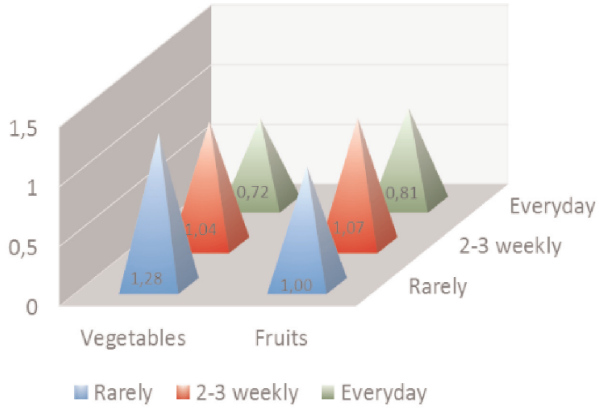


Graph 3. *Spearman correlation matrix for Gingival inflammation and citrus fruit intake*

Regarding gingival bleeding, group A showed lower values than groups C and B, with a statistical significance of p=0.001. (Graph 4, Graph 5)



Graph 4. *Gingival bleeding values for the three groups regarding vegetable intake*



Graph 5. *Gingival inflammation values for the three groups regarding vegetable and citrus fruit intake*

As expected no significant correlation was found for periodontal destruction and attachment loss for any of the investigated variables.

Discussion

The soft tissue of the oral cavity is made up of epithelial cells that have rapid rates of replication, metabolism and maturation which require a steady supply of essential nutrients. These cells turn over every

three to seven days, which makes the tissues of the oral cavity a sensitive indicator of adequate nutrition. Although the role of diet and nutritional factors in maintaining periodontal health is unclear, it is known that the defense mechanism of the gingival tissue and saliva can be affected by nutritional intake and status[1,14,15].

The influence of dietary intake of antioxidants, mainly vitamins, on reducing the oxidative stress damage in tissues should be considered.

The group of compounds known as antioxidants is the major defense against stress. Primarily, antioxidants which prevent the formation of new radical species include enzymes systems such as peroxide dismutase and glutathione peroxidase.

Secondary, antioxidants trap radical species and prevent chain reaction, include nutrition such as vitamin A, C and carotenoids.

The richest sources of vitamins are vegetables and fruits. Through the digestive tract, they reach all body tissues and cells, where they are essential for various metabolic processes, for example vitamin B for DNA synthesis, vitamin C for collagen synthesis, protection from oxidative damage, vitamin A for DNA replication[16, 17].

The importance of dietary intake food rich with vitamins for the maintenance of periodontal health was confirmed by the results of our study.

The participants who declared daily intake of vegetables and citrus fruits once or more than once a day had significantly lower index values of gingival bleeding and inflammation than those who took these types of food rarely.

Lower index values in the third group, in our opinion, may be due to antioxidant capability of vitamins in these types of food:

- Antioxidant effect: nutrients with antioxidant reaction help maintain cell integrity by reducing the free radical damage to host tissue that is initiated by the host's inflammatory and immune reactions. They also serve to protect the host from bacterial damage;
- Immuno-modulatory effect: nutritional vitamin enhance host immunological response in gingival tissue;
- Both vegetables and fruits are considered to be solid and abrasive food which results in diminished plaque accumulation.

