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University of Medicine - Sofia

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38 · 2012/2

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Conservative dentistry

Stimulated salivary indices in asthmatics treated with combined inhaled drugs

E. Karova

Summary

Different external factors, including drugs, can have positive or harmful effect on the oral cavity. Saliva carries out its protective functions thanks to its cleaning, remineralizing, buffering and antibacterial abilities.

Seventy patients – 30 asthmatics, treated with Seretide, Symbicort and Foster and 40 controls - were clinically evaluated. The flow rate, pH, buffering capacity and Str. Mutans quantity of stimulated whole saliva were determined on two visits in a 6-month period.

No considerable differences between asthmatics and controls were found out in all examined salivary indices. Their average levels increase inconsiderably on the second visit of asthmatic patients. Different drugs change the salivary indices insignificantly and almost in one and the same way in the course of the observation.

Key words: stimulated salivary flow rate, pH, buffer capacity, Str. Mutans quantity, asthma, inhaled corticosteroids

Introduction

Saliva is the main constituent of liquid oral environment and is a part of organism’s ability to maintain oral homeostasis. It is accepted as a critical factor that can induce and control progression or regression of dental caries. This is due to salivary cleaning, remineralizing, buffering and antimicrobial abilities [5,6,10,12,15,20,21,24].

Salivary function is predominantly protective and is associated with its constituents and physiology. Due to salivary buffering systems pH is held up between 6.1 and 7.0. Their activity differs among patients and depends on their caries activity. If the activity is low, unstimulated saliva is with pH 7.0, while in caries-active patients it decreases to levels of 5.5 [19,23]. Salivary flow rate and buffering activity are in inverse proportion to dental caries frequency. Low buffer capacity and low salivary flow rate are prerequisites for lower resistance to

1 Medical University – Sofia, Faculty of Dental Medicine, Department of Conservative Dentistry
microbial attacks. Buffer activity depends on hormonal and metabolitic changes and food insufficiency [3,11,23].

Many external factors, including drugs, can exhibit positive or negative local effect on oral cavity.

Bronchial asthma is an inflammatory disease of the respiratory tract with dynamic and variable nature. Anti-asthmatic drugs, taken daily for a long period of time, can control the disease. They improve respiratory function and patients’ quality of life though they don’t have immediate influence on it. Inhaled corticosteroids, alone or in combination with inhaled long-acting sympathicomimetics, are the most effective drugs in treatment of persisting asthma [2,4,8,25].

General practitioneres are aware of the systematic and local side effects of these drugs. Unfortunately, there are no enough data for their harmful effects on oral tissues or if we find such they are contradictory [7,9,13,16-18].

Ryberg at al. find out that prolonged use of β-blockers from asthmatics decreases significantly the amount of stimulated saliva, increases Lactobacillus titer and dental caries incidence. There are no substantial changes in Str. Mutans titer. Another investigators [9] come to the conclusion that inhaled drugs lead to a significant decrease in pH levels of saliva and dental plaque.

However, Hyypa and Paunio [7] don’t find out any significant changes in salivary quantity and composition, pH, buffer capacity and DMFS index in asthmatic children. Salem K. et al. [18] also come to the conclusion that inhaled corticosteroids can’t injure asthmatic children’ teeth. Just the opposite, their dental status is better when compared to controls because of their more frequent visits at the dentists’.

Analysis of scientific evidence reveals that most of the investigations are carried out with children and few of them are with adult asthmatics [7,9,13,14,16-18]. Additional and more profound knowledge is necessary in this field still more there is a world tendency in increasing the average life expectancy and “aging” of the population.

**Aim**

The objective of the investigation is to follow up the amount, pH, buffer capacity and Str. Mutans quantity in stimulated saliva in adult asthmatics, systematically treated with different combinations of inhaled corticosteroids and long-acting sympathicomimetics.

**Material and methods**
Seventy patients of both sexes, from 20 to 55 years old, participate in the study - 30 suffer from mild persistent asthma and are systematically treated with combinations of inhaled corticosteroids and long-acting sympathicomimetics and 40 are controls. All patients with accompanying diseases and treatment, affecting salivary quantity and acidity are excluded from the investigation. These with bad oral hygiene and parodontal diseases are excluded as well. All patients have made two visits in a six months period.

All asthmatics are examined by an allergologist, who determines the kind and dose of the drug. Asthmatic patients are not treated till now with the examined drugs and are divided in three groups, according to their medication (Seretide – Fluticasone propionate + Salmeterol, Symbicort - Budesonide +Formoterol and Foster- Beclometasone + Formoterol). The active component in all devices is inhaled in a dry powder form. The investigated medications have different quantity of gustatory correctors - 12,5 mg Lactose monohydrate in Fluticasone propionate + Salmeterol; 0.730 mg Lactose monohydrate in Budesonide + Formoterol and no correctors in Beclometasone + Formoterol.

Dental investigation and other procedures are carried out in a dental surgery in Faculty of Dental Medicine-Sofia by one and the same investigator. All patients are placed comfortably in a sitting position on a dental chair. All measurements are made in the morning, between 8 and 11 o’clock and patients are not allowed to eat, drink, smoke, clean their teeth and take medications 1 hour before the investigation.

Data analysis is made on the ground of comparison between: asthmatics and controls; first and second visit of asthmatics; the three inhaled drugs at the two visits.

Quantity and pH of stimulated saliva are measured following the instructions of GC Saliva-Check BUFFER, GC EUROPE N.V. The patient is instructed to chew a piece of wax and to spit in a spittoon after 30 seconds. After that he continues chewing for 5 minutes and spits in equal intervals of time in a graduated container. Quantity is measured in mL and is rated as: very low – less than 3.5 mL; low – 3.5-5.0 mL; normal – more than 5.0 mL.

pH levels are measured by pH test strips, placed into the saliva sample for 10 seconds. Strip colour is compared with the testing chart available in the package of GC Saliva-Check BUFFER, GC EUROPE N.V. Normally, pH of stimulated saliva exceeds that of unstimulated one with 1 unit.

Buffer capacity is tested with a special strip, part of the set GC Saliva-Check Buffer, GC EUROPE N.V.. Results are received in 2 minutes and they depend on the change of color of test pads. Buffer abilities of saliva are interpreted as: very low – values from 0 to 5; low - values from 6 to 9; normal/high – values between 10 and 12.
The investigation for availability and quantity of Str. Mutans is made with GC Saliva-Check Mutans, GC EUROPE N.V.. The result is positive and caries risk is increased if S. Mutans level is more than 500 000 CFU/ml. The result is negative if quantity of S. Mutans is less than 500 000 CFU/ml.

Statistical analysis is made with SPSS 15.0.

Results

1. Quantity of stimulated saliva

Mean quantity of stimulated saliva in asthmatic group is 8.36 mL and that in the control group is higher – 8.71 mL. All values are normal and there is no statistically significant difference between the two groups (t-test, \( p=0.599 \)). Table.1

Table 1. Comparative presentation of quantity of stimulated saliva in mL. (n=70)

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Mean values mL/5min</th>
<th>SD</th>
<th>SE Mean</th>
<th>Min</th>
<th>Max</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthmatics</td>
<td>30</td>
<td>8.36</td>
<td>3.07</td>
<td>0.56</td>
<td>4.00</td>
<td>16.50</td>
<td>0.599</td>
</tr>
<tr>
<td>Controls</td>
<td>40</td>
<td>8.71</td>
<td>3.25</td>
<td>0.52</td>
<td>3.50</td>
<td>18.00</td>
<td></td>
</tr>
</tbody>
</table>

A comparison of quantity of stimulated saliva from the two visits of asthmatic patients reveals a tendency of increase from 8.71 mL to 9.11 mL. The difference is statistically insignificant (paired simple t-test, \( p=0.201 \)). Table.2

Table 2. Changes of quantity of stimulated saliva in a six months period (n=30)

<table>
<thead>
<tr>
<th></th>
<th>Mean values mL/5min</th>
<th>SD</th>
<th>SE Mean</th>
<th>Min</th>
<th>Max</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>First visit</td>
<td>8.71</td>
<td>3.07</td>
<td>0.56</td>
<td>4.00</td>
<td>16.50</td>
<td>0.201</td>
</tr>
<tr>
<td>Second visit</td>
<td>9.11</td>
<td>2.50</td>
<td>0.46</td>
<td>4.00</td>
<td>15.00</td>
<td></td>
</tr>
</tbody>
</table>

A comparison is made between asthmatics treated with different drugs at each visit. Mean quantity values for patients treated with Foster (7.47 mL) are lower than these measured in asthmatics inhaling Seretide (9.30 mL) and Symbicort (9.37 mL). The same tendency is found out at the second visit: Foster – 8.67 mL, Seretide – 9.80 mL and Symbicort – 8.88 mL. Differences are statistically insignificant (ANOVA test, \( p_1=0.303, p_2=0.579 \)).
2. pH measurements

Mean pH value of stimulated saliva in asthmatics is 6.91 and is higher than that in the control group – 6.78. The difference is statistically insignificant (t-test, p=0.140). Table 3

Table 3. pH of stimulated saliva in asthmatics and controls (n=70)

|                  | Number | Mean values | SD  | SE Mean | Min | Max | |  
|------------------|--------|-------------|-----|---------|-----|-----| |  
| Asthmatics       | 30     | 6.91        | 0.42| 0.08    | 6.00| 8.00|0.140  
| Controls         | 40     | 6.78        | 0.37| 0.06    | 6.00| 7.00| |  

Mean salivary pH values from both visits of asthmatics are normal and are respectively 6.92 and 6.97, without any significant difference (paired simple t-test, p=0.375).

Analysis of mean pH values of saliva in the three groups at each visit doesn’t show any statistical difference (ANOVA test, p1=0.553, p2=0.436). Table 4.

Table 4. Analysis of pH of stimulated saliva according to the inhaled drug (n=30)

<table>
<thead>
<tr>
<th>Time of measurement</th>
<th>Inhaled drug</th>
<th>Mean</th>
<th>SD</th>
<th>SE Mean</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>First visit</td>
<td>Foster</td>
<td>6.80</td>
<td>0.35</td>
<td>0.11</td>
<td>0.553</td>
</tr>
<tr>
<td></td>
<td>Seretide</td>
<td>7.00</td>
<td>0.47</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Symbicort</td>
<td>6.95</td>
<td>0.44</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Second visit</td>
<td>Foster</td>
<td>6.90</td>
<td>0.32</td>
<td>0.10</td>
<td>0.436</td>
</tr>
<tr>
<td></td>
<td>Seretide</td>
<td>6.90</td>
<td>0.39</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Symbicort</td>
<td>7.10</td>
<td>0.46</td>
<td>0.15</td>
<td></td>
</tr>
</tbody>
</table>

3. Examination of buffer capacity

The mean values show that stimulated saliva in controls is with normal (high) buffer capacity. The mean value in asthmatics (9.40) is at the border of normal and low buffer abilities. There is no statistical difference between the two groups (t-test, p=0.226).

Buffer capacity of stimulated saliva is at the border of normal and low abilities at both visits of asthmatics, respectively 9.40 and 9.53 and is without any significant difference (paired simple t-test, p=0.573)

Measurements of buffer capacity at the first visit of patients treated with the three different inhaled drugs show almost one and the same values. At the second visit the levels for the groups treated with Foster and Seretide are a little bit higher (9.70) but without significant difference (ANOVA test, p1=0.941, p2=0.780). Table 5
Table 5. Analysis of buffer capacity of stimulated saliva in accordance to the inhaled drug 
(n=30)

<table>
<thead>
<tr>
<th>Time of measurement</th>
<th>Inhaled drug</th>
<th>Mean values</th>
<th>SD</th>
<th>SE  Mean</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Firest visit</td>
<td>Foster</td>
<td>9,30</td>
<td>2.41</td>
<td>0.76</td>
<td>0.941</td>
</tr>
<tr>
<td></td>
<td>Seretide</td>
<td>9.60</td>
<td>1.84</td>
<td>0.58</td>
<td>0.941</td>
</tr>
<tr>
<td></td>
<td>Symbicort</td>
<td>9.30</td>
<td>2.36</td>
<td>0.75</td>
<td>0.941</td>
</tr>
<tr>
<td>Second visit</td>
<td>Foster</td>
<td>9,70</td>
<td>2.11</td>
<td>0.67</td>
<td>0.780</td>
</tr>
<tr>
<td></td>
<td>Seretide</td>
<td>9.70</td>
<td>1.41</td>
<td>0.44</td>
<td>0.780</td>
</tr>
<tr>
<td></td>
<td>Symbicort</td>
<td>9.20</td>
<td>1.87</td>
<td>0.59</td>
<td>0.780</td>
</tr>
</tbody>
</table>

4. Examination of Str. Mutans availability

Mean value of quantity of Str. Mutans is higher than 500 000 CFU/ml in 20% of asthmatics and 17.5% of controls and they have higher risk of developing dental caries. The difference is insignificant (Fisher exact test, p=0.514). Table. 6

Table 6. Risk of developing dental caries according to the mean values of Str. Mutans in asthmatics and controls (n=70)

<table>
<thead>
<tr>
<th>Group</th>
<th>Risk of developing dental caries (RDDC)</th>
<th>Total in groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low risk</td>
<td>High risk</td>
</tr>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>Asthmatics</td>
<td>24</td>
<td>80.0</td>
</tr>
<tr>
<td>Controls</td>
<td>33</td>
<td>82.5</td>
</tr>
<tr>
<td>Total with RDDC</td>
<td>57</td>
<td>81.4</td>
</tr>
</tbody>
</table>

The percentage of asthmatics with high risk of developing dental caries increases at the second visit from 20% to 26.7%. The difference is statistically insignificant (McNemar, p=0.317).

Comparison of quantity of Str. Mutans in each asthmatic group doesn’t find out any significant difference at both visits (Kruskall Wallis Test, p=0.547, p=0.848).

Discussion

Quantity of stimulated saliva in asthmatic and control groups is normal, with minimal differences between them.

The stated data for increased quantity of stimulated saliva at the second visit of asthmatics are similar to that from another investigation on unstimulated saliva carried out by the same
investigators [1]. However, the differences between the groups are significant. Results from our study make us presume that we should not disregard organism’s potentialities to adapt to the new environment and to neutralize the harmful effects. Conclusions from our study differ from those received by the team of Ryberg [16,17] who find out significant decrease of quantity of stimulated saliva in asthmatics treated with inhaled corticosteroids.

Effect of Foster, Seretide and Symbicort on quantity of stimulated saliva is almost one and the same. However, the lowest results are reported on both visits of asthmatics treated with Foster. This can be due to the lack of gustatory correctors which can additionally increase salivary flow rate.

