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Immunohistochemical study of interrelation between ageing and dentin-pulp homeostasis

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Abstract

Interrelation between ageing and dentin-pulp homeostasis is a complex biological process characterized by analysis of fundamental events in the cells life span as apoptosis and autophagy, multilayered modulation and regulation of signals pathways. The research toolkit in our work include immunohistochemistry of tissue pulp-dentin sections by analyzing twelve (12) biologically active molecules associated with: non-collagen proteins with a structural, regulatory and signaling role for the mineralization and maturation of peritubular dentin (SIBLINGs); markers of vascularization and angiadaptation (CD34); pro-apoptotic and anti-apoptotic proteins (JAK1-STAT3); signal molecules-switches between different apoptosis pathways (BID-Caspasa 8); markers of cytoprotection by regulating the inhibitory threshold for apoptosis (NFkB); biomolecules for active autophagy, difficulty in mitophagy and organelle ageing (COX2, LAMP2, MAP LCII3). We performed molecular mapping of the senescent odontoblasts and compiled their molecular phenotype of ageing.

Introduction

In the ageing of dentin and pulp, fundamental biological processes, various bioactive molecules, signaling pathways, mediators, modulators, and regulations are involved. In view of the complex and diverse intracellular and intercellular communication mechanisms, much of the functions of biomarkers are unknown or incomplete. The aim of this study is to establish correlations between non-collagen proteins of the SIBLINGs family and lysosomal-mitochondrial path for apoptosis (BID-Caspasa-8), regulatory proteins for gene transcription (NFkB, JAK1-STAT3) and autophagy markers (COX2, LAMP2, MAP LC3II).

Materials and Methods

Ninety freshly extracted intact teeth of healthy individuals were enrolled in the study and arranged in three groups (n=30 in each group) regarding the patients' age: young (14-16 years old), adult (18-40 years old) and senescent (41-78 years old) groups. Conventional protocol of fixation, selective decalcination, dehydration, embedding in paraffin and sectioning to 2.5µm was followed. Immunohistochemistry of paraffin dentin-pulp sections was processed including: paraffin sections were dewaxed in xylene, rehydrated with distilled water, and then subjected to antigen retrieval (sodium citrate buffer, pH 6.0) and incubation with mouse monoclonal antibodies (Santa Cruz Biotechnology Inc., USA), using Leica-Bond Max automated system (Leica Biosystems, Germany). Statistical analysis including Kruskal-Wallis and Mann-Whitney tests, and Correlation analysis were performed (p<0.05).

Results and Discussion

By definition, the correlation analysis is applied to describe the strength and direction of the relationship between variables. The correlation analysis compared how the biomarkers studied changed with each other, showing the degree of connectivity in their expression by correlation index R or correlation factor. In a correlation analysis of data on the expression of biomolecules in dentin and odontoblasts with estimates 0, 1, 2, the strength of dependencies between variable pairs are interpreted in the range: from 0,6 to 0,5 strong correlation, from 0,49 to 0,4 mean correlation and from 0,39 to 0,3 weak correlation. The other co-indicators were not commented on due to a very weak and insignificant link between the indicators. The analysis of the results shows the presence of strong positive dependencies between

DMP-1/Ods-BID (0,505), OPN/Ods-STAT3, OPN/D-STAT3, CASP8-STAT3, CASP8-JAK1 (0,540), CASP8-COX2, SAT3-JAK1 (0,562), SAT3-COX2, JAK1-COX2 (0,576). The interpretation of the results of the correlation analysis shows that the strongest relationship between the biomolecules studied is between OPN expressed by odontoblasts and MAP LCII3, a marker of formed autophagosome and active autophagy (0,688). From the other representatives of the SIBLINGs family, the correlation DMP-1/Ods-BID is impressive, which means that the expression from the odontoblasts of DMP-1 increases in the same way as the expression of the pro-apoptotic protein BID. This means that the process of deposition of hydroxyapatite crystals around collagen fibril is a dynamic process that is not limited in time. Mineralization is active and in samples of old pulp and odontoblasts do not lose their ability to synthesis and secretion of DMP-1 over time, despite their phenotypic change. We can assume that DMP-1 plays a role in the formation and mineralization of peritubular and intratubular dentin, structurally and regulatory (1, 2, 3). The remaining members of non-collagen phosphorylated proteins do not demonstrate significant dependencies. Based on our previous results, the expression of DMP-1 and OPN from odontoblasts shows a similar trend of increase in cellular ageing. With age, we also found statistically significant more BID+ odontoblasts. Therefore, with age, the intracellular flow for apoptosis activation is mediated by BID-Caspasa-8 pathway and it connects the internal lysosomal-mitochondrial pathway and the external receptor-mediated pathway for apoptosis (4, 5, 6). All samples studied, regardless of age, exhibited positive immunoreactivity for MAP LCII3, a biomarker for formed autophagosome, a sign of organelle-active autophagy (7, 8). In the correlation analysis, both primary and secondary grouped data highlight strong dependencies between SAT3 and JAK1, which is in full sync with the fundamental concepts of biological binding of molecules in a single intracellular signaling route SAT3-JAK1. It is a receptor-mediated mechanism for signal transduction (activated with negative stimulation of cytokine receptors) and is a fast and direct pathway of transcription factor SAT3 to the nucleus with a variety of functional effects for the cell, including apoptosis (9, 10, 11). This signaling path for apoptosis is statistically significant more pronounced in old odontoblasts. Another interesting relationship is the correlation between JAK1 and COX2, which also indicates a link between organelle and cellular ageing of odontoblasts (12, 13). Also interesting is the correlation between CASP8 and JAK1, showing a similar change in protease and kinase, which are involved in two different pathways for apoptosis, but both receptor-mediated. They are activated in the absence of cytokines/growth factors and other mytogenic signals, and the presence of TNF α (14).

In summary of the results of the correlation analysis, we can conclude that the markers of cellular and organelle ageing of odontoblasts are DMP-1, OPN, COX2 and MAP LCII3. With age, the SAT3-JAK1 signal path is activated. Including CASP8 and BID indicates a link between the external and internal mitochondrial path for apoptosis.

Conclusions

The "molecular map of ageing" of the odontoblasts in the dental pulp includes:

1. Expression of non-collagen proteins from the family SIBLINGs: DMP-1 and OPN;
2. Difficulty in mitophagy (COX2 excess);
3. Active autophagy (MAP LCII3);
4. Active cytoprotection by NFkB;
5. Active signaling route for apoptosis by JAK1-SAT3;
6. Link between the external (receptor-mediated) and the internal (lysosomal-mitochondrial) pathway for apoptosis by BID-CASP8.
7. Gender determinism for OPN and JAK1-STAT3.

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Oral Pathological Processes Associated with Food Type Preferences and Anthropometrics in Children with Neuropsychiatric Disorders

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Abstract:

Mucosa lining oral cavity is a borderline area between the body and the external environment.

Objective: To study oral mucosa pathological changes in children with neuropsychiatric disorders (NPDs) as possible factors limiting their food choice and deteriorating nutritional status. **Materials and methods:** A comprehensive dental and nutritional examination was performed in a sample of 53 children with NPDs at the age of 5.67 ± 4.33 years in Varna. **Results:** Oral mucosa examination showed various clinical signs of inflammatory alterations in 66,1% of the children. Better mucosal condition is associated with improved chewing ability ($\rho = 0.35$, $p = 0.011$) and preference for solid foods ($\rho = 0.41$, $p = 0.002$). **Conclusion:** Pathological changes of oral mucosa in children with NPDs is a very frequent phenomenon and has a significant association with food choices and, which needs to be taken in consideration as this might affect long-term prognosis of NPDs children.

Key words: mucosal inflammation, food consistency, children with neuropsychiatric disorders, growth

Introduction:

Oral health problems contributing to eating disorders have high incidence and prevalence in children with neuropsychiatric disorders (NPDs) (1). According to Rusinova and co-authors, compromised masticatory function can lead to malnutrition or obesity in childhood (2). Oral mucosa lining the oral cavity is a borderline area between the body and the external environment. In case of soreness of the oral mucosa resulting from inflammatory processes or mechanical irritation, children experience discomfort and this disrupts the normal course of the chewing process. Food consistency choices results from various characteristics, mainly the subjective perception of the patient, as well as taste preferences. On the other hand, the consistency of food can positively impact the conditions in the oral cavity such as self-cleaning and inhibition of plaque progression of solid foods (fruits and vegetables), the structural role of cereals, nuts and legumes and the negative impact and cariogenic function of soft and mushy consistency of food (2).

Objective:

To study the presence of pathological processes in the oral mucosa in children with neuropsychiatric disorders (NPDs) as a possible factor limiting their choice of food consistency and deteriorating nutritional status.

Materials and methods:

We recruited 53 children (25 (47,2%) raised in a family environment (FE) and 28 (52,8%) in residential care homes (RC)) at the age of $5,67 \pm 4,33$ years for the period April-October 2017 in Northeastern Bulgaria. All children had an extra- and intraoral examination. The examination included a comprehensive dental exam, including determination of the bite type, state of the intraoral mucosa among others. The study was approved by the Medical ethical committee of Medical university of Varna

with a protocol № 60 from 23.02.2017. Signed informed consent was given by parents and legal guardians.

Statistical data processing was performed using the IBM SPSS v.23 statistical package. Chi square statistics (X^2) tested the differences in the oral and nutrition status of children living in residential care and home environment. Additionally, Spierman correlation analyses were applied to test the levels of associations between indicators of oral pathology and food preferences, as well as with different anthropometrical indicators.

Results:

Oral mucosa examination showed various clinical signs of inflammatory alterations such as redness and swelling, coated tongue and others in 35 (66,1%) of children. Boys and girls were equally affected – the prevalence was 18 (69,2%) and 17(63,0%) respectively. The analysis of the data resulting from the intraoral examinations has established that the relative share of children with inflammatory lesions of the oral mucosa (n = 35; 71,3%) is more than two times higher in the institutional care facilities (n=25; 89,4%) compared to children who live in a family environment (n=10; 40,0%). The calculated percentage differences between the child care environment of children with NPDs and inflammatory alterations of the oral mucosa are statistically significant ($X^2=16,1$; $p<0.001$). The correlation is positive and significant ($\rho=0,37$, $p=0.006$) showing a higher risk for children living in residential care environment.

The analysis of the data shows a normal level of saliva in the majority of children with NPDs (83%), which suggests a proper course of the first 3 phases of food processing in the oral cavity.

Profuse salivation is observed in 7.50% of the examined children, which is probably an accompanying symptom of the underlying disease. Deep prognathic bite (76%) is observed in the majority of children with profuse salivation. A detailed statistical analysis reveals a statistically significant relationship ($\rho=0.431$, $p <0.04$) between the amount of saliva and the type of bite in the studied children.

It is notable that in children with an orthognathic bite normal salivation is also found. Statistics on the preferences for food consistency of the examined children show a high relative share of the patients consuming mushy consistency food (72.20%), which does not require chewing efforts during their processing for swallowing. Children chewing solid food are about 1/4 of all examined (24.10%). From the cohort of children with orthognathic bite and open bite, those who prefer mushy food are 77.27% and 92.00%, respectively. In children with a deep prognathic and progenic bite, the distribution is equal. A statistically significant correlation has also been found between the state of the oral mucosa and the consistency of the food consumed, namely the improved chewing ability for solid food is associated with better state of the mucosa ($\rho = 0.41$, $p = 0.002$).

The analysis of the data has established a statistically significant relationship between the state of the oral mucosa, consistency of the food, chewing ability on one hand and most of the anthropometric indicators, including BMI, weight and height-for-age, as well as indicators like skinfold thickness, which are markers of adipose tissue insufficiency.

Discussion:

Oral-motor dysfunction is a major contributory factor in the pathogenesis of malnutrition and usually correlates with the severity of motor impairment (7).

It can occur by inadequate lip closure due to pain, increased salivation and constant pushing of the tongue which leads to food loss. The impact of the delayed development of oral motor skills on the solid food chewing ability according to our data affects the majority of studied children who prefer eating food with a mushy consistency – 71.70%, easily processed for swallowing no chewing effort required. These results coincide with the statement of Rusinova and co-authors that compromised masticatory function can lead to childhood malnutrition (2, 9, 10), which we have found to be widespread among children with NPDs.