The role of dietary vitamin C as a contributing risk factor for periodontal disease has been investigated using data from the Third National Health and Nutrition Examination Survey (NHANES III), and the results have been compared between smokers and nonsmokers[(18)]. The periodontal health of 12.419 adults aged 20 or older was compared to their dietary vitamin C intake. The dietary intake of vitamin C showed a statistically significant relationship to periodontal disease in current and former smokers as measured by clinical attachment. It was concluded that those with the lowest intake of vitamin C, and who also smoke, are likely to show the greatest clinical effect on the periodontal tissues. Thus, a weak but significant dose–response increase of risk for periodontal disease in lower vitamin C intake groups was found. The effect of mega-doses of vitamin C on non-deficient individuals has received little attention. A relationship between low levels of vitamin C and impaired wound healing has been demonstrated, and it has been suggested that gingival tissues undergoing healing could benefit from increased levels of vitamin C. In healthy young adult males classified according to periodontal status, one single intravenous dose of 500 mg of ascorbic acid resulted in statistically significant correlations between gingival status and ascorbic acid levels in whole blood and urine[19]. This is in contrast to a more recent study by Woolfe et al.[19], who evaluated the relationship of vitamin C supplementation to gingival clinical parameters. An intake of 1 g vitamin C per day for 6 wk in normal human subjects did not have an effect on the gingival response to the initial therapy; identical gingival responses to periodontal therapy were found in control and vitamin C–supplemented patients. Final blood vitamin C levels appeared to have increased minimally, suggesting that excesses of the vitamin were excreted in the urine. On the basis of the best available evidence, there are no benefits to the periodontal patient of taking vitamin C supplements and the dietary reference intake may be easily met through consumption of a healthy, balanced diet[20, 21, 22, 23].

Conclusion

From the results of our research we can conclude that patients should be advised, along with oral hygiene procedure, to consume vegetables and citrus fruits on daily basis in order to maintain periodontal health or to reduce disease severity.

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ANALYSIS OF IN SITU MATRIX METALLOPROTEINASE-1 CONCENTRATIONS AND CLINICAL PARAMETERS IN CHRONIC ADULT PERIODONTITIS PATIENTS

АНАЛИЗА НА КОНЦЕНТРАЦИИ НА МАТРИКСМЕТАЛОПРОТЕИНАЗА-1 И КЛИНИЧКИ ПАРАМЕТРИ КАЈ ВОЗРАСНИ ПАЦИЕНТИ СО ХРОНИЧНА ПАРОДОНТОПАТИЈА

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Abstract

Aim: to determine the correlation between the concentration of the collagenase MMP-1 in the inflammatory gingival bounding tissue in patients with a chronic form of periodontal disease with the clinical parameters. **Material and methods:** 32 patients with a chronic form of periodontal disease were monitored, according to the criteria set by AAP 1999. The index of dental plaque Silness-Loe was clinically followed, the index of gingival inflammation Loe-Silness, the index of clinical attachment loss and the Miller-Pelzer index of bone resorption. For setting the concentrations of MMP-1, the quantitative enzyme method was used, with the commercial set Colorimetic SensoLyte MMP-1 ELISA Kit. **Results:** We found the presence of a positive correlation between IDP and the examined MMP-1 (r =0.55). Significantly positive correlation was present between IGI and MMP-1. (r =0.77). The loss of attachment and resorption of the alveolar bone were strongly correlated with the concentrations of MMP-1 (r=0.83 and r=0.76). **Conclusion:** The microorganisms from the biofilm initiate the production of the collagenase MMPs-1 and their concentrations rise with the development of the inflammatory processes, leading to the loss of attachment and resorption of the alveolar bone. **Key words:** MMP-1, chronic periodontal disease, biofilm, inflammation.

Апстракт

Цел: да ги утврдиме корелациите помеѓу концентрациите на ММП-1 во инфламираните гингивални ткивни исечоци кај пациентите со хронична форма на пародонталната болест, со клиничките параметри. **Материјал и метод:** во студијата беа вклучени 32 пациенти со хронична форма на пародонталната болест во согласност со критериумите на ААП од 1999. Клинички ги проследивме индексот на дентален плак по Silness-Loe, индексот на гингивална инфламација по Loe-Silness, клиничкиот губиток на припој (CAL), како и Miller-Pelzer-овиот индекс на ресорпција на алвеоларната коска. За утврдување на концентрациите на ММП-1 беше користен квантитативен ензимски метод со комерцијалниот сет Colorimetic SensoLyte MMP-1 ELISA Kit. **Резултати:** утврдивме присуство на позитивна корелација помеѓу ИДП и испитуваната ММП-1 (r =0.55). Значајна, силно изразена позитивна корелација постоеше помеѓу ИГИ и ММП-1 (r=0.77). Клиничкиот губиток на припој, како и ресорпцијата на алвеоларната коска беа исто така силно позитивно корелирани со концентрациите на ММП-1 кај пациентите со хронична пародонтопатија (r =0.83 and r =0.76). **Заклучок:** микроорганизмите од денталниот плак (биофилмот) ја иницираат продукцијата на ММП-1 и нејзините концентрации се зголемуваат со напредувањето на инфламаторните процеси, доведувајќи до губиток на припој и ресорпција на алвеоларната коска. **Клучни зборови:** ММП-1, хронична пародонтална болест, биофилм, инфламација.

