



# MACEDONIAN DENTAL REVIEW

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<b>Comparison of two types of bonded Nickel titanium retainers in changes of Little irregularity index and inter canine width in twelve months of retention period.</b> <i>Kiseri Kubati J., Xharra Budima P., Qilerxhiu G., Demjaha M., Kjurchieva Chuchkova G., Kiseri B.</i> .....	89
<b>Incidence of iron-deficiency anemia in patients with geographic tongue.</b> <i>Stefanovska E., Georgieva S., Ristoska S., Zabokova Bilbilova E., Mindova S., Dirjanska K., Mitikj K., Mitikj J., Poposki B.</i> .....	97
<b>Therapeutic possibilities of the second generation of platelet-rich fibrin (A-PRF) in the treatment of oroantral communications OAC.</b> <i>Veska Stevkovska D., Apostolova G., Ivanovski K., Trajculeski S., Aleksova P., Anastasovska M.</i> .....	103
<b>Clinical relevance of tumor-associated macrophages (TAMS) and carcinoma-associated fibroblasts (CAFS) in oral squamous cell carcinoma (OSCC): immunohistochemical study.</b> <i>Idoska S., Popovik-Monevska D., Petrussevska G., Popovski V., Grcev A., Bozovic S., Koneski F.</i> .....	109
<b>Epulis fissuratum - class III: case report .</b> <i>Angeleski S., Karamanov I., Kacarska M., Idoska S.</i> .....	116

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<b>Споредба на два типа на никел титаниумски ретејнери во однос на промени на индексот на неправилности според Little и интерканината ширина во тек на дванаесетмесечен период на ретенција. Кисери Кубаџи Ј., Царра Будима П., Љилерџиу Г., Демјаха М., Курчиева Чучкова Г., Кисери Б. ....</b>	<b>89</b>
<b>Инциденца на сидеропенична анемија кај пациенти со географски јазик. Сџефановска Е., Георѓиева С., Ристоска С., Забокова Билбилова Е., Миндова С., Дирјанска К., Миџиќ К., Миџиќ Ј., Појоски Б. ....</b>	<b>97</b>
<b>Тераписки можности на втората генерација на тромбоцитно збогатен фибрин (А-PRF) во третманот на ороантралните комуникации ОАК. Велеска Сџевковска Д., Ајосџолова Г., Ивановски К., Трајчулески С., Алексова П., Анастасовска М. ....</b>	<b>103</b>
<b>Клиничка релевантност на макрофаги-асоцирани со тумор (TAMS) и фибробласти-асоцирани со карцином (CAFS) кај орален планоцелуларен карцином (OSCC): имунохистохемиска студија. Идоска С., Појовиќ-Моневска Д., Пејрушевска Г., Појовски В., Грчев А., Божовиќ С., Конески Ф. ....</b>	<b>109</b>
<b>Erulis fissuratum - класа III: приказ на случај. Анѓелески С., Караманов И., Кацарска М., Идоска С. ....</b>	<b>116</b>

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# COMPARISON OF TWO TYPES OF BONDED NICKEL TITANIUM RETAINERS IN CHANGES OF LITTLE IRREGULARITY INDEX AND INTER CANINE WIDTH IN TWELVE MONTHS OF RETENTION PERIOD

## СПОРЕДБА НА ДВА ТИПА НА НИКЕЛ ТИТАНИУМСКИ РЕТЕЈНЕРИ ВО ОДНОС НА ПРОМЕНИ НА ИНДЕКСОТ НА НЕПРАВИЛНОСТИ СПОРЕД LITTLE И ИНТЕРКАНИНТА ШИРИНА ВО ТЕК НА ДВАНАЕСЕТМЕСЕЧЕН ПЕРИОД НА РЕТЕНЦИЈА

Kiseri Kubati J.<sup>1</sup>, Xharra Budima P.<sup>2</sup>, Qilerxhiu G.<sup>3</sup>, Demjaha M.<sup>3</sup>, Kjurchieva Chuchkova G.<sup>4</sup>, Kiseri B.<sup>5</sup>

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### Abstract

**Introduction:** Fixed retainers bonded to the lingual surfaces of the mandibular canines (3-3 retainer) are commonly used type of retention after orthodontic treatment is finished. **Purpose** of this study is to assess two types of Nickel titanium fixed retainers, flat versus round shaped, for the effectiveness in maintaining the stability of the alignment of the mandibular anterior teeth after orthodontic treatment. **Material and methods:** the sample consisted of the dental casts of 60 consecutively treated subjects (18 male and 42 female) age 16-25, previously treated for mild crowding in Class I. 30 subjects received a flat Nickel-titanium Retainer, thickness .010" x .029" four-strand twisted in one, manufactured by Forestadent and 30 other patients had bonded multistrained round, co-axial retainer  $\varnothing 0.44\text{mm}/17''$ , manufactured by Dentaaurum. Retainers were bonded to all anterior teeth, at the end of active orthodontic treatment. The Little irregularity index and intercanine width were measured on dental casts immediately after treatment (T0), and 12 months (T1) post treatment. **Results:** The main Little irregularity index not significantly increased during the post treatment period from 0 mm to 0.18 mm (SD, 0.24) at (T1) in flat retainer group and 0.34 mm (SD, 0.47) in round retainer group. The intercanine distance increased from (T0) measured values in 0.34 (SD 0.31) in flat retainer group and 0.44 (SD 0.40) in round retainer group. In 4 patients (13.3%), of round group, unexpected post treatment complications (torque differences of the incisors) was recorded. **Conclusions:** Two types of fixed retainers, maintain the stability of teeth alignment after finished orthodontic treatment, during 12 months of retention phase. Round retainers have the tendency to incline the position of at least one incisor during 12 months of follow up. **Key words:** Fixed retainers, Little irregularity index, intercanine distance, retention

### Апстракт

**Вовед:** По завршување на ортодонтичкиот третман со фиксни ортодонтички апарати за ретенција и стабилност на постигнатите резултати најчесто се употребува фиксен ретејнер бондиран на лингвалните површини на мандибуларните канини. **Цел:** Целта на оваа студија е да се процени ефикасноста во одржувањето на стабилноста на постигнатите тераписки резултати, споредувајќи го ефектот од примената на двата типа на фиксни никел титаниумски ретејнери, рамен наспроти округол ретејнер. **Материјал и методи:** примерокот се состоеше од 60 студио модели од пациенти со умерена форма на малоклузија збиеност класа I (18 машки и 42 женски) на возраст од 16-25 години, претходно третирани со фиксен ортодонтички третман. Кај првата група од 30 пациенти беше поставен рамен префабрикуван никел-титаниумски ретејнер (од 4 пати плетена жица во едно), со дебелина .010"x .029", произведен од Forestadent, додека кај втората група од 30 пациенти поставен беше округол, коаксијален ретејнер (од 6 пати плетена жица во едно), со дебелина 0.44mm/17" произведен од Dentaaurum. Анализата на индексот на неправилност според Little и интерканината ширина беше мерена на студио моделите веднаш по третманот (T0) и 12 месеци (T1) по третманот. **Резултати:** Индексот на неправилност според Little не се зголеми значително за време во периодот по третманот (T0), односно од 0 mm до 0,18 mm (SD, 0,24) кај испитаниците кај кои беше поставен рамен ретејнер, и за 0,34 mm (SD, 0,47) кај групата кај кои беше поставен округол ретејнер. Интерканинското растојание од измерените вредности на почетокот на третманот (T0) кај првата групата испитаници со бондиран рамен ретејнер беше незначително зголемена за 0,34 mm (SD 0,31), додека кај втората група со бондиран округол ретејнер зголемувањето изнесуваше 0,44 mm (SD 0,40). Кај 4 пациенти (13,3%), од втората група со поставен округол ретејнер, евидентирани се неочекувани компликации по



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третманот (разлики во торкот на корените на инцизивите). **Заклучоци:** Обата модели на фиксен ретејнер, ја одржуваат стабилноста на положбата на забите по завршен ортодонтски третман, во текот на 12 месеци постретенционен период. Резултатите сугерираат дека округлиот ретејнер покажува минимално влијание на инклинацијата на мандибуларните инцизиви во текот на 12-месечното следење. **Клучни зборови:** фиксен ретејнер, индекс на неправилности според Little, интерканинско растојание, ретенција

## Introduction

Orthodontic retention is commonly defined as maintaining teeth in optimal aesthetic and functional position after orthodontic treatment is finished. Ideally, the correction should remain stable but there is often a tendency for teeth to relapse or to return to their primary position. Therefore, post treatment corrected tooth position generally requires maintenance for a period in order to prevent relapse<sup>1</sup>.

For this purpose fixed retainers are recommended. Retention is one of the most important final stages of treatment phase in which orthodontists have not come to a common agreement and also decided of which shapes and materials are more superior for this purpose<sup>2</sup>. Post treatment stability is unpredictable at the individual level and there are various factors that contribute to relapse; therefore, most patients are provided with a bonded lingual retainer in anterior teeth when the orthodontic appliance is removed in order to try minimize the chances of relapse<sup>3</sup>. After finishing orthodontic treatment the retention phase is very challenging both for orthodontists and patients. Pandis et al. concluded that at least 232 days of retention is needed to insure stability after an orthodontic treatment, furthermore several longitudinal studies evaluated post-treatment records and stated tremendous relapses in some occlusal relations, especially in the alignment of the mandibular anterior teeth<sup>4-7</sup>.

In majority studies related to fixed retainers, orthodontist researches believe that the only way to maintain the ideal alignment after orthodontic treatment would be a form of permanent retention. This can be a fixed retainer bonded in lingual area of anterior teeth, left in the mouth for a long period of time.

But some of the influencing factors that are associated with a relapse that are seen as occlusal factors, soft tissue pressures and further growth, while the biggest importance is given to the supragingival and transseptal fibers<sup>8-9</sup>

There are different types of methods that have been used for the retention of post treatment tooth position. Most of them have their advantages and disadvantages. The first appliances proposed were based on banded fixed appliances, then various removable retainers were advocated and most often the use of bonded fixed retainers have been suggested.

In this sense, bonded retainers consist of a various size and wire material bonded to the teeth with acid-etch retained composite. The main idea was to help prevent relapse in the lower incisor area<sup>10</sup>.

Retention is not a separate problem or phase in orthodontics, and the type of retention and retainers planned to be used should be considered during treatment planning.

Little's Irregularity Index (LII) is a commonly used Index to measure mandibular incisor crowding used in epidemiological studies by providing a guide to quantify mandibular anterior crowding<sup>11</sup>.

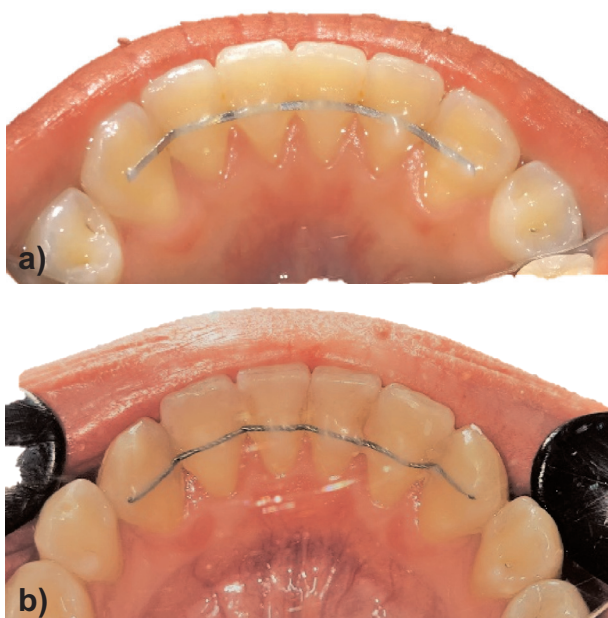
However, still there is no concrete conclusions about the post treatment stability of the mandibular anterior teeth with a bonded lingual retainer, since literature shows quite controversy results.

In recent years, flat easy banded nickel-titanium wire of thickness .010"x.029" for retention purpose has been introduced. Searching for stable and comfortable solution for patients, **the aim** of this study was to assess two types of Nickel titanium fixed retainers, flat and round, for the effectiveness in maintaining the stability of the alignment of the mandibular anterior teeth after orthodontic treatment is finished.

## Materials and methods

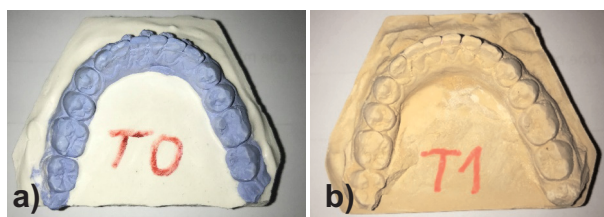
Our material consisted of the dental casts of 60 consecutively treated patients (18 male and 42 female) age 16-25, previously treated for mild crowding in Class I according to Cannut A, in Private Dental Practice Confident in Prishtina. A non-extraction treatment protocol was approved for all patients with straight wire appliances (0.022-in slot, Roth prescription). All subjects had previous Bolton Analyze within a standard range of values. After the orthodontic treatment finished for selected subjects; they were divided in two groups of 30 each. Also the groups were divided in subgroups of growing subjects (age 16-19) and adults subjects (age 20-25). The reason behind this subgroup division was to evaluate, if changes in growing patients, are being recorded with more significance. Subjects received a bonding mandibular lingual nickel titanium retainer from canine to canine in all anterior segment, at the end of active orthodontic treatment. They were randomly selected upon finishing orthodontic treatment. To 30 patients were bonded flat Nickel-titanium Retainer, thickness

.010" x .029" four-strand twisted in one, manufactured by Forestadent and 30 other patients had bonded multi-strained round, co-axial retainer  $\varnothing 0.44\text{mm}/17''$ , manufactured by Dentaaurum. Figure 1.



**Figure 1.** (a) Bonded Flat Retainer, (b) Bonded Round Retainer

First impressions are taken one week after fixed retainer is bonded marked with (T0) and initial measurements are conducted in study model. Second impression marked with (T1) is taken after 12 months of observatory period and final measurements were conducted at that point. Figure 2.



