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CONTENTS

EVALUATION OF VESTIBULAR BONE THICKNESS OF THE MANDIBLE IN RELATION TO THE MANDIBULAR CANAL: RETROSPECTIVE CBCT STUDY

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ABSTRACT

Introduction: In implantology, in the area of the mandible, during the implantation and the collection of autogenous bone structures of the lateral part of the mandible, it is necessary to pay attention to the position of the mandibular canal, nervus alveolaris inferior and foramen mentale. The best radiographic technique of imaging during various analyses is cone beam computed tomography (CBCT) providing the exact identification of anatomic structures and having the capability to provide accurate measurement and enlargement, high resolution without artefacts. The aim of the study was to evaluate the vestibular bone thickness of the mandible in relation to the mandibular canal and to determine is there dental status-related difference.

Methods: Out of 700 examined CBCT images, 217 CBCT images that satisfied inclusion criteria of the study were analyzed. The measurement was conducted by Sidexis program on the cross section of the CBCT image. The measurement of vestibular bone thickness was performed by measuring the distance from the lateral wall of the mandibular canal to the buccal surface of the mandibular compact bone covering the region of the second premolar, the first and the second molar.

Results: T-test revealed a statistically significant difference in vestibular bone thickness on the left side ($p = 0.028$) between dentate and partially edentulous patients in the area of distal root region of the second molar, where dentate patients (5.825) had, on average, larger vestibular bone thickness compared to partially edentulous patients (5.326).

Keywords: mandible, vestibular bone thickness, CBCT

Introduction

Surgical interventions in the area of the mandible demand certain precaution measures considering the anatomic structure of the mandible, as well as its vascularization and innervation. In implantology, in the area of the mandible, during the implantation and the collection of autogenous bone structures of the lateral part of the mandible, it is necessary to pay attention to the position of the mandibular canal, nervus alveolaris inferior and foramen mentale, as well as arteries and veins going through the mandibular canal so that, during the intervention, iatrogenic injury of these structures would not occur. (1-7) During surgical placement of implants, if the size of the bone is not sufficient for the implantation, numerous surgical interventions are conducted in order to compensate the bone for the placement of implant. One of the ways of bone compensation is compensation with the help of autogenous bone graft which can be harvested intraorally and extraorally. (8) Other ways of compensation of the volume of bone are guided bone regeneration as well as osteogenesis of alveolar distraction. (9)

The extraoral autogenous bone graft harvesting implies getting bones the most frequently from the area of iliac crest and it entails numerous disadvantages such as: higher costs, greater time consumption, hospitalization of patients, general anaesthesia, walking difficulties, pain, scars in the bone removal area. (8,10) The extraoral bone harvesting may involve taking of bone graft from other parts of the body such as the calvary, tibia or ribs. The intraoral bone harvesting offers more advantages in relation to the extraoral bone harvesting. The advantages are: better surgical approach, shorter time of surgery, general anesthesia not needed, elimination of the need of hospitalization, no scars, more comfortable for a patient. Also, bone harvesting from the area of maxillofacial region provides better biological benefits for augmentation. (8) This implies bone harvesting, most frequently from the area of the ramus of the mandible, but also the corpus, the mandibular symphysis, the residual ridge of the mandible and the process of the coronoideus. (8,11-14) Disadvantages of intraoral bone grafts are mainly temporary, among which the following are described: short-term disorder of the sensibility of lips, mucosa, skin or teeth, limited opening of the mouth, pain, changes in facial contours. Complications differ depending on the mandible area from which the bone graft is harvested. (15-20)

Considering the benefits of intraoral bone grafts, this manner of bone formation is increasingly used. However, by inadequate recognition of vital structures such as the lower alveolar nerve and mental opening, an injury of these structures can occur entailing certain consequences such as neurosensory changes of chin, lower lip, pain, stiffness, changes in senses; during the harvesting of bone grafts from the mandible or with osteotomy, as the alveolar nerve is closer to the buccal cortex of the mandible, the quality of the bone is smaller, the neurosensory disorders are more expressed, and women are especially more susceptible to the occurrence of these disorders. (7, 21-23) In order not to cause an injury of nerves, arteries and veins by intraoral bone harvesting, implantations and numerous other surgical interventions in the mandible area, a doctor of dental medicine must be familiar with the anatomic characteristics of the mandible as well as with pathological conditions and traumatic sequelae creating unfavorable conditions for the implantation that can lead to compromised physiological relations of the anatomic structures of the mandible. Prior to any surgical technique planned to be performed in the area of the mandible, with the aim of identification of the exact location of the anatomic structures, it is necessary to make appropriate radiographic imaging. (24, 25)