Introduction

Periodontal disease is a common, bacterial induced, chronic inflammatory disease that affects the supporting tissues of the teeth, leading to progressive destruction of connective tissue attachment and the alveolar bone. The bacterial etiology of the disease was discovered and confirmed a long time ago, and the bacteria and pathogenet-

ic mechanisms that lead to progression of the disease were identified[1]. However, tissue destruction as a dominating process is caused by the cascade of proteolytical enzymes originating from bacteria of the biofilm, as well as from the enzymes originating from the host-responsive cells[2,3]. The host initiates and controls the release of enzymes, including matrix-metalloproteinases (MMPs), to allow the tissues to retreat from the microbial destructive lesions. Recently, there has been increasing evidence implicating MMPs as key mediators in the tissue destruction associated with the various forms of periodontal disease, including the progression from gingivitis to periodontitis[4].

Matrix metalloproteinases are a family of 25 genetically various, but structurally related zinc and calcium-dependent endopeptidases that degrade the extracellular matrix (ECM) of interstitial stroma and the basement membrane components[5]. MMPs are expressed by various types of cells, inflammatory cells (monocytes, macrophages, lymphocytes and polymorphonuclear cells), and by resident cells (fibroblasts, epithelial and endothelial cells).

MMP-1 is synthesized and secreted by the tissue fibroblasts and macrophages in the chronic inflammation, but it has also been associated with normal tissue remodelling[6]. In vitro MMP-1 is expressed in numerous cells, such as hondrocytes, osteoblasts, keratocytes and various tumour cells[7,8]. MMP-1 can initiate extracellular matrix destruction and cooperates with other MMPs in collagen degradation[9,10]. MMP-1 (and MMP-13) are the key enzymes responsible for degradation of type I and III collagen.

The **aim** of this study was to evaluate the correlations between concentrations of MMP-1 in the inflammatory gingival bounding tissue at patients with a chronic form of periodontal disease with the clinical parameters.

Material and methods

In order to realize this goal, at the Clinic of Oral Pathology and Periodontology were studied 32 patients of both sexes, male and female, aged from 21 to 65 years, with moderate to severe expression of a chronic form of periodontal disease: pocket depth over 4mm according to the criteria proposed by the AAP, 1999. In all subjects, after their history was taken, we conducted a clinical examination to determine the level of periodontal destruction. We noted the dental plaque by using index IDP - Silness-Loe; gingival inflammation by using index IGI - Loe-Silness; clinical attachment loss – CAL, and index of bone resorption by Miller-Pelzer. All subjects were healthy and none of them had used antibiotics within the 6 months preceding the study. The test material was taken with the incision of inflamed gingival tissue during conventional treatment – curettage of the periodontal pockets. Gingival biopsies contained epithelium, connective tissue, proliferated sulcular epithelium and pathologically altered epithelium from the bottom of the pockets. Samples were stored in sterile plastic tubes, then in the shortest period of time were frozen and kept at - 80^0 C until further laboratory analysis, but not for more than 6 months. For setting the concentrations of MMP-1 a quantitative enzymatic method was used, the commercial SensoLyte MMP- 1 ELISA Kit Colorimetric from AnaSpec Inc, and with the help of this we obtained a quick, safe and sensitive determination of concentrations of MMP - 1 in the gingival tissue substrate.