**Figure 2.** (a) dental cast in beginning of retention phase (T0); (b) dental cast in the end of observatory period 12 months of retention (T1)

Subject inclusion criteria were as follows: (1) treated with full fixed appliances; (2) all had been treated for mild crowding at the beginning of the treatment in class I; (3) treated without extraction of any of lower teeth; (4) no caries, restorations, crown or bridges presented. (4) absence of habits and occlusal interference (5) canine

guidance bilaterally, (6) No interproximal enamel reduction or circumferential supracrestal fiberotomy that was systematically performed, (7) no presence of any syndrome.

Excluded subjects were: (1) The ones that failed to be present at requested follow up periods, (2) subjects that during follow up period did any kind of prosthetic restoration, (3) smokers.

Subjects got information letter with clear aim and purpose of the study and signed the consent letter to participate in the study. Ethical Committee of Kosova Dental Chamber approved the study.

The measurements for the Little irregularity index and intercanine width were made with an electronic caliper (digital 6, Mauser, Winterthur, Switzerland) with an accuracy of 0.01 mm. Figure 3



**Figure 3.** Digital Vernier Caliper 6 Inch 150mm Stainless Steel

Since in the stage T(0) all the casts were at 0 mm irregularity values it was easy to assess the difference in 12 months. At the point where anatomic contact points of adjacent teeth are touching, the measurement is considered zero. If there was increased measured irregularity or greater displacement it led to an increased index score. At the dental casts, the anatomic contact areas of the mandibular incisors were marked and the mesial anatomic contact areas of the canines. The linear distance between the markings was measured and the 5 values were added. The measurements were made at (T0) and (T1). Patients with scores less than 0.25 mm were considered to have good alignment. The intercanine distance was measured from the middle of the cusp of the mandibular right canine to the middle of the cusp of the mandibular left canine. All retainers were bonded directly by the same investigator. The same etching agent (Etch-Royale.), adhesive primer (Transbond XT primer, 3M Unitek) and flow composite (Transbond LR, 3M Unitek) were used to bond all retainers.

## Statistical analyses

The data obtained with the research were processed in SPSS software package, version 22.0 for Windows, and presented in tables and graphs.

- The qualitative series were processed by determining the coefficient of relations, proportions, and rates, and were shown as absolute and relative numbers.
- Quantitative series were analyzed with measures of central tendency (mean, median, minimum /maximum values), as well as by dispersion measures (standard deviation). -The Shapiro-Wilk W test was used to determine the normality of frequency distribution of investigated variables.
- Pearson Chi square test was used to determine the association between certain variables in the groups of subjects.
- Two independent samples were compared with the Mann Whitney U test. A two-sided analysis with a significance level of  $p < 0,05$  was used to determine the statistical significance.

## Results

In our investigation of the total sample of 60 cast models of 30 subjects with bonded flat Retainer (FR), gender

distribution was 11 (36.7%) Male and 19 (63.3%) Female, whereas in Round retainer group (RR) 7(23.3%) were Male and 23 (76.7%) female. The results of Chi-Square=1,269 and the  $df=1$ ;  $p=0,259$ . Table 1.

Observatory period was 12 months post retention. None of the indices revealed any significant differences between the two groups of study at the baseline in the mandibular arch, which implements similar optimal good alignment of mandibular dental arch in all individuals at the beginning of the trial according to the cast models.

At the beginning of retention phase (T0), there were all 60 patients with an irregularity index value of zero. 12 months after the treatment, the average LII in FR group was  $0.18 \pm 0.24$  with 50% patients with lower value than 0.01. In RR group the average LII value was  $0.34 \pm 0.47$  with 50% patients who had value 0.00. In FR/RR subgroup the average LII was  $0.26 \pm 0.26$  vs.  $0.41 \pm 0.58$  respectively with 50% patients with lower value than 0.02 in FR subgroup and 0.00 value in RR subgroup. Table 2.

The Little irregularity index rebounded slightly from the beginning of retention period to the follow-up phase in both groups, but the patients with bonded round multi-strained retainer showed slightly more incisor irregularity in lower arches than those having flat retainers at the 12 month follow-up stage; however, this difference was not statistically significant ( $p > 0.05$ ).

**Table 1.** Demographic data of the study patients by Group Flat Retainers (FR) and Round Retainers (RR).

Parameters	FR	RR	P-value
<b>Gender</b>			
Male	11 (36,7%)	7 (23,3%)	Chi-Square=1,269; df=1; p=0,259
Female	19 (63,3%)	23 (76,7%)	
<b>Age</b>			
Mean $\pm$ SD	20.09 $\pm$ 3.36	20.52 $\pm$ 3.38	Z=-0.658; p=0.510
Min/Max	16,1/25	16,1/225	
Median (IQR)	19,7 (16,8-23,4)	20,7 (17,2-23,5)	
Z=Mann-Whitney U Test      X <sup>2</sup> = Chi-Square      *significant for $p < 0,05$ Flat Retainers - FR;    Round Retainers - RR			

**Table 2.** Analysis of Little Irregularity Index (LII) by FR / RR groups and subgroups

LII	N	Mean ± SD	Min/Max	Median (IQR)	P-value
<b>Groups</b>					
FR	30	0.18 ± 0.24	0.0/0.8	0.01 (0.0-0.4)	Z=-0.680; p=0.496
RR	15	0.34 ± 0.47	0.0/0.8	0.00 (0.0-0.5)	
<b>Subgroup: 16-19 years</b>					
FR	30	0.26 ± 0.26	0.0/0.8	0.02 (0.0-0.5)	Z=0.124; p=0.901
RR	15	0.41 ± 0.58	0.0/0.2	0.00 (0.0-0.8)	
Z=Mann-Whitney U Test Flat Retainers - FR ; Round Retainers - RR					*significant for p<0,05



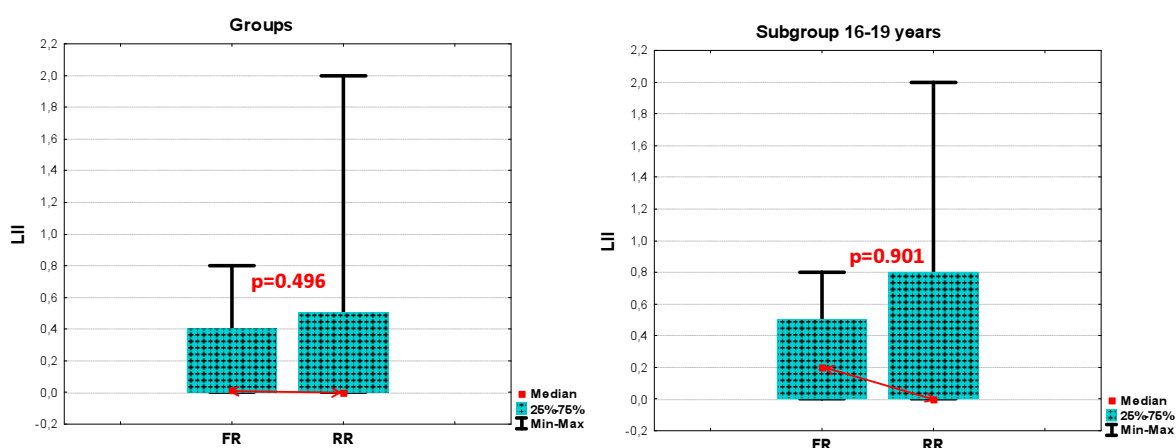
**Figure 3.** Proinlined d.41



**Figure 4.** Proinlined d.41



**Figure 5.** Torque change in d.42



**Graph 1.** Little Irregularity Index (LII) by FR / RR groups and subgroups

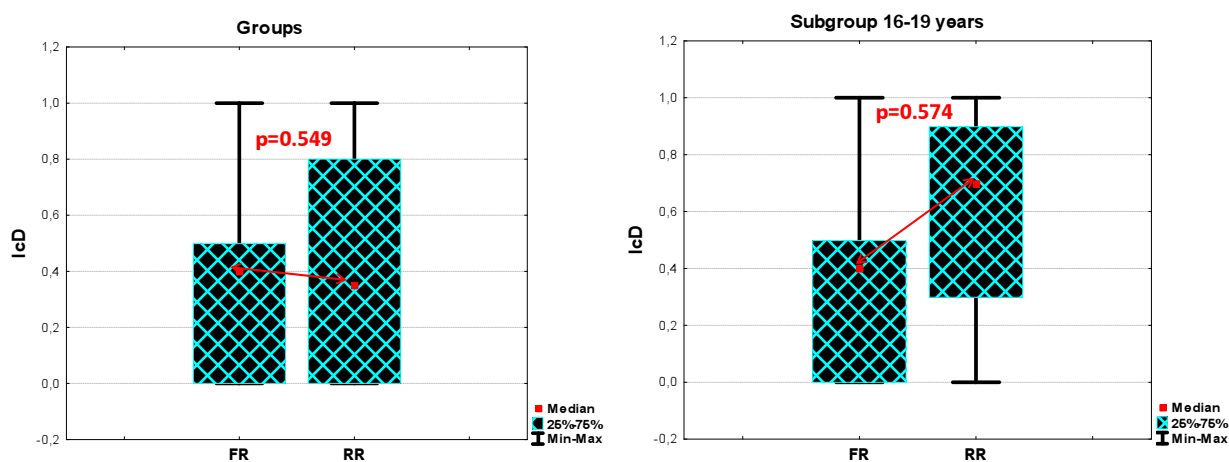
The minimum one lower incisor in three cases had torque difference (X effect). Figure 3. 4. 5.

In our study we recorded 4 subjects in a RR group

with one incisor minor inclination and recorded it only as clinically significant, but not statistically significant. Graph 1.

**Table 2.** Analysis of Little Irregularity Index (LII) by FR / RR groups and subgroups

IcD	N	Mean ± SD	Min/Max	Median (IQR)	P-value
<b>Groups</b>					
FR	30	0.34 ± 0.31	0.0/1.0	0.4 (0.0-0.5)	Z=-0,599; p=0.549
RR	30	0.44 ± 0.40	0.0/1.0	0.3 (0.0-0.8)	
<b>Subgroup: 16-19 years</b>					
FR	15	0.34 ± 0.31	0.0/1.0	0.4 (0.0-0.5)	Z=-1.783; p=0.074
RR	15	0.59 ± 0.36	0.0/1.0	0.7 (0.3-0.9)	
Z=Mann-Whitney U Test Flat Retainers - FR; Round Retainers - RR					*significant for p<0,05



**Graph 2.** Analysis of Intercanine distance (IcD) by FR / RR groups and subgroups.

In this study, Intercanine distance (IcD) remained stable during 12 months of retention period. 12 months after the treatment, the average IcD values in FR and RR groups, were  $0.34 \pm 0.31$  vs.  $0.44 \pm 0.40$  respectively. About 50% of the patients in this groups had IcD value lower than 0.4 vs. 0.3. The average IcD value in the FR/RR subgroup of 16-19 years old was  $0.34 \pm 0.31$  vs.  $0.59 \pm 0.36$  respectively with 50% patients with lower value than 0.4 in FR subgroup and 0.7 value in RR sub-

group. The mean values of the Intercanine distance showed stability from (T0) throughout follow up 12 months (T1) in both groups. Table 2.

As this study uses cast models of subjects treated for mild crowding in Class I, Intercanine distance was preserved and very slightly decreased therefore we assume that it was a favorable factor in maintaining stability of the Intercanine distance during 12 months of follow up. Graph 2.



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## Discussion

While patients are exclusively perceptive to the alignment of their incisors and canines, orthodontists are also sensitive to changes in tooth positions, from an aesthetic point of view, but also the relapse of the anterior teeth alone prey on any assessment for the stability of a treatment result.

Zachrisson made a longitudinal study during follow up period of 20 years and concluded that 0.0215 inch 5-stranded wires show better results based on failure rates and serve better to stability observed in follow-up sessions<sup>12</sup>.

Scribante et al. in a longitudinal prospective randomized study, made clinical comparison between Multi-stranded Wires and Direct-Bond Glass Fiber-Reinforced Composite Splints. They found no statistically significant difference between the two types of bonded retainers concerning the stability<sup>13</sup>.

Retention phase and possible relapse of previously stable results after orthodontic treatment has been issue of concern for orthodontists.

Different studies have investigated different modules of retention in comparison to standard wires with different properties<sup>14-17</sup>. In our study, anterior crowding was evaluated with Little's irregularity index and Intercanine distance and also these two parameters were used to evaluate the stability of lower anterior teeth in cast models taken after orthodontic treatment is finished.

Some studies show that greater increase in incisor irregularity was noted in the growing subjects compared with the adult group in both different wire groups, which could also be explained by influence of growth in young subjects<sup>5</sup>. In this study our results reveal no significant value among two groups with different type of retainers.

Similar result was described in the study of Kucera et al. where they found out 0.1% to 5% of patients experienced unexpected complication as X effect of lower incisors<sup>18</sup>.

In our study a mean Little Irregularity Index score in FR group was  $0.18 \pm 0.24$  and in RR group was  $0.34 \pm 0.47$  for 12 months post retention is regarded satisfactory, as we deliberately corrected Intercanine region in the tolerated dimension.

Renkema et al. in their study concluded that it is very important to avoid over expansion of the Intercanine area of lower arch during treatment, which would help maintain stability with fixed retainers during retention. [19] We preserved intercanine distance in all subjects during treatment, however in 4 subjects in a RR group was noticed minor inclination of one lower incisor. We stated it as clinically significant but not statistically. Early studies presented the advantages of using multi-stranded wires as

bonded retainers to avoid post treatment inclination of incisors, but also there were few reported cases with stability issues<sup>20</sup>. Later several studies, introduced the technique of bonding multi-stranded wires to canines only<sup>16-18, 20, 21</sup>. Al Nimri et al. reported in follow up observatory period of one year significant incisor irregularity with round SS retainer as compared to multistranded SS retainer bonded to lower anterior teeth<sup>15</sup>. Therefore, studies suggest that any over expansion beyond the original pre-treatment status will have the potential to increase the chances for relapse during post-treatment phase<sup>22</sup>.