The best radiographic technique of imaging in implantology during various analyses is cone beam computed tomography (CBCT) providing the exact identification of anatomic structures, having the capability to provide accurate measurement and enlargement, high resolution without artefacts. The radiation dose is lower compared to the usual CT scans, while the conventional radiography interprets a two-dimensional representation of the mandibular canal, with shortcomings of the third dimension by limited enlargement, reproducibility of details and distortion. (26-29) The accuracy of CBCT imaging has been confirmed by the use of different CBCT scanners. (30-34)

The aim of the study was to evaluate the vestibular bone thickness of the mandible in relation

to the mandibular canal and to determine whether there is dental status-related difference.

Materials and methods

It was accessed the database of CBCT images taken at the School of Dental Medicine at the University of Sarajevo in the period from 2017 to April 2020, taken for various dental purposes. Out of 700 examined CBCT images, 217 CBCT images from 110 females and 107 males were analyzed satisfying inclusion criteria of the study.

Inclusion criteria included the following:

1. acceptable image quality

2. representation of the entire mandible

3. clearly detectable and represented nervus alveolaris inferior

The exclusion included the following:

1. irregular volume of the bone and presence of pathological changes in the measurement region such as cysts, tumors, periapical lesions

2. presence of the mandible fracture

3. disturbed course and continuity of nervus alveolaris inferior

4. Impacted and semi-impacted teeth in the measurement region

5. Patients younger than 14 years

Patients were divided in two groups: fully dentate patients and partially edentulous patients.

CBCT images were taken by using an ORTHOPHOS SLX imaging unit. The nominal power output of this device is 2kW at 90 kV / 16mA, nominal frequency 50Hz / 60Hz. The tube voltage is 60-90kV (for 90kV max.12mA) and the power in tube is 3-16mA (for 16mA max.69kV). The frequency of generation of creating a high voltage is 40-120kHz. The time of exposure to image is a maximum 14.9 s. Entire filtration of X-ray tube is > 2.5 mm Al / 90 IEC 60522 0.3 mm Cu. The size of the focal point towards IEC 60336, measured in the central X-ray, is 0.5 mm.

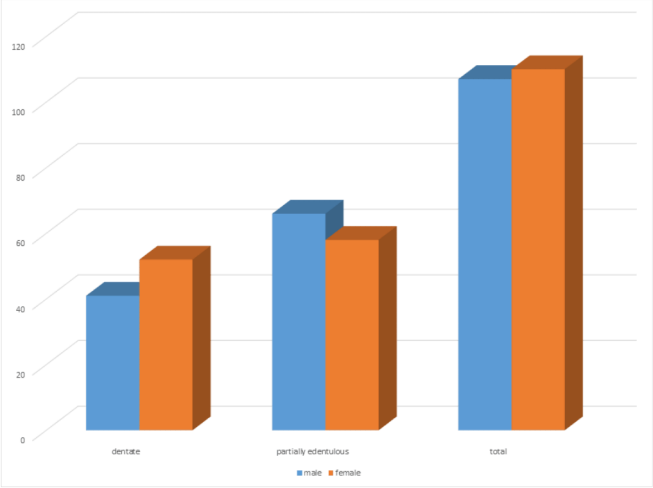
The measurement was conducted by Sidexis program on the cross section of the CBCT image. The measurement procedure involved previous mapping of the nervus alveolaris inferior, and subsequently in the region of the second premolar, of the first and second molar with the help of ruler and protractors on the cross-sectional intersection, following the tooth axis and / or roots axis, the vestibular bone thickness was measured (spongiosis + cortical plate), by measuring the distance from the lateral wall of the mandibular canal to the buccal surface of the mandibular compact bone. The measuring points along the alveolar nerve of interest were: the center of the second premolar (35 and 45) as well as the centers of the first and second molars, but also the areas of the mesial and distal roots of the first (36M, 46M, 36D and 46 D) and second molars on both sides of the mandible (37D and 47D). With partially edentulous patients in areas where teeth were missing, measurements were conducted only at measuring points that marked the “center of the tooth” and they were determined by reconstruction methods used in implantology. The smallest vestibular thickness of the mandible was recorded as the shortest possible value between the two mentioned distances, that is, the measurement was carried out under the angle of 90 degrees.