The relation of saliva to dental diseases depends on its composition and quantity. According to Hegde, the prevalence of sialorrhea in children with NPDs is 10% to 58% (8). Data from our study have reported that 7.5% of the studied children have hypersalivation. The highest is the relative share of children with abundant salivation in deep prognathous bite.

Conclusion:

The state of the oral mucosa in children with NPDs has a significant association with the consistency and type of consumed food, as well as the nutritional status. In this regard, pathologic changes in oral soft tissues are to be considered and treated as this might affect growth of NPDs children and their overall health and long-term complications.

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The prevalence of sleep bruxism in special needs children with ring chromosome 14 syndrome

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Abstract

Objective: To investigate the prevalence of sleep bruxism (SB) in children with ring chromosome 14 syndrome (r14).

Methods: A voluntary survey was administered to the caretakers of 13 r14 patients. Using clinical criteria recommended by the International Classification of Sleep Disorders (ICSD), bruxism was diagnosed as “possible,” “probable,” and “definite” bruxism and further distinguished as “sleep” or “awake” bruxism.

Results: Muscle fatigue, temporal headaches, and other ICSD criteria were associated with symptoms in >50% of the children. Jaw locking, muscle pain, and the criterion of “probable SB” were associated with 23%, 46%, and 54% of subjects, respectively. About one third suffered from daytime symptoms, including sleepiness, irritability, and morning headache. Hypotonia, together with distinct neuromuscular craniofacial anomalies, may predispose r14 children to apnea-hypopnea and, consequently, SB.

Conclusion: The pilot study suggests that SB is two to three times more prevalent in children with r14 syndrome than the general population children.

Keywords:

Ring Chromosome 14 Syndrome, Children, Sleep Bruxism, Dental Management, Special Needs

Introduction

Ring chromosome 14 syndrome (r14) is a rare genetic disorder characterized by distinctive facial features, intractable epilepsy, delayed development, intellectual disability, and autism spectrum disorder.¹ With less than 100 cases reported worldwide,² there are currently no studies to assess r14 children for sleep bruxism (SB).

Bruxism is a repetitive activity of the masticatory muscles, characterized by clenching the teeth or teeth grinding and/or by bracing or thrusting of the mandible.³ Bruxism is a sleep related movement disorder with two distinct circadian manifestations: occurring during sleep (SB) or during wakefulness (awake bruxism).

With an estimated prevalence of 14% in general population children, SB can lead to tooth wear, tooth mobility, tongue/cheek indentation, masticatory muscle hypertrophy, temporomandibular joint pain, headaches, and masticatory muscle pain or muscle fatigue.⁴ The dentist is responsible for its early detection, diagnosis, management, and prevention of possible consequences.

Chairside detection of SB involves the use of subjective reports, clinical examinations, and trial oral splints.³ Definitive diagnosis of SB can only be achieved by electrophysiological means, namely polysomnography. There is currently no effective treatment to stop SB permanently. Management generally focuses on tooth/restoration protection, reduction of bruxism activity, and pain relief.

In the absence of literature discussing the risk factors of SB in r14 children, the pilot study aims to investigate the prevalence of sleep bruxism (SB) in special needs children with ring chromosome 14 syndrome.

Methods

A voluntary survey was administered to the caretakers of 13 r14 patients who as of 2019 were registered in the National Organization for Rare Diseases (NORD) global data bank (Ring 14 USA Outreach). The diagnosis of bruxism was based on clinical criteria recommended by the International Classification of Sleep Disorders (ICSD).

A diagnostic criterion includes the report of regular or frequent tooth grinding sounds occurring during sleep and the presence of one or more of the following clinical signs and symptoms: (1) abnormal tooth wear consistent with reports of tooth grinding during sleep; (2) transient morning jaw muscle pain or fatigue; and/or temporal headaches; and/or jaw locking upon awakening consistent with reports of tooth grinding during sleep.⁵

The bruxism was classified by subcategory (“possible,” “probable,” and “definite” bruxism) and further distinguished as “sleep” or “awake” bruxism.⁶ “Possible bruxism” is based on self-report or by parent report (questionnaire or interview); “probable bruxism” is based on self-report plus inspection during clinical examination; and “definite bruxism” is based on polysomnography, preferably with video/audio recording.

Results

The majority of the children were diagnosed with r14 as infants, shortly after commencement of uncontrollable seizures. The majority of subjects was unable to speak, suffered from intractable seizures, and frequently exhibited behavioral outbursts. All the patients had microcephaly and presented with Class II malocclusions. Though 85% presented with attrition-type tooth wear, none of the subjects had been previously evaluated for possible SB.

Muscle fatigue, temporal headaches, and other ICSD criteria were associated with symptoms in >50% of the children. Jaw locking, muscle pain, and the criterion of “probable SB” were associated with 23%, 46%, and 54% of subjects, respectively.

Of the 13 children assessed for SB, about one third were reported to suffer from daytime symptoms of sleepiness, irritability, tiredness, morning headache, thirstiness in the morning, oral breathing and nasal stuffiness and night-time symptoms (habitual snoring, restless sleep and nightmares).

More than half the children presented with hypotonia, which together with certain neuromuscular and craniofacial anomalies, including dolicocephaly and high palate, may predispose the r14 children to apnea-hypopnea and, consequently, SB.

The risk factors include: low muscle tone in the mouth and upper airway, poor coordination of airway movements, narrowed air passages in the midface and throat, and enlargement of adenoid and tonsillar tissues.

As most of the r14 children suffered from frequent upper respiratory infections, increased upper airway infections and nasal secretions may further contribute to collapse and obstruction of both the oropharynx and the hypopharynx when the r14 children are sleeping.

Conclusion

The pilot study suggests that SB is two to three times more prevalent in children with ring 14 chromosome syndrome than the general population children. Further studies with validated diagnostic criteria are necessary to assess the prevalence of SB in r14 children more accurately. The presence of muscle fatigue and temporal headaches can be considered good tools to screen r14 children for SB. However, they do not replace the current gold standard, polysomnography, which may identify SB more accurately.

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Assessment of the effect of untreated hyperdontia on the adjacent teeth

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Abstract:

Supernumerary teeth (ST) cause developmental abnormalities and pathological changes in neighboring teeth.

Objective: To assess the effect of untreated hyperdontia on the adjacent teeth by studying the presence and degree of dilaceration, resorption of the associated permanent tooth root, and caries and its complications.

Material and methods: A case-control, observational, retrospective study of 106 patients with hyperdontia was conducted.

Results: Patients underwent a clinical study and radiography. Mild to moderate root dilaceration was most common, usually in distal direction. Teeth with oblique resorption predominated compared to horizontal resorption. In the teeth adjacent to the supernumerary tooth, caries was found in 18.9% of the cases.

Conclusions: In our study, the results showed that ST did not have a significant effect on the structure and development of the adjacent teeth.

Keywords: supernumerary teeth, degree of dilaceration, resorption of the root, caries and its complications.

Introduction:

In 2007, Ashkenazi et al. introduced the term “superlative” teeth, which various literatures acknowledge as polyphyodontism, third dentition, superdentition, abnormal, conical, duplicated or excess teeth [1]. Supernumerary teeth (ST) cause developmental abnormalities and pathological changes in neighboring teeth.

Material and methods:

A case-control, observational, retrospective study of 106 patients with hyperdontia was conducted.

Method for examination of root dilaceration. Patients underwent OPTG and/or periapical radiography by Dieck. Patients with a formed root length below 2/3 were excluded from the study. The radiographs were interpreted in the dark, using a magnifying glass (2x magnification), on an X-ray illuminator, with a black cardboard mask, with dimensions of the OPTG of 15/30 cm and of the periapical radiograph - 2.5/5 cm. Dilaceration of the tooth root is a deviation which is greater than 20° relative to the axial axis of the tooth. Each tooth adjacent to the supernumerary tooth was examined for root dilaceration according to Santana, Consolaro and Tavano [2], and was classified into one of three groups depending on the angle formed between the axial axis of the tooth and its root: (1) mild (20-40°), (2) moderate (41-60°), (3) severe (over 61°). According to the affected root length, dilacerations were further divided into cervical, middle and apical. The direction of deviation of the root subdivides the deviations into mesial, distal, buccal or lingual.

Method for examination of root resorption. According to the literature (M. Peneva), the root of the permanent tooth completes its formation within a period of 4 years after the eruption [3]. Root resorption is described as a horizontal shortening of the tooth root [4]. Segmental radiography by Dieck and OPTG,

were performed. Additional examinations were performed using CBCT (in some patients), examining the palatal root surface of the teeth adjacent to the supernumerary tooth for detection of impairment of its integrity. The severity of root resorption was determined radiographically according to its location and type (oblique, horizontal) using the method of Ericson and Kurol [56]: **grade 1** - no resorption (intact root surface); **grade 2** - mild resorption (affects up to ½ of the thickness of the dentin layer adjacent to the pulp); **grade 3** - moderate resorption (affects more than ½ of the thickness of the dentin layer adjacent to the pulp, but the pulp is not affected); **grade 4** - severe resorption (the pulp is affected by the process).

Methods for diagnosing caries and its complications in adjacent teeth: clinical methods - clinical examination; routine X-ray examinations.

Results and conclusions:

106 patients with 168 ST diagnosed clinically and radiologically were examined. The mean age was 16.84 ± 1.15 years. Of these, 68.9% were males and 31.1% - females, in a ratio of 2.21:1. In the mixed and primary dentition, the proportion of mesiodens (41.5%) and lateral incisor (7.5%) was the highest, while in the permanent dentition, distomolars (15.1%) and parapremolars (12.3%) predominated. Males most frequently had mesiodens (39.48%) followed by lateral incisor (23.68%), while females most frequently had mesiodens (59.8%) and distomolar (13%). We diagnosed 65.47% (110 ST) in the maxilla, 14.29% (24 ST) in the mandible, and 20.24% (34 ST) affecting both jaws. Mesiodens and lateral incisor were more common in the maxilla, and parapremolar was more common in the mandible.

Assessment of the presence and degree of dilaceration. Of the examined teeth, 67 (67%) teeth from the medially located teeth or tooth 21 in mesiodens and 53 (59.6%) teeth from the distally located teeth or tooth 11 in mesiodens, respectively, had complete root development, and we examined the root for detection of dilaceration. Mild to moderate root dilaceration was most common, usually in distal direction. Dilaceration was found in mesial and distal directions. The proportion of root dilaceration in the apical third of the root was the highest, followed by the middle third. No statistical significance of the results was found (p -value > 0.05). In most cases, root deviation was due to eumorphic teeth, followed by conical teeth, mainly in the distal direction.

Resorption of the root of the permanent tooth. 61.98% (119 of 192 teeth) of all teeth adjacent to the ST which had complete root development were examined for detection of root resorption. Grade I resorption was more common. Teeth with oblique resorption predominated (18.2% - distal; 13.6% - medial teeth), compared to horizontal (12.1% - distal; 11.4% - medial teeth) resorption. Severe (grade IV) resorption was most often due to eumorphic teeth. No statistical significance of the results was found (p -value > 0.05).

Caries and its complications. In the teeth adjacent to the supernumerary tooth, caries was found in 18.9% (20 patients) of the cases, with statistical significance of the results (p -value < 0.001). The proportion of patients with caries of the adjacent teeth was the highest in the age group 31 to 40. Adjacent of ST are also examined for caries, which found that 1/5 of patients have caries. Öztas B et al. gives slightly higher results than ours - ¼ of the teeth adjacent to the ST have caries [6]. Probably this is due to the fact that in the study of this author the patients have mostly distally situated ST.

De Oliveira Gomes et al. [5] found that 88.5% of ST caused complications, usually with tooth displacement (55.7%), followed by delayed eruption (50.8%), diastema (21%), tooth rotation (18.7%), retention of primary teeth (7.9%) and root resorption (0.3%). This shows that ST are the cause of various complications that require adequate behavior of the dentist for their prevention and treatment. Öztas B et al. [6] reported 2.34% of teeth with root resorption and 1.17% devitalization of the tooth in mesiodens. Bereket et al. [7] reported that 61.6% of patients with ST had no complications. In the remaining cases

(38.4%) the authors [7] reported: delayed eruption (46.2%), diastema/rotations (31.99%), crowding (13.27%), root resorption (3.55%), dental caries and pericoronitis (3.31%) and cystic formations (1.65%).