Forde et al. in bonded 3-strand stainless steel wire 0.0195 for retention found an increase in irregularity (0.77 mm). They assumed that it is related to higher rate of failure observed (50%)<sup>23</sup>. The study conducted by Zachrisson, reported that thinner wires demonstrated more distortion, and 0.0215-in multistrand dead-soft or heat-treated wires were considered more week for maintaining stability of anterior teeth<sup>20</sup>.

However, Renkema et al. evaluated the long-term effectiveness of a 0.0195-in 3-strand wire and found stable outcomes in mandibular anterior alignment in most patients<sup>5</sup>.

In our study, Intercanine distance (IcD) remained stable during 12 months of retention period, in agreement with previous studies<sup>5, 24-26</sup>.

The study conducted by Shapiro yielded interesting results. In a sample of 80 subjects of 10 years after retention of mixed malocclusions, the mandibular intercanine width has a high propensity to return to its pretreatment dimension<sup>27</sup>. On the other hand, a lot of studies have showed that multiple factors influence the stability of teeth after orthodontic treatment. Those factors as periodontal and gingival, soft tissue, occlusal, and growth factors cause teeth to revert to their pre-treatment positions. Changes may occur also as a result of normal dentofacial aging and are highly variable<sup>28-30</sup>. Burke et al. suggests that regardless of pretreatment classification or whether treatment was extraction or non-extraction, mandibular intercanine width tends to exhibit a net shift in post retention on the order of 0.5 mm expansion to 0.6 mm constriction. Their study strongly supports the concept of maintaining original intercanine width in orthodontic treatment because the net change in mandibular intercanine width was determined to be about zero in a total of 1,233 participants in their study, which is in agreement with our study<sup>31</sup>.

## Conclusion

Two types of fixed retainers maintain the stability of teeth alignment after finished orthodontic treatment during 12 months of retention phase.



Post treatment stability is not only dependent from the retention choice but also from the careful treatment planning, mechanics, periodontal and biomechanical considerations.

Retention phase should be monitored for extended period of time.

As this is a randomized clinical trial, bias is minimized. The prospective character of the study allows for a trustworthy and precise evaluation of the results. However, there are certain drawbacks, such as a small sample size and also because the study was conducted on limited post-orthodontic patients treated only for mild crowding, while other malocclusions such as Class II or III and also extraction protocol was excluded.

Furthermore, the duration of follow-up was limited to one year. Similar research with a larger sample size and longer follow up should be conducted in the future.

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# INCIDENCE OF IRON-DEFICIENCY ANEMIA IN PATIENTS WITH GEOGRAPHIC TONGUE

## ИНЦИДЕНЦА НА СИДЕРОПЕНИЧНА АНЕМИЈА КАЈ ПАЦИЕНТИ СО ГЕОГРАФСКИ ЈАЗИК

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### Abstract

**Aim:** To detect the incidence of iron deficiency anemia in patients with geographic tongue and patients with healthy oral mucosa, as well as its frequency in terms of sex and age and smoking-related association. **Material and method:** The study involved 25 subjects (13 women and 12 men) with satisfactory criteria for geographic tongue diagnosis, with an average age of 49 years. The control group consisted of 26 subjects with completely healthy oral mucosa (14 women and 11 men), with an average age of 52 years. The levels of hemoglobin, hematocrit, erythrocyte count, MCV, MCH, MCHC, and serum iron were determined for all subjects, to evaluate if there were any differences in the incidence of iron deficiency anemia among the subjects. **Results:** The collected data were analyzed using descriptive statistics. Our results indicated a statistically significant association between the finding of iron deficiency anemia and geographic tongue ( $p = 0.007661$ ), as well as a statistically higher prevalence of this condition among female subjects, ( $p = 0.013328$ ). There was no statistically significant difference ( $p = 1.00$ ) in the subjects with geographic tongue and iron deficiency anemia in relation to smoking. **Conclusion:** Iron deficiency anemia is more common among subjects with geographic tongue than those with healthy oral mucosa, with female predominance, which suggests that geographic tongue is one of the possible oral manifestations of this condition. **Key words:** Iron deficiency anemia, geographic tongue, oral mucosa.

### Апстракт

**Цел:** Да се детектира инциденцата на сидеропеничната анемија кај пациенти со географски јазик во однос на пациенти со здрава орална слузница, како и нејзината зачестеност во врска со полот и возраста и поврзаноста во зависност со пушењето. **Материјал и метод:** Во студијата партиципираа 25 испитаници (13 жени и 12 мажи) со задоволителни критериуми за дијагноза географски јазик, со просечна возраст од 49 години. Контролната група ја сочинуваа 26 испитаници со потполно здрава орална слузница, (14 жени и 11 мажи), со просечна возраст од 52 години. Кај сите испитаници беа одредени нивоата на хемоглобин, хематокрит, број на еритроцитите, MCV, MCH, MCHC и серумско железо, како би утврдиле дали постои разлика во инциденцата на феродефицитната анемија помеѓу испитаниците. **Резултати:** Собраните податоци беа анализирани со помош на дескриптивните статистички методи. Нашите резултати укажаа на статистички значајна поврзаност помеѓу наодот на сидеропенична анемија и географски јазик ( $p = 0,007661$ ), како и на статистички поголема зачестеност на оваа состојба кај испитаниците од женскиот пол ( $p = 0,013328$ ). Во однос на поврзаноста на сидеропеничната анемија кај испитаниците со географски јазик во однос на пушењето, не добивме статистички значајна разлика ( $p = 1,00$ ). **Заклучок:** Сидеропеничната анемија е почеста кај испитаниците со географски јазик во однос на оние со здрава орална слузница, со преодминантност кај женскиот пол, со што би можеле да заклучиме дека географскиот јазик е една од можните орални манифестации на оваа состојба. **Клучни зборови:** сидеропенична анемија, географски јазик, орална слузница.

### Introduction

The vast majority of systemic diseases oftentimes manifest themselves in the oral cavity, the tongue being the organ upon which these disease-related changes most frequently occur.

One of such conditions is the geographic tongue which happens to be among the most common. Geographic tongue is an inflammatory, benign condition which predominantly occurs on the dorsal lingual surface and is characterized by depapillated areas surrounded by

white keratotic lesions. The lesions are distinctly separated from the surrounding healthy mucosa and can vary from a few millimeters to a few centimeters in size<sup>1,2</sup>.

The atrophic changes on the lingual surface, which are frequently accompanied by glossitis, are characterized by a partial or total loss of the filiform papillae and sometimes, the fungiform papillae. The anatomic structure of the filiform papillae, which by itself integrates the keratinized layer of the squamous epithelium, also protects the tongue from mechanical, chemical, thermic and similar other extrinsic factors. Consequently, these

changes are generally registered by patients as asymptomatic and do not necessitate treatment<sup>3</sup>.

However, in patients where the filiform papillae are absent, sensitivity changes on the tongue's surface have been registered as well as sensations such as burning and stinging. A dysfunction in the taste has frequently been reported especially after consumption of a specific type of food and alcoholic beverages<sup>4,5</sup>.

Numerous studies have found the percentage of this condition amongst the population to be between 1% and 5.2% regardless of gender. The incidence of this condition varies around 2% in the American population, while in the rest of the world, it varies between 11 and 16%. Alternatively, some studies put forward data suggesting a larger presence of geographic tongue in women; 1:1.5 regardless of age<sup>6,7,8</sup>.

Different etiopathogenetic causes contribute to the occurrence of geographic tongue such as genetic factors<sup>9</sup>, systemic diseases, psoriasis<sup>10,11,12</sup>, atopy, diabetes, coeliac disease, avitaminosis (pernicious and iron deficiency anemia), psychosomatic conditions, lichen planus, Down syndrome, mental illnesses, and a tight association to a fissured tongue<sup>13,14,15</sup>.

Iron-deficiency anemia is a hypochromic, microcytic anemia caused by insufficient iron level in the body mostly due to an insufficient intake of iron and is often-times associated with the occurrence of geographic tongue. Contributors to this condition in women are menstruations, pregnancy and breastfeeding, whereas chronic bleeding from the digestive tract is a common contributor in men<sup>16,17,18</sup>.

Atrophic glossitis may be present in certain nutritional deficiencies, such as riboflavin deficiency, niacin, pyridoxine, folic acid, iron, zinc, B12, Vitamin E<sup>19,20</sup>.

Nevertheless, the true etiology of this condition remains unclear.

## Aim

The aim of this study is to detect the incidence of iron-deficiency anemia in patients with geographic tongue compared to patients with a healthy oral mucosa, relative to gender, age and associative to smoking.

## Material and method

Our survey included 25 patients (13 women and 12 men) with diagnosed iron-deficiency anemia, which averaged 49 years of age and made up the examined group, and another 25 patients with a healthy oral mucosa (14 women and 11 men) which averaged 52 years of age and made up the control group. A blood analysis was done for each of the examined patients with

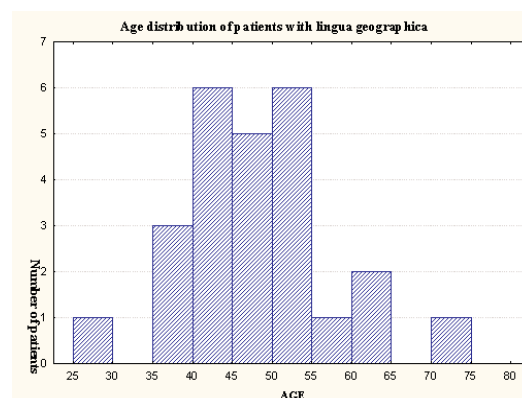
the aim of detecting iron-deficiency anemia, considering the following parameters: hemoglobin, serum iron, hematocrit, RBC, MCV, MCH, MCHC. General anamnestic data was also registered relative to the general systemic health of the patients and smoking habits. The registered data was statistically analyzed using descriptive statistical methods such as the Fischer, exact test and the Mann-Whitney test.

## Results

A total number of 50 patients participated in the study, 25 in each group, 13 women and 12 men in the examined group of patients with clinically diagnosed geographic tongue, whilst 14 women and 11 men took part in the control group with a healthy oral mucosa. The average age of the surveyed patients with a healthy oral mucosa was 49, whereas the average age for surveyed patients with geographic tongue was 52 (Table 1, Graph 1, 2).

**Table.1** Comparison between the surveyed group and the control group relative to age

	Number of patients	Mean	Min.	Max.	Std Dev
Patients with geographic tongue	25	51,52	43	62	5,41
Patients with healthy oral mucosa	25	48,96	30	72	9,25



**Graph 1.** Age distribution of patients with geographic tongue

Three patients in the examined group had a systemic illness (2 men with diabetes, 1 woman with Hashimoto thyroiditis), whilst in the control group 1 man had diabetes and 2 women had cardiac-related issues.

Patients with iron-deficiency anemia were identified through a blood analysis test where lower results were



**Graph 2.** Age distribution of patients with healthy oral mucosa

detected for the specific reference-parameters of iron-deficiency anemia (hemoglobin, serum iron, hematocrit, RBC, MCV, MCH, MCHC), (Table 2, 3, 4).

**Table.2** Referent levels (values) for blood parameters

	FEMALE	MALE
<b>RBC</b>	3,86-5,08 X 10/12 L	4,34-5,72 X 10/12 L
<b>HGB</b>	119-157 (g/l)	138-175(g/l)
<b>HCT</b>	0,356-0, 470(L/L)	0,415-0,530(L/L)
<b>MCV</b>	83,0-97,2 (fl)	83,0-97,2 (fl)
<b>MCH</b>	27,4-33,9 (g/dl)	27,4-33,9 (g/dl)
<b>MCHC</b>	320-345pq	320-345pq
<b>Fe</b>	8-30 (µmol/l)	11-32

**Table 3.** Average results of blood analysis parameters in the control group (healthy oral mucosa)

Patients with healthy oral mucosa (control group)	Mean	Std Dev	N	Std Err	t	p &Fe
<b>RBC</b>	4,90	0,71	25	0,14	34,47	0,000002
<b>HGB</b>	13,39	2,09	25	0,41	31,97	0,83
<b>HCT</b>	40,26	5,70	25	1,14	35,30	0,000002
<b>MCV</b>	85,24	7,19	25	1,43	59,23	0,000002
<b>MCH</b>	28,78	2,36	25	0,47	60,73	0,000002
<b>MCHC</b>	32,10	1,58	25	0,31	101,22	0,000002
<b>Fe</b>	15,20	5,46	25	1,09	13,89	0,000002

**Table 4.** Average results of blood analysis parameters in the surveyed group (geographic tongue)

Patients with geographic tongue (surveyed group)	Mean	Std Dev	N	Std Err	t	p &Fe
<b>RBC</b>	4,81	0,63	25	0,12	37,82	0,000002
<b>HGB</b>	13,63	1,34	25	0,26	50,56	0,003
<b>HCT</b>	42,12	5,28	25	1,05	39,83	0,000002
<b>MCV</b>	87,88	5,22	25	1,044	84,16	0,000002
<b>MCH</b>	28,69	2,02	25	0,40	70,79	0,000002
<b>MCHC</b>	32,58	3,04	25	0,60	53,43	0,000002
<b>Fe</b>	13,34	2,52	25	0,50	26,37	0,000002

**Table 5.** Comparison of occurrence of iron-deficiency anemia in surveyed and control group

	Iron deficiency anemia NO	Iron deficiency anemia YES	Total
Patients with geographic tongue (examined group)	16	9	25
%	64%	36%	
Patients with healthy oral mucosa (control group)	22	3	25
%	88%	12%	

**Table 6.** Comparison of occurrence of iron-deficiency anemia in patients with geographic tongue relative to gender

	Female	Male	Total
Without Iron deficiency anemia	5	11	16
%	31,25%	68,75%	
With Iron deficiency anemia	8	1	9
%	88,88%	11,11%	
Total	13	12	25

**Table 7.** Comparison of occurrence of iron-deficiency anemia in patients with geographic tongue relative to smoking

	Smoking YES	Smoking NO	Total
Geographic tongue without Iron deficiency anemia	2	14	16
%	12,5%	87,5%	
Geographic tongue with Iron deficiency anemia	3	6	9
%	33,3%	66,6%	

When comparing two variables through a Fischer test, a statistically significant link was established between iron-deficiency anemia and geographic tongue,  $p=0,007661$ . Similar significance was not detected between the same two variables in patients with healthy oral mucosa,  $p=0,248$  (Table 5).