The measurements of pH of stimulated saliva are in agreement with that from the study of Hyypa and Paunio [7] and are opposite to the results received from Kargul et al. [9]. The mean pH values suggest normal acidity of oral environment. No significant differences are found out in all comparisons between asthmatics and controls and between both visits of asthmatics and inhaled drugs.

pH values of stimulated saliva should be interpreted in conjunction with the results of salivary buffer capacity. Buffer abilities in the control group are normal, high, while they are at the border of normal and low abilities in asthmatics. This tendency is preserved at their second visit. Similarly to the results of Hyypa and Paunio all comparisons don’t show significant differences. We presume that inhaled drugs do not modify significantly buffer abilities of stimulated saliva because they can’t decrease substantially its quantity. Bicarbonate concentration in saliva depends on salivary flow rate and when it is increased, concentration of bicarbonate ions increases as well and pH isn’t low [20,23]. Probably, preserved buffer abilities of stimulated saliva can explain the lack of considerable pH changes.

Quantity of Str. Mutans in stimulated saliva of asthmatics is more often above 500 000 CFU/ml but there is no significant difference when compared with controls. Our results do not coincide with these of Mazzoleni et al. [13] and Salem et al. [18] who find out considerable increase of the quantity of these cariogenic microorganisms.

Str. Mutans quantity increases insignificantly at the second visit of asthmatics. Having in mind only this indicator we can state that there is no high risk for dental health of asthmatics. At the same time, we know that dental caries is a multifactorial disease and a lot of factors don’t act alone but simultaneously.

Conclusions
There are no significant differences between asthmatics and controls in relation to quantity, pH, buffer capacity and Str. Mutans of stimulated saliva.

The mean values of all examined indices of stimulated saliva increase insignificantly at the second visit of asthmatics.

Different combinations of inhaled drugs change insignificantly and almost in one and the same way all indices of stimulated saliva during the treatment course.

Because of their continuous systematic treatment, all asthmatics need special dental cares, despite the lack of considerable changes in stimulated saliva indices.

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Pediatric Dentistry

Interleukin-1β levels in gingival crevicular fluid during orthodontic movement of the teeth

L. Ribagin¹, M. Rashkova¹

Summary

Orthodontic treatment with fixed appliances alters oral environment and influences on the oral hygiene and gingival status. IL-1β is an important marker of the initial tissue reaction in the process of remodeling during orthodontic treatment.

Clinical and laboratory periodontal parameters OHI, PBI, GCF volume, IL-1β levels in GCF (analyzed by Enzyme-linked imunosorbent assay – ELISA) were followed for 18 months in a prospective study on 31 children /11-18 years / with orthodontic treatment with fixed appliances. 15 of the following patients had treatment with an active force brackets and 16 with passive operating system.

The results show that: (1) In the patients studied, oral hygiene status during orthodontic treatment is maintained at satisfactory levels, (2) gingival inflammation is negligible, despite the increased plaque quantity, (3) IL-1β in the GCF ranged from 51.989 to 100,766 pg / ml and can be considered as a marker of tissue changes during treatment, (4) Different systems brackets act on differently on the periodontal structures, as demonstrated by the different levels of IL-1b in treatment.

Key words

Interleukin-1β, bone remodeling, orthodontic treatment.

Introduction

The first studies of bone remodeling in humans date back to 1892 when the German surgeon Julius Wolff explains the transformation of bone architecture in response to the long-term mechanical impact on it [3].

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Since that time the "law" of Wolff has been updated, modified and serves as a basis for research, continuing to explore the complex field of bone biology that underlies the orthodontic movement of teeth [2]. Under normal conditions, the pattern of alveolar bone remodeling is maintaining homeostatic state. Conducting orthodontic treatment dynamically changes the balance between bone resorption and apposition [22]. When orthodontic forces are applied on the teeth, they are transmitted to the periodontal ligaments (PDL) and alveolar bone adjacent. These forces, in turn, are the beginning of a complex cascade of events that lead to remodeling and eventual tooth movement [14]. The sequence of tissue and cellular level processes in bone remodeling are well described, but there is no comprehensive understanding in the coordination of biochemical reactions at the molecular level [10]. Therefore, current research is geared towards a better clarification of the molecular biology of bone remodeling during orthodontic tooth movement [2, 4, 6, 7, 9, 14, 18, 20, 23].

There are multiple interconnected systems that control levels of mature osteoclasts and osteoblasts, which are active during bone remodeling. Most of them are implemented with the group of cytokines [4].

In recent years, gingival crevicular fluid (GCF) was used as a means of measuring different molecules and microorganisms in the gingival sulcus. GCF is mediated osmotic transudat in the gingival sulcus, which tends to increase the volume and change in composition during inflammation and increased capillary permeability [23]. In the complex composition of gingival fluid can be detected markers involved in bone remodeling during orthodontic treatment [11, 15, 17, 18]. Analysis of samples of GCF can provide a better understanding and control of biochemical processes associated with tooth movement in orthodontic treatment and may help clinicians to make treatment decisions in the study of symbolic markers of bone remodeling.

**Purpose**

The aim of this study was to trace the dynamics of the cytokine IL-1β in the course of orthodontic treatment with fixed appliance.

**Tasks**

1. To examine the oral hygiene and gingival status during orthodontic treatment in children for a period of 18 months.

2. To examine the levels of IL-1β in the GCF of representative teeth during the research period.

**Materials and Methods**

The object of the study were 31 children (from 11 to 18 years), 17 girls and 14 boys on which was carried out orthodontic treatment with fixed appliance. In the prospective study were followed basic periodontal clinical parameters for a period of 18 months. In the begging of the research, before
placing the brackets children were chosen in good health: no antibiotic therapy during the previous six months, without taking anti-inflammatory drugs in the month preceding the survey, without active periodontal pathology (with parameters in probe of the gingival sulcus depth ≤ 3 mm) and without x-ray evidence of periodontal bone loss.

The study has permission from KENIMUS (Commission of ethics of science research’s, MU-Sofia) and informed consent from each patient or the parents of the under age patients.

For the period of 18 months, patients were followed for nine visits as follows: first visit – before the orthodontic treatment (up to 1 week), the second visit - 24 hours after placing of the brackets; 3rd visit - after 1 week, 4th visit - after 3 weeks; fifth visit - after 6 weeks 6th visit - on the third month, 7th visit - the sixth month, the 8 visit - 12 -months, 9th visit - the 18th month.

Oral status of the children was examined and recorded using a specially prepared card for evaluation, prepared based on accepted in Pediatric Dentistry Department - Sofia card for children examination and included – the risk of caries development, risk of periodontal disease development, dental and periodontal status. The following indices were used - oral hygiene index (OHI of Green - Vermillion, simplified) based on the presence of dental plaque after staining based on information from the entire dentition, Papilla Bleeding Index (PBI) of Saxer Mulhemen and determining periodontal depth through probe (PD). For each of the 9 following visits were recorded: OHI, PBI, gingival fluid volume of the five representative teeth [16, 11, 23, 31, 43].

Quantitative analysis for the presence of IL-1β was performed on two of representative teeth (11, 23) at 1, 2, 3, 6, 7 and 8th visit. Teeth whose gingival fluid was studied were selected according to their predominant use in other similar studies, because of the easy accessibility of the collection of gingival fluid with less risk of contamination with saliva, and especially because in most patients these teeth are active participants in the leveling phase of treatment.

Methods for collecting GCF.

It was used strips of filter paper (FILPAP sro, CZ-411 08 Steti, Czech Republic; medium fast) - rectangular sizes - 2mm/12mm. Before sampling, dry strips with polypropylene appendorf type capsules, in which they were placed were measured by an analytical balance. The strips were placed in the medio-vestibular sections of the gingival sulcus till a sensation of slight resistance in it for 3 minutes. It was isolated from saliva and the teeth were thoroughly dried. To avoid contamination of the samples of blood, saliva and plaque, all clinical measurements were carried out after the samples collection. After collecting the samples with the strips, they were placed in appendorf mini capsules, measured again on an analytical balance and the amount of GCF was registered. Samples were frozen in -30 ° until analysis.
Methods for the Study of IL-1β.

After thawing and extraction of GCF using methodology created by us (to the samples was added buffer, and then each sample was placed on a vortex for 15 seconds, centrifugation is performed at 13 000 g for 9 minutes), was conducted quantitative analysis of the studied markers using solid-enzyme method - ELISA in clinical laboratory USBALE "Academic Ivan Penchev". It was used highly sensitive kits for human IL-1β in biological fluids (Human IL-1β Platinum ELISA BMS224 / 2 / BMS224/2TEN Bioscience). The results were recorded on ELISA reader "Multiscan plus" with 450 nm wavelength.

Statistical processing of data was performed with SPSS-19 and using the Paired Samples T-test for comparison of averages.

Results

1. Oral hygiene and gingival status of the studied children. The results of OHI and PBI registered during orthodontic treatment are presented in the following diagrams 1 and 2.

Diag.1. Oral hygiene index during orthodontic treatment

Due to the conducted, before the placement of brackets, professional oral hygiene, relatively high initial values of OHI fell sharply after one week (P <0.05). Then during treatment oral hygiene deteriorated again and maintained at a level close to the initial values, regardless of the professional oral hygiene and motivation that has taken place in every subsequent visit (P>0.05).
During the first week PBI falling sharply after the conducted professional oral hygiene before the treatment ($t_{1,2}=5.72; P <0.05$). Throughout treatment levels of the index remain twice lower than the initial values ($t_{1,3}=4.39; P <0.05$). This indicates that orthodontic treatment does not provoke an inflammatory response in the gingiva, despite poor oral hygiene during treatment.

2. Amounts of IL-1β in GCF during the course of orthodontic treatment.

Mean values of IL-1β isolated from GCF of the representative teeth - upper right central incisor and upper left canine on 1, 2, 3, 6, 7 and 8th visit are presented in the following table 1.

*Tab. № 1 Mean values of IL-1β (pg / ml) in different visits of examined children.*

<table>
<thead>
<tr>
<th>Results</th>
<th>n</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; visit</td>
<td>31</td>
<td>80,201 ± 51,16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1 week before</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; visit</td>
<td>31</td>
<td>51,989 ± 33,39&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>After 24 hours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; visit</td>
<td>31</td>
<td>53,477 ± 42,80&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>After 1 week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6&lt;sup&gt;th&lt;/sup&gt; visit</td>
<td>31</td>
<td>100,766 ± 65,38&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>After 3 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7&lt;sup&gt;th&lt;/sup&gt; visit</td>
<td>31</td>
<td>81,061 ± 51,44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>on the 6&lt;sup&gt;th&lt;/sup&gt; month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8&lt;sup&gt;th&lt;/sup&gt; visit</td>
<td>31</td>
<td>77,001±72,08&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>on the 12&lt;sup&gt;th&lt;/sup&gt; month</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Paired Samples           |    | $t_{1,2}=2.36 P=0.03$ $t_{1,3}=2.11 P=0.04$ $t_{1,6}=1.43 P=0.16$
<p>|                          |    | $t_{1,7}=0.33$ $t_{1,8}=0.32 P=0.75$ $t_{1,9}=0.21 P=0.84$ |</p>
<table>
<thead>
<tr>
<th>T-test</th>
<th>$t_{2,6}$=4,96 P=0,00</th>
<th>$t_{2,7}$=2,42 P=0,02</th>
<th>$t_{2,8}$=1,83 P=0,08</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{3,6}$=4,19 P=0,04</td>
<td>$t_{3,7}$=2,17 P=0,04</td>
<td>$t_{3,8}$=1,6 P=0,12</td>
<td></td>
</tr>
<tr>
<td>$t_{6,6}$=1,38 P=0,18</td>
<td>$t_{6,7}$=1,53 P=0,14</td>
<td>$t_{6,8}$=0,41 P=0,68</td>
<td></td>
</tr>
</tbody>
</table>

NB. Identical letters indicate no statistically significant difference, and the different one the presence of such difference ($p <0.05$).

The resulting initial mean values of IL-1β were 80,201 ± 51,17 pg / ml, which is to support the results of other studies [9, 13, 21,24], most authors have reported rates based on the total protein in the fraction - 0.88 ± 0.11 pg / μg [21]; 0.58 ± 0.08 pg / μg [24]. However, the numerical values of the studied biomarkers from different studies are difficult to compare, because in different studies are used different methodologies - immunofluorescent method is considered more sensitive than ELISA, different types of ELISA kits and others.

During the first week the average IL-1β levels decreased to 53,477 (± 42,8 PG / ml) (P <0.05) and rose twice in the third month of treatment (100,766 (± 65,38 PG / ml)) (P <0.05). After the third month the interleukin levels fell slightly, but remain within the baseline to one year of treatment (P> 0.05).

**Discussion**

With the help of immunohistochemical methods before more than twenty years was shown that IL-1 can be detected in the periodontal tissues of canine of cats after application of mechanical force [1]. This is one of the first experimental evidence to support the opinion that cytokines regulate remodeling processes during orthodontic treatment.

The key position of the IL-1β among cytokines as mediators involved in bone remodeling process induced by orthodontic tooth movement has been shown in studies in recent years [1, 5, 6, 7, 9, 12, 17, 18, 20, 21, 23]. Our study also presents results that support that statement, based on research on a larger number of patients followed for a significantly longer period of time than those quoted in the literature.

The prevalence of studies in experimental conditions and on patients for testing tissue remodeling mediators (IL-1β) are immediately after the beggining of orthodontic treatment (24 hours, 1 week to 1 month after the start of treatment). Most results showed an increase of tissue mediators during this period [5, 18, 19, 21, 24]. The results we obtained show increased cytokine levels at a later stage on the third month of treatment.

In a study of 10 patients with orthodontic treatment are reported levels of two mediators of bone resorption - IL-1β and prostaglandin E (PGE) in GCF, for 5 consecutive visits (before placement of brackets, one hour later, on the 24th, on the 48th, and 168 hours). Radioimmunoassay was used for testing and found that the levels of IL-1β were highest at 24 hours (19,2 pg). For PGE
also reported a peak at the 24th hour retentive and at the 48 hours. So the increase of both mediators is maximum one day after the begging of the treatment [5].

In another study of 12 adolescents for up to 1 week after the start of treatment with fixed technique, using ELISA are measured quantities of IL-1β, IL-6, TNF-a, EGF, β2-MG, and total protein in samples of GCF. Samples were isolated from the gingival sulcus of the upper canine tooth. There have been registered the highest levels of cytokines at the 24th hour. At the 48th and 168th hour are also reported higher levels but with tend to establish a new balance on the new level. The results for IL-1β (0.88 pg of total protein) at the 24 hours, correspond with those obtained in a previous study [21]. We do not consider such a peak at 24 hours, but there is a slight increase in the values at 168 hours.

Unfortunately, most studies are for a short period of time and can’t be compared with the obtained from us results in half, and one year after the beginning of treatment.

In a study of 12 patients, the cytokine profile is followed from the 24th hour to the fourth month after the application of orthodontic force. Levels of IL-1β and 6-TNF-α reached its maximum at the 24th hour, which indicates that the studied mediators play an important role during the early stages of tooth movement, while in the later stages of the leveling phase, it decreases. After leveling of the dental arches, periodontal system is stabilized at a new physiologic, homeostatic condition. The authors note that the levels of studied mediators are associated with the level of mechanical stress and offer the use of a mild and prolonged impact forces during orthodontic treatment [18]. Exactly using of such forces in the treatment of patients in this study was probably the reason for the gradual increase in the expression of cytokines, without a sharp rise at the beginning. After leveling of the arches in our study was also found a reduce of IL-1β in GCF.