Conclusion:

In our study, the results showed that ST did not have a significant effect on the structure and development of the adjacent teeth.

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Dental operating microscope in removing gutta-percha from root canals during endodontic retreatment

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Abstract:

The study compares the effectiveness of gutta-percha removal during retreatment with and without the aid of dental microscope. 54 extracted teeth are prepared, obturated and then retreated with H-Files, ProTaper Retreatment files (PTR) and combination of PTR and microscope. The cleaning efficiency is evaluated with 3D CBCT. All the treatment techniques leaved a certain amount of gutta-percha in the root canal. The largest amount of residual gutta-percha is left after PTR, followed by H-files. There is no statistically significant difference between the H-files and PTR groups. Such a difference exists between them and the group with microscope ($p < 0.05$). In the conditions of this study, using operating microscope leads to better gutta-percha removal from the root canal system during endodontic retreatment.

Introduction:

Rotary nickel–titanium instruments have been widely investigated for effectiveness of gutta-percha removal^{1,2}. None of the tested systems so far was able to eliminate the residual gutta-percha and root canal infection associated with it. Thus, additional techniques should be considered.

Nowadays dental microscope is considered as a golden standard in a routine treatment. It is successfully applied in everyday clinical practice such as conservative preparation of endodontic access and localization of orifices, as well as in much more complicated cases^{3,4}. Whether using operating microscope leads to better cleaning of the root canal system from the gutta-percha during endodontic retreatment is still unclear, which motivated the **purpose** of the present study: To compare the effectiveness of gutta-percha removal from the root canals during endodontic retreatment with and without the aid of dental operating microscope.

Materials and methods

Fifty four (54) standardized canal fillings with length between 15 cm and 17 cm, constant taper .06 and apical diameter #30 are used for the study. They were divided in three groups. First group: gutta percha is removed with H – files. Second group - with ProTaper Retreatment Files (PTR). Third group - microscope: same protocol as PTR, visual inspection under DOM, repetition of the protocol if remnants of GP are observed. The residual gutta-percha is registered with 3D CBCT (three-dimensional cone-beam computed tomography). Each canal is examined for the presence of gutta-percha in the coronal, middle and apical part . The residual gutta-percha is evaluated with the following indexes: 0 – no gutta-percha; 1 – presence of gutta-percha up to 1/2 of the canal wall; 2 – presence of gutta-percha more than 1/2 of the canal wall; 3 – the presence of gutta-percha on the entire wall. The results are processed by alternative analysis, comparison of two relative shares with u-criterion, as the level of statistical significance was $P = 0.05$.

Results:

All the treatment techniques leaved a certain amount of gutta-percha in the root canal. (**Table 1**). The largest amount of residual gutta-percha is left after treatment with PTR, followed by H-files. The values obtained from the DOM group are smaller. A comparison between the different techniques with respect

to the residual gutta-percha in the whole canal is presented in **Table 2**. The analysis showed that there is no statistically significant difference between the two groups with the highest amount of residual gutta-percha – H-files and PTR. Such a difference exists between them and the group with microscope ($p < 0.05$).

Table 1. Remaining gutta-percha in the canal for each technique (as a percentage from the possible maximal numerical value)

Technique	N	% ± Sp
PTR	459	52.51±2.33
H	459	50.76±2.33
PTR with microscope	459	22.14±1.95

Table 2. Tukey post Hoc multiple range test comparing different techniques for removal of remaining gutta-percha in the canal

Technique	u	P(u)
PTR – H	0.53	>0.05
PTR – PTR with microscope	9.90	<0.0001*
H - PTR with microscope	9.32	<0.0001*

* with significant difference

Discussion:

In the present study, files designed specifically for retreatment (PTR) perform equally poorly with H-files. This may come as a surprise, but the results of other studies are similar⁵. PTRs are designed especially for removal of the gutta-percha from the root canal. The manufacturer recommends that the canal be further prepared with ProTaper or another Ni-Ti system. The apical diameter of the file D3, which reaches the working length is 20, and the taper is 07. For the other group without microscope – H files, the apical diameter is 30 and the taper is .02. Considering that there is no statistically significant difference between these two groups, it can lead to the conclusion that differences in apical diameter and taper do not play role in cleaning ability of the instruments during gutta-percha removal.

De Mello Jr et al. compare the effectiveness of gutta-percha removal with and without the use of an operating microscope in combination with ultrasonic tips. In the conventional removal group, the average residual percentage was 25.21, and in the microscope removal group – 9.31. There is a statistically significant difference. The authors conclude that the operating microscope in combination with ultrasonic tips removes gutta-percha better, although both groups lack completely cleaned canals⁶. It can be said that to a large extent the results of our study coincide with the above. However, there are opposite results. There are authors who do not report statistically significant difference when removing gutta-percha using a microscope (7.3% residual material) compared to the group without using magnification (8.3%)⁷. This study is much older - since 1999 and we assume that then the possibilities of the offered microscopes were smaller and their use in endodontic practice was not so widespread. Perhaps the most recent study on the benefits of using DOM to remove old canal filings is by Chauhan R et al.⁸. The authors conclude that clean canal walls are best achieved when the treatment is performed under the supervision of DOM.

Obviously, there is not enough data obtained concerning the benefits of gutta-percha removal with the aid of DOM. Additional in vitro and in vivo studies should be conducted to clarify if this tool, per se, improves the quality of this procedure or better results are due to the instruments used in the canal during retreatment.

Conclusion: In the context of this study, using operating microscope leads to better cleaning of the root canal system from the gutta-percha during endodontic retreatment.

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The effect of topical application of 0.2% chlorhexidine digluconate on tooth color in smokers and non-smokers

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Abstract:

Background:

The color of an object is determined by the spectral composition and intensity of the light falling on it, by the changes that light undergoes in contact with it. The aim of this study was to determine the effect of topical application of mouthwash containing 0.2% chlorhexidine digluconate on tooth color.

Material and methods:

The study included 60 clinically healthy dental patients who had not used mouthwash containing 0.2% chlorhexidine digluconate in the past 6 months. Patients were instructed to rinse their mouth with the mouthwash provided twice per day for 14 days in compliance with their usual habits of cleaning the oral cavity. 2% chlorhexidine digluconate, on the 7th day after the start of administration and on the 14th day.

Results:

The study included 60 people - 38 women (63.3%) and 22 men (36.7%). The average age of the subjects was 28.85 ± 10.63 years. The average age of women was 27.95 ± 1.79 years, and of men 30.41 ± 2.13 . No statistically significant difference in age between the sexes was found ($p = 0.392$). Our study did not find a statistically significant change in tooth color in smokers and non-smokers, both in the preliminary study and on the 7th day and 14 days. In contrast, however, we found a statistically significant change in the color of the examined teeth on the 7th day of the application of the mouthwash, compared to baseline and on the 14th day, in comparison with both the original and the registered color on the 7th day.

Conclusion: The fourteen-day administration of 0.2% chlorhexidine resulted in a statistically significant change in the color of the examined teeth compared to baseline and compared to the values established on the 7th day after the start of the use of 0.2% chlorhexidine digluconate.

Introduction:

The color of an object is determined by the spectral composition and intensity of the light incident on it, by the changes that light undergoes upon contact with it (refraction, reflection, absorption) and therefore by the composition of the light reflected by it. The composition of the reflected light depends on the light source (artificial or natural lighting). A body that reflects all types of light in daylight looks white, and what absorbs all the light falling on it - black. The spectrum of white light is colored by the color of the reflected light, and the rest is absorbed. Many authors compare the change in the color of hard dental tissues after application of 0.2% chlorhexidine gluconate (CHX) with dental plaque

surfaces. Intergroup comparisons show a statistically higher intensity of staining on dental surfaces that have not been previously cleaned of plaque. The presence of plaque increases the side effects of CHX.

Objective:

The aim of this study was to determine the effect of topical application of mouthwash containing 0.2% chlorhexidine digluconate on tooth color.

Material and methods:

The study included 60 clinically healthy dental patients who had not used mouthwash containing 0.2% chlorhexidine digluconate in the past 6 months. The subjects were divided into two groups of 30 depending on whether they are smokers or not. A smoker is a person who smokes at least 10 cigarettes a day for the last 5 years. Patients were instructed to use the provided mouthwash twice daily for 14 days in accordance with their usual oral cleansing habits. The rinsing technique involved using 10 ml of 0.2% chlorhexidine digluconate solution for 1 minute twice.

Spectro Shade Micro (MHT, Verona, Italy) was used to determine the color of hard dental tissues. It uses a combination of a digital device and an LED spectrophotometer. from the device radiation varies from 410 to 680 nm. The device can give a medium color to the whole tooth, divide it into thirds or present its zonal distribution on the tooth surface.

Results: The probants were 38 women (63.3%) and 22 men (36.7%). The average age of the persons was 28.85 ± 10.63 years. The average age of women was 27.95 ± 1.79 years, and of men 30.41 ± 2.13 years. No statistically significant difference in age between the sexes ($p = 0.392$) was found. Teeth located in the right maxillary half of the studied patients were previously cleaned of plaque and dental plaque, and those located in the left half of the maxilla were not manipulated. The technique of applying the mouthwash also includes gurgling, twice and consecutively with 10 ml of 0.2% chlorhexidine digluconate. On the 7th day and on the 14th day the mouthwash is used. In contrast, however, we found statistical significance of the change in color at the level of the study. and on the 14th day, compared to both the original and the registration, the color of the 7th day.

Discussion:

Many authors study the side effects of chlorhexidine associated with plaque. Their aim is to compare the change in color of hard dental tissues after the application of 0.2% chlorhexidine digluconate on pre-cleaned dental plaque and the effects on plaque-coated surfaces.

Intergroup comparisons show a statistically higher intensity of staining of tooth surfaces that have not been previously cleaned of plaque. These results confirm the need to remove the biofilm before using mouthwash containing chlorhexidine digluconate to reduce side effects. associated with discoloration.

Stratul et al. investigated whether smoking affected the effects of chlorhexidine digluconate and found a statistically significant difference in smokers and non-smokers. Our study did not find statistically significant differences in the change in the color of hard dental tissues in the two groups of patients - smokers and non-smokers, after administration of 0.2% chlorhexidine. Haydari et al. investigated the effects of mouthwashes containing different concentrations of chlorhexidine digluconate (0.06%; 0.12%; 0.2%) and found that the concentration of CHX had no effect on side effects. Bagis et al. found the most pronounced change in color in the first few days of administration of 0.2% chlorhexidine digluconate. In order to avoid staining of tooth surfaces as a side effect and at the same time to favorably affect the accumulation of plaque and the development of gingivitis in patients, there is also 0.2%

chlorhexidine with anti-discoloration (ADS) system on the market. Many authors have studied the degree of staining and clinical efficacy of 0.2% chlorhexidine with anti-staining system (ADS) compared to 0.2% chlorhexidine (traditional) after 15 days of use. Mouthwash containing chlorhexidine digluconate ADS stains teeth less, and has the same therapeutic effect at 15 days of use in patients with chronic periodontitis. Yogesh et al. studied the effect of mouthwashes with and without ADS on tooth discoloration and their effect on reducing plaque after surgery after 15 days of use. It is recommended that CHX mouthwash be used for a short period of time because it is more effective but has the ability to color, and CHX + ADS to be used for longer periods of time when there is no serious periodontal disease or oral hygiene on high level.

Conclusion: The fourteen-day administration of 0.2% chlorhexidine resulted in a statistically significant change in the color of the examined teeth compared to baseline and compared to the values established on the 7th day from the start of the use of 0.2% chlorhexidine digluconate, which does not depend on whether patients are smokers or non-smokers.

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Quality of root canal obturation in two different preparation techniques

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Abstract

When there is an indication for endodontic treatment, one of the steps is to enlarge the existing root canal, in harmony with the principles for root-canal preparation. During the phase of preparation, endodontic instruments are used, but the instruments themselves vary by taper, cross-section and diameter at the tip. Some teeth have gentle roots and therefore, the preparation step is crucial not to be overdone in order to preserve as much sound dentin as possible.