According to the results from the Fischer test, a conclusion could be drawn that there is a statistical significance ( $p=0,013328$ ) for the frequency of iron-deficiency anemia in female patients with geographic tongue (Table 6).

Considering the Mann-Whitney test ( $p=1,00$ ), no statistically significant relation could be detected between the frequency of iron-deficiency anemia in patients with geographic tongue and smoking (Table 7).

## Discussion

One of the most occurring types of anemia is the iron-deficiency anemia, which occurs due to iron deficiency. The World Health Organization (WHO) defines anemia as a condition in which the concentration of hemoglobin falls below 130g/l in adult men, under 120 g/l in adult women and under 110g/l in pregnant women<sup>21</sup>.

Hypochromic blood analysis shows lower coefficients for serum iron and red blood cell index (MCV), average red blood cell volume (MCH), average weight of hemoglobin in red blood cells, MCHC, hemoglobin and others<sup>22</sup>.

The aim of our study was establishing whether the occurrence of iron-deficiency anemia is higher in patients with geographic tongue relative to those with healthy oral mucosa. The results of our study present the fact that iron-deficiency anemia is more frequent in patients with geographic tongue (36%) in relation to those with healthy oral mucosa (12%). The discrepancy between the examined groups showed a statistically significant difference ( $p= 0,007661$ ).

Although the available data in academic literature suggests that the incidence of geographic tongue is highest in patients at the age of 20-29 years<sup>4,9</sup>, our survey has found the highest incidence to be in patients between 43 and 62 years of age, or an average age of 52<sup>9,14</sup>.

Geographic tongue was detected in 88,88% of the surveyed female patients which, in turn, is in accordance with the academic data that suggests that geographic tongue is more prevalent in female population<sup>13,23</sup>.

The fact that iron-deficiency anemia is more frequent in women might be associated with heavy menstrual bleeding<sup>23</sup>, heightened physiological necessity of iron during pregnancy and higher blood volume<sup>24</sup>.

Many manifestations on the tongue occur due to poor oral hygiene as well as poor health habits such as smoking<sup>25</sup>.



During this survey we detected the association between iron-deficiency anemia and smoking in patients with a geographic tongue. 9 patients with a detected geographic tongue and iron-deficiency anemia were examined, 3 of whom were smokers (13,3%) and 6 were non-smokers (66,6%). The other 16 patients with geographic tongue but without iron-deficiency anemia included 2 smokers (12,5%) and 14 non-smokers (87,56%). We could not identify a significant association to smoking in the aforementioned group ( $p=1,00$ ).

Some studies point in the opposite direction, suggesting a reverse relationship between geographic tongue and smoking, or in other words, smokers have a lesser tendency to develop geographic tongue manifestations<sup>26, 27</sup>.

## Conclusion

Pathological changes that appear on the tongue may sometimes be the sole indicator of a systemic illness. For that reason, the tongue is considered to denote oral and general health.

Among the most common conditions is the geographic tongue, which happens to occur often and at any age in the human population. The goal of our study was to determine whether iron-deficiency anemia occurs more frequently in patients with geographic tongue relative to those with healthy oral mucosa.

The results corroborated the notion that iron-deficiency anemia is statistically more prevalent in patients with geographic tongue as opposed to those with healthy oral mucosa ( $p=0,007661$ ).

A higher percentage of geographic tongue was noted in female patients (88,88%) in comparison to male patients ( $p=0,013328$ ).

Regarding the association to smoking in patients with a geographic tongue and iron-deficiency anemia and those without iron-deficiency anemia, which posed as a lateral goal of ours, a statistically significant discrepancy was not established.

Based on the results from our survey, a conclusion could be made that geographic tongue is one of the possible oral manifestations of iron-deficiency anemia.

Considering the fact that geographic tongue might provoke symptoms such as pain and burning after consuming certain types of food and beverages, which consequently interferes with the quality of life, early detection and treatment is of crucial importance.

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# THE THERAPEUTIC POSSIBILITIES OF THE SECOND GENERATION OF PLATELET-RICH FIBRIN (A-PRF) IN THE TREATMENT OF OROANTRAL COMMUNICATIONS OAC

## ТЕРАПИСКИ МОЖНОСТИ НА ВТОРАТА ГЕНЕРАЦИЈА НА ТРОМБОЦИТНО ЗБОГАТЕН ФИБРИН (А- PRF) ВО ТРЕТМАНОТ НА ОРОАНТРАЛНИТЕ КОМУНИКАЦИИ ОАК

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### Abstract

OAC is a pathological communication created between the maxillary sinus and the oral cavity, which if left untreated will progress to oroantral fistula or chronic sinusitis. It usually occurs after extraction of the first maxillary molar because it is closely related to the sinus and is a common indication for extraction. PRF is an autologous product derived from the patient's blood (Choukroun's platelet-rich second-generation fibrin). The fibrin matrix is one of the major components of PRF. The fibrin matrix contains platelets (which secrete growth factors, a key factor in the mechanism of action of PRF), leukocytes, and stem cells. There are several different ways of repairing OAC, whereas different factors play a role in choosing one of them. The aim of this paper is to describe clinical cases of OAC treatment with the application of platelet-rich fibrin in the form of a plug (PRF plug) and membrane (PRF membrane). For this purpose, in two patients at the Clinic and the Department of Oral Surgery at the Faculty of Dentistry in Skopje, UKIM, PRF-plug and PRF-membrane was applied, following clinically and radiologically confirmed OAC, after tooth extraction #16 and #25. In the first case, PRF was used as a support for the surgical method, while in the second, as a conservative method for repairing the OAC. Patients noted a calm postoperative course after 24 hours, 72 hours, 7 days and 4 weeks, with no postoperative pain, presence of minimal edema, without postoperative complications. Two months postoperatively, patients underwent clinical and X-ray evaluation of the alveolar socket. Based on the results obtained from these two case studies, we can conclude that PRF-assisted repair of oro-antral communications can be a method of choice. **Key words:** oro-antral communication, platelet-rich fibrin, conservative treatment, surgical treatment.

### Апстракт

ОАК претставува патолошка комуникација создадена помеѓу максиларниот синус и усната празнина која доколку не се лекува ќе премине во ороантрална фистула или хроничен синусит. Најчесто настанува после екстракција на првиот максиларен молар бидејќи е во најблизок сооднос со синусот и претставува честа индикација за екстракција. PRF претставува аутологен производ добиен од крвта на пациентот (тромбоцитно збогатен фибрин од втора генерација на Choukroun). Фибринската матрица како една од главните компоненти содржи: тромбоцити (кои секретираат фактори на раст, клучен фактор во механизмот на дејство на PRF), леукоцити и матични клетки. Постојат повеќе различни начини за санирање на ОАК, чиј избор зависи од различни фактори. Целта на овој труд е да се опишат клинички случаи на третман на ОАК со апликација на тромбоцитно збогатен фибрин во вид на чеп (PRF plug) и мембрана (PRF membrane). За таа цел, кај двајца пациенти, на Клиниката и Катедрата за орална хирургија при Стоматолошкиот факултет во Скопје, УКИМ, беше аплициран PRF-чеп и PRF-мембрана, после клинички и рендгенолошки утврдената ОАК, по екстракција на забите #16 и #25. Во првиот случај PRF беше употребен како поддршка на хируршкиот метод, додека во вториот, како конзервативен метод за санирање на ОАК. Кај пациентите беше забележан мирен постоперативен тек по 24 часа, 72 часа, 7 дена и по 4 недели, со отсуство на постоперативна болка, присуство на минимален едем, без постоперативни компликации. Два месеца постоперативно, кај пациентите беше направена клиничка и рендгенолошка евалуација на алвеолата. Врз основа на резултатите добиени од овие два прикази на случаи, можеме да заклучиме дека PRF поддржаното санирање на ороантралните комуникации може да биде метода на избор. **Клучни зборови:** оро-антрална комуникација, тромбоцитно збогатен фибрин, конзервативен третман, хируршки третман.

## Introduction

The maxillary sinus is the largest of all the bilateral paranasal cavities that begins to develop in the third month of embryonic development. It is located in the body of the maxilla and has a pyramidal shape with a base medially to the nasal cavity and apex towards the zygomatic extension. The maxillary sinus is separated from the roots of the molars by a thin layer of trabecular or spongy bone called "cancellous bone", although in some anatomical projections that bone layer is absent so that the apical tops of the roots of the molar dentition are in close correlation or projection with the floor of the sinus cavity. This condition allows the infection to spread rapidly and can enter the maxillary sinus. In such cases, the act of extracting the molar teeth may be the reason for the creation of oro-antral communication OAC with or without the presence of infection.

OAC is a communication created between the maxillary sinus and the oral cavity which, if left untreated, will turn into oroantral fistula or chronic sinusitis<sup>1</sup>. The close anatomical ratio of the tooth roots, especially the molars and premolars, contributes to the occurrence of this complication during their extraction.

After OAC diagnosis, clinical (positive nose-Valsalva blow test, rhinolalia-nasal speech, saline bubbles protrude through the alveolar socket) and X-ray verification, selection of the most appropriate OAC treatment is considered, considering the localization of OAC, its size, the possible presence of an infection or foreign body (corpus alienum) in the sinus.

There are two types of classic OAC treatment: conservative and surgical.

Conservative treatment is applied when the diameter of the communication is less than or equal to 2 mm; when the sinus is not infected and when there is no foreign

body in it, the postoperative alveolar socket is narrow and deep. The goal of the conservative treatment is to form a stable clot in the apical part of the alveolar socket.

Surgical treatment is indicated for communication greater than 2 mm and when the post-extraction alveolar socket is shallow. Several types of incisions are used in surgical treatment (the most common is the Wassmund-Rerhman trapezoidal buccal mucoperiosteal incision).

PRF is an autologous product derived from the patient's blood (Choukroun second-generation platelet-rich fibrin). The fibrin matrix is one of the major components of PRF. The fibrin matrix contains platelets (which secrete growth factors, a key factor in the mechanism of action of PRF), leukocytes, and stem cells.

Platelets play a role in hemostasis and in the wound healing process. Activated platelets form a platelet plug, degranulate and release growth factors: cytokines (PDGF, EGF, IGF, TGF- $\beta$ 1, VEGF) and chemokines that attract certain cells to the injured tissue, stimulate neoangiogenesis and healing.

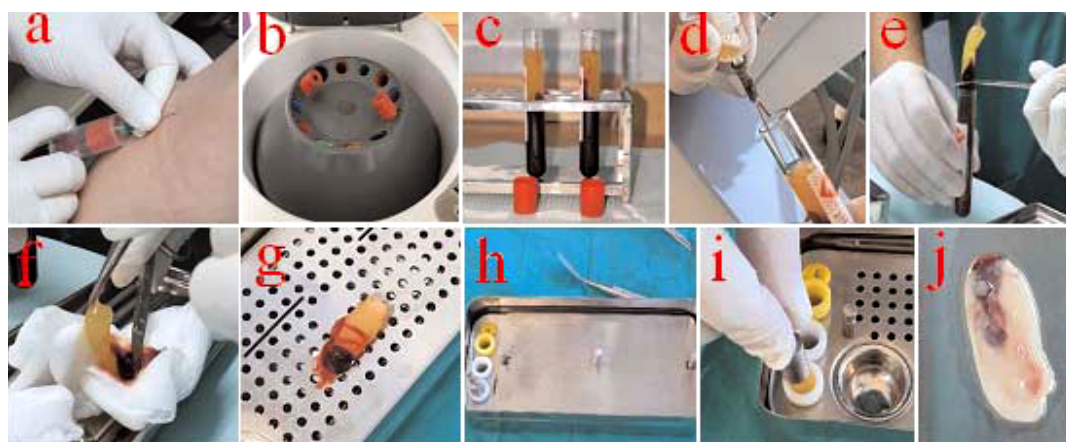
The clinical applications of PRF and the benefits of its use in post-extraction socket preservation, augmentative procedures for horizontal and vertical enlargement of the residual alveolar ridge, implant procedures, and periodontal regeneration have been described by many authors<sup>2</sup>. Gülsen et al.<sup>3</sup>, Asaad et al.<sup>4</sup> analyze the effectiveness of PRF in the treatment of OAC.

The aim of this paper is to describe clinical cases of OAC treatment by applying platelet-rich fibrin in the form of PRF plug and PRF membrane.