Other authors as Tzannetou S at al., compare the levels of mediators IL-1β and β-glucuronidase in GCF of molars, premolars and incisors during rapid palatal expansion. The authors reported reduced initial levels of the studied mediators in previously conducted oral-hygiene prophylaxis [19]. These results coincide with those obtained in our study.

In the literature is reported for a significant correlation between plaque accumulation, gingival inflammation and gingival fluid volume in orthodontic treatment [8]. In this study, PBI levels remain lower than the initial values, indicating that orthodontic treatment does not provoke an inflammatory response in the gingiva despite poor oral hygiene during treatment.

**Conclusions**

1. Oral hygiene status during orthodontic treatment is maintained in relatively satisfactory levels;
2. In the course of orthodontic treatment, gingival inflammation is negligible, despite increased plaque accumulation.
3. In orthodontic treatment IL-1β in GCF is between 51.99 to 100.77 pg/ml and can be considered as a marker of tissue changes during treatment and the follow-up of it in samples of GCF is noninvasive method suitable for controlling these processes.

4. Opportunities provided by modern molecular and genetic methods for studying cellular mediators in microquantities of GCF, open new horizons for the study of remodeling processes in modern orthodontics.

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References


2. Faulkner, Mathue Gene, "Gingival crevicular fluid (GCF) levels of interleukin-6 (IL-6), soluble glycoprotein 130 (sgp130), and soluble interleukin-6 receptor (sIL-6R) during orthodontic tooth movement" (2011). UNLV Theses/Dissertations/Professional Papers/Capstones. Paper 1278.


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A Study of the Composite Restorations’ Marginal Adaptation in Er-YAG Laser and Turbine Drill Prepared Cavities

Zhegová G¹, Rashkova M¹, Vasileva R², E. Tarasova³

Summary
Er-YAG laser-assisted cavity preparation is a modern and an effective strategy which has penetrated, widely used in dental practice and has become an element of the modern caries treatment with minimal intervention model.

Purpose of the recent study was to evaluate and compare the micro leakage of cavities prepared with a Er-YAG laser and turbine drill and restored with a nano-sized hybrid composite resin and a flow able composite resin.

Materials and Methods
One hundred and twenty standardized cervical cavities were prepared respectively: 90 with an Er-YAG laser (300mJ/20 Hz) and 30 with a turbine drill. A half of the patterns were restored with a nano-sized hybrid composite resin and the rest of them with a flow able composite resin. Laser-prepared patterns were divided into 3 subgroups according to the additional treatment of the dental surfaces (before restoration): (1) with Er-YAG laser finishing and beveling of the enamel edges (100 mJ/20 Hz); dentinal surfaces pretreatment with 5,25% NaOCl and acid etching with 37% orthophosphoric acid. After thermo cycling the specimens were immersed in a 0.5% aqueous basic fuchsine dye and then were sectioned longitudinally (buccally-to-lingually) through their centers. We investigated the gingival and occlusal length and average length of micro infiltration using optical microscope.

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Results

Er-YAG laser cavity preparation without additional pretreatment of dental surfaces results to similar micro leakage values compared to the conventional cavity preparation. Er-YAG laser finishing and beveling of the enamel edges of the laser-prepared cavities reduces micro leakage values of the hybrid composite resins. The additional dentinal surfaces pretreatment with NaOCl reduces micro leakage of hybrid composite resins as well as flowable composite resins in Er-YAG laser prepared cavities.

Key words: Er-YAG laser, Micro leakage, Nano-sized Hybride Composite resin, Flow able Composite resin, Cavity preparation.

Introduction

In the last few years new technologies have been proposed as an alternative of the conventional mechanical methods for cavity preparation. For a long period these methods used high-intensity lasers [22] have been widely used and approved by professionals. Within these lasers the Er:YAG laser has been especially introduced in dental practice for treating hard dental tissues [12, 20, 22, 27, 35, 40].

The Er-YAG laser wavelength (2940 nm) matches the absorption peak of water in the process of this wavelength’s interaction with hard dental tissues. The energy is converted into heat, leading to water vapor formation, which expands and produces high pressure inside the target tissue and induces instantaneous micro-explosions and ejection of particles of tissue in a process called thermo-mechanical ablation [1, 6, 8, 20, 21, 27, 33, 34].

According to many authors applying of the proper Er-YAG laser parameters and the exact working protocol leads to micro retentive hard dental surfaces, no smear layer and reduced bacterial colonization without thermal damages of surrounding tissues. [1, 14, 17, 20, 31, 35, 40].

The cavity restorations’ microleakage is under consideration of many factors like polymerization shrinkage, differences in thermal expansion coefficients of dental structures and restorative materials, as well the mechanical stress [36]. In order to improve composite materials adhesion it is necessary to study not only the properties of materials but the interface dental hard tissues-restorative materials characteristics as well [32].

SEM studies showed that an Er-YAG laser cavity preparation led to micro retentive enamel surfaces, opened enamel prisms with preserved form and structures but ablated in undifferentiated way (Silverstone type III) [37] and abruptly cut enamel prisms’ extremities. Dentinal surface is retentive and free of smear layer. Dentinal tubules are opened and protruded. They can be seen
loosely bound particles ejected and additionally adhered to the dental surfaces [12, 20, 40]. It has been established that the Er-YAG laser beam applied on dentinal surface led to thermally affected layer (thick about 3-5µm). This layer consists of denaturated collagen fibers and makes the adhesion of composite resins worse especially in the intertubular dentin [7, 29, 38].

Some authors recommend additional lased hard dental surfaces pretreatment with 37% orthophosphoric acid in order to approve micro mechanical retention of composite materials [3, 4, 5, 9, 15, 40].

In our country there are no studies concerning the influence of Er-YAG laser radiation on microleakage and marginal adaptation of composite materials.

Studies with regard to morphological effects on the permanent and primary enamel and dentin after applying of one of the best laser systems (Er-YAG) introduced in dental practice for hard dental tissues as to the effect of orthophosphoric acid, followed Er-YAG laser cavity preparation are extremely poor [41, 42].

Only a few studies in the literature evaluated the impact of Er-YAG laser irradiated dentin treated with 5% sodium hypochlorite on composite resins’ microleakage [29]. Limited studies on the role of Er-YAG laser cavity margins’ beveling on quality of composite restorations’ marginal adaptation have also been conducted [15].

**Purpose:** Comparative microleakage study at an Er-YAG laser and a turbine drill prepared cavities, filled with a nanohybride and a flowable composite resin.

Following **tasks** have been formulated:

1. Evaluation of microleakage at Er-YAG laser and a turbine drill prepared cavities, filled with a nanohybride composite resin;
2. Evaluation of microleakage at Er-YAG laser and a turbine drill prepared cavities, filled with a flowable composite resin.

**Material and methods**

One hundred and twenty samples of extracted teeth prepared using the following method were evaluated: "60 extracted permanent molars were treated with a scalpel or scaler to remove dental calculus and soft tissues, then polished, washed with water and examined under an optical microscope with a magnification for exclusion from the study with defects in enamel and dentin. The teeth were stored in distilled water at 4 °C until start of the experiment."

One hundred and twenty cervical cavities on vestibular and oral surfaces of 60 teeth were prepared by an Er-YAG laser and a turbine drill. The preparations were standardized using a template with dimensions of 4.00 x 3.00 mm, and the gingival cavity wall was located 1,5 mm
occlusal direction from enamel-cement junction. The depth of the cavities was 2 mm, determined with a calibrated periodontal probe.

Nineteen of the cavities were prepared with an Er-YAG laser and the laser beam was directed at an angle of 90 ° to the tooth surface. A sapphire tip with a diameter of 1.3 mm and a length of 19 mm was used. The Er-YAG laser parameters used for cavity preparation are shown in Table 1.

Table 1. Er-YAG laser parameters used for cavity preparation

<table>
<thead>
<tr>
<th>Laser power</th>
<th>Energy/ pulse frequency</th>
<th>Theoretical energy fluence</th>
<th>Pulse duration</th>
<th>Water-air cooling</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 W</td>
<td>300 mJ/20 Hz</td>
<td>22.61 J/cm²</td>
<td>50µs</td>
<td>39 ml/min</td>
</tr>
<tr>
<td>0.5-1.0 mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Regardless of the used adhesive system and filling material the specific features of Er-YAG laser irradiated enamel and dentin surfaces and the absence of smear layer require a different approach in the preparation of both the enamel and the dentin surface [3, 4, 9, 26, 29, 40].

In order to reduce the effect of microleakage, tooth surfaces of a part of the Er-YAG laser prepared cavities were further processed. The cavity enamel edges have been beveled with a sapphire tip (diameter of 1.0 mm and length 19 mm; theoretical fluence -12.74 J/cm²) with a low laser energy (100mJ/20Hz).

It was applied an acid etching on enamel surfaces with 37% orthophosphoric acid in order to reduce the enamel roughness after Er-YAG laser ablation.

Dentinal surfaces have been treated with 5.25% sodium hypochlorite solution for 20 seconds in order to remove the denatured collagen, due to laser ablation and then washed with distilled water. The dentin has been further etched with 37% orthophosphoric acid for 15 sec. in order to correct the strongly marked relief of the surface. Object of our previous studies was SEM analysis of irradiated with different Er-YAG laser parameters hard dental tissues, and their further treatments [41].

The remaining 30 cavities were prepared with a diamond fissure turbine bur (N-ISO 1090) with air and water cooling. Burs have been changed after every 4 preparation. Finishing of enamel edges of the cavity was performed at an angle of 45 ° with a turbine bur.

All cavities were filled according to the requirements of the manufacturer of the used filling materials (Kaloret GC and Gradia Direct Loflow GC).

The polymerization was conducted with halogen light for 40 sec at a distance of 0-1 mm. All restorations were defined with diamond burs and polished with paper discs. One operator performed all preparation, recovery, finishing and polishing.

Grouping of the samples in the experiment is shown on the following table (Table 2).
Table 2. Grouping of the samples in the experiment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cavity preparation</th>
<th>Additional treatment before restoration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A - 15 cavities with a hybrid composite</td>
<td>Laser 300 mJ /20 Hz;</td>
<td>enamel: edges fining with a laser 100mJ/20Hz; 37% orthophosphoric acid; dentin: treatment with 5,25% NaOCl; enamel and dentin: 37% orthophosphoric acid.</td>
</tr>
<tr>
<td>1B - 15 cavities with a flowable composite</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2A - 15 cavities with a hybrid composite</td>
<td>Laser 300 mJ /20 Hz;</td>
<td>enamel: 37% orthophosphoric acid; dentin: treatment with 5,25% NaOCl; enamel and dentin: 37% orthophosphoric acid.</td>
</tr>
<tr>
<td>2B - 15 cavities with a flowable composite</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3A - 15 cavities with a hybrid composite</td>
<td>Laser 300 mJ /20 Hz;</td>
<td>enamel: 37% orthophosphoric acid; dentin: 37% orthophosphoric acid.</td>
</tr>
<tr>
<td>3B - 15 cavities with a flowable composite</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4A - 15 cavities with a hybrid composite</td>
<td>Turbine drill</td>
<td>enamel: 37% orthophosphoric acid; dentin: 37% orthophosphoric acid.</td>
</tr>
<tr>
<td>4B - 15 cavities with a flowable composite</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All samples were stored in distilled water (37° C for 24 hrs), followed by a thermocycling 1000 times each at 5° and 55° C (Thermocycler THE 1100/1200). The cycle duration was 30 seconds. Transmission time between each bath was 10 sec.

Then the samples were coated with a composite resin in the field of root surfaces and 2 drops nail (nail) 1 mm from the edge of the filling were applicated. The teeth were immersed in 0.5% aqueous basic fuchsin for 24 hrs, followed by washing with running water to remove excess dye and dried at room temperature.

Samples were separated longitudinally in the middle of the filling in buccal-lingual direction with a low speed separator and water cooling.

The microleakage of samples was examined using a light microscopy Orthoplan Leitz, in reflected light, with the aid of an eyepiece equipped with a micrometer line. Dental samples were fixed with plasticine on a glass slide and they were leveled by a hand press. The studies were performed at magnification 50 and 100 X. Measurement accuracy was ± 10⁻² mm.
Gingival, occlusal and average length of dye penetration were examined. Two researchers reported results independently. Measures were average. For each filling the sector with greatest penetration was recorded (photographed).

Mean contact lengths on which microleakage was measured, compared between all groups were and presented in the following table 3.

Table 3. Average values of contact lengths

<table>
<thead>
<tr>
<th>Groups</th>
<th>A n</th>
<th>A length of the contact Kalore (mm)</th>
<th>B n</th>
<th>A length of the contact Gradia LoFlo (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ± SD</td>
<td>mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-st group</td>
<td>15</td>
<td>6.82 ±0.26 a</td>
<td>15</td>
<td>6.81 ±0.23 a</td>
</tr>
<tr>
<td>2-nd group</td>
<td>15</td>
<td>6.79 ±0.26 a</td>
<td>15</td>
<td>6.84 ±0.23 a</td>
</tr>
<tr>
<td>3-rd group</td>
<td>15</td>
<td>6.85 ±0.27 a</td>
<td>15</td>
<td>6.82 ±0.24 a</td>
</tr>
<tr>
<td>4-th group</td>
<td>15</td>
<td>6.83 ±0.27 a</td>
<td>15</td>
<td>6.86 ±0.25 a</td>
</tr>
<tr>
<td>Independent t- test</td>
<td>t1,2=0.24; t2,3=0.54</td>
<td>t1,2=0.41; t3,4=0.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>t1,4=0.17</td>
<td>t1,4=0.45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The table shows that there are no statistical significant differences between the mean values, which is a prerequisite for objectivity of the comparative analysis between the evaluated groups.

Statistical analysis. Microleakage results were compared using the statistical program SPSS-19 (comparing averages by Independent T-test) and the adoption of statistical reliability at P<0.05. Microscopic examination was carried out at the Institute of Mineralogy and Crystallography, Bulgarian Academy of Sciences.