The test material (samples) was frozen and kept in the Laboratory of the Faculty of Pharmacy in Skopje. The laboratory assays were performed at the Institute of Molecular Biology and Genetics at the Faculty of Natural Sciences and Mathematics at the "Ss. Cyril and Methodius" University, Skopje. Obtained data were statistically processed using Statistics 6.1 computer programs for Windows.

The protocol for the collection of samples from human subjects was approved by the ethical committee for medical-dental investigations of the Faculty of Dentistry at the Ss. Cyril and Methodius University, Skopje, according to the Helsinki Declaration on Human rights.

Results and discussion

Although research investigations into the pathogenesis of periodontal disease traditionally focused on the impact of bacterial infection, in the last two decades more attention has been directed towards the study of host response factors (mechanisms) that determine the disease[11]. Molecular and cellular studies on the pathogenesis of the disease confirm that biofilm is a primary etiologic factor, but the disease still occurs as a result of the interaction between specific pathogens and sensitive immune and immune-inflammatory host-responses[12], which are modulated by a number of intrinsic (genetic) and externally induced factors[13].

Table 1 shows mean values of clinical parameters - IDP, IGI, CAL, the index of bone resorption and concentration of MMPs-1 in inflamed gingival samples in patients with a chronic form of periodontal disease. The mean value for the index of dental plaque (IDP) ranged from 1 to 3 (2.37 ± 0.61). The gingival inflammation index (IGI) ranged from 1 to 3, mean 2.31±0.59. Clinical attachment loss (CAL) ranged from 4 to 7mm; the average value was 5.25 ± 1.18 mm. The average value for the index of bone resorption was 3 ± 0.92mm, ranging from

2 to 4mm. Concentration of MMP-1 ranged from 301.36 to 1456.76 pg/100 microg. protein in inflamed gingival tissues, the average value was 749.02 ± 338.03 pg/100 microg.protein.

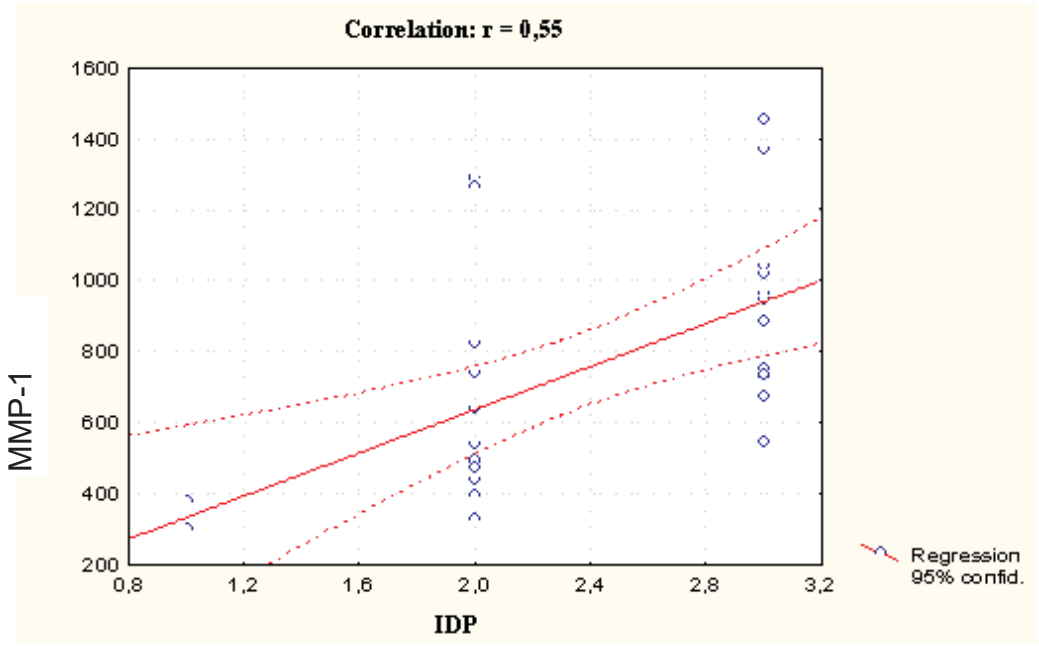
Table 1. Average values of examined clinical parameters IDP, IGI, CAL and the index of bone resorption and concentration of MMPs-1 in patients with a chronic form of periodontal disease