The statistic software IBM SPSS Statistics 23 was used to conduct the statistic tests, as well as for the calculation of descriptive statistics. Excel 2013 was used to create graphs in descriptive statistics. Considering different variables, comparisons and size of samples of certain groups and sub-groups, test used within the study included: t-test for independent variables, post-hoc (Bonferroni).

Results

A total of 217 examinees participated in research, out of which 93 examinees were fully dentate and 124 examinees were partially edentulous. The average age of dentate patients was 26.31 years (minimum 14 and maximum 67 years), partially edentulous 44.36 years (minimum 19 and maximum 72 years).

Graph 1 shows the number of patients divided according to sex and dental status.



Graph 1.
Number of patients divided according to sex and dental status

Tables 1 shows the mean values of vestibular bone thickness in dentate and partially edentulous patients.

As shown in Table 2., the vestibular bone thickness did not differ between dentate and partially edentulous patients for following measuring points: second premolar (35 and 45), mesial root of the first molar (36M and 46M), the distal root of the first molar (36D and 46 D), and the mesial root of the second molar (47M and 37M).

T-test (Table 2) revealed a statistically significant difference in vestibular bone thickness on the left side ($p = 0.028$) between dentate and partially edentulous patients in the area of distal root region of the left second molar, where dentate patients (5.825) had larger vestibular bone thickness compared to partially edentulous patients (5.326).

Based on results of the post hoc test shown in Table 3, a statistically significant difference ($p<0.0001$) exists in the values of vestibular bone thickness, in dentate patients, except for groups of teeth: 46M-47C, 46C-46D, 46D-47D, 36M-36C, 36M-37M, 36M-37C and 36C-36D.

In partially edentulous patients, the vestibular bone thickness differs ($p<0.0001$) between the area of second premolar and mesial root of the first molar and between the area second premolar and center of the first molar, on both sides of mandible. Also, there is statistically significant difference for vestibular bone thickness between measuring points: second premolar and distal root of the first molar on the left side.

Patients		Vestibular thickness on the right side		Vestibular thickness on the left side	
	Measuring points	Mean	Std. dev	Mean	Std. dev
Dentate	tooth center 45	4.2653	1.13226	4.3161	1.03599
	mesial root 46	5.6805	1.14493	5.7149	1.12388
	tooth center 46	6.0142	1.12357	5.9941	1.23003
	distal root 46	6.2126	1.20777	6.1988	1.26778
	mesial root 47	6.0444	1.45977	6.1900	1.34601
	tooth center 47	6.1232	1.50903	6.1612	1.40054
	distal root 47	5.6895	1.47323	5.8251	1.42357
Partially edentulous	tooth center 45	4.5950	1.48346	4.1355	1.27427
	mesial root 46	5.7928	1.26833	5.6535	1.48611
	tooth center 46	6.2094	1.25022	5.8090	1.34210
	distal root 46	6.1056	1.36646	5.9695	1.34263
	mesial root 47	5.4611	1.49810	5.7525	1.57368
	tooth center 47	5.5911	1.29879	5.8610	1.58062
	distal root 47	4.7989	1.35929	5.4540	1.49830

Table 1.
Mean values of vestibular thickness of the mandible on the both side according to dental status

Measuring points	t - statistics	p - value
right - tooth center 45	- 1.145	0.254
left - tooth center 35	- 0.709	0.479
right - mesial root 46M	- 0.307	0.759
left - mesial root 36M	0.062	0.951
right - distal root 46D	0.509	0.612
left - distal root 36D	0.900	0.370
right - mesial root 47M	0.557	0.578
left - mesial root 37M	1.750	0.082
right - distal root 47D	1.456	0.147
left - distal root 37D	2.223	0.028*

Table 2.
Results of t – test (differences between vestibular bone thickness between dentate and partially edentulous patients); * Statistical significance at the level of 5 %

Groups of teeth - Dentate	p - value
45 46M	<0.0001
45-46C	<0.0001
45-46D	<0.0001
45 47M	<0.0001
45-47C	<0.0001
45-47D	<0.0001
46M-46C	<0.0001
46M-46D	<0.0001
46M-47C	0.023
46C-46D	0.014
46D-47D	0.001
47M-47D	<0.0001
47C-47D	<0.0001
35-36M	<0.0001
35-36C	<0.0001
35-36D	<0.0001
35-37M	<0.0001
35-37C	<0.0001
35-37D	<0.0001
36M-36C	0.006
36M-36D	<0.0001
36M-37M	0.005
36M-37C	0.021
36C-36D	0.004
37M-37D	<0.0001
37C-37D	<0.0001