Results

1. Microleakage of Er:YAG laser and turbine drill prepared cavities, filled with a nanohybride composite Kalore GC

The results of gingival, occlusal and average microleakage in Er:YAG laser (300mJ/20Hz) prepared cavities filled with a GC Kalore are presented in Table. 4, Figures 1, 2.
Table 4. Microleakage of Er:YAG laser and turbine drill prepared cavities, filled with a nanohybride composite Kalore GC

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Gingival leakage mm</th>
<th>Occlusal leakage mm</th>
<th>Average leakage mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean ± SD</td>
<td>mean ± SD</td>
<td>mean ± SD</td>
</tr>
<tr>
<td>1-st group</td>
<td>15</td>
<td>0.004 ±0.011a</td>
<td>0.002 ±0.008a</td>
<td>0.003±0.006a</td>
</tr>
<tr>
<td>2-nd group</td>
<td>15</td>
<td>0.094 ±0.160b</td>
<td>0.002 ±0.006a</td>
<td>0.048±0.081b</td>
</tr>
<tr>
<td>3-rd group</td>
<td>15</td>
<td>0.217±0.150c</td>
<td>0.011±0.018a</td>
<td>0.114±0.080c</td>
</tr>
<tr>
<td>4-th group</td>
<td>15</td>
<td>0.227±0.167c</td>
<td>0.014±0.027a</td>
<td>0.121±0.088c</td>
</tr>
<tr>
<td>Independent t- test</td>
<td></td>
<td>t1,2=2.17; t1,3=2.18; t1,4=5.50; t1,4=0.17</td>
<td>t1,2=0.00; t2,3=1.75; t2,4=1.67; t3,4=1.69; t4,4=0.39</td>
<td>t1,2=2.15; t2,3=2.24; t2,4=2.35; t3,4=5.33; t4,4=0.22</td>
</tr>
</tbody>
</table>

Note. Identical letters indicate no statistically significant difference, and different presence of it (p<0.05).

The table shows that the values of microleakage in turbine prepared cavities (Group 4) compared to Er-YAG laser prepared cavities (without further treatment with NaOCl - Group 3) showed no difference in terms of average, gingival and occlusal permeability (p> 0.05).

Comparing the three groups of samples of Er-YAG laser prepared cavities, demonstrated a microleakage reduction in cavities after treatment with sodium hypochlorite (Group 2), compared to untreated samples ( Group 3 ) (p <0.05). In the group of additional finishing of cavity margins (with laser parameters 100mJ/20Hz) (Group 1 ) microleakage decreased significantly, and showed a statistical significant difference compared to with turbine drill prepared cavities (p <0.05). Occlusal permeability revealed no differences between the four groups, and the differences are mainly due to infiltration of the gingival area.

It can be concluded that an additional treatment of the Er-YAG laser prepared cavities with sodium hypochlorite and the laser finishing of the cavity margins forms a good surface for bonding of the composite material and greater than that of the conventional preparation with a turbine drill.

The following figures illustrate the obtained results Figure 1 and 2.
2. Microleakage of Er:YAG laser and turbine drill prepared cavities, filled with a flowable composite Gradia GC

Comparing of gingival, occlusal and average microleakage in Er:YAG laser (300mJ/20Hz) prepared cavities filled with a GC Gradia Direct Loflow, are presented in the following Table. 5, Figure 3, 4.

Table 5. Microleakage of Er:YAG laser and turbine drill prepared cavities, filled with Gradia Direct Loflow GC

<table>
<thead>
<tr>
<th>Groups</th>
<th>Gingival leakage mm</th>
<th>Occlusal leakage mm</th>
<th>Average leakage mm</th>
</tr>
</thead>
</table>
The results showed that the microleakage values of a turbine drill prepared samples, (Group 4) did not differ from the Er-YAG laser prepared samples without additional treatment with NaOCl (Group 3) in terms of gingival, occlusal and average dye penetration. There was no statistical significant difference (p> 0.05). In all groups occlusal permeability was minimal and did not differ significantly.

The additional dentinal surface treatment with sodium hypochlorite after Er-YAG laser cavity preparation with leads to reliably lower (gingival and average) microleakage. It is more than twice less than that of a laser prepared samples without the treatment with sodium hypochlorite and even smaller compared to the permeability obtained after the conventional preparation (p<0.05).

The additional dentinal surface treatment with sodium hypochlorite after Er-YAG laser irradiation removed denatured collagen, which could be a likely cause of improving the contact with the filling material and microleakage reducing in Er-YAG laser cavity preparation compared to conventional preparation.

Additional laser fining (100mJ/20Hz) of enamel edges for laser prepared cavities did not substantially alter the degree of microleakage (p>0.05). The following figures illustrate the obtained results (Fig.3 and 4).
Figure 3A. Er-YAG laser (300 mJ/20 Hz) preparation, fining (100mJ/20Hz); 5,25% NaOCl; 37% orthophosphoric acid, bond, Gradia Direct Loflow GC, Magnification:50X

Figure 3B. Er-YAG laser (300 mJ/20 Hz) preparation, 5,25% NaOCl; 37% orthophosphoric acid, bond, Gradia Direct Loflow GC, Magnification:50X

Figure 4A. Er-YAG laser (300 mJ/20 Hz) preparation, 37% orthophosphoric acid, bond, Gradia Direct Loflow GC, Magnification:50X

Figure 4B. Turbine drill preparation, 37% orthophosphoric acid, bond, Gradia Direct Loflow GC, Magnification:50X

Discussion

Quality of contact between the Er-YAG laser irradiated hard dental tissues and composite filling materials is a subject of various investigations [3, 4, 5, 6, 7, 8, 15, 17, 18, 26, 29, 32]. Studies have found that Er-YAG laser prepared cavities, and filled with composite materials showed lower bond strength as compared to conventional prepared cavities and filled with the same material [11, 19]. Other authors found no differences between Er-YAG laser and bur-prepared cavities, regardless of the different adhesive systems with total-etch or self-etch adhesive systems [3, 6]. Krmek et al. reported the lowest microleakage for Er-YAG laser preparation followed by an additional acid etching [26].

The results of the present study showed that the microleakage in Er-YAG laser prepared samples without additional treatment with NaOCl for both composite materials, did not differ from those prepared with a turbine drill. The additional laser finishing of cavity margins with parameters
100mJ/20Hz, decreased significantly the microleakage of laser prepared cavities (filled with a nanohybrid composite) compared to turbine drill prepared samples. Similar results reported in the literature and other authors [15].

Probably finishing of the cavity margins with low laser energy corrected uneven ablated enamel prisms, in a sense of prism edges "rounding" and reduced the effect of the "sharp cut" prism edges, described in the literature. Thereby providing better penetration of nanohybrid composites it decreased the microleakage at cavity margins [5, 15].

Laser finishing of the cavity margins did not lead to significant differences in permeability of flowable composites. The most likely explanation for this result is that due to its liquid consistency, flowable composites were better adapted to the roughness of laser-treated enamel surface and no further processing of cavity margins required [18].

At present, the effects of Er-YAG laser on the organic components (collagen fibers) of the dentine remains still unclear. Soares et al. reported that Er-YAG laser radiation applied on dentinal surface affected the organic matter [38]. Using transmission electron microscopy, Ceballos et al. showed a thick 3-4 μm laser-modified layer, an alteration of the dentin subsurface, beneath which collagen fibrils seemed mixed and appear to have lost crossbanding and are fused together, thereby eliminating interfibrillar spaces. Er-YAG laser irradiation did not create smear layer in laser prepared cavities, compared to the conventional preparation where its removal could be achieved by etching, and then application of NaOCl removed the described above dentinal layer of denatured collagen [7].

NaOCl disrupts the pyridinoline cross-links that occur in collagen I and II [16]. Effects of NaOCl on the organic matter of the dentin occur depending on the applied concentration [2, 13]. In our study administration of 5,25% NaOCl for 20 sec. on dentinal surfaces irradiated with Er-YAG laser, followed by washing with water (20 sec.) before acid treatment of cavities showed lower average microleakage at both composite materials. Similar results reported other authors in the literature, after the same method’s application [29].

However, NaOCl does not affect inorganic substances, thus it cannot act on the roughness of laser-treated dentinal surface as dentinal tubules [24, 33].

Er-YAG laser irradiation applied on hard dental tissues during the cavity preparation leads to uneven strongly retentive surfaces in both enamel and dentin [1, 12, 20, 31, 35, 40, 41]. In the present study after Er-YAG treatment of dentinal surfaces it was applied 37% orthophosphoric acid. It corrects the strongly expressed enamel and dentin retentiveness removing the effect of “abruptly cut prism edges” which can be broken during polymerization process of composites, partially removes the highly mineralized peritubular dentin, enlarges dentinal tubules orifices, and reveals collagen fibrils. The orthophosphoric acid favors the creation of a better hybrid layer at Er-YAG
laser prepared cavities. On the other hand it can remove loosely bound particles- consequence of the laser treatment [3, 4, 9, 29, 40].

**Conclusions**

1. Er-YAG laser cavity preparation without additional cavity treatment with NaOCl leads to microleakage values similar to those at the conventional cavity preparation with a turbine drill.

2. Enamel edges laser fining during Er-YAG laser cavity preparation decreases the microleakage at a hybrid composite material and does not influence the microleakage at flowable composites.

3. The additional dentinal treatment with NaOCl at Er-YAG laser prepared cavities decreases the microleakage in both hybrid and flowable composite materials.

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Orthodontics

Open bite in children between 7 and 14 years of age – distribution and clinical expression

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Summary

Open bite is malocclusion, causing significant functional and aesthetical disturbances. Most commonly, the presence of an open bite is due to the action of unhealthy habits or of the divergent pattern of facial growth.

Objective: The aim of our study was to determine the frequency and age distribution of the open bite in children aged between 7 – 14 years.

Materials and methods: 1300 children aged between 7 – 14 years, who had not been treated orthodontically, were examined. For evaluating the dynamics of their malocclusions progress, the children were divided into seven age groups by using a 1-year age interval. For assessing the deviations graduated periodontal probes was used.

Results: Anterior open bite were observed in 2% of the studied children, while the buccal open bite was 0.9%. In 54% of the children with anterior open bite it was up to 1mm and 46% was from 1 to 3mm. There were no children with open bite more than 3mm.

Conclusion: The results obtained show that the maximum presentation of these malocclusion correlates with the time of action of some unhealthy habits and the type of facial growth. This is important for the prevention of open bite and the time to start the orthodontic treatment.

Key words: epidemiology, malocclusion, open bite

Introduction

Open bite is a malocclusion, which severely affects function and impairs aesthetics. Subtelney and Sakuda have described the open bite as a vertical distance between the incisal edges of the maxillary and mandibular incisors or as a vertical loss of the inter-dental contact [10]. Moyers has subdivided the open bite into incisal and buccal open bites (Fig. 1) [4]. Sassouni has divided the open bite into dento-alveolar and skeletal subtypes [8]. The first subtype is due to the effects of unhealthy habits, such as sucking the fingers, tongue or any objects, incorrect swallowing, obstruction of the upper

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respiratory passages [6]. The second subtype is associated with peculiarities of the skeletal growth [5], which are largely genetically predetermined [2].

Objective
The aim of our study was to determine the frequency of the anterior and posterior open, as well as the severity of its clinical expression.

Material and methods
In our study, 1,300 children aged between 7 and 14 years, were examined. These, who were on treatment or had completed successfully their orthodontic treatment at the time of entering the study, were not enrolled in the group of examined children. To follow-up the dynamics in the development of the malocclusion, the examined children were assigned to seven age groups with an 1-year interval between them, as shown in Figure 1.

Figure 1. Distribution of the children by gender and age

The examination was performed under entirely clinical conditions and sufficient light. Each child was examined by using a sterile individual dental kit and single-use gloves. For the measurements, a
graduated periodontal probe (American Eagle Instruments Inc.) was used. Measurement accuracy was determined as ±1mm.

Anterior open bite was assessed, when the clinical crowns of the incisors were completely erupted and a vertical distance was available between the incisal edges of the upper and lower incisors. The measurements were done in the central occlusion state, as the graduated portion of the periodontal probe was placed perpendicularly, in a way to stay parallel against the vestibular surfaces of the incisors, at the shortest distance to them. The examiner’s sight was at the level of the upper incisors and the vertical distance between the incisal edges was assessed, depending on the dividing lines (Fig. 2).

![Fig. 2](image)

Anterior open bite assessment

The open bite was measured in mm, as the distance was divided into three degrees: up to 1mm, from 1 to 3mm, and above 4mm. The cases with missing incisors in one of the dental arches, were registered as “cannot be estimated”.

Posterior open bite was assessed, when the posterior teeth were completely erupted and a vertical distance was available between their occlusal surfaces. Depending on the number of affected antagonists, the open bite was divided into the following subgroups: open bite in one pair of antagonists; in 2 to 3 pairs of antagonists; in 4 to 5 pairs of antagonists and in more than 5 pairs of antagonists.

Statistical processing of the data was performed by using the SPSS 15 statistical software for Windows (SPSS Inc., Chicago, Illinois, USA).

The following methods were used:
- Descriptive analysis – tabulated and graphical representation of the distribution of variables – absolute and relative frequencies.
- Cross tables for examining the correlations between the qualitative variables.
- \( \chi^2 \)-test and Fisher’s exact test for testing hypotheses of independence.
- Graphical analysis – graphical representation of the statistical data for illustration and analysis.

**Results and discussion**
Out of the 1,300 examined children, anterior open bite were found in 26 (2%). This constitutes 7.5% of the children with vertical occlusion deviations.

Table 1 Anterior open bite by gender and age

<table>
<thead>
<tr>
<th>Age groups</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Number</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of the children with open bite in the group</td>
<td>11,1%</td>
<td>55,6%</td>
<td>0%</td>
<td>0%</td>
<td>11,1%</td>
<td>0%</td>
<td>22,2%</td>
</tr>
<tr>
<td>% of all children in the group</td>
<td>1,1%</td>
<td>4%</td>
<td>0%</td>
<td>0%</td>
<td>0,9%</td>
<td>0%</td>
<td>2,3%</td>
</tr>
</tbody>
</table>

| Girls      | 6 | 3  | 1   | 2  | 2 | 0  | 3   |
| Number     |   |    |     |    |   |    |     |
| % of the children with open bite in the group | 35,3% | 17,6% | 5,9% | 11,8% | 11,8% | 0%  | 17,6% |
| % of all children in the group | 7,3% | 2,6% | 1,2% | 2,3% | 2,3% | 0%  | 3%  |
| Total number | 7 | 8  | 1   | 2  | 3 | 0  | 5   |
| % of the children with open bite in the group | 26,9% | 30,8% | 3,8% | 7,7% | 11,5% | 0%  | 19,2% |
| Total % of the children in the group | 4% | 3,3% | 0,6% | 1,1% | 1,5% | 0%  | 2,7% |

No statistically significant correlation was found between the anterior open bite and the age of the children ($x^2$=3.44, p=0.75).

From the data in Table 1 and Figure 2, it is obvious that the peak of this malocclusion presents in the early mixed dentition and the number of children with open bites significantly decreases in the stage of late mixed dentition. We suggest that this is on the account of the children with this type of deformity caused by unhealthy habits. In the period of transition from the late mixed to permanent dentition, there is a relatively persistent level of incidence of this malocclusion, and in the period of permanent dentition, a slight increase of the number of children with open bite is observed. We suggest that this is on the account of the children with accelerated vertical facial growth, particularly intensified during puberty.
Figure 2. Anterior open bite: distribution by gender and age

From the data in Figure 3, it is obvious that there is a relatively equal number of children with open bites of up to 1mm and from 1 to 3mm. No children with open bites above 3mm were found.