Introduction

The prognosis of the tooth is dependent not only on the quality of the endodontic treatment itself, but on the quality and quantity of the remaining dentin as well. Root canals are sometimes depicted as smooth hollow tubes that are tapered in shape. These misleading images do not reflect the intricate anatomical structure and complexity of root canal systems. They are often asymmetrical or oval in cross section, they branch, dilacerate and divide and the canal walls show concavities and convexities. Complex root canal anatomy should be considered one of the most significant challenges in creating root canal shapes that will support good obturation outcomes and leave enough remaining strength in the root. After biomechanical instrumentation, the completed root canal shapes need to withstand the internal compressive forces of obturation; provide enough resistance form to contain softened and compressible filling materials and retain enough strength for mastication^{1,2}. This is the reason for modern endodontics to be focused on minimally invasive shaping of the root canals.

Materials and Methods

20 extracted teeth are examined (Mandibular incisors) and divided into two groups. Conventional endodontic access is prepared in all the examined specimens. The first group (n=10) is treated with The Pathfile System (Pathfile 1(13/02); Pathfile 2(16/02); Pathfile 3(19/02) /Dentsply/ and after that, all of the examined root-canals from this group are prepared with K-file (20/02). The second group (n=10) is treated with The Protaper Universal System (S1(18/02), S2(20/04), F1(20/07) /Dentsply/, using endomotor X-Smart Plus (Dentsply). After preparation, all root canals are irrigated with 2,5% NaOCl and activated with ultrasonic (Mini - Piezon EMS). Paper points are used (20/02 for group 1 and 20/06 for group 2), the root-canal systems of the examined teeth are obturated, using the single cone technique with bioactive mineral sealer (BioRoot RCS/Septodont) and gutta-percha cones (20/02 for group 1 and 20/06 for group 2). After the root-canal obturation, all the examined teeth are left in 0,9% NaCl for 72 hours. The teeth are examined, using CBCT to evaluate the quality of the root-canal obturation.

Results

In group 1(Fig. 1), the coronal and mid-root sealing is adequate, but unfortunately the apical sealing is not hermetic in 70% of the examined teeth. In group 2(Fig. 2), however, the sealing is much better showing 70% of the cases with adequate root-canal filling quality on the coronal, mid-root and apical sealing.

Discussion

Unfortunately, even if our root canal obturation is hermetic and there are virtually no bacteria left, the tooth might be compromised. The cause of this is the big coronal taper of the instruments used during the preparation phase and the little to no pericervical sound dentin left^{3,4,5,6,7}. This is the motive and reason for the idea of minimally invasive shaping, using instruments with 2% or 4% taper. This however should not be done in a way that prevents the adequate three-dimensional root-canal obturation, which itself could compromise the tooth prognosis by being the etiologic factor of periodontitis^{8,9,10}.

Conclusion

From the research conducted it is established that the used endodontic protocol of work does not allow full 3D obturation of the root canals from the first group. From the CBCT projections, the apical third portion is hollow. This gives us the reason to conclude that the processing of the root canals with 2% tapered files to apical size #20 is insufficient.

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Controlled Enzyme Excavation with Brix 3000 and Photodynamic Therapy in the Treatment of Closed Asymptomatic Pulpitis in Primary Teeth

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Summary

Aim

Microbiological research of controlled enzyme excavation with Brix 3000 and photodynamic therapy using FotoSan in the treatment of reversible pulpitis in primary teeth.

Results

The microflora in carious dentin in relation to closed asymptomatic pulpitis contains *S. mutans* (91%), *Lactobacillus spp.* (45%), *S. mitis* (18%) and *S. sanguis*, *S. parasanguis*, *S. epidermidis* и *Neisseria*. After the excavation to partially infected dentin, the types and quantities of microorganisms are significantly lowered with *S. mutans* being preserved; Following the FotoSan procedure, the residual bacterial flora is entirely reduced.

Key Words: Brix 3000, photodynamic therapy, *S. mutans*, reversible pulpitis

Introduction

The minimally invasive method is successfully applied in treating closed asymptomatic pulpitis in primary teeth through indirect pulp capping [1].

Brix 3000 is a new material for enzyme excavation. Its main component is papain which is bio-encapsulated via EBE Technology (Encapsulating Buffer Emulsion). This technology releases the enzyme thus generating proteolysis of the carious dentin [2].

The photodynamic therapy is based on the application of a light source for activating the photosensitizer, which in the presence of oxygen, produces nascent oxygen and free radicals. The latter change the metabolic features of the cell and lead to its death [3].

The fluorescence is an indirect measure for determining the degree of demineralization of the rigid dental structures [4].

Aim

Microbiological research of controlled enzyme excavation with Brix 3000 and photodynamic therapy using FotoSan in the treatment of reversible pulpitis in primary teeth.

Material and Methods

22 closed asymptomatic pulpitis in primary molars have been examined (ICDAS II code 06) in children aged 4-7, divided into two groups: group 1 – 10 pulpitis, excavated with Brix 3000 to partially infected dentin; group 2 – 12 pulpitis, excavated with Brix 3000 to partially infected dentin and an additional disinfection procedure. The excavation was controlled with ProFace. All clinical cases were treated through indirect pulp capping.

Microbiological samples were taken from carious dentin, partially infected dentin and partially infected dentin before and after disinfection with FotoSan. The sowing was performed on a blood agar, a selective agar for lactobacilli and on a Brain-Heart infusion broth.

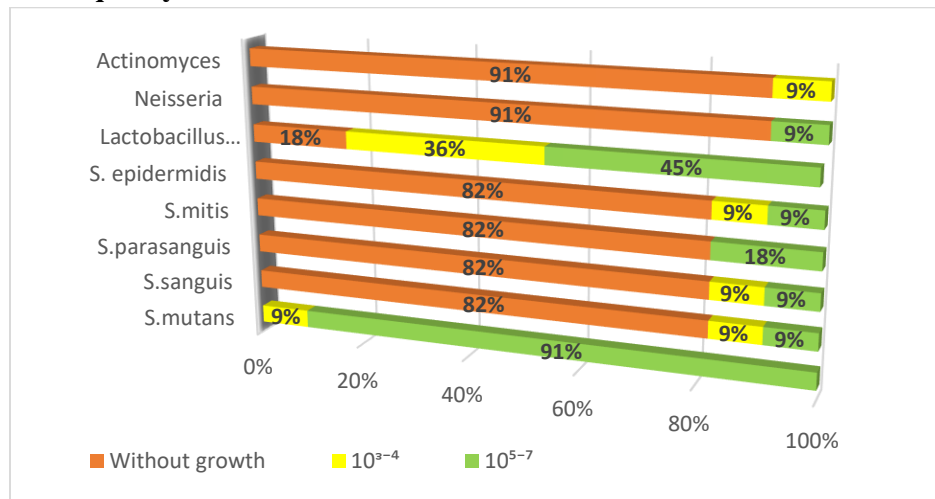
Methodology of Controlled Excavation with Brix 3000 includes:

First stage – exposure of the carious lesion; first microbiological sample; ProFace – red fluorescence; *Second stage* – cleaning the EDJ; ProFace – lack of fluorescence; *Third stage of excavation*, differs according to the group - *Group 1* – placement of Brix 3000 in the cavity. Excavation with manual

excavator. ProFace – to presence of limited red fields only in the area of the upper pulp dentin; taking a microbiological sample. *Group 2* – the methodology from group 1 is repeated followed by a disinfection procedure with PDT; placement of toluidine blue in the cavity and activation with FotoSan two times for 10 seconds; flushing with saline; taking a microbiological sample; *Stage four – follow up and obturation* – placement of Calcium Hydroxide and temporary obturation from GIC. After 1 month – opening of the tooth, taking a microbiological sample. After 2 months – final compomer obturation.

Results

Microorganisms Frequency Isolated from Carious Dentin Before Excavation with Brix 3000



Graph № 1 Microorganisms frequency isolated from carious dentin before excavation with Brix 3000

Most resilient and widespread microorganism is *S. mutans* (91%), followed by *Lactobacillus spp.* (45%) and *S. mitis* (18%). In single cases *S. sanguis*, *S. parasanguis*, *S. epidermidis* and *Neisseria* could be found.

Microorganisms in Partially Infected Dentin Before and After PDT in group 2

Table № 1 Microorganisms in Partially Infected Dentin Before and After PDT

Type of Microorganisms	Before FotoSan				After FotoSan			
	10 ³⁻⁴		<10 ³		10 ³⁻⁴		<10 ³	
	N	%	N	%	N	%	N	%
<i>S. Mutans</i>	12	100%	0	0%	0	0%	0	0%
<i>S. Sanguis</i>	2	16.7%	0	0%	0	0%	0	0%
<i>S. Mitis</i>	2	16.7%	0	0%	0	0%	0	0%
<i>S. Epidermidis</i>	0	0%	2	16.7%	0	0%	0	0%

NB: % are above 100% since more than one microorganisms could be seen in a case

The existing microorganisms in the preserved partially infected dentin, after the FotoSan procedure, are not isolated.

Discussion

Our results demonstrate that a great variety of microorganisms are isolated from the carious dentin. The most common are *S. mutans*, *Lactobacillus spp.*, followed by *S. mitis*. In lower quantities *S. sanguis*, *S. parasanguis*, *S. epidermidis* and *Neisseria* are isolated.

S. mutans is isolated from partially infected dentin in all cases of closed asymptomatic pulpitis. In single cases *S. sanguis*, *S. mitis*, *S. epidermidis* are isolated. After disinfection with FotoSan microorganisms are not isolated.

Goyal *et al* compare the conventional method of preparation with the enzyme method while researching 25 children aged between 5-9. Microbiological samples are taken before and after excavation of the carious dentin. The results reveal a significant reduction in the amount and quantities of *S. mutans* and *Lactobacillus spp* [5].

Contradicting data in relation to photodynamic therapy could be observed in the literature. Alves *et al* e.g. study 20 children with active caries lesions in primary molars. The results demonstrate statistically significant difference in the quantities of *S. mutans* after the selective removal of the infected dentin and after using the PDT [6]. This research supports our results.

Conclusions:

1. The microflora in carious dentin in closed asymptomatic pulpitis include *S. mutans* (91%), *Lactobacillus spp.* (45%), *S. mitis* (18%) and *S. sanguis*, *S. parasanguis*, *S. epidermidis* and *Neisseria*;
2. After the excavation to partially infected dentin, the types and quantities of microorganisms are significantly lowered with *S. mutans* being preserved;
3. Following the FotoSan procedure, the residual bacterial flora is entirely reduced.

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Manifestation of sleep bruxism in children - clinical cases

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Abstract

Bruxism in children is a common parafunction, which is accompanied by different symptoms and effects on the orofacial structures. A wide variety of risk factors can contribute to teeth clenching. By using specific diagnostic criteria, we can analyze the condition of every patient and make a proper treatment plan according to the established etiology and pathology of bruxism.

In this case report, we compare two cases of bruxism, which have different symptoms and different effects on the teeth of the patients. The first patient presents wide loss of tooth structures, while the second patient's main complaint is pain in the jaw and teeth after waking up. The aim of this comparison is to show how divergent can bruxism be in its manifestation. This requires an in-depth approach in the diagnostics of this parafunction in order for its proper treatment and follow-up.

Keywords: bruxism, tooth wear, jaw pain

Introduction

Bruxism is “a repetitive jaw-muscle activity, characterized by clenching, grinding and/or gnashing the teeth, as well as medialization of the lower jaw”. It can be classified as sleep/nocturnal or awake bruxism depending the time of occurrence [1]. One of the most common consequence of this parafunction is tooth wear [2, 3]. Nocturnal bruxism can lead to some morning discomforts, like bad mood, difficult awakening and fatigue, pain in the muscles, jaws and teeth [4]. This diversity of symptoms and risk factors requires a thorough examination, using established diagnostic criteria – anamnesis, clinical examination of the oral cavity, electromyographic examination or complete audio-video polysomnographic examination [1]. Today we have also the Bitestrip, which is a kind of a portable electromyograph, which has a built-in computer chip that records the number of contractions of the masseter for 5 hours during sleep [5]. In this case, report we present two children with bruxism but with different symptoms.