Table 3.
Results of post hoc tests: differences between vestibular bone thickness between different measuring points for dentate patients

Groups of teeth - Partially edentulous	p - value
45-46M	<0.001
45-46C	<0.0001
45-46D	0.002
47M-47D	0.007
47C-47D	0.004
35-36M	<0.0001
35-36C	<0.0001
35-36D	<0.0001
35-37M	.009
35-37C	0.006
35-37D	0.071
37C-37D	0.002

Table 4.
Results of post hoc tests: differences between vestibular bone thickness between different measuring points for partially edentulous patients

Discussion

The position of the mandibular canal within the mandible is highly variable and a clear consensus regarding its position does not exist. Different authors have observed its course considering different reference points, different age, sex, dental status (6, 35, 36)

This research, by measuring the value of vestibular bone thickness as distance from the lateral wall of the mandibular canal to the buccal mandibular compact bone, indicates to us the course of the mandibular canal. The higher the values of vestibular bone thickness at a certain measuring point are, the more distant is the mandibular canal from the outer (vestibular) surface of the mandible. The value of vestibular bone thickness is the highest in the area of the first molar, and the thinnest in the area of the second premolar. This finding is in agreement to some extent with those of Sghaireen *et al.* who also found the lowest values of vestibular bone thickness in the area of the second premolar, but the highest ones in the area of the second molar. These authors measured the distance from the buccal

and lingual surface of the mandibular compact bone to the nervus alveolaris inferior, and the distance from the top of the alveolar ridge to the nerve. They found that the mean values of vestibular bone thickness in the area of the second premolar in partially edentulous totalled 2.08 mm on the right side and 1.70 mm on the left side, and in dentate patients 2.14 mm on the right and 1.74 mm on the left side. (37)

The highest thickness of the vestibular bone in dentate patients considering the right side is in the area of the distal root of the first molar (6.21 mm), being the same on the left side (6.19 mm). In partially edentulous patients, the highest value of vestibular bone thickness is also in the area of the second molar, specifically on the right side in the area of the tooth center (6.20 mm) and on the left side in the area of the distal root (5.96 mm). The values of vestibular bone thickness are increasing going distally then falling down from the distal root of the second molar. Regardless of dentate or partially edentulous patients, the alveolar nerve is at the distal root of the first molar slightly more distant from the outer surface of the bone of the mandible in relation to other areas. The research conducted by Koivisto *et al* shows that vestibular bone thickness is also the highest in the area of the second molar. The highest mean vestibular bone thickness totalled 5.4 mm in the area of the mesial root of the second molar, being the smallest at the position of the second premolar with a value of 2.6 mm. (37) The maximum mean value in this study is higher compared to the results of the maximum mean value in the research by Koivisto *et al*. These differences of mean values at the same positions of the measuring points among different researchers may be a consequence of racial differences, age and other differences as well. (38) Al-Siweedi concluded that ethnic affiliation impacts all measurements in his study, inclucing the buccolingual position of the mandibular canal, and Levine *et al.* drew the same conclusion (39,6)

Safaee *et al* measured the distance of the mandibular canal from the outer cortex of the lingual and vestibular sides of the mandible. They have concluded that the canal approaches the lingual side by going posteriorly and that the highest vestibular bone thickness is in the area of the second molar. (40)

In their research, Valdec *et al* found that the mean value of bone thickness between the mandibular canal and the buccal surface of the mandibular cortical plate was approximately 4 mm directly posterior to the mental foramen. That distance increased up to the value of about 6 mm in the first 30 mm distally from the mental foramen and then decreased to about 3 mm at the most posterior measurement at the level of the mandibular foramen. According to those results, it is clearly seen that the mandibular canal is moving away from the buccal cortex by going from the mental foramen and then probably at the position of the first or second molar (as it is the case in this study) it reaches maximum values of distance from the buccal mandibular cortex and then it approaches the buccal cortex in lateral segments. (41)