The literature analysis has shown that the open bite incidence, found in our study, is lower than these in several other countries (1; 3; 7; 9; 11).

Posterior open bite were found in 12 (0.9%) of the children (7 boys and 5 girls). These were children with completed eruption of the posterior teeth and the absence of a inter-dental contact was not due to a therapeutic intervention. Because of the small number of children with this deformity, these children were not subdivided by gender, but by age and severity of open bite manifestation only (Table 2). It seems again that this deformity was presented mainly in the early mixed dentition, in relation with the action of unhealthy habits.

Table 2 Open bite in the buccal region by age and severity of manifestation

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Open bite in 1 pair of antagonists</th>
<th>Open bite in 2-3 pairs of antagonists</th>
<th>Open bite in 4-5 pairs of antagonists</th>
<th>Open bite in more than 5 pairs of antagonists</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0(0%)</td>
<td>4(2.3%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>4(2.3%)</td>
</tr>
<tr>
<td>II</td>
<td>1(0.4%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>1(0.4%)</td>
<td>2(0.8%)</td>
</tr>
<tr>
<td>III</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>IV</td>
<td>0(0%)</td>
<td>1(0.5%)</td>
<td>1(0.5%)</td>
<td>0(0%)</td>
<td>2(1.1%)</td>
</tr>
<tr>
<td>V</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>VI</td>
<td>0(0%)</td>
<td>2(1.2%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>2(1.2%)</td>
</tr>
<tr>
<td>VII</td>
<td>0(0%)</td>
<td>1(0.5%)</td>
<td>1(0.5%)</td>
<td>0(0%)</td>
<td>2(1.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>1(0.1%)</td>
<td>8(0.6%)</td>
<td>2(0.2%)</td>
<td>1(0.1%)</td>
<td>12(0.9%)</td>
</tr>
</tbody>
</table>

Out of 12 children with posterior open bite, anterior open bite were simultaneously observed in 9 and edge-to-edge bite in 1 child. The number of cases with open bite was the highest in the period of early mixed dentition, and this is probably associated with some unhealthy habits, such as infantile
swallowing. In the majority of the cases, two to three pairs of antagonists were affected, most commonly, unilaterally.

**Conclusion**

Profound knowledge of the incidence and dynamics in the development of the open bite is of importance with regard to our practice as clinicians. The period of early mixed dentition is particularly appropriate for primary and secondary prophylaxis. Thus, preconditions for deformity autocorrection are created, in addition to the tendency of its improvement with increasing the age in the cases, where the aetiology is due to any unhealthy habit. When the reason for the open bite occurrence is a vertical type of facial growth, the early treatment is aiming at change and modification of the growth direction in the periods of its highest intensity. It is important to take into consideration that during this type of treatment, a relapse may occur in the period of puberty. In such cases, planning of retention, appropriate with regard to the type and duration of its application, is of importance to completion of the growth of the facial skeleton.

**References**


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Electronic protocol for oral focal diagnostics

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Summary

Oral diagnostics as an interdisciplinary problem requires teamwork of different medical specialists. Each of them must have all medical records of the patient and must be facilitated in the communication with other team members. In today’s society of electronic communications in which computers are playing key role, the creation of an electronic record of oral diagnostics would meet the needs of the experts’ team.

As a result of the conducted research with a specialist programmer in our team we have developed software product “EFD v. 1”. The computer program is an electronic protocol for integrated oral diagnostic and has the features of a consultation module, assisting the dentist during the process of focal diagnosis.

The creation of the electronic medical record of the patient, which is easily portable and can be transmitted electronically between physicians is a step forward in modern oral diagnosis and would save valuable time for diagnosis and treatment.

Key words: electronic diagnostic protocol, oral diagnosis, dental informatics

Introduction

An integral part of modern dental practice are computer technologies that play a major role in the comprehensive care of the patient [13].

One of the most promising area of dental informatics is a computer-aided diagnostics where throughout an appropriately developed electronic system is possible to imitate the process of data collection, analysis and reaching accurate diagnoses [2].

Computer-aided diagnostics is used mainly in the development of [3]:

– Electronic disease history

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⁴ Programmer
Jahanbani et al. found that computer-assisted training in oral pathology is a promising tool for advanced learning and can complement traditional forms of teaching [5].

Borra et al. developed a consultation computer program in the field of oral pathology to assist students in dental diagnosis of oral bone disease [2].

In the last two decades decision support systems in the field of dental medicine has been actively developing and progressing [4, 10, 17, 14, 15]. In the field of oral medicine systems that assist decision making in the diagnosis of oral diseases have been developed.

One of the most well-developed consultation programs for oral pathology is the system DART (Diagnostic Aid Resource Tool), which contains a database of clinical and radiographic manifestations of more than 600 diseases [11]. Of particular interest is the diagnostic module of oral medicine integrated into electronic dental disease history ICOHR (Intelligent Computer-Based Electronic Health Record). The program is image-based algorithm system diagnostics of oral mucosal lesions [16, 17].

The application of computer technology in thermodiagnosis is a necessary condition for registration and analysis of the findings [6, 7, 9]. Anbar is of the opinion that modern thermography technique is based on non-contact infrared monitoring, digitizing of the registered signal and computer systems to process the resulting images for analysis and interpretation. The author states that imaging methods has the potential to become a major diagnostic tool in many clinical situations [1].

By increasing the number of decision support programs in the field of oral diagnostics arises the necessity of their accreditation. The future development of these systems is determined by the need for their efficient implementation in practice. This concept requires the integration of the consultation program with the electronic disease history [8, 12, 18].

Aim

Creation of an electronic protocol for complex oral diagnostics to facilitate the communication of the focal team for the needs of everyday clinical practice.

Material and methods

For the design and implementation of a computer program the following has been done:

1. Examination and analysis of the possibility of creation computer program that satisfies the following requirements:
To store and process data from clinical and paraclinical tests
To be possible for the computer program to be used easily and comfortably by beginners.
To allow smooth integration into the clinical setting and in the treatment process without requiring extra effort on the part of the physician.

2. Investigation of the practice and experience of existing systems or those under construction in oral diagnosis has been done.

3. The minimum requirements for hardware, software and computer knowledge of the user has been determined.

4. The scientific literature has been investigated and processed in order to create a protocol for examination and diagnostics, that will serve as the basis of the user (graphical) interface of the computer program.

5. To create a computer program programming language of high level Visual C++ has been used. The drawing of the special elements has been performed using interface OpenGL.

For the work with application data (database) a standard SQL has been used, as a format of a standard database - Microsoft Access. This allows for future expansion of the functionality of the program and a potential of the so created data to be used with other software applications.

Results
As a result of the conducted studies and in a team with IT - specialist a software "EFD v.1" has been developed. The computer program is an electronic protocol for the purposes of the complex oral diagnostics and has the features of a consultation module assisting the dentist during the process of focal diagnostics.

1. Logical model of the computer program "EFD"

The logic model for software development is based on the use of interrelated and comprehensive electronic protocol for recording and analysis of data necessary for focal diagnostics. The scheme of the computer program allows to input data from the patient’s history, clinical and para-clinical surveys, results from the specific focal targeted diagnostic methods and the integration of digital images from imaging investigations.

Basic structural unit of the consumer environment in which the clinician uses the created graphic interface is a dialog page. Basic form of the user interface is interactive graphics, by which the user can change and manage the graphical display using a routing device like a computer mouse or keyboard.
The developed computer program has the characteristics of a consultation system in order to assist the dentist in the process of focal diagnostics. The Consultation Module allows a comparison between the data entry, analysis and focus on the user in solving specific diagnostic problem. It consists of two parts: a knowledge base and a logical machine. To formalize the knowledge in the Knowledge Base production rules have been used - a conclusion is accepted as true, if one or more assertions (rules) of the type 'if ... then ... " are valid. For example, the statement "if the measured temperature in the periapical region of symmetric to the sagittal plane teeth differ by more than 0.4 ° C, then there is evidence of an active focus" is formalized to a production rule in the following way: if the measured temperature difference is more 0.4 C + symmetrical teeth → there is data for active foci (Fig. 1).

![Production Rule Diagram](image1)

**Fig. 1** Graphical representation of a production rule used to formalize the knowledge in the consultation module of the computer program

The system perceives as data the information recorded in medical history, clinical and paraclinical examinations of the patient. Consultation program supports the process of decision-making at focal diagnostics by displaying the appropriate graphic symbols or messages.

1. **Structure of the computer program "EFD"**

The main user environment in which the operation with the program takes place is the dialog box (Figure 2).

![Dialog Box Diagram](image2)

**Fig. 2** Dialog box of the computer program "EFD"

Electronic record of importing the necessary data from the complex oral diagnostics is organized as a set of worksheets. Each page corresponds to the stage of the diagnostic process.

For registration of the necessary diagnostic information are created eight worksheets:
1. "Find entered patient."
2. "Registering the patient."
3. "Medical history".
4. "Focal diagnostics and dental status."
5. "Diagnostic images and X-ray imaging."
6. "Regulation thermography."
7. "Infrared Camera".
8. "Electrocutaneous test of Gehlen."
9. "Test of blood, saliva, urine".

In worksheet "Dental focal diagnostics" the information received from the "Vitality tooth test", "Corrosion potential measurement" and "Digital or infrared thermometry" are directly being recorded (Fig. 3).

*Fig. 3 Worksheet for entering information about the state of HDT / endodont and the data from the focal diagnostics*

A special electronic chart for interactive registration of the state of the hard dental tissues and endodont of the existing teeth has been developed. An appropriate chart of the dentition has been created, wherein each tooth has a schematic representation (Figure 4).
For data analysis of intraoral thermometry the following rules have been used:

1. IF the symmetrical teeth + temperature difference more than 0.4 ° C, THEN there is an active focus → red color value (Fig. 5).

2. If the adjacent teeth + temperature difference more than 0.4 ° C, THEN there is an active focus → red color value (Fig. 5).

3. If the adjacent teeth + no temperature rise in distal direction, THEN there is a potential focus → green color value (Fig. 5).

Due to the specifics of focal targeted diagnostic methods "Regulatory Thermography", "Infrared Camera", "Electrocutaneous test of Gehlen " separate worksheets have been developed. To avoid double data entry, the recording is done in the method of diagnostics page and the computer program will automatically duplicate the page for "Dental focal diagnostics" (Fig. 6 and 7)
Fig. 6 Worksheet for entering and analyzing the results of the regulation thermography

Fig. 7 Worksheet for registration and analysis of the survey results with the infrared camera
Discussion

The development of our own, unique computer program targeted in the focal dental diagnostics to assist the acquisition of knowledge in the field of oral pathology and possess the characteristics of the consultation system was motivated by the following reasons:

- in the review of the scientific literature in the field of focal oral diagnostics was not discovered any developed software or such in process of developing with consultation character.
- possibility for continuous improvement and updating of the software product with the development of knowledge and practice in the field of focal diagnostics.
- implementation of the software in the educational program of the Faculty of Dental Medicine, Sofia.

Created computer program "EFD" meets the requirements specified in the methods and uses both classic and latest research methods in the field of focal diagnostics. The developed program allows continuous product development, flexible and dynamic improvement to the requirements of the intended user (student, teacher, general practitioner dentists). The system can be used as a model and basis for similar developments in the field of dental medicine.

In establishing the organization of electronic diagnostic protocol several facilities has been created. For the data registration mainly templates have been designed or dropdown list has to be activated. For this purpose, marking with the cursor of the mouse has to be used. The introduction of free text using the keyboard is minimized, especially in cases where it is necessary to use qualifying information or recording numerical values. Image character codes and colors for application of diagnostic data in electronic charts have been widely used. This allows a considerable array of information to be located in a relatively limited workspace. A major advantage is the possibility of integration of digital diagnostic images in the pages of the operating system.

For the development of consultation module of the computer program production rules have been used because specific knowledge of focal diagnostics can easily be formalized to claims of the type "if ... then ..."). On the other hand, this approach allows the addition of new knowledge, relevant rules, which do not contradict the previous ones.
This approach meets the modern concept of integrating the consultation program with electronic medical history in order to complete application in practice [8, 12, 18]. Educational value of the created computer program "EFD" comes from the implementation of a standardized protocol focal diagnostics, thereby acquire skills to conduct a comprehensive and structured study for precise analysis of the collected data and formulating a treatment plan.

**Conclusion**

From the implementation of the intended objective the following conclusions can be withdrawn.

1. The developed computer program for complex oral diagnostics - Electronic Focal Diagnostics is multifunctional:
   - can serve as an interactive electronic protocol which significantly facilitates the communication between the different professionals involved in the diagnosis and treatment of patients with focal infection;
   - has educational value and can properly be applied in computer-aided training of students and general dentistry.
   - provides the ability to store and add a universal database for the current state of the epidemic problem in Bulgaria, which may be useful for a variety of future studies.

**References**


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Additional treatment of renal transplanted patients with chronic periodontitis with diode and Nd:YAG laser – a pilot study

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Summary

Aim

This study aimed to investigate and evaluate long term changes in periodontal condition and periodontal microbiota of renal transplanted patients, after diode and Nd:YAG laser application.

Methods

There were ten renal transplant patients of average age of 40 years with chronic periodontitis enrolled in the present study, from February till October 2012. Presence of chronic periodontitis was clinically and radiographically (panoramic x-ray) assessed. Clinical periodontal examination was performed with Florida Probe®. Nonsurgical periodontal therapy including debridement of the dentition with ultrasonic scaler and scaling and root planning with hand instruments were performed. Two of the deepest periodontal pockets in different quadrants of the dentition were chosen in every patient. After SRP, the first pocket was treated with laser as supplementary therapy. The second pocket was for control. The patients were randomly distributed in two groups: first group – supplementary laser therapy of one deep periodontal pocket with diode laser – n=4; second group – supplementary laser therapy of one deep periodontal pocket with Nd:YAG laser – n=6. Classical cultural method and polymerase chain reaction (PCR) method were used to identify the microbiological profile of the laser treated periodontal pockets.

Results

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² Department of medical microbiology, Faculty of Medicine
A statistically-significant change in OHI was found at the third month (p=0.011). There were no significant changes in HI, BOP, PPD, CAL (p>0.05) between the first and the third month of the study. A reduction of the bacterial count after diode and Nd:YAG laser application was observed.

**Conclusion**

As already established, supplementary laser therapy with diode and Nd:YAG lasers contributes for the support of the periodontal health of renal transplanted patients. Longer observation of the above mentioned criteria in both groups is required, to understand and characterize the distinct features of diode (808 nm) and Nd:YAG laser effects. It turns out that for the improvement of oral hygiene habits of the patients, crucial role has motivation and clinical oral hygiene procedures performed by members of the study team.

**Keywords:** Nd:Yag laser, diode laser, periodontal treatment, renal transplanted patients, periodontal pathogens

**Introduction**

In the present scientific literature the two-way connection between periodontitis as focus of infection and finely balanced immune profile of transplant patients is increasingly discussed. [1,2,3,6,7,8,13].

Organ transplant patients and in particular kidney transplant patients are prone to micro inflammations as a result of transitional uremia. [8,17].