Clinical cases

We have examined two children – a male, aged 6 years old (K.D.) and a female, aged 5 years old (I.P.). The data we gathered is from the anamnesis, behavioral questionnaire, and clinical examination, photometric analysis of clinical crown length and assessment of muscle activity during sleep. Based on the acquired data we diagnosed both children with nocturnal bruxism.

Anamnestic data

We have two children, which are in good health, without any general diseases and not suffering often from rhinitis, tonsillitis or allergies. The parents of both children reported bruxism onset at the age of 4, with a frequency of around 3-5 nights a week. K.D. visits the dental office because his parents are concerned of the abrasion of his teeth, while I.P. complains of pain in the jaws and teeth after awaking and morning fatigue due to poor sleep. Their psycho-emotional development is similar and they did not present significant differences, based on a questionnaire, the parents completed.

Clinical examination

K.D. has a mixed dentition and I.P. – primary dentition. Both children present a high caries risk. During the examination of the masticatory muscles, we found that I.P. had hypotrophy of m.masseter, while K.D. did not present such. We completed a thorough examination of the teeth abrasion and the results showed that K.D. has tooth wear on all primary teeth in different stages, with the severest presenting loss of coronary length from 1/3 to 2/3 (fig.1). I.P. has tooth wear on teeth 53, 52, 73, 72,

71, 81 and 82. The most severe loss in her teeth is visible reduction of tubercles and incisal edges in the enamel (fig. 1). Both children present different degrees of abrasion, but we had to determine whether it is physiological (due to the masticatory function) or pathological (due to bruxism).

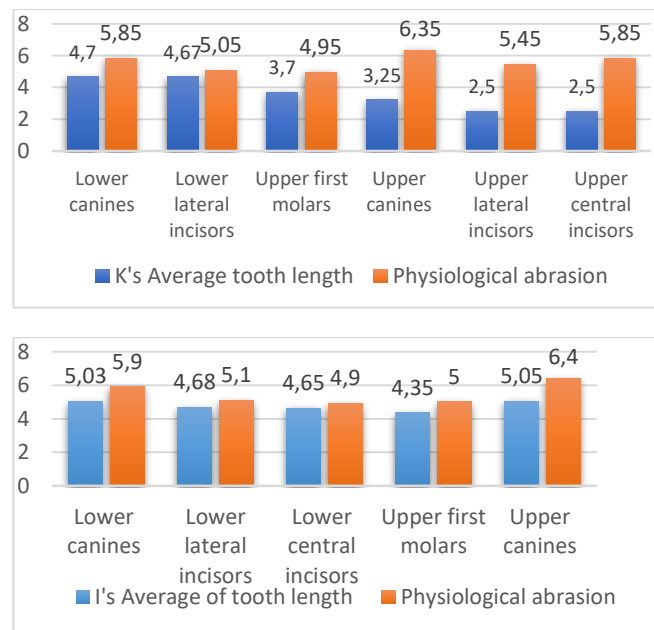
Fig. 1 *The dentitions of the patients*



Photometric analysis of clinical crown length

We can determine the degree of structure loss with digital photography and photometric analysis of the pictures. By comparing the average clinical crown’s lengths that we found with the average length of teeth and their physiological abrasion for the age [6], we can find the difference, which is the pathological tooth loss. The results are presented in figure 2.

Fig. 2. *Comparison between the average tooth length and the physiological abrasion lengths for K.D. and for I.P.*



The two charts show that the average values of tooth lengths found in K.D. are quite different from the average length of the teeth with physiological abrasion for the age, which means that we observe pathological abrasion as well. The values in I.P.’s chart have minor differences, meaning that pathological tooth loss is not so significant in her case.

Assessment of the sleep and muscle activity

We examined the muscle activity of m.masseter during sleep with the Bitestrip device and audio-video recording. K.D.’s muscle activity value according to Bitestrip was “3”, which means over 100 muscle contractions over a period of 5 hours, while I.P.’s result was “2” - 61 – 100 muscle

contractions over a period of 5 hours. Both children slept around 10 hours, did not have snoring or difficulty breathing.

Discussion

The presented two cases have similarities in the anamnestic data and behavioral assessment, but clinically they differ from each other by the manifestations of bruxism. In K.D. we observe a very severe overall tooth abrasion, while such is absent in I.P. On the other hand, I.P. has morning complaints, as jaw and teeth pain and we found hypertrophy in the m.massesteri while examining her. Tooth wear is often associated with bruxism [7] but some studies do not find this connection [8]. Other studies confirm the connection between bruxism and morning pain and fatigue [4]. The Bitestrip device reported different values of muscle activity for each child – a more severe value in K.D., than in I.P. We must always examine our patients comprehensively, and after receiving the initial data, focus our efforts on resolving the identified individual risk factors [9].

Conclusion

Bruxism is a parafunction with a multifactorial etiology and the condition can manifest in different ways in each patient. Bruxism can have long-term consequences for the dentition and orofacial structures. When it is diagnosed in early childhood, measures can be taken to address the identified individual risk factors and limit these effects in the future.

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Covid-19 among students and dental personnel in Dental Faculty, Plovdiv, Bulgaria

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Abstract:

Purpose: To analyze the cases of Covid-19 infected patients and contacts among students and staff of Faculty of Dental medicine.

Materials and methods: Prospective study was conducted in the period September 2020- July 2021 and information for 136 people was recorded.

Results: There were 57 confirmed cases and 79 contact people. Most of the confirmed cases were among the students of the Dental Medicine Faculty (n=41, 71.9%). Almost all the COVID-19 cases were diagnosed with a PCR test (n=52, 91.2%). 13 (22.8%) of the confirmed cases stated that they had a previous contact with a known case of COVID-19.

Conclusion: Strict observance and regular control of anti-epidemic measures since the beginning of the pandemic, prevented the widespread spread of this infection during the work process.

Keywords: dentistry, COVID-19, anti-epidemic measures, pandemic

Introduction:

After rapid transmission on 11th March 2020 the World Health Organization (WHO) declared a pandemic of COVID-19 [1].

Dental health care personnel (DHCP) have daily direct and indirect contact with saliva, blood and instruments, that are contaminated with saliva and blood, which determines the dental office as a place at increased risk of spreading viruses, including SARS-CoV-2 [2,3,4]. Prolonged exposure more than 15 minutes necessitates regular ventilation of the dental office, due to the risk of inhalation, both by patients and staff when working without or with improperly placed face mask [5,6].

In the pandemic spread of COVID -19, it is imperative that the dental personnel and dental students have knowledge and observe the rules for disinfection of the workplace, hands, dental impressions, and prevention of the spread of airborne infections. [7, 8].

In Faculty of Dental Medicine -Plovdiv (FDM) a protocol for registering the cases and contacts of COVID-19 was prepared and strictly observed. Anti-epidemic measures (quarantine) were taken. Compliance with the anti-epidemic regime was controlled by the staff of FDM, as well as by an epidemiologist from the Department of Epidemiology.

The aim was to analyze the cases of Covid-19 infected patients and contacts among students and staff of FDM to assess the applied anti-epidemic measures, identify possible omissions and make recommendations for optimizing the work process in FDM.

Materials and methods:

During the study period September 2020- July 2021 information for 106 students and 30 personnel was recorded. A card for epidemiologic investigation was created.

Quantitative variables were presented by mean and standard deviation (mean±SD) or median (25th percentile; 75th percentile), based on the sample distribution. Qualitative variables were presented as number absolute/relative frequencies totals and percentages (n,%).

Results:

Information for 136 people was analyzed (106 students and 30 DHCP from FDM). Among them - 57 confirmed COVID-19 cases and 79 contact people. Regarding all the prospectively studied confirmed cases and contacts of a known case of COVID-19 anti-epidemic measures were imposed (quarantine). (table 1)

Table 1. Data for confirmed cases and contact

Variables	Confirmed case (n=57)	Contact person (n=79)	p-value
Gender n, (%)			
Male	23 (40.4)	36 (45.6)	0.545
Female	34 (59.6)	43 (54.4)	
Occupation n, (%)			
Healthcare personnel	16 (28.1)	14 (17.7)	0.316
Student	41 (71.9)	65 (82.3)	
Duration of the contact with a confirmed case			
No information			0.000
0-7 days	46 (80.7)	6 (7.6)	
7-14 days	7 (12.3)	52 (65.8)	
>14 days	2 (3.5)	2 (2.5)	
	2 (3.5)	19 (24.1)	
Observance of the hand hygiene protocol			
Yes	13(22.8)	70 (88.6)	0.000
No	44 (77.2)	9 (11.4)	

Almost all the COVID-19 cases were diagnosed with a PCR test (n=52, 91.2%). In 1 (1.75%) of the 57 confirmed cases admission to a hospital was required. (table 2)

Table 2 . The most commonly reported symptoms in COVID-19 cases.

Variables	Yes n, (%)	No n, (%)	p-value
Symptoms	53 (92.9)	4 (7.1)	0.000
Elevated temperature	35 (61.4)	2 (38.6)	0.000
Headache	34 (59.6)	23 (40.4)	0.000
Cough	26 (45.6)	31 (54.4)	0.0005
Shortness of breath	10 (17.5)	47 (82.5)	0.053
Loss of taste/smell	26 (45.6)	31 (54.4)	0.0005

Discussion:

DHCP are at a very high risk of acquiring this infection from patients who carry the virus. That virus transmission can occur through aerosols generated during medical and dental procedures [9]. Infection control strategies for dentistry recommended by the Center for Disease Control (CDC) have been focused mainly on blood-borne infections, but the guidelines about airborne diseases are not as strong [10-12]. At least one individual with training in infection prevention should be responsible for developing written infection prevention policies and procedures based on evidence-based guidelines, regulations, or standards [13]. There was a statistically significant difference between cases and contacts of COVID-19 regarding the observation of hand hygiene protocols (Pearson χ^2 test= 60.276, $p=0.000$). The importance of hand hygiene as one of the most critical factors for reducing the risk of transmission of COVID-19 has been previously reported [14,15]. It is a simple but effective measure that dissolves the lipid coat surrounding the virus and kills or inactivates the virus if the hands have been contaminated [14,15].

Conclusion:

We believe that the strict observance and regular control of anti-epidemic measures in the FDM since the beginning of the pandemic, prevented the widespread spread of this infection during the work

process. The preparation of an optimized protocol for work during the pandemic of SARS-CoV-2/ Covid-19 and the meticulous monitoring of compliance with this protocol through regular inspections.

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Comparison of microleakage between 4% and 6% taper bioceramic endodontic obturation

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Abstract:

Purpose: To compare the microleakage between 4% and 6% taper bioceramic endodontic obturation, achieved by hydraulic condensation and bioceramic sealer BioRoot RCS.

Materials and Methods: Thirty samples with 12 mm length are obtained from single rooted human extracted teeth. The root canals of the samples are prepared and obturated with single cone hydraulic condensation technique and BioRoot RCS.

Results: The test results show respectively 0.39% penetration for group BC4, and 0.17% for group PT6. Decreased permeability of dye is reported for all samples compared to the positive control (C +) ($p < 0.05$), as well as insignificant difference compared to the negative control (C-) ($p > 0.05$).

Conclusion: Both tapers of the endodontic obturation show low level of dye penetration for all the samples in both experimental groups included in the current study.

Key words: bioceramic sealer, BioRoot, microleakage, hydraulic condensation, taper

Introduction:

One of the commonly used methods for assessing the sealing ability of various materials and techniques for filling root canals is based on the quantitative measurement of the dye passed through the obturation. Methylene blue was used in this study because its molecular size is similar to bacterial metabolic products such as butyric acid, which can leak from infected root canals and irritate periapical tissues. It also has the potential to enter through the obstructed canals into the complex anatomies of the apical third or into the space between the elements of the obturation - dentin-sealer-gutta-percha [1, 2, 3]. Dye penetration should be considered as an indicator of permeability potential, as the filler material, which prevents the penetration of small molecules such as dyes, has the ability to prevent the leakage of larger molecules, such as bacteria and their by-products [2, 4].