Parameters	mean	SD	min	max
IDP	2.37	0.61	1	3
IGI	2.31	0.59	1	3
CAL	5.25	1.18	4	7
Bone resorp. index	3	0.92	2	4
MMPs-1	749.02	338.03	301.36	1456.76

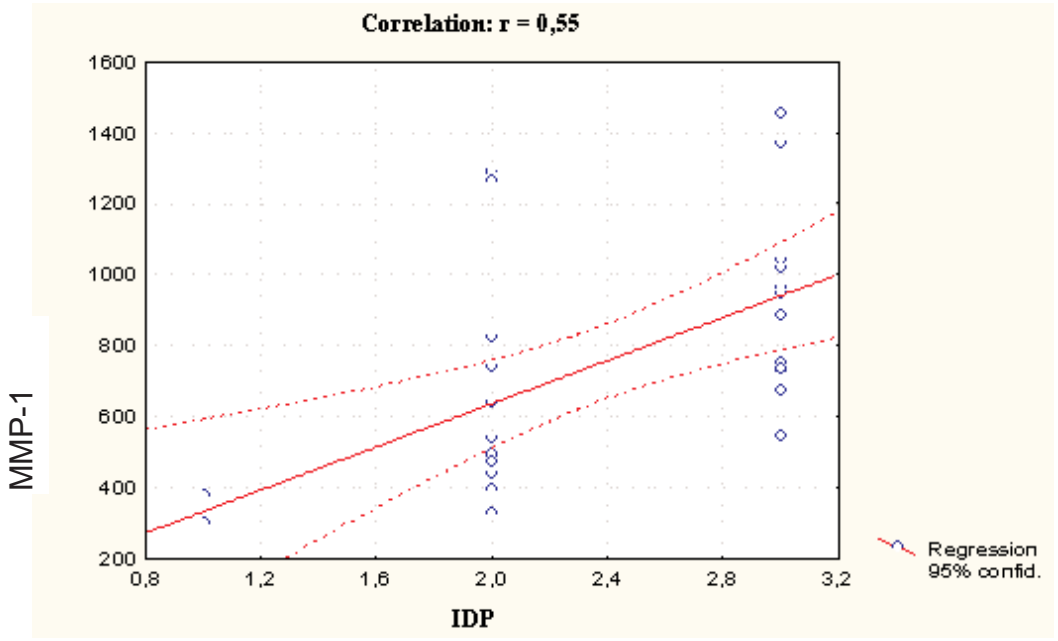
Analysing the correlations between the concentration of MMP-1 in inflamed gingival tissues and clinical parameters, we noted a significant positive correlation between the mean values of the index of dental plaque and MMP-1 (r =0.55). This means that large amounts of dental plaque cause increased concentrations of examined MMP-1. Numerous studies suggest that the interaction of bacteria present in the biofilms with inflammatory cells (monocytes/macrophages, polymorphonuclear cells), and with residential cells (fibroblasts) results in the release of proteases from the host-cells. Bacterial adhesion activates secretion of proinflammatory mediators, such as (IL-1α, IL-1β, TNF-α) from epithelial cells, which diffuse into connective tissue and stimulate host-cells to produce and release matrix metalloproteinase[14]. Hence, increased levels of interstitial collagenase were detected in inflamed human gingival tissues, gingival fluid and inflammatory exudates from gingiva[15]. MMP-1 that is present in these tissues is proven to be converted from a latent to an active, destructive form of this enzyme during the inflammatory processes in periodontal disease. Domeij and co-workers[16] suggest that gingival fibroblasts are capable of producing MMP-1 (and MMP-3), as a result of IL-1β and TNF-α stimulation.