Odontogenic bacteremia was observed in 17-100% of patients received periodontal or endodontic treatment [10]. Oral bacteria cultivated from the blood of these patients included periodontal pathogens as Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Porphyromonas gingivalis and Bacterioides forsythus. These microorganisms (MO) are included in certain periodontal microbial complexes [16].

In modern literature periodontal diseases are considered as infectious diseases caused by microorganisms reside in oral plaque biofilms. Periodontal lesions are constantly renewable source of bacterial antigens, cytokines, Gram-negative bacteria and other mediators of inflammation, which disseminate systematically and affect different organs and tissues of the body [12].

Recent studies shows that biofilm bacteria are organized in complex structural and functional system, not observed if the same bacteria are in planktonic, free state. Good understanding of biofilm biology is of considerable importance for understanding pathogenesis of biofilm associated diseases such as periodontitis. Biofilms provide significant benefits for bacteria such as: an increased ability to attach to different types of surfaces; metabolic cooperations in which waste metabolic products of
one species are used for nutrition to another (food chain and food web); increased resistance to antibiotics and antimicrobial agents; synergetic pathological effects of different species; more effective mechanisms to overcome host immune system. All these features of bacterial biofilms make treatment of periodontitis and biofilm associated diseases particularly difficult [5,11].

The above mention reasons underlie numerous scientific searches for the introduction of more effective methods of impact on periodontal disease such as: antimicrobial therapy, host modulation therapy, photodynamic therapy, probiotics (Lactobacillus salivarius containing linguettes), and effects of laser radiation. Since transplant patients are extremely fine balanced between positive aspects of immunosuppressive therapy and its detrimental side effects, we chose the opportunity to explore in detail the effects of two different types of laser radiation in combination with conventional periodontal therapy, without including any type of medicament.

Laser devices are one of the most promising new systems for non-surgical periodontal therapy with their prominent haemostatic, detoxifying and bactericidal effects, tissue ablation capacity and effect of photo-bio-modification of the tissue. Laser treatment can be used as an adjunct or alternative mean of conventional mechanical periodontal therapy. Lasers can reach areas inaccessible to conventional instruments. Mechanical treatment usually forms smear layer on the root surface containing bacteria, debris of infected cement and particles of calculus. Laser treatment does not produce smear layer and along with detoxifying and bactericidal action creates favorable conditions for better healing process and attachment of periodontal tissues. Ablation of soft tissue wall of periodontal pocket with removal of the epithelial layer and inflammatory lesions creates good conditions for rapid healing process. Bleeding after laser treatment is significantly less compared to conventional SRP. Patients report little or no postoperative discomfort [4,9,14,15].

**Materials and methods**

Ten kidney transplant patients of average age 40 years with clinically and radiographically (panoramic x-ray) established chronic periodontitis, were enrolled in the present study, from February to October 2012. Definition of Page and Eke (2007) was used as clinical criteria for presence of periodontitis. According to this definition periodontitis is established if there are at least two not adjacent sites with interproximal loss of attachment ≥ 4 mm or at least two not adjacent sites with probing periodontal depths ≥ 4 mm. Inclusion criteria for patients in the project are:

- At least 10 natural teeth;
- No history of periodontal treatment in the last six months;
- Not received antimicrobial drugs in the last four months.
Patients were randomly distributed into two groups: first group – adjunctive laser therapy of one of the deepest periodontal pocket with PPD≥4mm with diode laser – n=4; second group – adjunctive laser therapy of one of the deepest periodontal pocket with PPD≥4mm with Nd:YAG laser – n=6.

At the initial examination all teeth of the dentition were examined and registered. Clinical periodontal examination was conducted with computerized periodontal probe (Florida probe ®) by trained examiners. Periodontal pockets were probed at six sites around each tooth, including the third molars. Measured data included probing pocket depth (PPD); clinical attachment loss (CAL); bleeding on probing (BOP). Data were recorded and analyzed in electronic file for each patient in the software of the computerized periodontal probe. Evaluation of the oral-hygiene status was done by oral-hygiene index OHI included in the computer software. After periodontal examination the two deepest periodontal pockets in two different quadrants of the dentition was determined for each patient. Samples were taken from each pocket for microbiological examination. Microbiological samples were taken from each of the selected pockets with three sterile paper pins, after cleaning of supragingival plaque. Each pin was for one of the three microbiological tests – cultural methods for aerobe and anaerobe bacteria, and diagnostic PCR. Each patient received mechanical periodontal therapy including removing of plaque and calculus with ultrasonic scaler and scaling and root planning (SRP) with hand instruments. After debridement of the dentition as adjunctive therapy in one of the two selected periodontal pockets was applied a diode laser with parameters of radiation as follows (1W, 20 Hz, ¼, 20 s, Ø=320) in the first group, and the Nd:YAG laser with parameters of radiation as follows (20Hz; 2W; MSP – 4 applications in 20 seconds each) at second group. The second periodontal pocket serves as control. All patients received detailed instructions on methods and means of oral hygiene for improving and maintain good oral health. Appropriate brush and toothpaste as well as interdental hygiene tools, chlorhexidine digluconate containing mouthwash were recommended.

Patients were invited for periodontal reevaluation three months later when new microbiological diagnosis of selected periodontal pockets was conducted.

**Statistical analyses**

1. Descriptive analysis.
2. Non-parametric test of Mann-Whitney – to verify the hypothesis testing of difference of two independent samples.
3. Non-parametric Wilcoxon-test – to verify the hypothesis of a difference of two dependent samples.
Results

According to the calculated average loss of clinical attachment, examined patients can be distributed into the following groups: two patients with mild generalized chronic periodontitis (CAL 1-2мм); six patients with moderate generalized chronic periodontitis (CAL 3-4мм); two patients with severe generalized chronic periodontitis (CAL>5мм).

Table 1 Comparative presentation of indexes OHI, CAL, BOP, characterizing the periodontal state: initial status and reevaluation at the 3 month of the study

<table>
<thead>
<tr>
<th>Index</th>
<th>n</th>
<th>Time of registration of the index</th>
<th>Min</th>
<th>Max</th>
<th>$\bar{X}$</th>
<th>SD</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>OHI</td>
<td>10</td>
<td>The initial status</td>
<td>1,0</td>
<td>2,2</td>
<td>1,5</td>
<td>0,35</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 month</td>
<td>0</td>
<td>2,0</td>
<td>0,94</td>
<td>0,58</td>
<td></td>
</tr>
<tr>
<td>BOP</td>
<td>10</td>
<td>The initial status</td>
<td>6%</td>
<td>92%</td>
<td>34,4%</td>
<td>25,3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 month</td>
<td>11%</td>
<td>75%</td>
<td>33,6%</td>
<td>17,2</td>
<td></td>
</tr>
<tr>
<td>CAL</td>
<td>10</td>
<td>The initial status</td>
<td>1,9%</td>
<td>6,9</td>
<td>3,8</td>
<td>1,51</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 month</td>
<td>2,0</td>
<td>7,7</td>
<td>3,69</td>
<td>1,85</td>
<td></td>
</tr>
</tbody>
</table>

Significantly lower average values for OHI was established at the reevaluation at the 3 month compared to the initial values of the index (p=0.011). According to other indices there were no significant differences between initial values and reevaluation values (p>0,05).

**OHI:**

Oral hygiene index of patients (OHI) is presented as an average values for the initial status and reevaluation status at the third month of the survey, separately for both study groups. All patients observed improvement in oral hygiene in varying degrees – Table 1.

**BOP (bleeding on probing):**

Bleeding on probing is calculated as a percentage of bleeding on probing sites to the bottom of the periodontal pocket or gingival sulcus, from all sites that been probed (six sites per tooth), in each patient and as average value for each group, separately for the initial and reevaluation statuses. In some patients a higher prevalence of bleeding sites is observed despite absence of plaque, maintaining a good oral hygiene and a general improvement in the periodontal condition. This could
be attributed to the immunosuppressive therapy of transplant patients, administrating new drugs or changes of intake regime of present drugs, etc.

**PPD: pocket probing depths**

**Figure 1. Percentage distribution of initial and reevaluation values of pocket probing depths (PPD)**

Pocket probing depth is calculated as a percentage of different measured pocket depths (sites with PPD < 3.4 mm; PPD 3.4-5.4 mm and PPD > 5.4 mm) for each group of patients. Same data were calculated at re-evaluation of the patients at the third month of the study. Comparing the results for PPD of the initial study and re-evaluation in both groups is found increase in the percentage of sites with PPD < 3.4 mm and reduction in sites with PPD 3.4-5.4 mm (figure 1).

When reviewing patients files there are two cases that stand out, with severe periodontitis and bad compliance. In these patients performed periodontal therapy does not lead to good results. They even show deterioration of the periodontal condition – areas with deepened periodontal pockets and continuous bleeding on probing. One of the patients reported at the reevaluation visit for a spontaneously exfoliated lower incisor. Both patients had unsatisfactory oral hygiene with clearly visible dental plaque that covered most of the tooth surfaces and filled the interdental spaces.

**CAL (clinical attachment loss):**

Table 2 Comparative presentation of CAL values: initial and reevaluation values at the third month

<table>
<thead>
<tr>
<th>Index</th>
<th>n</th>
<th>Method of registration</th>
<th>Time of registration</th>
<th>Min</th>
<th>Max</th>
<th>X</th>
<th>2,10</th>
<th>Level of significance</th>
</tr>
</thead>
</table>

57
There are no significant differences in mean values of CAL between initial values and reevaluation values 3 months later, both in laser treated and in control teeth.

Clinical attachment loss is calculated as average value for each study group. The results show decreased in the mean loss of attachment without statistical significance in both groups of patients (table 2). In both groups is observed gain of attachment. To this point, there are no marked differences in the studied parameters between diode laser group and Nd: YAG laser group. In both groups of patients laser treated periodontal pockets (diode laser and Nd: YAG) showed no statistically significant change in mean PPD and CAL. Exceptions to this are two patients with very good oral hygiene standard, which showed decrease in probing pocket depth and gain in attachment.

**Conclusion**

The intermediate results from this study are promising and tend to improve periodontal health of medical compromised patients such as kidney transplant patients.

**Acknowledgment**

This study is performed thanks to financed by Scientific Research Fund project - MU 03-24/12.12.2011 “Comparative study of microbial reduction of periodontium after disinfection with diode laser and Nd: Yag laser of renal transplant patients”

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<table>
<thead>
<tr>
<th></th>
<th>treatment of teeth</th>
<th>of the index</th>
<th>3 month</th>
<th>6 month</th>
<th>12 month</th>
<th>18 month</th>
<th>24 month</th>
<th>30 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL</td>
<td>10</td>
<td>L</td>
<td>3,4</td>
<td>13,0</td>
<td>6,96</td>
<td>3,38</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>The initial values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 month</td>
<td>2,0</td>
<td>13,0</td>
<td>6,68</td>
<td>2,13</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>K</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 month</td>
<td>3,0</td>
<td>9,0</td>
<td>5,52</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*L- laser treated tooth, K- Control tooth*

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Laser bioenergetics and bio synergetic approach in prosthetic dental medicine

Kamenova J.¹

Summary

Background

Prosthodontics is a very responsible process in dental medicine due to its main task - to achieve full aesthetic, functional and sensory neuro-rehabilitation of the masticatory apparatus in which the most important condition is to harmonize and align teeth contact elements of the mandibular joints, muscle activity and movements of the jaw without damaging periodontal and bone structures and maintain strength and integrity of soft tissue in contact with the prosthetic constructions. Laser dentistry is developing in many new directions one of which is Biological medicine.

Purpose and Objectives

The author has set the main task to explore the use of biological laser therapy to achieve complete rehabilitation of the masticatory apparatus in cases of manifest functional pathology. The main goal is to provide fundamental, clinical and practical situation for to create a system of standards of frequency therapy, consistent with generally accepted international Curriculum guidelines for Laser Education, ALD, USA and comply with country conditions. The author also has made a thorough analysis of the theoretical achievements so far and presented her own clinical observations with the aim to determine which doses are most acceptable to the climatic and demographic conditions in Bulgaria and they can be recommended as most effective for the needs of prosthetic dentistry.

Material and methods

The author has developed two research projects on the application of PDT in prosthetic dentistry during six years. A long – lasting clinical experiment on 1500 (800 women and 700 men of middle age - 47 years) patients with known functional pathology of the masticatory apparatus is made. The new paradigm of modern BIOSINERGETICS is applied – simultaneous application of different sources of laser light with different physical parameters such as overall treatment effect can be

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obtained from their complex usage. The method of Medicine based on Evidence is preferred - collecting clinical data from randomized studies and construction of experimental models, creating own original hypothesis on which is formed an individual model for optimal laser stimulation for each clinical case.

Results and Discussion

Investigations on models of situations in the mouth and extensive clinical observations are provided on the basis for recommendation of some new optimal doses of operating modes. The contribution of this new original proposed paradigm is the application of combined laser therapy for rehabilitation of the maxillofacial area in reflexive processes and harmonic function between the masticatory apparatus, joint elements, muscle activity and nerve sensory control of movement of the mandible during function, and prevention and treatment of trauma from occlusion and parafunktions. The author offers the following outlook for the application of biological medicine as a new branch in the dental practice that takes place on several levels: PDT - red / infrared laser Biostimulation - intraoral photodynamic laser therapy, infrared PDT - resonance fotobiomodulation red / infrared laser acupuncture - laser bioenergetics.

Conclusions

Complex PDT in bioenergetic and biosinergic aspect so far is the most effective, fast and efficient method that the author is recommend for use in Prosthodontics in the case of functional pathology of the masticatory apparatus.

Introduction

Prosthetic treatment is a highly responsible process in the Dental medicine because of its main purpose – the achievement of a full aesthetic functional and neurosensory rehabilitation of the masticatory system, where the most important task is to be harmonized and synchronized the interdental contacts, the temporo – mandibular joint (TMJ) elements, the muscle activity and the mandibular movement, without to be damaged the periodontal and the bone structures and to be saved the health and the integrity of the soft tissues, which have contact with the prosthetic appliances. This task can be solved with the help of the laser photodynamic therapy (PDT) in one or another degree, in dependence from the degree of the achieved PDT biostimulating effect.

Contemporary tendencies in the scientific and the experimental work about the laser biostimulation effectiveness provoked by PDT, are in the field of the bioenergerties achievement not only in the target and regions, but also in the transfer of the inserted in the human organism laser light throughout the whole human body. It is well known that the laser light even if applied locally has the opportunity to be transported throughout the human body in deferent levels. A large number
of experimental data has been collected and many authors have discussed the depth of the laser beam penetration in the targeted tissues and about its transmission, which is essential for the determining of the PDT dose. [1-18] The newest tendencies of this development are targeted to the clinical application of the Bioenergetics and Biosynergetic phenomenon of the laser radiation over the whole human organism at all levels of its biological organization. The base for the new paradigm development are the following mostly important up to this moment theories – The Biosynergetics theory of Aging, proposed by – Prigogine about energydonored PDT to the biological phenomena, [13,14]; the Cable Hypothesis, as well as the theories of Grothuss Mechanism ,[4,11]; and Soliton Mechanism, [2,3,18]; which are referred to the autooscilating energetic and wave processes in the human organism, the receptor regulation of the cellular mitochondria and the phenomenon of energy accumulation in dissipative systems in the human body, and also energy emission from different organs.