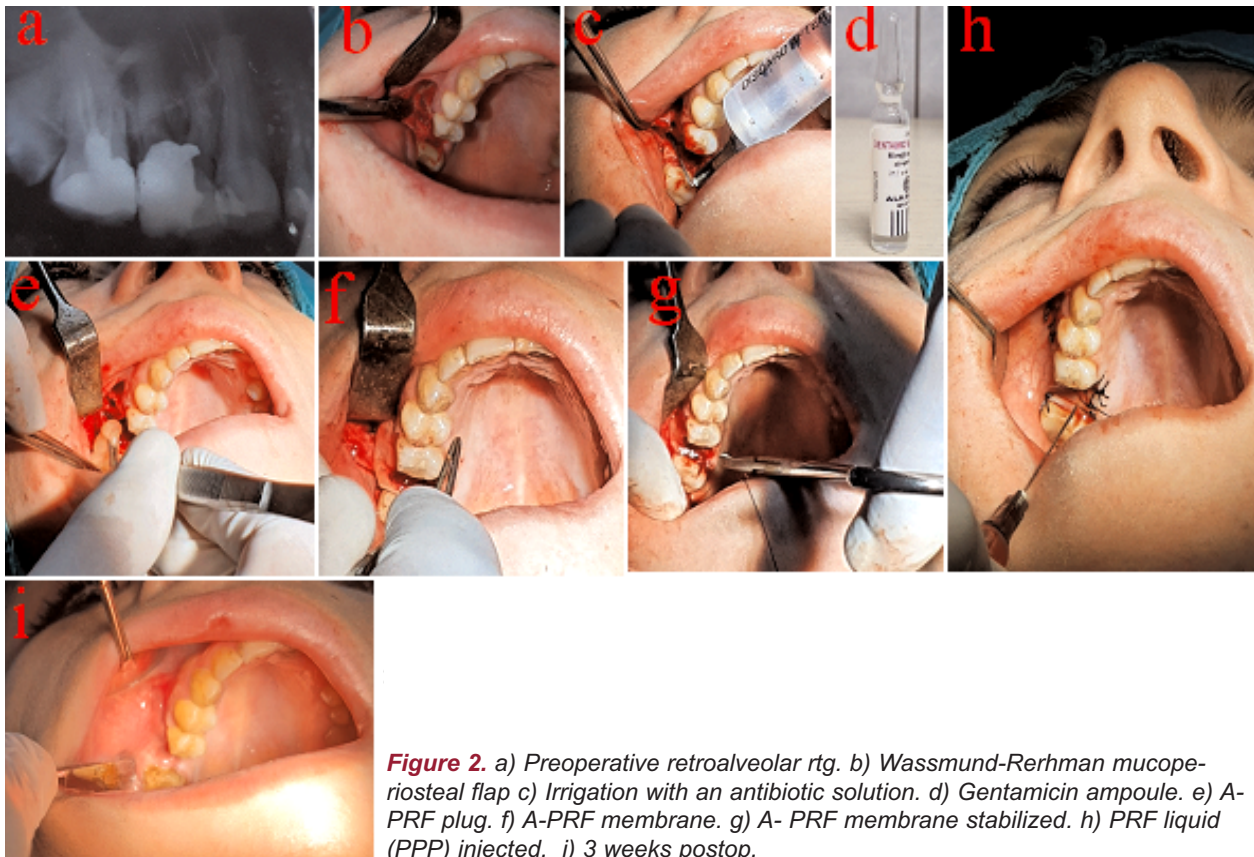
## Case reports

### *A-PRF protocol (advance -PRF)*

Venous blood with venipuncture was collected from the medial cubital vein for the preparation of A-PRF in



**Figure 1.** a) Venipuncture. b) A-PRF tubes in Centrifuge. c) A-PRF centrifuged d)PPP evacuated. e) A-PRF clot. f) erythrocyte-rich red thrombus separated g) A-PRF in PRF box. h) A-PRF covered with a metal lid. i) PRF plug. j) A- PRF membrane



**Figure 2.** a) Preoperative retroalveolar rtg. b) Wassmund-Rerhman mucoperiosteal flap c) Irrigation with an antibiotic solution. d) Gentamicin ampoule. e) A-PRF plug. f) A-PRF membrane stabilized. h) PRF liquid (PPP) injected. i) 3 weeks postop.

patients. The samples were collected in two sterile 12 ml A-PRF tubes without added anticoagulant, which were immediately centrifuged in a Duo Quattro Centrifuge at 1300 rpm for 8 min. After centrifugation, the tubes were left on a stand to stabilize for about 5 minutes. With the help of a sterile needle and syringe, the upper liquid fraction - PPP (platelet poor plasma) was evacuated from the tubes, which was injected perioperatively later after the end of the surgery to condition the soft tissues and reduce the oxidative stress. Using a sterile forceps, the middle fraction (A-PRF) of platelet-rich fibrin was removed from the tubes and placed on sterile gauze where the red erythrocyte-rich red thrombus (third layer) was carefully separated using closed sterile scissors. One PRF sample was placed on the rack in the PRF box and then covered with a metal lid that ensures the formation of a well-hydrated A-PRF membrane of equal thickness, while the other PRF sample is placed in a separate PTFE compartment from the PRF box to form a PRF plug (figure 1).

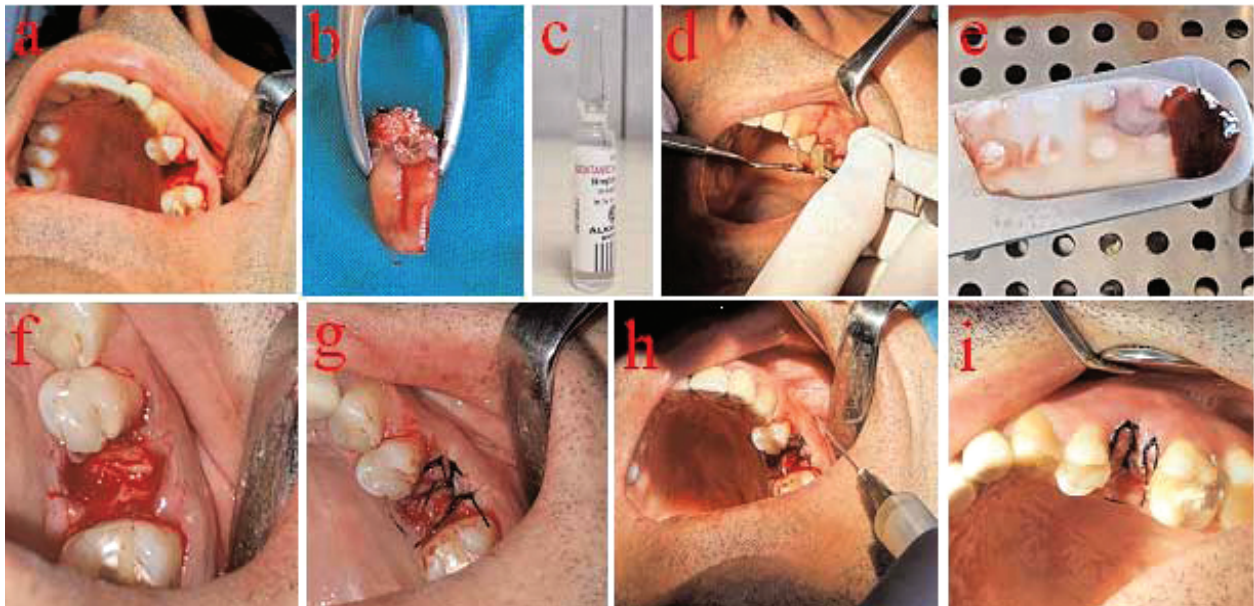
### **Clinical case 1**

Patient aged 29 was admitted to the Clinic and the Department of Oral Surgery at the Faculty of Dentistry

in Skopje, UKIM for extraction of the upper right first molar 16 (Dg. Chronic apical periodontitis 16). After the performed clinical and radiographic examinations, the patient was indicated for extraction by informing the patient about possible collision with the maxillary sinus. After the extraction and performance of the Valsalva test, the OAC from the alveolar socket of the bucodistal root was ascertained at 16 (discomfort, Valsalva +, rhinolalia). The patient was excluded from all systemic diseases that may interfere with the chosen method of repairing oroantral communication (PRF-based surgical treatment).

In the first clinical case, a Wassmund-Rerhman mucoperiosteal flap was created and additional mobility of the vestibular flap was obtained by periosteal incision. After localization and visualization of the OAC at the bucodistal part of the post-extraction alveolar socket, surgical debridement, curettage and excessive irrigation with an antibiotic solution (gentamicin ampoule) was performed. The first PRF plug was applied with bucodistal localization, and on the entire post-extraction alveolar socket, A-PRF membrane was applied and stabilized under the flap. After repositioning of the mucoperiosteal flap, 4/0 non-resorbable silk sutures were placed (horizontal matrix sutures) and the remaining liquid fraction





**Figure 3.** a) Postextraction socket; b) Radix; c) Gentamicin ampoule d) A-PRF plug; e) A-PRF membrane; f) A-PRF membrane placed; g) A-PRF membrane stabilization with single interrupted and x- suture; h) liquid fraction – PPP injected; i) 72 hours postop

- PPP was injected perioperatively at the vestibular aspect of the surgical field. The patient was advised to use oral antiseptics, avoid activities that may increase intranasal pressure, and antibiotic prophylaxis was administered in her postoperative care. A calm postoperative course was noted in the patient after 24 hours, 72 hours, 7 days and 4 weeks, with no postoperative pain, presence of minimal edema, without postoperative complications (surgical wound dehiscence or symptoms of maxillary sinusitis). The sutures were removed after 14 days (figure 2).

### Clinical case 2

Patient aged 55 was admitted to the Clinic and the Department of Oral Surgery at the Faculty of Dentistry in Skopje, UKIM for extraction of the upper left second premolar 25. After clinical and radiographic examinations, the patient was indicated for extraction (Dg. Gangrene radix 25) with information about possible collision with the maxillary sinus. After extraction and performance of the Valsava test, the OAC from the post-extraction alveolar socket was concluded (discomfort, Valsava +, rhinolalia). The patient excluded all systemic diseases that may interfere with the chosen method of repairing oroantral communication (PRF based conservative treatment - flapless).

In the second clinical case, after tooth extraction 25 and localization / visualization of the OAC on the most

apical part of the post-extraction alveolar socket, surgical debridement, curettage and abundant irrigation with antibiotic solution (gentamicin ampoule) followed. The first PRF plug is adapted to the post-extraction alveolar socket with subsequent placement and stabilization of the A-PRF membrane. A stabilizing 4/0 non-resorbable silk suture was placed and the remaining liquid fraction - PPP was injected perioperatively. The patient was advised to use oral antiseptics, avoid activities that may increase intranasal pressure, and to administer antibiotic prophylaxis in his postoperative care. A calm postoperative course was noted in the patient after 24 hours, 72 hours, 7 days and 4 weeks, in the absence of postoperative pain or discomfort. The sutures were removed after 10 days (figure 3).

### Discussion

The sinus membrane covers the periosteum with a thin layer of ciliated pseudostratified respiratory epithelium, an important factor in protection and defense of the sinus cavity<sup>5,6</sup>. The mucociliary apparatus protects the sinus from infection, and the membrane acts as a biological barrier. In conditions of present perforation of the membrane, it is a front door for penetration and bacterial invasion.

The ciliary cells with their cilia tremble at a rate of 700-800 times per minute and allow drainage of mucous and serous secretions to the ostium, and due to its superior location, they must overcome the force of gravity.

Calyx cells produce glycoproteins that are responsible for the viscosity and elasticity of the mucosa.

The function of basal cells is unknown, but according to some authors they may serve as differentiation-capable stem cells stimulated by growth factors found in PRF.

The distance between the floor of the maxillary sinus and the apical tops of the roots of the maxillary distal teeth is approximately 1-1.25 cm<sup>1,6</sup>.

The most common cause of OAC is the first maxillary molar because it is closely related to the sinus and is a common indication for extraction. It is the cause of OAC in 51% of cases<sup>7</sup>. The roots of the second maxillary molar are also positioned quite close, which contributes to frequent injury of the mucosa of the maxillary sinus.

OAC is also much more common if a solitary tooth is extracted due to physiological atrophy of the alveolar ridge than when the dentition is intact. Other risk factors, besides anatomical, that can cause OAC include the following: pathological lesions in bone, progressive periodontitis, iatrogenic factors such as inadequate extraction force or atypical separation extractions.

In a detailed review of world literature, there are a small number of authors who analyze the effectiveness of application of second generation of platelet-rich fibrin. Gülsen et al.<sup>3</sup>, Asaad et al.<sup>4</sup> analyze the effectiveness of PRF in the OAC treatment.

Surgical wound healing takes place in 4 overlapping phases: hemostasis, inflammation, proliferation and remodeling. Platelets are shown to be important cells that regulate the phase of haemostasis through formation of blood clots; capable of stimulating proliferation and activation of fibroblasts, neutrophils, macrophages, and mesenchymal stem cells, which are actively involved in wound healing (cell recruitment, proliferation, differentiation, and remodeling).

The regenerative potential of PRF can be explained by the three-dimensional structure of the fibrin matrix containing a number of growth factors and cytokines (PDGF, TGF- $\beta$ 1, IGF and VEGF), embedded in the fibrin matrix with a proven role in the healing process of wounds and soft and hard tissues, which gradually release over a period of more than 10 days.

PRF-releasing growth factors stimulate the proliferation and differentiation of osteoblasts, chondroblasts, endothelial cells, and fibroblasts, important in the healing processes of damaged tissues.

Furthermore, the fibrin matrix stimulates the expression of integrin  $\alpha$ v $\beta$ 3 which allows cells to adhere to fibrin, fibronectin, and vitronectin. This cascade of events is of particular importance for initiating the process of neoangiogenesis, and thus accelerating the healing of tissue wounds<sup>8,9,10</sup>.

VEGF is a key factor in the growth of endothelial cells in neoangiogenesis that stimulates the proliferation and migration of vascular endothelial cells. VEGFR-2 is an endothelial cell receptor for VEGF.

The primary growth factor for fibroblasts in the second stage of healing is FGF-2, which acts on the recruitment, stimulation, and migration of fibroblasts that synthesize the collagen matrix.

In addition to the positive effect on soft and hard tissue healing, PRF has antihemorrhagic effects. These effects are important in reducing postoperative edema<sup>11,12</sup>.

## Conclusion

The key to the regenerative potential of PRF lies in its angiogenic potential, control of the immune system, recruitment of circulating stem cells and stimulation of epithelialization of surgical wounds. Taking into account all these advantages of the second generation of platelet-rich fibrin PRF, such as: safety, natural autologous bio-material, long-term release of growth factors, ease of operation and economy, we can conclude that PRF-assisted repair of oro-antral communications can be a method of choice.