Graph 1 shows the correlation between the dental plaque index and MMP-1 in patients with a chronic form of periodontal disease. Pearson's coefficient of correlation suggests that there is a significant positive correlation between these parameters (r=0.55).

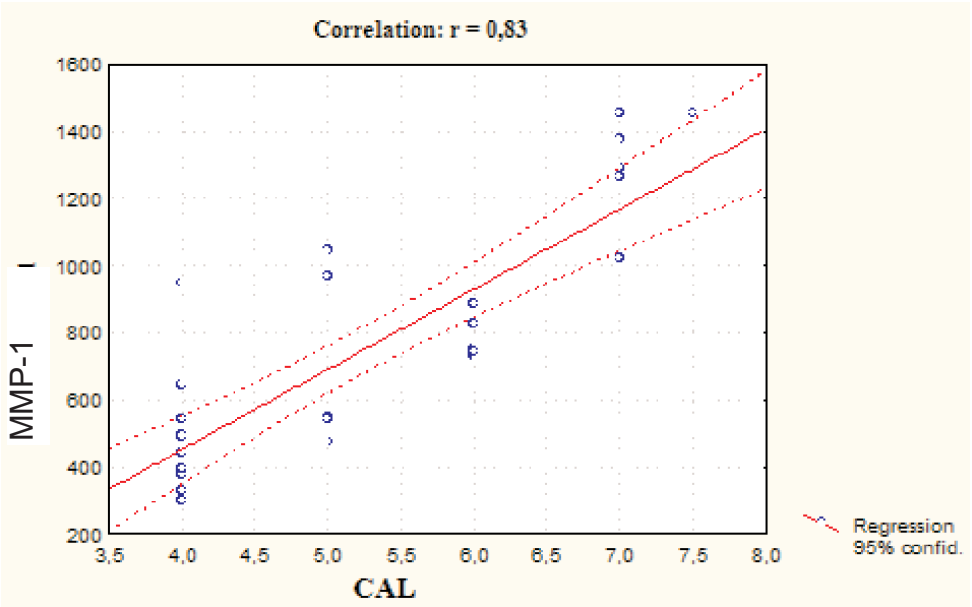
We noted a significant positive correlation between the IGI and MMP-1 in patients with chronic periodontitis (r=0.77). These results suggest that the intensification of gingival inflammation leads to increased values of MMP-1. Our findings comply with the results of Alfant *et al.*[17]. In their studies they detected a statistically significant reduction of the collagenases MMP-1 (-8,-13) 6 weeks after periodontal therapy, and they observed a



Graph 1. *Correlation between IDP and MMP-1 in patients with chronic periodontitis*



Graph 2. *Correlation between IGI and MMP-1 in patients with chronic periodontitis*