An important advantage of this new approach is the resonance photoacustic effect of the laser radiation over the bioenergetic organ structures, which main effect is the provoking of the corresponding biological response. Still, however, the mechanisms of the laser beam organ and system interactions are not sufficiently researched in order to be determined the exact dosage in each clinical case and the patient’s risk. The biochemical mechanisms, that lie in the base of the positive effects of the laser radiation are not completely cleared and understood, which is a reason for the complexity of the exact choice of the physical parameters of the different laser systems. The classification and the specification of the sources of the healing light in wide spectrum is systematized (Various sources of radiation technical classification and specification) [1] The Golden standards are being suggested under the form of therapeutic protocols. They are only a direction for an incipient approach in the laser therapy. It is known, that the human body has an individual optic characteristics and because of that the search is targeted to improvement and unification of the standards, not to discussions of doses and their complex calculation. Until recently the Golden standards for the dosing of the laser Energy for PDT where categorical – optimal dose =4J/cm² for intraoral application in CW mode, 8J/cm² for the TMG dysfunctions treatment and 0,1J/cm² for laser acupuncture therapy. These standards where build from the Sweden school of Jan Tuner and Lars Hode, as well as from the Norway Laser Academy from Marita Luomanen. [1,6,7,9,10,15,17] . Tiina Karu revised these values and pointed that these doses are not sufficient enough for achievement of an effective biostimulation and recommended they to be raised. [1,6, 7, 17].

Many of the achieved experimental and clinical results are already published and discussed in our earlier publications. [19, 20, 21]. In the recent work we will pay special attention to the choice as the most correct approach in the laser therapy.
Goal and tasks

The aim of this clinical study was to build our own original scientific and working hypotheses and to suggest a new approach for treatment of the functional pathology of the masticatory system, that to guarantee a PDT high healing effectiveness for the achievement of a full aesthetic, functional and neurosensory mouth and masticatory system rehabilitation and restoration.

For this goal we build the following tasks:

1. To build our own working hypothesis about the mechanisms of laser radiation interaction effect over the human organism accordingly to its individual optical and bioenergetics characteristics, based on established theories and paradigms.
2. To create our own method of frequency – resonance laser therapy that to be used for prophylactics and treatment of the functional pathology of the masticatory system.
3. One of our main tasks was to build a new method for combining and synergetic action of the Bioenergetic phenomenon of several laser sources with different wavelengths for provoking of a specific biological response.
4. To be created fundamental clinical and practical situation for building of frequency - therapy standards system, based on the Curriculum guidelines for Laser Education, ALD, USA in consideration with the conditions in our country. [15]

Materials and Methods

For a period of 6 years we have been working for 2 scientific projects on the PDT application in Prosthodontics. We examined and treated 1500 patients (757[50,4%] female and 743[49,6%] male in average age of 47 years) suffering from functional pathology disorders of the masticatory system. The patients were divided into 5 groups accordingly to the specific pathological functional disorders. The patients in each group were further distributed into two - working and control groups. The main clinical criteria for all groups were the occlusal trauma in its different manifestations. In each group of patients were included approximately 300 people (at round 150 for the working group and 150 – for the control group with the same diagnosis). The patients in all working groups (750 people [50,0% of all patients ] – 385 female [25,7%] and 365 [24,3%] male) were treated with resonance LT using our original new approach. The patients from the control group (750 people [50,0 %] - 372 [24,8%] female and 378[25,2%] male ) were treated with the classical healing methods, without the usage of laser radiation.

The patients groups distribution according to two clinical signs – the type of manifested pathology in working and control groups and according to their sex is presented in Tabl.1
Distribution of the examined and treated patients in groups according their pathology and sex.

In group 1 are included approximately 300 randomly gathered patients (149 female [9.93% for all 1500 people] and 151 male [10.07% from all 1500 people]), who were distributed in two groups of about 150 people [10% of all 1500 people] each. These patients were diagnosed and treated for occlusal trauma, clinically manifested with perio damage in a moderate degree and presence of periapeical bone lesions. For the second group the main clinical sign was occlusal trauma with manifested horizontal, vertical and combined bone resorption and alveolar ridge atrophy. As seen from Tabl.1 in these group were classified 154 female [10.26% from all 1500 people] from all two subgroups – working and control and 146 male [10.74% from all 1500 people]. In Group 3 were included patients with diagnose occlusal trauma combined with myofascial pain, migraine type of headache, and reflected neck and back pain. From them 146 female [10.74% from all 1500 people] and 154 male [10.26% from all 1500 people]. The patients distribution in group 4 was conducted according to the clinical sign of OT and consequent trigeminal nerve neuralgia. In this group were included 156 female [10.4% from all 1500 people] all together for the two subgroups working and control and 144 male [9.6% from all 1500 people]. In Group 5 were placed patients with manifested OT combined with TMJ disorders. It was consisted from approximately 300 people for the two subgroups – working and control, and similarly to the other groups – 152 female [10.13% from all 1500 people] and 148 male [9.87% from all 1500 people].

For a greater accuracy in the determination of the doses for optimal laser stimulation the patients from the working groups - 750 души [(50, 0 %) from the whole number] were divided in 2 groups according to characteristic clinical symptoms – the first group consisted patients with manifested acute inflammatory reactions and the other one with chronic disorders. The patient’s distribution is represented on Fig.1 and Fig.2.
The first task that we solved was the creation of a working hypothesis for the bioenergetics transformation and the biosynergetics effects of the laser radiation over the whole organism.

We built our original hypothesis experimentally. During the 6 years we created several our original experimental models of the situation of the oral cavity and investigated the influence of the intrinsic and extrinsic factors, as well as the electrochemical processes, which might influence the direction of distribution and transmition of the laser beam – electrolytic model, model for measurements of the magnetic induction and homogenometry and also a model for investigation of the trancemembrane transport which we have described in details in our previous publications. [18,19,20]

The second task was solved by the construction of a model for achievement of optimal laser stimulation. Our approach was individual for each of the laser treated 750 patients [50, 0 % from all number] and the optimal treatment dose was determined in several stages byphysiological, therapeutic, functionally – diagnostic and etiologic model until the most appropriate model for optimal laser stimulation is built. The investigated process included biosynergetics estimation of three types of laser systems with different laser light sources, as follows: LLLT diode system – Six Touch Screen, (Atlantis, Plovdiv, BG) - Red probe (\(\lambda=680\) nm , output power - 30mW, in CW mode of emission, coherent light), IR probe (\(\lambda=904\) nm, 18W per pulse, in pulse mode up to 4000Hz, coherent light), multiyidiode magnetic cluster probe , (\(\lambda=904\) nm IR coherent light \, output power - 18W, noncoherent light sources – 3 LED - \(\lambda=830\)nm, 3 LED - \(\lambda=740\) nm ). High power diode laser system – Six Lancet (Atlantis, Plovdiv, BG) - \(\lambda=980\)nm, output power - 7W. High power Er:Cr/ YSGG - Biolase, USA and output power - 6W.
The greatest attention in our scientific works was paid to the photoinfrared photobiomodulation with LLLT diode Ga-As 904nm Six Touch Screen, (Atlantis, Plovdiv, BG).

On the Fig.3 and Fig.4 are shown pictures of the used laser devices, arranged by the way of their combined usage for achievement of bioenergetics stimulation and biosynergetics effect.

All the 750 people [50,0 % from the whole number ] from the five working groups were treated as follows: extraoral laser magnet therapy – magnet ring 50H, optimal dose OD = 8,5 J/cm² – sedation of the biosynergetics effect of the coherent red laser light in combination with 3 IR non-coherent diode light sources. The radiation was directed from right to left following the Wolkevitz lines or the Fall’s fields. The next stage was a neuromuscular relacsation of the masticatory muscles by applying TENS – trigger electric acupuncture with frequency of - 60Hz. [20]

Before the main prosthetic treatment we performed a laser assisted prophylaxis of the soft tissues with two main purposes: pain management and minimal bleeding during the process of abutments preparation or preprosthetic treatment. For the purpose of the preprosthetic treatment we used 3 types of laser systems – high power laser acupuncture for micro invasive painless and wound free and bloodless procedures oral hygiene prophylaxy Er:Cr/ YSGG, Biolase Whitening, USA: treatment of caries lesions , endotreatment of abutments which are with periapical lesions , laser assisted perio management ( in accordance with the Golden standard - LANAP), laser surgical gingival correction , esthetical shaping of the gingival line, frenectomies, no flap crown lengthening, alveolar ridges correction and others. [1,15]

The third task was solved by the following approach – leading sign for solving this main task was the newest paradigm of the modern biosynergetics – application of different laser light sources with different wavelengths so that the common treatment effect to be achieved by their combined usage. On the all 750 patients from the five working groups we applied a combined LT, using low
POWER AND HIGH POWER DIODE LASER SYSTEMS IN BIOSYNERGETICS EFFECT ACCORDING TO OUR OWN CLINICAL APPROACH FOR INTRAORAL SEDATION RED LASER LIGHT IN CW MODE FOR 7’ ALONG ONE OF THE JAWS AND LASER IR TONIFICATION WITH 40 Hz - 3’ PER JAW – TOTAL DOSE – RED SEDATION BY SCANNING METHOD FOR 14’ FOR THE BOTH JAWS – OPTIMAL DOSE = 3.5 - 5J/cm² AND IR TONIFICATION – OД = 2-4 J/cm².

Two months after the described procedures we applied vibration photoinfrared photobiomodulation in two stages – 20,40,400, and 900Hz, 7’ for a jaw with scanning movements for each of them always from right to left, for the maxilla, and after that in the same order for the mandible with an optimal dose = 3.5 – 7.5J/cm². For the next four days we applied vibration photoinfrared photobiomodulation on acupuncture method. For each patient we prepared an individual recipe for combined of different types of acupuncture points – the acupuncture of the far body points we made with 20Hz,20” in each point – optimal dose = 0.8 -1.0J/cm². The face acupuncture points were treated with 40Hz, 40” each. We examined the healing effectiveness of the following wavelengths – 680, 780, 830, 904 and 980 nm.[19, 20]

Our clinical observations were randomized and systematized in an exact order and this gave as the opportunity to solve the forth task according following sings: changes of the functional indicators dynamics, produced by the resonance effects of stimulation of the electromagnetic waves on the human body; we also investigated the healing effect of the different frequencies of the laser radiation in the visible spectrum and the near IR light over the main powerful subjective symptoms of OT – pain, dysfunction, myalgia, neuralgia and periodontal damage.[18,19,20]

We registered the corresponding biological response of the separated working patients groups with manifested characteristic symptomatic by instrumental analysis and we evaluated it in accordance of the necessity of medication treatment, immunosuppressive and local treatment – anti-inflammatory, antimicrobial, poly – vitamins therapy and others. We performed scientific researches over the effectiveness of the laser radiation in accordance to the biological biochemical indicators before, during and after the treatment, which allowed us to do a diagnostic prognosis for the direction of the healing biostimulative processes in accordance of two clinical signs – early and late evaluation of the healing effect of the laser light in the different regions of the light spectrum. [19]

Results and discussion

Based on the Skulachev W., Prigogine I., Pogodin S. i Sovage S., we had the knowledge and the experience, necessary for the construction of a model for an optimal laser stimulation for treatment of functional pathology of the masticatory system. [12,13,14,16, 18, 19, 20] The new in our paradigm is the application of the combined laser therapy for rehabilitation of the maxillo – facial
region in terms of the reflex processes and the harmonic function between the masticatory system, the temporo–mandibular joint elements, the muscle activity and the neuro–sensory regulation of the movements of the mandible during their function, as well as for prophylactics and treatment of the occlusal trauma and the trauma, resulting from parafunctions. [19,20] With the use of the photoinfrared resonance influence of the laser light the reparative processes and the lymph circulation are being accelerated and autooscilating processes in the brain and the other human organs are being modulated. While Tiina Karu accepts, that the most – important mechanism of the healing effect of the laser radiation is the cascade energy transformation in the oxidation–reduction system and the increase of the energy content in the mitochondria, we accept, that the high effectiveness of the laser therapy can be achieved only if a cascade energy transformation of the laser beam over the whole body is produced.

The received results are giving us ground to point the combinative laser therapy, which includes resonance and informational PDT as a highly effective approach, which provokes a fast and an adequate clinical response and is distinguished with a durable healing effect. For the upper mentioned 6 years we achieved in all of the patients in the five working groups a full rehabilitation of the masticatory system, a harmonization of the oclusal relationships between the teeth rows of both of the jaws and a synchronization of the function of the elements of the TMJ. [18,19,20] After only 4 procedure we observed complete healing, successful epithelisation after the laser preprosthetic surgical treatment and attenuation of the powerful subjective symptoms in all of the 750 patients. The randomized clinical studies showed, that up to the fifth year after the treatment are not observed recedives or secondary damage. [19,20, 21]

The results showed that if we apply only intraoral methods of radiation during the PDT we cannot perform the maximal photobiostimulation. Only with the combined laser therapy with different light sources, and by combining the effect of multiple light sources with a coherent and a non–coherent radiation, and with the usage of the synergetic application of a suitable resonance regime, we can achieve a high efficiency for short time with long lasting effect.

On figures 5, 6 and 7 are presented the therapeutic protocols for PDT of the gingiva and the periodontium of the teeth during a full prosthetic and aesthetic rehabilitation of the masticatory system.
Fig. 5, 6 and 7

On the schemes are shown the methods of radiation – scanning, in points, garland – shaped, as well as the regions of application of the laser beam. In the tables are represented some of the most – important recommended physical parameters of the laser radiation, such as output power (P)[mW]), regime of radiation and the fluence [J/cm²], which we accepted as an optimal dose. As seen from the figures above the recommended from us doses are a bit higher than the Golden standard, but are very well tolerated by the patients. We support the position given from Tiina Karu and accept, that the optimal dose for PDT in the prosthodontics must be higher than the golden standard (optimal dose = 2-4[J/cm²]) or optimal dose at around 3,5 -8,5 J/cm² for intraoral therapy along the two jaws – scanning radiation or garland – shaped, radiating the gingival from right to left and optimal dose = 8,5 -12,5 J/cm² for the treatment of the TMJ. As the most appropriate dose for the PDT of the muscles in cases of muscle pain and contractures with a light from the visual red spectrum, we can recommend the optimal dose of 4-6 J/cm². If for the purposes of the prosthodontics is necessary to be achieved muscle relaxation, the optimal dose for the near infrared laser light can be increased to 7,5 J/cm².