Materials and methods:

For this study 30 extracted human single-rooted teeth are used without endodontic treatment. On each of them, the coronary part is removed by a diamond separator so as to obtain specimens with a standardized length of 12 mm. A standard endodontic cavity is prepared and the working length is measured using an apex locator. For groups PT6 (n=12), C- (n=3), and C+ (n=3) files from the Pro Taper Gold system are used sequentially until the working length is reached with file F3 (D0 = 0.3 mm; 9% taper in the apical 3 mm), and for group BC4 (n=12), files from the iRace system are used sequentially until file R3 (D0 = 0.3 mm; 4% permanent taper along the entire length of the root canal) reaches working length. Irrigation is performed with 2.5% sodium hypochlorite each time the instrument is changed. The BioRoot RCS endodontic sealer (Septodont, France) is mixed according to the manufacturer's instructions, and applied in the root canal using Lentulo for all the groups. After inserting the sealer, the gutta-percha point from the respective system is also inserted. The positive control group C+ (n=3) is obturated with only a 6% gutta-percha cone and no sealer. The negative control group C- (n=3) is obturated just like the positive one, but each sample is afterwards fully coated with nail varnish externally to endure tight seal as a negative control. The endodontic cavity is closed with a temporary filling (i-Pro, I-Dental, Italy) and the sample are placed in saline with 0.5% thymol, where

they remain for 4 weeks for final setting of the sealer. Each of the prepared specimens, after drying with lignin paper, is fixed in an Eppendorf tube (1.5 ml) with the bottom cut off (A) so that at least 6 mm from its root is protruded beyond the end of the vessel. Methylene blue dye (2%, 0.5 ml) is placed in the same Eppendorf tube (A). Another Eppendorf tube (C) containing 1 ml of distilled water is placed beneath (A) so that the apical 3 mm of the root is immersed in the water. Epoxy resin applied in two coats was used for fixation, each of which cured independently in 20 minutes [5]. All experimental units are connected together in a common system to which a constant pressure of 1 atm is applied for a period of 60 minutes. At the end of the specified time, container (C) is separated from container (A) without contaminating the solution and the lids of the lower Eppendorf tube (C) are closed. Samples are analyzed on a TECAN spectrophotometer, Sunrise. The relationships are assessed by Kruskal-Wallis test and a subsequent Dunn's Multiple Comparison test.

Results:

In determining the results of the present study, the solutions in the lower Eppendorf tube (C) are diluted 300-fold. The (C+) control group is equated to 100% at a dye concentration of 0.0066%. For the negative control group (C-), a dye concentration of 0.000004% (0.054%) is determined. The test results show respectively 0.39% penetration for group BC4, and 0.17% for group PT6. Decreased permeability of dye is reported for all samples compared to the positive control (C+) ($p < 0.05$), as well as insignificant difference compared to the negative control (C-) ($p > 0.05$). All samples from the positive control leaked 100% of the dye. Therefore, there is no retention of dye molecules in parts of the root canal or in the lateral and dentinal tubules. The negative control group shows absolute resistance to dye penetration. After identifying differences between the groups (Kruskal-Wallis test), Dunn's Multiple Comparison test is performed to verify the statistical significance of these differences. Differences with statistical significance are found both between the positive (C+) and negative control (C-) and between both of the experimental groups with the positive control (C+) ($p < 0.05$). The variation analysis do not show an intergroup statistically significant difference in micropermeability for the experimental groups. For both experimental groups the results are less than 0.000005% and unreliable ($p > 0.05$), although our expectations were that we would receive a statistically significant difference between the two groups. The differences between the negative control group (C-) with each of the experimental groups are without statistical significance ($p > 0.05$). Thanks to this analysis, it can be stated categorically that both obturation techniques ensure good seal of the root canal.

Discussion:

The BioRoot RCS bioceramic sealer showed a low degree of permeability in all samples in the experimental groups of the present study. Due to the recent introduction of this material in the literature, only 3 scientific articles have been published examining the sealing abilities of this material [6, 7, 8]. Their results correspond to those obtained by us, showing a low micropermeability of canals obturated with BioRoot RCS, although the filling techniques used are different. In group PT6 we report less leakage than the other two groups. We attribute this to the better hydraulics of the material obtained from more widely prepared canals, leading to deeper penetration of the sealer into the dentinal tubules and less likelihood of the formation of pores in the obturation. Germain et al. compare the amount of voids (pores) when using a new bioceramic sealer ("New Bioceramic Sealer", St Joseph University, Beirut, Lebanon) in 4% and 6% prepared canals and the technique of hydraulic condensation (calibrated single gutta-percha cone). They found much more pores in 4% of the preparation than in 6% with the new material ($p < 0.001$) [6]. Eymirli et al. compared the penetration depth of the bioceramic sealer (EndoSequence BC Sealer) into the dentinal tubules using 2% and 4% gutta-percha points in a 4% prepared canal and found significant differences between the groups ($p < 0.05$) [7]. Because the gutta-

percha point acts as a plunger for the sealer, the better the match between the preparation and the point, the more efficient the sealer will be pushed into the dentinal tubules. In the Eymirli experiment, a 2% gutta-percha point applied in a 4% conical canal did not fit snugly against the canal walls and therefore did not exert sufficient pressure on the endodontic sealer. This is probably the reason why the authors get differences with statistical significance.

Conclusions:

1. Preparation techniques, used in this research, do not affect the degree of dye penetration in the root canal system.
2. Root canals, obturated with bioceramic sealer BioRoot RCS and gutta percha, show low level of dye penetration for all the samples in both experimental groups included in the current study.
3. The obturation with bioceramic impregnated gutta-percha cones (BC Points, FKG) and bioceramic sealer BioRoot RCS does not influence significantly the level of dye penetration of the endodontic obturation.

Abbreviations:

HC – hydraulic condensation

LC – lateral condensation

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Association of the Salivary sIgA and Dextran with Clinical Parameters of Plaque-induced Gingivitis in Children

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Abstract Purpose: Study of the association between salivary secretory immunoglobulin A (sIg A) and dextran levels with clinical parameters of plaque-induced gingivitis in children. **Materials and methods:** Four groups of participants based on gingival status. Plaque index and gingival index were determined. ELISA-methods were used for quantification of the salivary markers. **Results:** The average value of salivary dextran among these with severe gingivitis was 2.93-fold higher in comparison to the control group. A significant positive correlation between PLI and salivary dextran was established among the participants with moderate and severe gingivitis. The average value of salivary sIg A in the participants with severe gingivitis was 2.25-fold higher compared to the same parameter among the controls. A high positive correlation between GI and salivary dextran was recorded in the group with mild gingivitis. **Conclusion:** Salivary sIg A and dextran levels are associated with clinically manifested degrees of plaque-induced gingivitis in children.

Key words: plaque-induced gingivitis, saliva, sIg A, dextran, children

INTRODUCTION Plaque-induced gingivitis is widely spread among children and is characterized with significant long term social-medical impacts on individual and community level in worldwide scales. That is a non-specific inflammation of gingiva initiated and maintained by dental plaque accumulation [1]. The accumulation of bacterial dental plaque causes inflammatory reaction in gingival tissues, afflicting the immune system reactivity of the macro organism [2]. Dental plaque accumulation is mediated by bacterial extracellular polysaccharides and salivary constituents. Dextran produced by the catalytic activity of dextransucrase is considered as one of the basic factors in plaque-induced gingivitis. Salivary secretory immunoglobulin A (sIg A) serves as a tool of the protective local immunity mechanisms against dental plaque accumulation in the oral cavity [3].

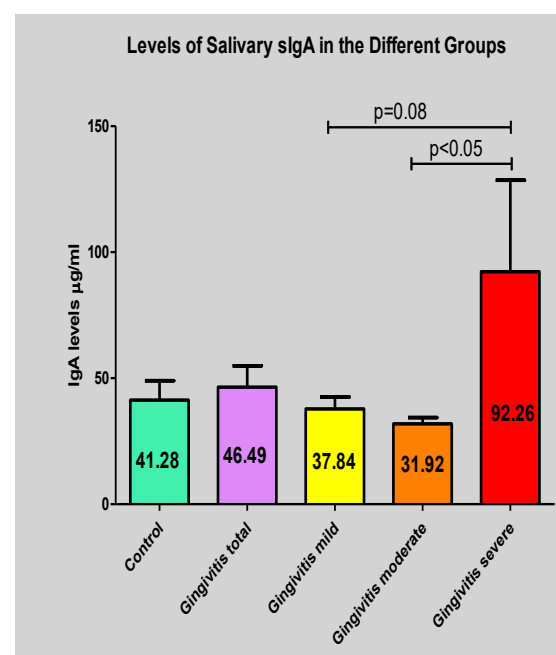
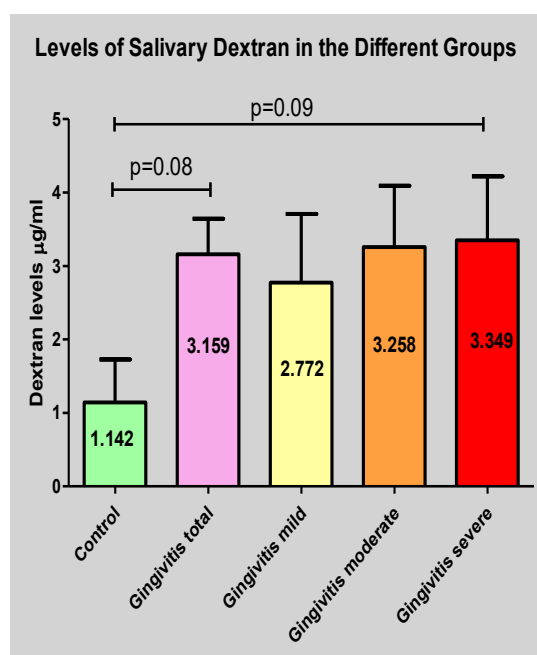
AIM Study of the association between salivary sIg A and dextran levels with clinical parameters of plaque-induced gingivitis in children.

MATERIALS AND METHODS The study included 44 children with no common diseases, divided into four groups: a control group of 6 children without plaque-induced gingivitis; a group of 12 children with a mild degree of plaque-induced gingivitis; 18 children with a moderate degree and 8 children with a severe degree, based on the application of the clinical indices of Plaque index Silness-Löe (PLI) and Gingival Index Löe -Silness (GI). The study was approved by the Ethics Committee at the Medical University-Varna. A parent or guardian of each participant in the study signed an informed consent. The clinical dental examination was performed at the University Dental Medicine Center at the Faculty of Dental Medicine, Medical University-Varna. The examined data were collected for a period of approximately one year. PLI and GI were implemented for the evaluation of the dental plaque level and gingival state. Dextran ELISA kit was used for quantitative determination of dextran in saliva (Lifespan Technologies, US). As a standard dextran with molecular weight 40 kDa was used (Sigma-Aldrich). ELISA for quantification of sIg A in saliva was performed using Human sIg A ELISA kit (Shanghai Sunred Biological Technology Co., Ltd, China) according to the manufacturer's instructions. The following statistical methods were applied in the study: descriptive analysis, correlation analysis, t-test.

RESULTS A statistically significant difference was established between the PLI levels among the groups in the study (control group-mild degree, $p= 0.0012$; control group-moderate degree, $p= 0.0004$; control group-severe degree, $p=0.0024$; mild-moderate, $p<0.0001$; mild-severe, $p= 0.0002$; moderate-severe, $p=0.0258$). A positive correlation was recorded between the clinical indices PLI and GI in all groups of children suffering from plaque-induced gingivitis. Among the participants with a moderate degree was established a statistically significant positive correlation between both of these parameters PLI and GI, with coefficient of correlation equal to 0.5245 (p value= 0.0255). There was a definite tendency of an increase of the average values (AV) of salivary dextran parallel to the progression of the gingival inflammation: AV=1.142 for the controls; AV=2.772 for the group with mild gingivitis; AV=3.258 for these with moderate gingivitis and AV=3.349 for the group with severe gingivitis. (Graph 1) The average value of salivary dextran in the group with severe gingivitis was 2.93-fold higher in comparison to the control group. (Graph 1) A significant positive correlation between PLI and salivary dextran level equal to 0.6667 was ascertained among the representatives with a moderate degree of plaque-induced gingivitis. A similar coefficient of correlation (0.6669) between the same markers was recorded among the participants suffering from a severe degree of that oral disorder. A great positive correlation between the clinical index GI and salivary dextran level, with a coefficient of correlation of 0.7182, was registered in the group of children with diagnosed mild degree of plaque-induced gingivitis. A moderate positive correlation between GI and salivary dextran level, with a coefficient of correlation that amounts to 0.4671, was established among the participants with a moderate degree of plaque-induced gingivitis. A moderate positive correlation between GI and salivary sIg A was ascertained in the groups of participants with a moderate and severe degree of gingivitis, with a coefficient of correlation respectively equal to 0.4932 and 0.4486. A slight positive correlation between the salivary markers dextran and sIg A was registered in the group of representatives with mild gingivitis and among these with severe gingivitis. The coefficient of correlation amounted to 0.2000 and 0.1429, respectively. A negative moderate correlation between both of these salivary indicators, with a coefficient of correlation equal to -0.4524, was recorded among the children suffering from a moderate degree of plaque-induced gingivitis.