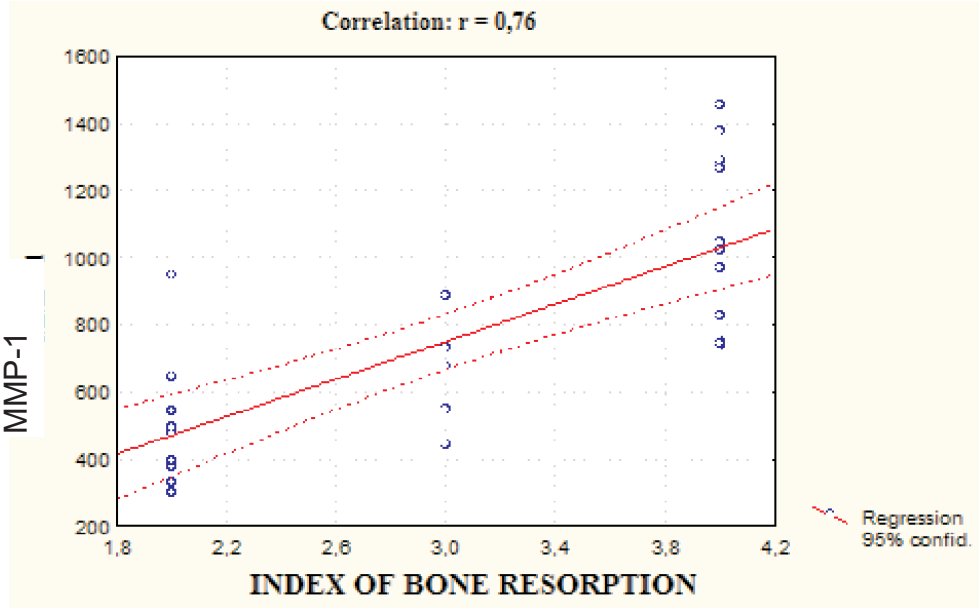


Graph 3. *Correlation between CAL and MMP-1 in patients with chronic periodontitis*

post-therapy reduction in gingival bleeding index, indicating reductions in gingival inflammation. Similar results were noted by Buduneli, Tuter and Sorsa[18,19,20] in their previous studies, investigating MMP changes associated with treatment of chronic periodontitis. Some other authors suggest that tissue extracts and cultured tissue samples from inflamed gingiva increase the collagenase activity, compared with healthy gingiva[21].

Graph 2 shows the correlation between the IGI (index of gingival inflammation) and MMP-1 in patients with chronic periodontitis. Pearson's coefficient of correlation suggests that there is a significant, strong, positive correlation between these parameters (r =0.77).

The loss of attachment was strongly, positively correlated with the concentrations of examined MMP-1 in patients with a chronic form of periodontal disease



Graph 4. *Correlation between the index of bone resorption and MMP-1 in patients with a chronic form of periodontal disease*

(r=0.83). This means that increasing levels of MMP-1 in inflamed gingival tissues lead to a severe loss of epithelial attachment. Chronic periodontal inflammation is characterized by increased apical proliferation and migration of gingival sulcular epithelial cells. This results in degradation of periodontal ligament collagen fibers and finally irreversible loss of tooth attachment. In periodontal diseases, inflamed gingival sulcular epithelium exhibits local loss of integration, evidently associated with epithelial migration into underlying periodontal connective tissue[22].

Graph 3 shows the correlation between CAL (clinical attachment loss) and MMP-1 in patients with chronic periodontitis. Pearson's coefficient of correlation suggests that there is a significant, very strong positive correlation between these parameters (r = 0.83).

Pearson's coefficient of correlation suggests that the resorption of the alveolar bone is strongly, positively correlated with the concentrations of examined MMP-1 in patients with a chronic form of periodontal disease (r=0.76). Our findings agree with Hayami *et al.*[23]. In their studies they indicate that inhibition of collagenases with dexamethasone increases the formation of mineralized nodules in the PDL. During the periodontal disease, destruction and loss of extracellular matrix (ECM) occurs which is mediated by MMP originating from inflammatory and resident cells present in the periodontal ligament complex. PDL cells are crucial for the regeneration of lost periodontal and mineralized tissues, through processes due to differentiation of precursor cells into osteoblasts. Hajami *et al.*[24] suggest that with the presence of elevated levels of MMP-1 (or MMP-13), such differentiation can be inhibited, because of the reduced amount of osteoblasts which are able to mend and repair the bone tissue. Moreover, they suggest further *in vivo* studies on animal models, aiming to get more information about the mechanisms that involve MMP-1 and/or MMP-13 in the inhibition of osteoblastogenesis and limited bone repair.

Graph 4 shows the correlation between the bone resorption index and the concentration of MMP-1 in patients with chronic periodontitis. Pearson's coefficient of correlation suggests that there is a significant positive correlation between these parameters (r = 0.76).

Conclusion

The microorganisms from the biofilm initiate the production of the collagenase-MMP-1 and their concentrations rise with the development of the inflammatory processes, leading to the loss of attachment and resorption of the alveolar bone.

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