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# CLINICAL RELEVANCE OF TUMOR-ASSOCIATED MACROPHAGES (TAMs) AND CARCINOMA-ASSOCIATED FIBROBLASTS (CAFs) IN ORAL SQUAMOUS CELL CARCINOMA (OSCC): IMMUNOHISTOCHEMICAL STUDY

## КЛИНИЧКА РЕЛЕВАНТНОСТ НА МАКРОФАГИ-АСОЦИРАНИ СО ТУМОР (TAMs) И ФИБРОБЛАСТИ-АСОЦИРАНИ СО КАРЦИНОМ (CAFs) КАЈ ОРАЛЕН ПЛАНОЦЕЛУЛАРЕН КАРЦИНОМ (OSCC): ИМУНОХИСТОХЕМИСКА СТУДИЈА

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### Abstract

**Introduction:** The factors, which influence or play a key role in tumor invasion and metastasis of the squamous cell carcinoma of the oral mucosa (OSCC), are subject of many scientific researches. **Objectives:** The aim of this study was to investigate the clinical relevance of OSCC infiltration with TAMs and CAFs. **Materials and methods:** Immunohistochemical analysis of both stromal/tumoral CD68+ TAMs and  $\alpha$ -SMA positive CAFs was performed in paraffin-embedded tissue specimens from 23 OSCC patients and correlated with the clinical stage and degree of malignant cell differentiation. **Results and conclusions:** The presence of CAFs was not detected in the specimens of all 23 patients, but TAMs were found in all of them. Stromal myofibroblasts are heterogeneously detected in the OSCC, and the lowest density of CAFs is in the first clinical stage. There is a statistically significant correlation between clinical stage 1 and the degree of density of CAFs compared to other clinical stages ( $p=0.006474$ ). There is a statistically significant difference between clinical stage 1 and other stages 2, 3 and 4, and the presence of TAMs in the tumor stroma ( $p<0.033179$ ) and in the tumor nests also ( $p<0.033179$ ). There is no statistically significant difference between the density of CAFs and the degree of differentiation of tumor cells, and also the degree of differentiation of tumor cells does not correlate with the expression of CD68+ TAMs neither in the tumor stroma ( $p=0.438807$ ), nor in the tumor nest ( $p=0.9488644$ ). **Key words:** Tumor microenvironment, carcinoma-associated fibroblasts, tumor-associated macrophages, oral squamous cell carcinoma.

### Апстракт

**Вовед:** Кои фактори влијаат или имаат клучна улога во туморската инвазија и метастазирање на планоцелуларниот карцином на оралната мукоза (OSCC) се сеуште предмет на многу научни истражувања. **Цели:** Оваа студија има за цел истражување на клиничката релевантност на инфилтрација на OSCC со TAMs и CAFs, евалуирајќи ги туморските маркери CD68 и  $\alpha$ -SMA во туморската строма. **Материјали и методи:** Направена е имунохистохемиска анализа на стромални/интратуморски CD68-позитивни TAMs и  $\alpha$ -SMA-позитивни CAFs на ткивни примероци од орален планоцелуларен карцином кај 23 пациенти, а потоа резултатите се корелирани со клиничкиот стадиум на болеста и со степенот на диференцијација на малигните клетки. **Резултати и заклучоци:** Не е утврдено присуство на CAFs кај сите 23 пациенти, но кај сите 23 пациенти се пронајдени TAMs. Стромалните миофибробласти се хетерогено детектирани во OSCC, а најмала густина на CAFs има во првиот клинички стадиум. Постои значајна статистичка корелација помеѓу густината на CAFs во првиот клинички стадиум на болеста споредено со останати клинички стадиуми ( $p=0.006474$ ). Исто така, постои статистички значајна разлика помеѓу клинички стадиум 1 и останатите стадиуми 2, 3 и 4 и присуство на CD68+ TAMs во туморска строма ( $p<0.033179$ ) и туморското гнездо ( $p<0.033179$ ). Не постои статистички значајна разлика помеѓу густината на CAFs и степенот на диференцијација на туморските клетки за  $p=0.72158$ , и исто така степенот на диференцијација на туморските клетки не корелира со експресија на CD68+ TAMs ниту во туморската строма ( $p=0.438807$ ), ниту во туморското гнездо ( $p=0.9488644$ ). **Клучни зборови:** Туморска микросредина, фибробласти-асоцирани со карцином, макрофаги-асоцирани со тумор, орален планоцелуларен карцином.

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## Introduction

Oral squamous cell carcinoma (OSCC) is one of the malignancies with a very high incidence and mortality and poses a significant health problem all around the world<sup>1</sup>. Despite advances in understanding the molecular biology of this cancer, diagnosis and treatment including multimodal tumor therapy, mortality remains below 50% as 30 years ago, due to locoregional relapses and metastasis.

Staging and grading of OSCC is necessary for determining the following surgical and oncologic treatment. The TNM system of cancer staging is based on assessment of the size of the primary tumor (T), involvement of locoregional lymph nodes (N), and the distant metastases (M). This classification is important not only for treatment planning, but for estimating the risk of tumour recurrence and assessment of the overall survival. However, this classification only considers the anatomic extension of the disease. OSCC staging and grading criteria are periodically changed and improved, introducing modifications that influence the risk stratification as well as the treatment of this type of malignancy<sup>2</sup>.

Histopathological grading for squamous cell carcinoma of the lip was first introduced by Broders, and was based on the differences in differentiation between tumors. Later, more complex grading systems were suggested. These multifactorial systems consider the differentiation of the tumor cells, the tumor invasion patterns and host reactions e.g. inflammatory response<sup>3</sup>. These systems should be updated by introducing the feature of cancer-associated fibroblasts (CAFs) assessed with the use of immunohistochemistry for  $\alpha$ -SMA<sup>4</sup>. Until now, the cause of cancer progression has been attributed to cumulative genetic changes in the oral epithelium, but this model, based only on alterations in the oral epithelium alone, has changed, and a new model is proposed, in which the tumor microenvironment (TME) has a significant contribution to cancer progression. In 1889, Paget proposed the "seed and soil" hypothesis, in which invasion of neoplastic tissue was explained by the fact that cancers induced changes in adjacent stromal cells<sup>5</sup>.

TME is a complex system composed of various stromal cells such as fibroblasts, endothelial cells, immune cells, and various components of the extracellular matrix (ECM). In such environment, the tumor cells reprograms the surrounding stromal cells for tumorigenesis, cancer progression and invasion of the surrounding tissue. The stromal cells in TME, unlike cancer cells, are not prone to mutations and their behavior is modulated by several cytokines. The most numerous and important immune cells in TME are tumor-associated macrophages (TAMs);

in solid tumors these cells make up 5-40% of the tumor mass<sup>6</sup>.

TAMs are a mixed population of macrophages constituting both M1 and M2, but mainly composed by M2 macrophages which are recruited and educated by cancer cells<sup>7</sup>. TAMs are identified by immunohistochemistry. The antibody against CD68 is a pan-macrophage marker that is widely used to identify all macrophages regardless of their phenotype<sup>8</sup> (Macrophages in the microenvironment). Under physiological conditions, macrophages are polarized into proinflammatory and anti-tumor M1 phenotype; however, tumor cells may influence redirection of the macrophage polarization to an alternatively activated M2 phenotype. The M2 phenotype, in turn, secretes cytokines, chemokines, enzymes, growth factors, and several matrix metalloproteinases, thereby intensifying inflammation as well as promoting tumor progression, immunosuppression, angiogenesis, as well as resistance to the treatment also<sup>6</sup>. In vitro studies have shown that, depending on the chemokine stimuli, the polarization from M1 to M2 can be reversed<sup>9</sup>. The clinical relevance of macrophage subpopulations in cancer is not clear, yet. Recent studies show that a high degree of TAM infiltration in tumor tissues has been correlated with poor prognosis in many cancers such as lymphoma, cervical cancer, bladder cancer, and breast cancer. In that manner, many studies have also confirmed that a high degree of TAM infiltration is correlated with tumor metastasis. The results of some studies on animal models show that low TAM infiltration can inhibit tumor growth and metastasis<sup>10</sup>.

Local residents i.e. normal fibroblasts (NFs) are the major source of cancer-associated fibroblasts (CAFs). CAFs are distinguishable from NFs by their tumor-supportive properties. However, the mechanism underlying the transition of NFs to CAFs in OSCC remains still unclear.  $\alpha$ -SMA reflects the expression of CAFs in the tumor mesenchyme and is the most common marker of CAFs. Normal mesenchymal fibroblasts do not express  $\alpha$ -SMA. Activated fibroblasts, which are mostly  $\alpha$ -smooth muscle actin-positive fibroblasts termed myofibroblasts, are one of the major cellular constituents of the tumor stroma. Stromal fibroblasts are not only activated in injured tissue, but are activated by cancer cells also. Myofibroblasts synthesize ECM components, several proteins, growth factors, and cytokines, thus creating a permissive environment that supports tumor growth. Unlike wound healing changes, tumor fibroblasts that are adjacent to the tumor remain activated, as in chronic inflammation. The results of many studies define CAF as very important element of the TME in OSCC due to their significant role in tumor invasion and development, suggesting a poor disease outcome<sup>11</sup>.

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## Objectives

This study aims to determine the presence of TAMs and CAFs in OSCC and also investigates the clinical relevance of tumor infiltration with these two cell types. TAMs and CAFs are immunohistochemically analyzed with two tumor markers: CD68 (tumor marker for both M1 and M2 macrophages) and  $\alpha$ -SMA (tumor marker for CAFs). Stromal/tumoral expression of CD68 and stromal expression of  $\alpha$ -SMA were evaluated, as well as possible relation with the clinical stage of the disease and the degree of tumor cell differentiation.

## Materials and methods

### *Patients and tissue samples*

Tissue samples from 23 patients confirmed for OSCC were obtained and stored at the Institut for Pathology. The patients underwent surgery at the University Clinic for Maxillofacial surgery in Skopje between 2016 and 2018. None of the patients received preoperative chemotherapy or radiotherapy. Patient and tumor characteristics, including clinical stage of the disease and the histologic grade, were determined from the patient's medical record and pathology reports. The stage of the disease was classified according to the criteria of the 7<sup>th</sup> Edition of AJCC/UICC TNM classification system of the OSCC. Tumor differentiation was classified into 4 levels: good, moderate, poorly differentiated, and undifferentiated (anaplastic) according to Broder's histological classification of tumor cell differentiation in OSCC (Broder's grading descriptive system)<sup>12</sup>.

### *Immunohistochemistry (IHC) and IHC evaluation*

Tissue samples underwent IHC and were analyzed at the Institute of Pathology, within the University Clinical Center "Mother Teresa" in Skopje. Immunohistochemistry was performed using Dako EnVision flex system. The following primary mouse monoclonal antibodies were used: anti- $\alpha$ -SMA and CD68. Nikon 80 digital microscope was used.

### *CAFs and TAMs Assessment*

CAFs were evaluated by immunohistochemistry. Positive or negative fibroblasts were identified on the basis of  $\alpha$ -SMA expression. CAFs were defined as large spindle-shaped fibroblasts that express  $\alpha$ -SMA. A modification of the classification system of Kellerman et al. was used to determine the density of CAFs<sup>13</sup>. Density levels were categorized into 4 categories: Negative (0), Rare (1), Focal (2) and Abundant (3). Samples in which no stromal myofibroblasts were detected were classified

as negative. Samples showing sporadic stromal myofibroblasts were classified as rare. Samples showing focal arrangement were classified as focal. Samples showing numerous and densely arranged stromal myofibroblasts were classified as abundant.

CD68 expression was determined by counting the number of CD68-positive macrophages (TAMs) in the invasive areas of the OSCC specimens. Macrophages were defined as stromal cells larger than 10 $\mu$ m in diameter, which express CD68. Each section was displayed on a low power magnification (low-power field with 100x magnification) to identify the areas with the highest macrophage density, then the macrophages were counted in three fields on high power magnification (high-power field on 400x magnification) and the mean number of macrophages per HPF was obtained<sup>14</sup>. Additionally, we assessed the number of TAMs in TS (tumor stroma) and TN (tumor nest).

### *Statistical analysis*

All collected statistical data are processed using the following statistical methods:

- All statistical series are tabulated
- Analysis of relationships between attributive statistical series is done with  $\chi^2$  – Test
- Testing the significance of differences between two arithmetic means in independent samples (between groups) was done with Student's t-test
- Samples are divided into groups with low and high number of CD68+ TAMs in tumor stroma (TS) and tumor nest (TN) based on their number, using cut-off values of 55 for TAMs in tumor stroma and 6 for TAMs in the tumor nest.

## Results

The presence of  $\alpha$ -SMA + CAFs was not detected in all patients with OSCC. Out of 23 patients, in 5 of them CAFs were not detected, and in the remaining 18 CAFs were present. Out of 23 patients, 7 patients are in the first clinical stage (Stage 1), of which 4 have no CAFs, and the other three patients have CAFs but scanty CAFs (grade 1). Of the remaining 16 patients, which are in more advanced clinical stages 2, 3, and 4, just one patient had no CAFs, and that patient was in the second clinical stage (Stage 2); CAFs were found in the other 15 patients. Therefore, there is a statistically significant correlation between clinical stage 1 and the degree of density of  $\alpha$ -SMA-positive CAFs, compared to other clinical stages. There is a statistically significant difference between the density of CAFs in stage 1 and other stages (2, 3 and 4) ( $p = 0.006474$ ).  $\chi^2$  e **7.4134**. (see Table 1).

**Table 1.** Correlation of  $\alpha$ -SMA-positive CAFs degree with the pathological stages of the patients with OSCC.

Pathological stage (pTNM)	$\alpha$ -SMA+ CAFs (degrees)		p-value <0.05
	0	1, 2, 3	
pTNM 1	4	3 (degree 1)	<b>0.006474</b>
pTNM 2, 3, 4	1	15	

Pathological stages of OSCC: Pathological stage 1, 2, 3 and 4 (pTNM 1, 2, 3, 4). Density of CAFs in TS (degrees): 0 - no CAFs, 1.) scanty, 2.) focal, 3). abundant.