Graph 1. Average Values of Salivary Dextran in the Different Groups

Graph 2. Average Values of Salivary sIgA in the Different Groups



The average value of the indicator sIg A in non-stimulated saliva is highest in the group of children suffering from severe gingivitis, namely AV severe= 92.26 µg/ml. Among the participants with diagnosed mild and moderate degree of gingivitis are recorded similar levels of the variable of average value, respectively AV mild= 37.84 µg/ml and AV moderate= 31.92 µg/ml. The average value of salivary sIgA in the participants with severe gingivitis, AV=92.26 µg/ml, was 2.25-fold higher compared to the same parameter in the control group (AV control= 41.28 µg/ml). (Graph 2)

DISCUSSION The contemporary diagnostics relies on the potentials of saliva as a diagnostic medium implemented for the purposes of detection of oral-dental diseases, especially for children [4]. Exopolysaccharides produced by oral pathogen *Streptococcus mutans* are characterized as water-soluble and water-insoluble glucans. Water-soluble glucans are also known as dextran rich in α -1,6-glucosidic linkages. Production of water-soluble dextrans in the oral cavity affects the bacterial growth, colonization and dental plaque formation [5]. Our results also demonstrate that increased dextran levels in saliva are associated to the progression of the gingival inflammation and the degree of plaque-induced gingivitis. It is considered that an elevated oral antigenic load intensifies the synthesis of sIg A [6]. The increased rate of salivary sIg A with target orientation against pathologic bacteria in dental plaque can defend the gingival tissues from plaque-induced gingival inflammation by control of cytokines' and interleukins' response [7]. In parallel, the main protective trait of salivary secretory immunoglobulin A is associated to inhibition of adherence of pathogenic microorganisms, rather than disintegration and dysactivation of antigens [8]. That is associated to established interrelations between GI, salivary dextran and sIg A recorded in our study. Some bacterial species which are representatives of oral microbioma in patients with periodontal disorders are identified by researchers as producers of Ig A protease. That enzyme suppresses the activity of serum Ig A and salivary IgA, with an enhancement of the microbial adherence potential and pathogenic plaque overgrowth. Reduced level of sIg A leads to an elevated risk of periodontal diseases [8]. This establishment corresponds to our results of a negative moderate correlation between salivary dextran and sIg A in the group with a moderate degree of gingivitis.

Conclusion: Salivary sIg A and dextran levels are associated with clinically manifested degrees of plaque-induced gingivitis in children.

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Comparison of the transverse effects of class II elastics and myofunctional appliances during the treatment of class II 1 malocclusion

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Summary

Purpose: To compare the scope of transverse changes as a result of the treatment with class II elastics and myofunctional appliances.

Material and Methods: We studied the intercanine, interpremolar and intermolar distance on cast models of 70 growing patients before and after their sagittal treatment with the two modalities. **Results:** In the upper jaw – the intercanine distance decreased after the treatment with class II elastics and increased after the treatment with EF Braces; lower jaw - intercanine distance increased with 0,57 mm more due to the EF Braces treatment, the interpremolar and intermolar distances exhibited a significant difference of 0,77 mm and 1,36 mm between the two treatment modalities accordingly. **Conclusions:** As a result of the treatment with class II elastics the intercanine distance in the upper jaw and the intermolar distance in the lower jaw decrease. EF Braces causes expansion of both jaws, more pronounced in the lower one.

Introduction

Class II 1 malocclusion, albeit in the sagittal plane, is usually accompanied by various vertical [1] and transverse discrepancies which are often disregarded but need to be properly taken into account for the best clinical outcome. This is why we chose to focus our comparative study on the transverse effects of two of the most commonly used methods for class II correction – class II elastics and EF Braces myofunctional appliance [2,3]. Research on the sole usage of myofunctional appliances report expansion of the upper and lower dental arches [4]. On the other hand, the class II elastics exhibit some side effects which influence the transverse dimension of the arch such as lingual tipping and mesiolingual rotation of the lower molar [5]. Our aim was to examine and compare the scope of transverse changes as a result of the sagittal treatment with class II elastics and myofunctional appliances.

Materials and methods

Our study included 70 patients aged from 10 to 16 years divided into two groups of 35 patients each – first group was treated with class II elastics, second group - with EF Braces trainer. The inclusion criteria of the patients were the following: class II division 1 patients with $\alpha\text{ANB} \geq 4^\circ$, non-extraction treatment and no patients with hypodontia, only patients in permanent dentition. The exclusion criteria were asymmetric class II division 1, complete skeletal growth, patients with values of $\alpha\text{MP/Sn} > 40^\circ$, obstructive oral breathers. We took impressions from the patients just before the application of the two appliances with 0.016x0.022 stainless steel(SS) archwires inserted in both arches and after reaching class I relationships. For this period patients were instructed to wear the class II elastics (1/4', 6,5 oz) for the whole day except during eating and the EF Braces appliance for 12 hours a day with breaks. We measured the intercanine, interpremolar and intermolar distance in the upper and lower jaw with a digital caliper (Hammacher D-42699) with accuracy up to one decimal place on the cast models.

Statistical methods: The parameters were measured on metric scales and are presented with the mean value and standard deviation (\pm SD). The intergroup comparisons were made by the independent-samples t-test. In the tables these cases are marked with a sign! in the column for the value of p. All analyzes were performed at a permissible error level (alpha) of 5% ($p < 0.5$), but the results were graded according to statistical significance as follows: * - significant difference / change at $p < 0.05$; ** - significant difference / change at $p < 0.01$; *** - significant difference / change at $p < 0.001$. For analysis and graphical presentation of the data were used IBM SPSS, version 26 (2019), Minitab version 19 (2020) and MedCalc® version 20.008 (2021).

Results

As a result of the treatment with **class II elastics** only the intercanine distance in the upper jaw and the intermolar distance in the lower jaw significantly decreased with 0,28 mm and 0,76 mm accordingly. The group treated with **EF Braces**, on the other hand, showed an increase in all the values. There was a significant increase in the intercanine distance – 0,60 mm, interpremolar distance – 0,76 mm and intermolar distance – 0,60 mm in the lower jaw.

When comparing the transverse changes of both methods we found the following results: upper jaw – the intercanine distance decreased after the treatment with class II elastics and increased after the treatment with EF Braces with an overall difference of 0,47 mm; there were no significant changes in the interpremolar and intermolar distance; lower jaw – there was a significant difference in all three parameters – intercanine distance increased with 0,57 mm more due to the EF Braces treatment, the interpremolar and intermolar distances increased after the EF Braces treatment and decreased as a result of class II elastics application with a difference of 0,77 mm and 1,36 mm accordingly (Table 1).

Transverse dimensions	Treatment			t-test
	EF Braces (N = 35)	Class II intermaxillary elastics (N=35)	95% CI	p
Upper jaw				
C -C change \bar{X} (\pm SD) (mm)	+0.19 (\pm 0.86)	-0.28 (\pm 0.63)	0.47(0.10 to 0.82)	0.012*
P -P change \bar{X} (\pm SD) (mm)	+0.26 (\pm 0.86)	+0.03 (\pm 0.16)	0.23(-0.52 to 0.07)	0.137!
M -M change \bar{X} (\pm SD) (%)	0.00 (\pm 1.19)	+0.05 (\pm 0.73)	0.05(-0.43 to -2.90)	0.510
Lower jaw				
C-C change \bar{X} (\pm SD) (mm)	+0.60 (\pm 0.75)	+0.03 (\pm 0.11)	0.57(0.12 to 0.82)	0.000***!
P-P change \bar{X} (\pm SD) (mm)	+0.73 (\pm 0.96)	-0.04 (\pm 0.20)	0.77(0.16 to 1.10)	0.000***!
M-M change \bar{X} (\pm SD) (%)	+0.60 (\pm 0.75)	-0.76 (\pm 1.16)	1.36(0.89 to 1.83)	0.000***

Table 1: Comparison of the changes after the application of the two treatment modalities; ***: Statistically significant change at $p < 0.001$.

Discussion

The decrease of the intercanine distance in the upper jaw and the intermolar distance in the lower jaw after the elastics' application has its biomechanical foundation. Although their main effect is sagittal, they have a transverse effect as the line of action of the force passes vestibularly from the center of resistance of these teeth inclining the upper canines and lower first molars orally. The reported changes are slight as we apply the class II elastics on rectangular SS archwires.

The buccal shields of the EF Braces eliminate the action of the external muscular group and thus allow the tongue to widen the arches [6]. M. buccinator and m. orbicularis oris become stretched in the zone of their insertion. Hence, the periosteum is irritated and bone starts to deposit [7]. Ramirez [4] reports higher values of increase for all parameters. In his study, however, patients are treated only with the myofunctional device T4K without using fixed appliances. Our results illustrate the effect of EF Braces exclusively using rectangular stainless steel archwires. These results indicate that if a slight expansion of the dental arches is needed to compensate for the possible relapse [8] after completion of orthodontic treatment, the EF Braces myotrainer should be the appliance of choice.

Conclusion

As a result of the treatment with class II elastics the intercanine distance in the upper jaw and the intermolar distance in the lower jaw decrease. The application of EF Braces causes expansion of both jaws which is more pronounced in the lower one.

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Digital analysis of dental arch perimeter in upper arch after incisor protrusion

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The **aim** of our research is to find out the value of tooth-alveolar changes after protrusion of upper frontal teeth in cases with crossbite in early mixed dentition. **Materials and Methods:** In our research participate 37 patients on average age 7.68. We used fixed functional appliance with protruding spring, designed by us. We made linear measurements before and after treatment on cephalometric X-rays and digital models. **Results:** The average duration of treatment is 2, 85 months (from 2 to 5 months). We also measure dental arch perimeter before and after treatment. The dental arch perimeter average increase with 3.33 mm. **Conclusion:** The crossbite should be treated at the moment it has been find. This unblocks the jaws and helps them to follow their natural growth pattern.

Keywords: Crossbite, Digital models, Dental arch perimeter, Fixed functional appliance

Introduction

The crossbite in frontal area is oral disposition of upper incisors compared to lower. When this deformation is not complicated with skeletal problem, the most common manifestation is retrusion of upper frontal teeth. This deformation when occur in period of growth lead to complication in development of anatomy and function in facio-cranial area. For patient this is serious aesthetical problem. Anatomical and functional problems that can occur because of untreated cross bite in frontal area are: complicated and blocked growth processes in upper jaw, the tendency of developing III class malocclusion, gingival inflammation or recession in lower incisors; occlusal trauma; abrasion of email on incisal edges; the abnormal chewing is developed, because of untraditional movements of lower jaw; temporomandibular disorders. Different authors report that the frequency of crossbite in frontal area is from 1.6% to 7.9% [1-3]. In his research above untreated orthodontically children Karaiskos find out 10.5 % crossbite in frontal area in 6 years old children and increasing percent of 11.9% in 9 years old group [4].

In clinical practice, the orthodontist used fixed appliances such as: Bonded Resin-Composite Slopes, described by Bayrak [5]; 8 brackets used by Wiedel [6]; Dowsing used 2x4 appliance [7]; Bowman present Quick Fix technique for inclination of incisors through protruding arch [8]; Sockalingam and Tzatzakis are supporters of dental disocclusion with brackets on incisors [9, 10], Pinho use anterior composite ramps, posterior composite bite block and selective wear of temporary canines [11]. Other authors used removable appliances [12, 13]. Ilisulu a wide range of treatment protocols can be used to correct an anterior crossbite in the mixed dentition [14, 15]. In systematic review authors concludes that treatment with fixed appliances is more effective from treatment with removable appliances [16, 17]. On the base of years of accumulated clinical experience we invented appliance included: two plastic caps that cover the distal teeth. In the appliance designed the TPA which connected two plastic parts are also included; there is the protruding spring, which comes out from medial palatinal surfaces of plastic parts, touching palatinal surfaces of the incisors [18].