Conclusions

1. Our working hypothesis for the application of the laser light in the prosthodontics postulate that the laser beams by the mechanism of ion transformation through the biomembranes transfer the energy in the masticatory muscles, which decreases their muscle activity and consequently the masticatory pressure. These processes of biostimulation and muscle relaxation are favorable for the periodontal complex and the bone structure of the jaws and help for the harmonization and the synchronization of the elements of the TMJ.

2. The resonance and the informational PDT produces a fast and an adequate clinical response and is being distinguished with a very high treatment effectiveness. We recommend a new approach for application of the Biological medicine as a modern branch of the prosthodontics at several levels: PDT – red / infrared laser biostimulation – optimal dose = 4-6 J/cm², photo dynamic intraoral laser therapy – optimal dose = 3,5 -8,5 J/cm², infrared PDT – resonance photo bio
modulation – optimal dose = 8,5 -12,5 J/cm², red / infrared laser acupuncture up to 1 J/cm² – laser bioenergetics.

3. The combinative PDT in bioenergetic and biosynergetic aspect is the most effective, fast and successful method up to this moment, that we recommend for application in the prosthodontics for treatment of the occlusal trauma and the functional pathology of the masticatory system.

Further publications will follow, which will import more clarity and will reveal the new biosynergetic approach in all aspects of its application.

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Case report

Clinical case of retreatment of a lower first molar with five root canals

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Summary

The anatomic characteristics of the different teeth and their variations are a real clinic challenge. Literature data of the morphological features in the lower first molar is represented.

The aim of the article is to represent a clinical case of endodontic retreatment and treatment results of a chronic periodontitis with exacerbation in first lower molar with five root canals.

The retreatment protocol of tooth 36 with apical periodontitis and five root canals, diagnosed radiographically and with a microscope is being described. In the mesial root there are 3 root canals discovered and in the distal root two canals. The radiographic control during the retreatment and 3, 6 months after show the healing process of the bone lesions (periapical, lateral, furcation). The importance of knowing the root canal morphology and applying contemporary means for diagnostics (radiographic images with different projection and operative microscope) is being emphasized.

Key words: first lower molar, root canal morphology.

The first lower molars are the first one to erupt of the permanent human dentition and that’s why they need a special treatment.

According to the literature data these teeth have relatively big coronal part, two, seldom three roots and three or four root canals [22, 5]. Fundamental for the endodontic treatment success is the knowledge of the inner tooth anatomy - the pulp chamber and the root canal system. The root canal anatomy can be a real challenge directly related to the treatment approach. According to R. Slowey (1979) the root canal anatomy is a route map for the successful endodontics [23].

The general aim of the root canal treatment is preservation and healing of the apical and periapical structures. That means cleaning, shaping and disinfection, allowing tri-dimensional obturation of the canals [H. Schilder, 1967, 1974 – 5]. The main reasons for endodontic failure are poor shaping, cleaning and consequent inadequate root canal filling. The anatomical variations in the different teeth are a real challenge that we encounter daily and to great extent they define the endodontic success.
The proper access of the pulp chamber to open, relocate the orifices and the root canals are fundamental steps in the root canal treatment allowing identification of the variations, number and position of the root canals.

There are numerous investigations related to the root canal variations in the first lower molar. In 1925 W. Hess [1] performed an investigation with 512 first molars and reported that 0.3% have one root canal, 17.7% have two root canals, 78% have three root canals, 4% have four root canals. In 1992 De Deus (1992) investigated 75 teeth and found that 8% have two canals, 56% - three canals, 36% - four canals [1].

Other authors performing similar investigations are T. Okumura (1927), A. Skidmore and A. Bjorndal (1971), F. Pineda and Y. Kuttler (1972), G. Hartwell and R. Bellizzi (1982). They didn’t find cases with five root canals. According to Skidmore and Bjorndal [22] relatively one third of the lower first molars have four canals. These canals are separate and end with separate foramina, can have one mutual apical foramen or they have communicating transversal anastomoses. In the furcation area of the lower molars there are many other foramina.

A. Martinez-Berna in 1983 found five canals in 1.22% of the investigated teeth sample (29 of total 2362 teeth). A. Martinez-Berna and P. Badanelli [12] stress the importance of searching for a forth or even fifth canal. The authors describe the treatment of a symptomatic tooth in 28 year old patient with irreversible pulpitis in which they found 5 canals (2 mesial and 3 distal canals) with means of an operative microscope. H. Fabra-Campos in 1985 investigated 145 first lower molars and found in 2.75% - five root canals [6]. Only E. Jacobsen, K. Kick and R. Bodell (1994) found more cases - 12 from 100 molars with third mesial canal [1,10]. That’s why the area between the mesiobuccal and mesiolingual canals should be carefully investigated for a fifth canal.

The complex root canal anatomy shows also ethnic dependence [24, 7, 8, 9]. In investigation of the first lower molar in the Chinese people it is found that 51.4% of the teeth have four root canals and 25.8% are with separate distolinguual root [24]. According to another investigation of Chinese people the frequency of three roots in first lower molars is 31.97% [8, 9]. The morphological variety of the different roots and root canals in the tooth samples is also described [24, 8, 9]. According to publications [5], done on other populations, the frequency of the third rot is 13% and in 1% of the lower first molars there are 5 canals. Data is published of different clinical cases, also retreatment with the unusual root canal anatomy and morphology [3, 4, 10, 21, 2, 25, 24]. The third mesial root canal is between the buccomesial and linguomesial root canal [20, 3, 11]. These mesial canals are described to be narrow, sometimes separate or joining one of the main canals. For finding an extra root canal it is important to make good investigation of the pulp chamber with suitable diagnostic means [20, 3, 4, 11].
The clinical skills to localize, clean, decontaminate and fill all root canals are necessary, but one shouldn’t forget the importance of the clinical prediction of the complex root canal morphology and the use of contemporary treatment means, digital images, CBCT, microcomputer tomography [19, 13, 16, 7, 10, 11, 24]. With the help of the operative microscope the clinicians are able to find the fifth canal in 1-15% of the cases [20, 2]. The confirmed diagnosis should be combined with radiological and tomography images [10].

The aim is to represent rare clinical case of endodontic retreatment and treatment results of a chronical periodontitis in acute phase in a lower first molar with five root canals.

Clinical case

28 years old female patient was referred to the clinic for retreatment of tooth 36. The patient’s complained about permanent dull pain when touching with the tongue tooth 36. The patient reported to have undergone root canal treatment on the same tooth a year ago. After clinical and X-ray investigation periodontitis chronic granulomatosa diffusa exacerbate was diagnosed. (Fig.1). On the segmented Dick radiograph periapical lesions round the apical part of the distal root of tooth 36 was observed (5 PAI, Orstavik) as well as the middle third of the mesial root together with the furcation area was involved (4PAI, Orstavik-14,15).

During the first visit a defect distooclusal composite filling was removed. The pulp chamber was opened and investigated with a microscope (D.F Vasconcellos-Brazil). Precise investigation of the pulp chamber floor showed 3 mesial root canals and 2 distal canals. The entrance of the third mesial canal was between MB and ML orifices. The working length was measured with Raypex 4, VDW as
well as radiographically determined (Fig.3). The main mesial root canals were 21mm long and the extra mesial root canal was 2.5mm shorter (18.5mm) and pointing to the MB canal.

![Figure 3. X-ray determination of working length](image)

The root canals were cleaned and shaped with hand files and rotary K3, Sybron Endo according to the crown-down technique. The root canal treatment of this molar included the following irrigation protocol: 2.5% hypochlorite, destilled water, 3% hydrogen peroxide, destilled water after changing every instrument, ultrasound activation of the irrigation solution and ozoning with hypochlorite for 1 minute in each canal according to the method (Ozone DTA, Taiwan). The tooth was closed with sterile cotton pellet and temporary restoration.

In 2 days during the second treatment appointment the tooth had no symptoms. The irrigation protocol of hypochlorite ultrasound activation and ozone was repeated. For 14 days the root canals were filled with Ca(OH)$_2$ paste with iodoform Metapex, META, Korea and the tooth was closed again.

During the third visit the temporary root canal filling of Ca(OH)$_2$ was removed with hand files and irrigation. Additional cleaning of the canals with rotary files and irrigation was done in order to remove all temporary root canal rests. The root canals were filled with warm gutta-percha, Thermafil and Top SEAL, Maillefer (Figure 4 and 5).

The root canal filling is being controlled with radiographs with different angles (fig. 6, 7, 8). For visualizing of the five root canals the recommended centralizing is 30 degrees distal. For treatment assessment several radiographs are necessary. On some of them it is seen that the third mesial root canal is very close by to the mesio-bucal canal. In the zone of the periapical lesion round the mesial
root there is an accessory canal, filled with sealer. This lateral canal in between the middle and apical root canal third, probably is the reason for the atypical bone lesion engaging the middle of the mesial root and the top of the interradicular septum. Figures 7, 8 and 9 show the two separate root canals in the distal root end up with separate apical foramena. They are with small sealer extrusions. The treatment is finished with sandwich restoration with GIC Fuji IX, GC and composite Filtek P-60, 3M ESPE.

Figure 4. Pulp floor, 2 distal filled orifices with Termafil.

Figure 5. Pulp floor and 5 filled orifices

Figure 6. Postoperative X-ray of tooth 36

Figure 7. Postoperative X-ray of tooth 36
Clinical and radiological control of the tooth is being ensured. The control radiographs show big localizing of the bone lesion 3 months after the root canal filling (figure 10 and 11). The broad periapical lesion is highly affected and almost totally disappearing of the interradicular bone lesion of the middle third of the mesial root is to be seen. The sequence of segment radiographs of the first lower molar showed that the bone structure round the mesial root is completely recovered. The bone
lesions round the apical part of the distal root and the interradicular septum are immensely restricted (figure 12 and 13).

Figure 12. Postoperative X-ray of tooth 36
6 months after the root canal filling

Figure 13. Postoperative X-ray of tooth 36
6 months after the root canal filling with 30 degrees distal angle

According to the standards of the European endodontic association the root canal retreatment of the periapical periodontitis of the first lower molar is successful. The reasons for the relatively quick healing are most probably complex. Main role has finding the extra root canal in the mesial root, placed between the MB and the ML canal. Great influence has the mechanical root canal cleaning, filling and the contemporary irrigation protocol. Very interesting is the fact 5 months after the root canal filling and 6 months after the endodontic retreatment the extruded sealer has disappeared.

**Conclusions**

The root canal retreatment of the first lower molar with chronic periodontitis with unusual root canal morphology with an extra fifth canal is a real diagnostic and technical challenge.

This article represents the clinical management of a first lower molar with five canals under a microscope. In the mesial root there are three separate canals to be found. The control radiographs show three different mesial canals which are effectively cleaned and filled. The represented radiographs during and 6 months after the root canal treatment show the healing process of the bone lesions (periapical, lateral and furcational). This clinical result shows the necessity of radiograph images with different projection of the tooth, accurate diagnosis and management of the teeth with endodontic problems and unusual root canal morphology.
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Case report

X ray diagnostic problems in facial traumas – case report with a foreign body in facial regions

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Summary
A case concerning undiagnosed metal foreign body in soft tissues of facial skull is presented. The authors emphasize on the necessity of rendering the radiograms of patients with maxillo-facial trauma in Emergency Departments to be done by radiologist.

Key words: facial trauma, foreign body, X-ray diagnostic

Introduction
Traumatism is a world wide problem; maxillo-facial one is extremely serious because of the diagnostic and therapeutic problems.

Facial skull anatomically consists of many and with complex shape bones which is difficult to be visualized radiologically. That makes us use different and special X-ray projections. And that’s why the facial skull is one of the most difficult regions to explore in X-ray diagnostic.

Clinical practice shows that facial injures are the most serious ones. The most important factor concerning successful treatment is the well-timed and exact X-ray diagnose so that form, function and esthetics can be recovered. [3,6,7,11,12,13]

The question concerning diagnostic of localization and removal of foreign bodies migrating in facial soft tissues has a special place. Foreign bodies transparent for X-rays (Ro negative) are difficult for diagnostic. Using conventional X-ray methods it is relatively easy to find out the non transparent (Ro positive) foreign bodies [1,2,4,5,8,9,10].

It is a common fact that in Emergency Departments one of the first investigations of patients with maxillo-facial trauma is X-ray of skull with conventional X-ray unit in order to exclude bone fractures.

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It is also known that in Emergency Departments different specialists are on duty (orthopedics, neurologist, maxillo-facial surgeons etc.). They fix the X ray examination and usually analyze the information by themselves. They do not look for the conclusion of radiologist, so this often leads to diagnostic mistakes.

We present a case, confirming the existence of such practice in Emergency Departments.

**Case report**

From anamnesis:

41 year old man entered the clinic with pain and swelling in the area of the left zygoma. He complaints of formication in the infraorbital edge and pain in the left eye. The patient has had a car accident 4 months ago. He got a laceration in the left facial part; didn’t loose consciousness. In the Emergency departments he was examined by a neurologist; X ray of the skull were ordered (anterior and lateral view of skull)and he did not find any X ray changes. The wound was sewn.

Two months later the patient got pains in the left eye, tingle in the left infraorbital region. Due to this CT examination was done. The presence of X ray positive foreign body in the left zygoma region was found.

From examinations:

Extraoral examination shows facial asymmetry due to the swelling in the left infraorbital and zygoma regions; nasolabial fold was erased; presence of postoperative scars (fig.1)

![Image](fig. 1)

From the X rays done after the accident we can see:

X ray of the skull – posteroanterior radiograph shows that in the area of left zygoma there is a homogenous X ray positive shadow with metal characteristics which has indefinite polygonal form, size about 5 cm and margins expanding beyond the zygoma (fig.2)
Lateral radiograph of skull shows that X-ray positive object is seen in soft tissue above the roots of teeth and in the lower part of the maxillary sinus (fig. 3).

On CT slices we can observe X-ray positive foreign body – in the soft tissue of the described above facial region (fig. 4).
From operational protocol: Under anesthesia a cut in the base of left lower eyelid was done, the foreign body – a metal car fragment was localized and removed (fig. 5).

Discussion
This case shows that routine roentgenograms of the skull in anterior and lateral view give us very exact information about presence and localization of positive foreign body in soft tissues of the left facial part. The well expressed metal shadow is demonstrative; too typical and no radiologist can miss it. In this case neurologist didn’t recognize this typical X ray symptom for metal foreign body. This caused the development of complications (formication and pain in infraorbital region) and has led to the use of special, expensive and ionizing CT examination. Then the radiologist set the
diagnose, i.e. describe the obvious on conventional X rays typical symptom of metal foreign body in the left facial part.

**Conclusion**

The diagnose of metal foreign body in the facial region could be faultlessly set with conventional X ray methods in Emergency departments. Timely treatment prevents from the development of clinical complications; use of expensive special methods such as CT, recurring surgical treatment etc.

This case shows the necessity of applying of orders for regulating of requirements about authenticity of results of X ray examination of patients with maxillo-facial trauma:
- roentgenograms must be analyzed by radiologist and results declared by him too
- operation must be done only after the results of X ray examination are presented by radiologist or consulted with such one

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