**Table 2.** Correlation of  $\alpha$ -SMA-positive CAFs degree with the degrees of malignant cell differentiation in patients with OSCC.

Degree of malignant cell differentiation	$\alpha$ -SMA+ CAFs (degrees)		p-value <0.05
	0	1,2,3	
G1/G2	5	16	0.72158
G3/G4	0	2 (both patients have degree 1 CAFs (scanty))	

Density of CAFs in TS (degrees): 0 - no CAFs, 1 - scanty, 2 - focal, 3 - abundant. Degrees of malignant cell differentiation: G1 - well differentiated, G2 - moderately, G3 - poor, G4 - undifferentiated (anaplastic).

**Table 4.** Correlation between the number of CD68-positive TAMs and the degree of malignant cell differentiation. Degrees of malignant cell differentiation: G1 - well differentiated, G2 - moderately, G3 - poor, G4 - undifferentiated (anaplastic).

Degree of malignant cell differentiation	CD 68+ TAMs (TS)			CD68+ TAMs (TN)		
	Low	High	p-value	Low	High	p-value
G1/G2	12	9	<b>0.438807</b>	1	1	<b>0.9488644</b>
pTNM 2, 3, 4	0	2		11	10	

Degrees of malignant cell differentiation in OSCC: G1 - well differentiated, G2 - moderately, G3 - poor, G4 - undifferentiated (anaplastic).

**Table 3.** Correlation of number of CD68-positive TAMs with the pathological stages in patients with OSCC.

Pathological stage (pTNM)	CD 68+ TAMs (TS)			CD68+ TAMs (TN)		
	Low	High	p-value	Low	High	p-value
pTNM 1	6	1	<b>&lt; 0.033179</b>	6	1	<b>&lt; 0.033179</b>
pTNM 2, 3, 4	6	10		6	10	

As stated above in the text, out of 23 patients, CAFs were not found in 5 patients. All 5 patients had a G1/G2 degree of cell differentiation. CAFs were found in the other 18 patients, 16 of whom had G1/G2, and 2 had G3/G4 degree of differentiation. Both patients with G3/G4 degree of differentiation have little CAFs present, i.e. in oral carcinomas with poor cell differentiation and in anaplastic carcinomas there is a lower number of CAFs, thus worse differentiated tumors have fewer CAFs.  $\chi^2$  is **0.127** and for  $p = 0.72158$  there is no statistically significant difference between the density of CAFs and the degree of differentiation of tumor cells (see Table 2).

The presence of CD68-positive TAMs was found in both the tumor stroma (TS) and the nests (TN) in all 23 patients with OSCC. There is a statistically significant difference between clinical stage 1 and other stages (2, 3 and 4), and the presence of TAMs in the tumor stroma ( **$p < 0.033179$** ). In the same manner, there is a statistically significant difference in the presence of TAMs in tumor nests between clinical stage 1 and the other clinical stages.



**Table 5.** Expression of CD68 + TAMs in tumor stroma (TS) and tumor nest (TN). Student's t-test ( $t=-5.41148$ ).

Groups	Mean	SD	min	max	p-value
CD68+ TAMs (TS)	65.22	41.44762	9	156	< 0.00001
CD68+ TAMs (TN)	14.35	17.73443	0	55	

cal stages (2, 3 and 4) ( $p < 0.033179$ ). Namely, it is observed that out of 7 patients, who were in the first clinical stage, 6 patients have a low number of TAMs, and only one patient had a high number of TAMs. We got the same results in tumor nests.  $\chi^2 = 4.5365$ . (see Table 3).

The degree of differentiation of tumor cells does not correlate with the expression of TAMs neither in the stroma nor in the nests. There is no statistical significance between TAMs infiltration of tumor stroma ( $p=0.438807$ ) and tumor nests ( $p=0.9488644$ ) with the degree of malignant cell differentiation.  $\chi^2=4.5365$ . However, although there is no statistical significance, it is noted that out of 2 (two) patients with poorly differentiated/ anaplastic cancer, both patients have a high number of TAMs that infiltrate the tumor stroma (TS). (see Table 4).

There is a statistically significant difference in the expression of TAMs between stroma and nest for  $p < 0.00001$ . Testing of the significance of the differences between two arithmetic means between the tumor stroma and the tumor nest was performed with the Student's t-test ( $t=-5.41148$ ). (see Table 5).

## Discussion

It is widely accepted that tumor development of OSCC depends on a complex interaction between malignant cells and the tumor microenvironment (TME)<sup>15</sup>. In our group of 23 patients, among other, we investigated the presence of CD68-positive TAMs and  $\alpha$ -SMA-positive CAFs in the tumor stroma, and found that stromal myofibroblasts are not detected in all 23 patients, so the question arises whether CAFs play any role in oral carcinogenesis? A study by Eliene-Magda de-assis et al.<sup>16</sup>, which evaluates the presence of stromal myofibroblasts in oral leukoplakia (OL), normal mucosa and OSCC, found no presence of stromal myofibroblasts in all 30 patients with OL, and in 10 patients with normal oral mucosa as a control group. This indicates that these cells have no relevance during oral carcinogenesis. In the rest

of the 41 patients with OSCC, approximately one-third (26.8%) had no presence of stromal myofibroblasts, while two-thirds (73.2%) had them in the tumor stroma, so this result is identical to our finding where 21.8% of tumor stromas do not have stromal fibroblasts, and the remaining 78.2%, in which stromal fibroblasts have been identified, have different densities. However, this heterogeneous presence of stromal fibroblasts in OSCC suggests that myofibroblasts are associated with the creation of a conducive environment for tumor invasion in OSCC<sup>16</sup>. The presence of myofibroblasts in OSCC stroma in our study, but also their absence in epithelial dysplasias in the studies of numerous other authors, suggests the need for further investigation to elucidate the role of myofibroblasts in the carcinogenesis of OSCC.

The results of Etemad-Moghadam et al.<sup>17</sup> of the 40 OSCC study samples, showed positive immunostaining in myofibroblasts in all oral squamous cell carcinomas, i.e. the presence of myofibroblasts in the stroma of all OSCCs was confirmed, but not in the samples with oral epithelial dysplasia or in normal oral mucosa. In addition, the results of the study showed that there is no significant difference between the different degrees of tumor differentiation and the number of myofibroblasts<sup>17</sup>. Our results are consistent with Ethemad's.

If we compare the clinical stages of the patients in our study and the degree of differentiation of cancer cells with the number of CAFs, we come to the conclusion that the number of CAFs is lowest in the lowest clinical stage, and vice versa, the worse the cell differentiation is (G3/G4), the less CAFs.

Patients in lower clinical stages have a better prognosis and live longer and, according to our research, these patients have a lower number of CAFs; according to that, in order to come to the exact conclusion about the prognosis of our patients, we need to do further investigations. This difference in number and distribution of CAFs is confirmed by the difference between patients in the first clinical stage and patients in higher stages. Therefore, from the obtained results we can conclude that patients in the first clinical stage either do not have or have a very small number of CAFs which is consistent with the findings of Ibrahim O'Bello<sup>18</sup> and Kellerman et al.<sup>19</sup>. Ibrahim O. Bello's research on tongue cancer has shown that the density of CAFs is associated with the degree of tumor malignancy, tumor growth and progression. Kellerman's findings are similar, confirming that the higher degree of infiltration with CAFs is associated with more advanced TNM stage and lymph node metastasis.

Our results contradict the findings of Fujii N. et al.<sup>20</sup>, in his study of 108 respondents with OSCC they found



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that there is no significant correlation between the distribution of CAFs and the clinical stage of the disease. In a study conducted by Yahui Yu et al.<sup>21</sup>, the authors included 152 patients with nasopharyngeal carcinomas and examined the association between  $\alpha$ -SMA expression levels and clinical features of the patients<sup>21</sup>. The summarized results show that there is no significant correlation between the level of  $\alpha$ -SMA expression and the clinical stage of the disease.

The presence of CD68 + TAMs macrophages in tumor stroma was observed in all 23 patients with OSCC in our study. The results showed the presence of a small number of TAMs in the first clinical stage, in both TS and TN, i.e. a strong association of early-stage disease with the presence of small number of TAMs has been established. In addition, the results of some studies on animal models show that low TAM infiltration can inhibit tumor growth and metastasis, thus corresponding with our findings<sup>10</sup>.

Our results do not match the results of many authors such as Fujii N.<sup>20</sup> and Yahui Yu<sup>21</sup>. The results of Fujii N. et al., obtained from 108 patients with OSCC, testify that there is no significant correlation between the level of expression of CD68 with clinicopathological parameters such as the clinical stage and the degree of differentiation of cancer cells in oral squamous cell carcinomas<sup>20</sup>. In a study by Yahui Yu et al., the authors included 152 patients with nasopharyngeal cancers and examined the association between CD68 expression levels and clinical features of patients; the summarized results show that there is no significant correlation between CD68 expression levels and clinical stages of the disease<sup>21</sup>.

Faustino J. Suarez-Sanchez<sup>6</sup> investigated the clinical relevance of OSCC infiltration with TAMs. He and his colleagues evaluated the expression of CD68 as a pan-macrophage marker of both M1 and M2 macrophages, and CD163 as a tumor marker of M2 macrophages in tumor nests and the surrounding tumor stroma, and found that tumor infiltration with CD68-positive TAMs was not associated with none of the examined clinicopathological parameters, i.e. it is neither associated with the clinical stage of the disease nor with the degree of malignant cell differentiation. However, a large number of CD68-positive cells have been reported to infiltrate the stroma in larger tumors (T3 – T4), in more advanced stages (Stage 3 and 4), and in moderately/poorly differentiated tumors, although the differences are not statistically significant<sup>6</sup>. In our study, although there is no statistical significance, it is observed that patients with poorly differentiated/anaplastic cancer have a high number of stromal CD68-positive TAMs (out of two patients with G3/G4, both of them have a high number of CD68-positive TAMs in the tumor stroma (TS)).

Studying tumor infiltration with TAMs by assessing CD68-positive TAMs with immunohistochemistry, Lin et al.<sup>22</sup> in 84 patients with laryngeal carcinoma found that the infiltration of carcinomas with these cell types significantly correlated with poor prognosis. In contrast, Troiano et al.<sup>23</sup>, in the HNSCC meta-analysis, reports that there is no association between CD68-positive macrophage expression in tumor nests or stroma and survival. Therefore, these findings raise the question about the use of the panmacrophage marker CD68 in IHC analysis as it has no prognostic utility in patients with HNSCC.

The association of infiltration with TAMs and patient prognosis differs among tumors. As we find in literature, TAM infiltration predicts poor prognosis in breast cancer, uterine cervix cancer, and bladder cancer, but it predicts good prognosis in other tumors i.e. prostate cancer, lung cancer, and brain tumors, and these differences may be related to various factors which refer to the tumors, but perhaps also to the manner in which the research was conducted<sup>22</sup>.

Some results in literature suggest that TAM infiltration can be used to evaluate patients' condition and predict the prognosis of the carcinomas. Therefore, if we hypothesize that TAM infiltration may be an early, sensitive prognostic indicator, then expanded radical surgery or appropriate expansion of surgical range should be considered even for patients with early stage and well-differentiated OSCC once high TAM infiltration is identified. However, there is a general conclusion among investigators that further investigations are necessary to determine how to apply this potentially predictive indicator into everyday clinical work.

In addition, we expand the investigation for TAMs in our research as we found statements in literature for possible influence of TAMs infiltration of the tumor nests on the prognosis of the OSCC patients. According to our results, there is a statistically significant difference in the number of CD68-positive TAMs in tumor stroma and tumor nest in favor of tumor stroma. Searching for data in literature, we found that in endometrial cancer, infiltration with TAMs in TN shows a positive correlation with reduced recurrence, while in invasive breast cancer it suggests an unfavorable prognosis. If these results are taken into account, there are strong recommendations to examine TAMs localization, both in TN and TS<sup>24</sup>.

## Conclusion

More recent researches focused on the TME in OSCC aims to overcome the poor success of conventional treatment of this malignancy. Predicting the aggressiveness of the tumor and the prognosis of

patients with OSCC is still a weakness of the standard pathological report, and the introduction of new pathological parameters is a big step towards personalized treatment of patients with OSCC.

Our results for CAFs and TAMs are within the numerous limitations of this research, among others, and the small group of patients with OSCC. Therefore, to obtain more precise and relevant answers to the question of the clinical relevance of these two types of cells, we consider that it will be necessary to conduct further, far more extensive and better-designed studies in the future.

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# EPULIS FISSURATUM - CLASS III: CASE REPORT

## EPULIS FISSURATUM - КЛАСА III: ПРИКАЗ НА СЛУЧАЈ

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### Abstract

Epulis fissuratum, or denture fibrosis, is a benign hyperplasia of the fibrous connective tissue of the oral mucosa. It is a reactive lesion to chronic mechanical irritation produced by the flange of a loose and poorly fitting dentures. The therapy is surgical excision. Removal can be performed conventionally – with scalpel, electrocautery, or contemporary with laser. In this article, authors present an interesting case of epulis fissuratum class III rarely seen in every day oral surgery practice. A 61 year-old female was referred to the University Clinic of Oral Surgery for assessment of a soft tissue change in the maxillary front. The clinical examination revealed slight swelling in the central portion of the upper lip, from the vermillion, along the philtrum to the base of the nose, causing a noticeable cosmetic deformity. Intraorally, many overlapping hyperplastic gingival folds and gaps obliterated the fornix of the anterior maxilla. The prosthesis was old and unusable. Based on subjective and objective findings, diagnosis of epulis fissuratum class III was established. Owing to the size of the folds, their deep insertion, and primarily the need for cheiloplasty as cosmetic and functional surgery, the patient was referred to the University Clinic for Maxillofacial Surgery. Surgery was performed under local infiltrative anaesthesia, using an electrocautery for precise excisions. After full recovery, new prosthesis was made. There is no postoperative recurrence 2 years after surgery. **Key words:** epulis fissuratum, class III, surgical treatment, denture.