The aim of our study is to evaluate the protocol for treatment in crossbite in early mixed dentition, to find out the data for tooth-alveolar changes during protruding of upper frontal teeth, trough treatment with fixed functional appliance in upper jaw designed by us.

Materials and Methods. In our research 37 patients on average age 7.68 (from 6 to 10 years) are included. Across them 15 (40.5%) are female and 22 (59.5%) are male. Including criteria are crossbite in frontal area of one to four incisors, and each patient need protrusion of upper incisors, I skeletal class, in vertical direction lower frontal teeth covered upper from 2 mm to full clinical crown. The patients have given their consent that all the collected data can be use in science research. We used our fixed

functional appliance with protruding spring. We made linear measurements before and after treatment on cephalometric X-rays and digital models. We measure the distance between PTV line and tree points in incisor: Ie -incisor edge; Ic -tooth cervix; Ia-tooth apex. We measure the angle between axis of central incisor (I) and SN plane and the perimeter of dental arch on digital models create before and after treatment. We make the measurements in upper dental arch from the distal surfaces of first right permanent molar to distal surface of left first permanent molar (p1 – dental arch perimeter before treatment, p2- perimeter after treatment).

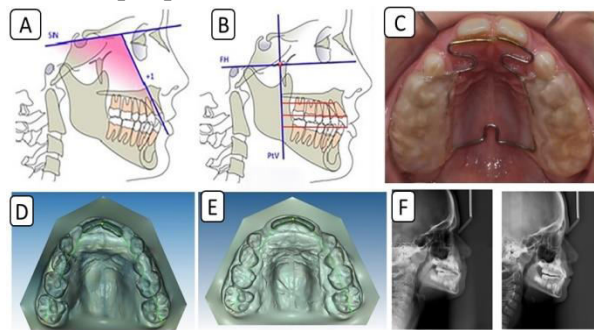


Fig. 1 Methods for established the result
 A The changes in upper incisor inclination; B The distance between PTV line and tree points in incisor; C Functional fixed appliance D,E Measurement on digital model before and after treatment; F X-ray

Comparison	Paired Differences					t	df	p	
	Mean	SD	Min	Max	95% CI of the Difference				
p1 - p2	3,33	2,28	0,10	12,00	4,15	2,50	8,25	37	<0,001
I/SN1 - I/SN2	8,91	6,46	-4,00	23,00	11,23	6,58	7,80	37	<0,001
Ie-PTV1 - Ie-PTV2	4,47	2,16	1,00	9,00	5,25	3,69	11,73	37	<0,001
Ia-PTV1 - Ia-PTV2	1,47	2,12	-1,00	7,00	2,23	0,70	3,91	37	<0,001
Ic-PTV1 - Ic-PTV2	2,22	1,41	0,00	6,00	2,73	1,71	8,91	37	<0,001
Comparison						t	df	p	
Difference (Ie-PTV)		Difference (Ia-PTV)				5,97	37	<0,001	
Difference (Ie-PTV)		Difference (Ic-PTV)				7,23	37	<0,001	
Difference (Ia-PTV)		Difference (Ic-PTV)				-1,25	37	0,221	

Tab. 1 The results showed changes in teeth position and dental arch perimeter

The teeth in distal segment are included in appliances fixed part, so the change in dental arch perimeter is due only to incisors movement. The survey data were processed with statistical system SPSS 15.00 for Windows statistical software.

Results. The average duration of treatment is 2.85 months (from 2 to 5 months). With Paired samples t-test have been established that the average value of protrusion of incisors is 8.91⁰ and average change in the perimeter of upper dental arch for the period of treatment is 3.33mm (from 1 to 5.02mm) with standard deviation (SD) of 2.28mm (the data are shown in Tab. 1). Most significant changes are in Incisal edge, which is moved vestibular with average vale of 4.47mm. The tooth apex is moved only with 1.47mm. This difference is sign of process of protrusion and minimal vestibular movement. The protrusion is tooth movement, which is possible for this shorth treatment period, while the corpus movement required time. The average change in tooth cervix region is closer to root movement and is with average value of 2.22mm. In comparison the significant difference between the data for tooth movement before and after treatment, the indicators that are observed are: Ie-PTV and Ia-PTV (incisal edge and apex) and between Ie-PTV and Ic- PTV (incisal edge and cervix). These differential relations one more time confirm the conclusion that with the appliance we achieved mostly protrusive movement, and that is the way to create space in dental arch.

The average proclination of incisors is 9.13° and the average increase in perimeter is 3.32mm. The correlation between this parameters is for every 3° proclination we increase the dental perimeter with 1 mm. We also measure tooth arch perimeter before and after treatment. The dental arch perimeter average increase with 3.32 mm (from 1 to 5.02 mm).

Discussion Crowding in frontal area disturb the aesthetic and when the distal segment is affected that lead to occlusal problems, which affect the chewing function. Through orthodontic analysis the discrepancies in tooth arch are frequent finding. We measure the available space (the tooth arch perimeter) and necessary space. When we know with how many mm the protruding method will increase the dental arch perimeter, we may make prognosis for future orthodontic treatment and we rely on this predictability. Therefore, the early treatment of anterior crossbite will unblock the growth in

upper jaw and will increase the perimeter of upper dental arch with average value from 1mm for 3⁰-3.5⁰ protrusion of incisors.

Conclusion. The crossbite should be treated at the moment it has been found. In all the cases the treatment with fixed functional appliance with protruding spring is first stage of interceptive treatment. This unblocks the jaws and helps them to follow their natural growth pattern. Contemporary method for measurements of dental arch perimeter with appropriate software eliminate the possibility of human mistake and give opportunity for visualization and prognosis of treatment results.

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Cross infection control awareness and practices in Bulgarian dental laboratories

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Abstract:

The aim of the present study was to assess the knowledge, practices of infection prevention measures and use of personal protective equipment (PPE) among dental technicians. A structured anonymous questionnaire consisting of 23 questions was distributed among dental technicians in Bulgaria. SPSS Statistics v.25.0 was used for statistical analysis, at a significance level $p < 0.05$. Overall 39 dental technicians took part in the study with a median age of 42.45 years. 66.67% of the respondents were informed if the impression were previously disinfected mainly through a phone call-51.28%. 58.97% were disinfecting impressions regardless preferably by spraying with alcohol disinfectants. Only 35.9 % of the respondents have received the vaccine for Hepatitis B. The lack of communication between dentists and dental technicians raises the need for increasing awareness and establishing educational programs.

Key-words: infection control, dental technician, disinfection, impression

Introduction:

Dental professionals, whether working with patients in clinics or in dental laboratories, are always at risk of exposure to a wide variety of microorganisms from blood and saliva of patients and also to airborne infections from aerosols and spatter during procedures [1]. A dental laboratory is an area where dental technicians can get infected mainly from soiled impressions received from the dental clinics [2,3]. On the other hand, cross infection may arise among dental staff and patients from contaminated items sent from the dental laboratories to dental clinics [4]. Implementation of infection control protocols and procedures, coupled with effective communication among the dental practitioner and technician, can help in creating a safe environment for personnel exposed to potential occupational risks [5]. The aim of the study was to assess the knowledge, practices of infection prevention measures and use of personal protective equipment (PPE) among dental technicians.

Materials and Methods:

The study was carried out in the period February- April 2017. A structured anonymous questionnaire was distributed among dental technicians across Bulgaria. 39 dental technicians from 6 districts in the country took part in it. The questionnaire consisted of 23 questions. The main part of the questions was related to type and mode of disinfection of impressions, working surfaces and handpieces, availability of and type of method for sterilization used for instruments, use of personal protective equipment (PPE), HBV vaccination. There were also demographic questions and questions that assess the workload and the working experience of the participants. Statistical analysis was conducted using SPSS statistics v 25.0.

Results:

39 dental technicians took part in the study. The median age of the respondents was 42.45 ± 11.02 years. The information regarding the workload in the laboratory and the working experience of the participants

is presented in **Table 1**. We were not able to find an association between the working experience and the disinfection practices of the respondents ($\chi^2=1.726$, $p=0.422$).

Table 1. Professional experience and major characteristics of the working process.

Question	Variable	n	%
What is your working experience?	Up to 5 years	9	23.1
	5-10 years	6	15.4
	More than 10 years	24	61.5
How many impressions do you receive weekly?	Up to 10	9	23.08
	10-20	18	46.16
	More than 20	12	30.76
Impressions from what material do you more commonly use?*	Alginate	32/39	82.05
	C-silicones	26/39	66.67
	A-silicones	14/39	35.90
	Polyether rubber	1/39	2.56

*Multiple choice

The study revealed that almost two thirds of the dental technicians- 66.67%, $n=26$ were aware whether the impressions they receive in the laboratory are disinfected. When asked about how they are informed if the impressions are disinfected prior to receive 51.28%, $n=20$ of the respondents answered that they are informed through a phone call. **Table 2** summarizes the practices for disinfection of the respondents, types of disinfectants used and the use of PPE. We found that most of the participants had more than 10 years of working experience (61.5%, $n=24$) but were not able to find an association between this variable and the disinfection practices of the respondents ($\chi^2=1.726$, $p=0.422$).

Table 2. Practices of disinfection in the dental laboratory and use of PPE and HBV vaccination status of the respondents

Question	Variable	n	%
Do you disinfect the impressions regardless of previous disinfection in the dental office when receiving them?	Yes	23	58.97
	No	16	41.03
How do you disinfect the impressions?	Spraying	22	56.4
	Immersion in a solution	11	28.2
	Cleaning agent and water	6	15.4
How do you disinfect the surfaces in the dental laboratory between procedures?	Only cleaning	18	46.16
	Cleaning and disinfection	21	53.84
How do you disinfect burs, stones, diagenes, sandpaper discs between procedures?	Disinfection	10	25.64
	Sterilization	4	10.27

	Disinfection and sterilization	6	15.38
	No measures	18	46.16
	No response	1	2.55
What PPE do you use when you cast?*	Protective eyewear	24/39	61.54
	High temperature resistant gloves	18/39	46.16
	Working garment	31/39	79.49
Have you been vaccinated against Hepatitis B?	Yes	14	35.9
	No	25	64.1
*Multiple choice			

We found out that the respondents with working experience under 5 years were predominantly vaccinated (66.67%, n=6) but we couldn't prove an association between the working experience of the participants and the HBV vaccination status ($\chi^2=9.029$, $p=0.06$).

Discussion:

The problem of inadequate communication between dentists and dental laboratory regarding the disinfection of impressions has been recognized widely in the literature [6,7]. In our study 51.28% of the respondents reported that they receive the information about disinfection of impressions through the telephone, 20.5% through a label on the impression and only 12.8% stated there is a written protocol. All three ways of coordinating disinfection actions are acceptable but building a protocol would help to better control infections.

Kugel et al [8] found a lack of confidence among dental technicians that impressions were disinfected resulting in repeat disinfection of some impressions. This result is in accordance with the results from our study in which 58.97% of the respondents stated that they disinfect the impressions regardless of previous disinfection. In our study the preferred method of disinfection was spraying-56.4%. Of the various methods of disinfectant application, immersion is considered to be the most reliable [9] because all exposed surfaces of the impression trays and materials are covered by the disinfectant [10].

PPE acts as an important safety barrier to prevent exposure of the skin and mucous membranes of the dental staff [11]. The adherence to PPE in our study was low, as only 53.8% were using protective eyeglasses, only 38.4% a mask and only 25.6% gloves. In our study only about one third (35.9%) of the respondents have received HBV vaccination which correlates with results from a Romanian study [12].

Conclusion: There is lack of communication between dentists and dental technicians, and this raises the need for increasing awareness and establishing educational programs. Furthermore, a campaign for addressing the low vaccination coverage with the Hepatitis B vaccine would be also beneficial.

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