### Апстракт

Epulis fissuratum, или протетска фиброза, претставува бенигна хиперплазија на фиброзното сврзно ткиво на оралната мукоза. Станува збор за реактивна промена која настанува како резултат на хронична механичка иритација предизвикана од крилото на лабава и несоодветна протеза. Терапијата е хируршка ексцизија. Отстранувањето може да се изврши конвенционално со скалпел, електрокаутер, или современа метода со ласер. Во овој труд, авторите презентираат интересен случај на epulis fissuratum, III класа кој ретко се гледа во секојдневната орално-хируршка пракса. Пациентка на возраст од 61 година се обрати на Универзитетската Клиниката за орална хирургија поради присутна мекоткивна промена во предел на максиларниот фронт. На клиничкиот преглед беше детектиран слабо назначен оток на средишниот предел на горната усна, од вермилионот по должина на филтрумот се до базата на носот, предизвикувајќи видлив естетски деформитет. Интраорално, поголем број на хиперпластични гингивални набори, а помеѓу нив усеци, го имаа обтурирано форниксот во фронталниот предел на максилата. Протезата беше во неупотреблива состојба. Врз основа на субјективниот и објективниот наод беше поставена дијагноза Epulis fissuratum класа III. Поради големината на пликите, нивната длабока распространетост и примарно поради потребата од хелиопластика од естетски и функционален аспект, на пациентката и беше посочено да се обрати на Универзитетската Клиника за максилофацијална хирургија. Хируршкиот третман беше извршен под локална анестезија со примена на електрокаутер поради потребата од прецизна ексцизија. По потполно заздравување се изработи нова протеза. Две години по операцијата не се јави рецидив. **Клучни зборови:** Epulis fissuratum, III класа, хируршки третман, протеза.

### Introduction

Epulis fissuratum, or denture fibrosis, is a reactive fibrous connective tissue inflammatory hyperplasia of the alveolar ridge oral mucosa and vestibular area. It is most frequently caused by mild and repeated chronic trauma of an ill-fitting prosthesis<sup>1</sup>. It can occur in any area of the upper or lower jaw<sup>2</sup>, but the most affected is the anterior maxilla. Fibrous hyperplasia is a consequence of a long-standing loose and illfitting denture. It

can be also seen in patients with edentate frontal maxilla and dentate mandibular front.

The results of a 5-year clinical study showed that patients treated with complete maxillary dentures and mandibular overdentures demonstrated less vertical alveolar bone reduction compared with the patients who had bimaxillary complete dentures<sup>3</sup>. Prosthetic base needs to undergo occasional revision and readaptation in order to optimally adhere to the changing alveolar ridge. Otherwise, the pressure and the chronic trauma caused by

ill-fitted prosthesis is a direct cause of vestibular mucosal hyperplastic overgrowths. In patients with total and partial dentures, the oral mucosa may exhibit acute or chronic reaction to microbial denture plaque. Also, an allergic reaction to denture base constituents may be elicited.

According to *Budtz-Jørgensen E.*<sup>4</sup> denture stomatitis is the most common condition of the palatal mucosa in about 50% of complete or partial removable dentures wearers. Angular cheilitis is present in 15% of the cases. Denture induced traumatic ulcers are seen in about 5% of denture wearers. The prevalence of denture induced hyperplasia caused by chronic injury of denture border is seen in about 12% of the denture wearers. Flabby ridge, as a replacement of the alveolar ridge by fibrous connective tissue, is present in 10 to 20%. The most important of all is the evidence of the development of an oral squamous cell carcinoma after chronic injuries of the oral mucosa in such patients<sup>4</sup>. Epidemiologically, since this phenomenon occurs as a consequence of the use of prosthesis, it is predominantly present in middle or more advanced adulthood. It is reported in 5-10% of dentures wearers and it occurs more frequently in the anterior part of the jaw compared with the posterior<sup>5</sup>, with 66-75% higher presence in females. Denture hyperplasia occurs predominantly in females and it is suggested that its formation may be affected by hormonal imbalance in the menopause<sup>6,7</sup>.

Surgery is a treatment of choice. The surgical excision can be done conventionally, by electrocautery, and with a laser surgery as an up to date treatment modality. In order to maintain the results of the surgery, it is very important to make a new, well-stabilized prosthesis.

The fibrous hyperplasia is classified into three classes according to the localization and extension of fibrous hyperplasia<sup>8</sup> (Table 1).

**Table 1.** Classification according to the localization and extension of epulis fissuratum

Epulis fissuratum Classes	Localisation
Class I	Hyperplasia completely attached to mucoperiosteum
Class II	Hyperplasia attached to the mucous membrane of the lips or cheek (soft wall of the vestibular fornix)
Class III	Hyperplasia involving the mucoperiosteum and mucosa, obliterating the entire fornix

In this article we present an interesting case of Epulis fissuratum - Class III, rarely seen in oral surgery practice.

## Case report

Hyperplastic mucogingival folds excessive proliferative changes of the upper vestibular mucosa associated with an old and loose, ill-fitting upper prosthesis. A 61 year old female was referred to the University department of oral surgery due to excessive proliferative changes of the upper vestibular mucosa associated with an old and loose, ill-fitting upper prosthesis. The clinical examination revealed slight swelling in the central portion of the upper lip, from the vermilion, along the philtrum to the base of the nose, causing a noticeable cosmetic deformity. There was a distinctive tightening of the free edge of the lip towards the right side making a fold that visibly deformed the width of the vermilion. Intraorally, many overlapping hyperplastic gingival folds and gaps obliterated the fornix and protruded beyond the upper lip on the right side. (Figure 1).

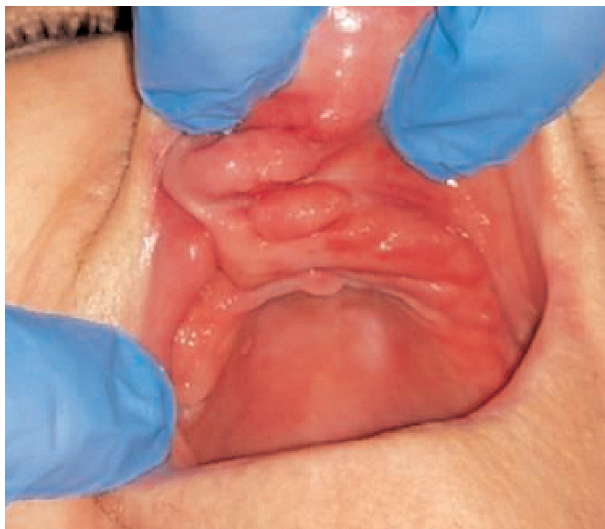


**Figure 1.** Hypertrophic folds that protrude beyond the upper lip on the right side.

The uniqueness of this case was the presence of extremely marked hyperplasia, many mucogingival folds and serpentine gaps widely spread, with intraoral and extraoral-cosmetic deformity (Figure 2, Table 1- III Class).

The overlying mucosa had normal colour and smooth surface, showing mild hyperaemia in some parts as a result of moderate inflammation. On palpation, the folds were painless and with firm consistency. The prosthesis was old and unusable. She reported that despite the occasional discomfort, pain and swelling that forced her not to use the denture for a limited time period, she restrained from visiting her dentist for 5 years. Based on subjective and objective findings. the diagnosis of epulis fissuratum class III





**Figure 2.** Hyperplastic mucogingival folds completely obliterating the upper right vestibule.



**Figure 4.** Intraoperative application of an iodoform packing in the alveolar ridge and the fornix to assist with the healing and to maintain the depth of the vestibular fornix.



**Figure 3.** Surgical treatment of epulis fissuratum with electrocautery.



**Figure 5.** The sutures were removed one week after surgery. Minimal labial swelling was a main postoperative symptom.

was established. Owing to the size of the folds, their deep insertion, and primarily the need for cheiloplasty as cosmetic and functional surgery, the patient was referred to the University Clinic for Maxillofacial surgery. According to the American Society of Anaesthesiologists classification, our patient was classified as ASA I (without systemic diseases) as a patient with uneventful medical history. The surgery was planned and performed under local infiltrative anaesthesia, using an electrocautery for precise excisions, layered ablation of the hyperplastic mucosal growths, and unimpaired visibility due to controlled haemostasis. (Figure 3).

After the surgery, the depth of the vestibular fornix was maintained with iodoform packing (Figure 4).

Postoperative period was without complications. The labial swelling was minimal and the pain was mild, as expected. Recovery was accelerated by topical application of NBF gel. Fibrin patches appeared on the first postoperative day. The secondary healing was regular, and the epithelization was an ongoing process. The sutures and iodoform packing were removed a week later. (Figure 5).

After two weeks, partial epithelialization was observed (Figure 6).



**Figure 6.** Postoperative clinical examination, (two weeks after surgery) with partial epithelization.



**Figure 7.** Complete recovery 3 months after surgery.

The treatment was finalized by referring the patient to the Clinic for dental mobile prosthetics for making a new prosthesis that is well-fitted to the new anatomic circumstances (Figure 7).

Complete epithelization of the remodelled vestibular surface was seen three months after surgery.

## Discussion

Epulis fissuratum develops slowly over a prolonged period in patients with ill-fitting dentures. It is associated with a denture flange of either a full or partial denture<sup>9</sup>. Typically, patients with epulis fissuratum are asymptomatic<sup>10</sup>. It is considered as an overgrowth of intraoral mucosal tissue resulting from chronic and prolonged irritations<sup>11</sup>. Chronic trauma to the oral mucosa is

a risk factor for the development of oral squamous cell carcinoma. Many studies have shown that the sharp edges of teeth or the ragged edges of ill-fitting dentures have potential to cause oral carcinoma<sup>12,13</sup> if persistent over a long period. The therapy of denture hyperplasia comprises of two options, conservative and surgical. The conservative approach is non-invasive and should be the treatment of choice suitable for early stages of mucosal hyperplasia, without fibrosis. It includes removal of the acrylic flange first, followed by relining and rebasing after the complete healing of the lesion<sup>11,12</sup>. The conservative approach was not considered in the presented case since the fibrous inflammatory hyperplasia was excessive and longstanding. The second treatment option is surgical removal of the excessive and hyperplastic tissue. It can be used as an adjunct to the conservative one, when the result is unsatisfactory, or as a stand-alone treatment. This was the treatment of choice that was considered for the case presented here. In the period before surgery, it is important to undertake necessary measures to reduce the irritation and mucosal inflammation. Therefore, our patient was strongly advised against denture use for 2-3 weeks prior to surgery. Additionally, during these period, daily mouthwash with oral antiseptics were part of our patient's daily routine. With these measures alone, there is a possibility for partial regression, and, very rarely, a complete regression of the hyperplastic proliferation. Surgical treatment can be conventional – with scalpel, electrosurgery and laser surgery. Which technique will be applied depends on the clinical situation, advances and disadvantages of each treatment, and on the surgeon's preferences. Scalpel surgery is treatment of choice for removal of epulis fissuratum class I and II, when the lesions are limited to the mucosa. Conventional surgery was not considered in this case because of the amount of the tissue to be removed, accompanied with abundant intraoperative bleeding. In such cases, electrosurgery is a better treatment option. Electrocautery is better than the stainless steel scalpel in relation to time taken for incision, intraoperative blood loss and early postoperative pain, but is inferior to scalpel in relation to wound healing<sup>14</sup>. Lasers are another contemporary surgical option to be considered. The following lasers are recommended for soft tissue surgery: CO<sub>2</sub> laser, Er:YAG laser, Nd:YAG laser, diode laser, argon laser and KTP laser. Many studies have shown that carbon dioxide lasers have more promising results than conventional surgery. The advantages of carbon dioxide laser are tissue protection, asepticity, rapid wound healing, minimal postoperative pain and tissue swelling, insignificant scarring, less than 6% recurrence rate, repeatability of the treatment, and minimal func-



tional impairment of the oral cavity<sup>15,16</sup>. Er:YAG laser is another top laser for soft tissue surgery. In order to effectively cut or ablate human mucosa, the Er:YAG laser targets the chromophore of water selectively instead of the extracellular matrix of collagen. A high absorption peak in the water minimizes the thermal damage to the tissue, which results in improved healing. The weak haemostatic effect of the Er:YAG laser as a disadvantage is neglected due to the large number of advantages it provides, such as: uneventful recovery, accelerated healing and predictable results<sup>17</sup>. During the Er:YAG and tissue interaction, the bacteria in the path of the beam are destroyed as the water within the bacterial cells undergoes the same instantaneous phase change, and surgical field is sterilized<sup>18</sup>. The liquid nitrogen cryosurgery has also been used successfully in a number of oral surgical procedures. It is widely implemented in the management of aggressive primary jaw lesions and minor soft tissue surgeries. It also provides excellent haemostasis, without direct contact with tissue while maintaining an aseptic environment, good healing with minimal postoperative oedema and pain<sup>19</sup>.

Electrosurgery was considered as first treatment option in the presented case. It enabled precise excisions, layered ablation of the hyperplastic mucosal growths, and unimpaired visibility due to controlled haemostasis. Besides, it was important to exclude other lesions in the differential diagnosis. Therefore, the excised tissue was sent for histopathology analysis, and the findings were in accordance with the preoperative diagnosis of epulis fissuratum, thus excluding a malignant proliferation. Postoperative period was uneventful and without complications. What is most important, the vestibule depth was maintained. The secondary healing is a slow healing process. Considering the extent of the wound, partial epithelialization was observed as expected after two weeks, and the epithelization was an ongoing process. The epulis fissuratum III class is a clinical manifestation rarely seen in oral surgery. The chronically enlarged oral mucosa necessitated surgery for functional, aesthetic and diagnostic reasons.

## Conclusion

Epulis fissuratum is a common soft tissue enlargement, but the class III presentation is very rare. The recommended therapy is surgical, providing good aesthetics and function. To maintain the postoperative results, a new, well adapted prosthesis is mandatory, as well as frequent

visits to the dentist to manage any trauma and to prevent occurrence of inflammatory hyperplasia.

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