Dentate patients have the highest mean values of vestibular bone thickness, and slightly lower values were found in partially edentulous patients. However, we have detected a statistically significant difference between dentate and partially edentulous patients only for vestibular bone thickness measured in the distal root of the second right and left molar. Kilic *et al* investigated different positions of the mandibular canal in relation to different reference points as well as the position in the buccolingual direction in different groups of patients according to dental status. They obtained results showing that the canal for dentate, partially edentulous and edentulous patients, varied considering different measuring points, and in some segments, there was a difference between the position of the canal in different patients. In some segments that difference did not exist, and no precise conclusion has been made regarding the exact position of the canal in the buccolingual direction and the thickness of the buccal wall of the mandible at the place of passing of the mandibular canal in three different groups of patients. (42)

Conclusion

"The safe zone" of surgical intervention does not exist because each patient is an individual for himself, but the area of the distal root of the first molar is a point of orientation in clinical conditions, whereby chances of iatrogenic injury of the nervus alveolaris inferior are smaller. Whenever possible before any mandible surgery, it would be desirable to do perform CBCT scan.

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ASSESSMENT OF CRESTAL BONE LOSS AND DEGREE OF SUCCESS OF RITTER SPIRAL DENTAL IMPLANTS BASED ON RADIOLOGICAL MEASUREMENTS

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ABSTRACT

Introduction: After the implantation of dental implants and especially after the prosthetic load, the resorption of the alveolar ridge often occurs impairing the osseointegration. After numerous studies, dental implant manufacturers realized that the platform switching technique can lead to a reduction in resorption.

The aim of study was to show the overall level of crestal bone loss around the Ritter spiral implant after one year.

Materials and methods: The database of CBCT images were taken at the Faculty of Dentistry of the University of Sarajevo in the period from 2021 to 2023, where 73 CBCT images meeting inclusive study criteria were analyzed. The images were analyzed in the GALILEOS program, where the measurements were made in an axial cross-section, and the reference points for the measurement were from one side of the implant to the furthest point of bone loss on that side.

Results: The annual loss of the crestal bone is 0.81 mm. The greatest loss of crestal bone is on the distal side of the implant (0.93 mm) and the smallest on the vestibular side (0.63mm) of the implant. The greatest loss of crestal bone on an annual level had implants in the area of molars in the upper jaw.

Conclusion: All Ritter spiral implants were successfully osseo-integrated. It was proven the bone loss towards the sides of the implant and position in the jaw.

Key words: Crestal bone loss, dental implants, osseointegration

Introduction

In order for the implant to have a functional durability, good osseointegration of the implant is necessary thus creating its stability. After the placement of dental implants, and especially after prosthetic loading, the resorption of the alveolar ridge frequently occurs compromises the osseointegration and stability of the implant. Exceptional importance regarding osseointegration is the crestal bone around the implant influencing its integrity.

The challenge for a dentist is the constantly increasing aesthetic demands in the area of front teeth, the so-called "aesthetic zone", with the renewal of the natural anatomy surrounding the implant. However, resorption of the crestal bone will cause the gingival recession by affecting the outcome of therapy and aesthetics. (1)

Post-restorative reduction of bone height around the implant has been known for a long time to be a normal consequence of implant therapy with regard to two-part implants. It has been described that the level of crestal bone is typically located approximately 1.5 to 2 mm below the implant-abutment junction (IAJ) one year after implantation. Several factors contribute to remodeling of the bone around the neck of the implant, more precisely biological width, infiltration of bacteria, mechanical factors, implant design, surgical trauma, surface structure of the implant and platform switching. (2)

The horizontal component of biological width represents the halo around the implant in its most coronal aspect, and research shows being approximately 1.4 mm. Due to this established mean regarding horizontal dimension of direct crestal bone loss around dental implants, a problem arises when implants are placed in adjacent places in the mouth. If the implants are placed too close together, the overlapping of the horizontal components of the biologic width of each implant serves for the increase of the effective vertical loss of the crestal bone between the implants. (3)

Research indicates that in implant systems with superstructures attached with screws, bacteria can penetrate into the internal cavity of the implant. (4-7) Hermann *et al* discovered that on bone loss at the alveolar ridge significantly impacts the micromovement between the platform of the implant and the implant abutment, but not by size of the micro-gap of interface between the implant platform and the superstructure. That may be the consequence of the fact that micromovement increases the flow of bacteria from and into the micro-space, by causing the inflammation of the connective tissue in the area of the micro-space thus leading to the bone resorption. Bone resorption will progress vertically and horizontally until biological width is created and stabilized. (8, 9)

Several studies have shown that with two-part implants, changes in the level of the bone crest appeared depending on the location of the interface between the platform of implant and the supra-structure, if the interface was moved coronally from the alveolar bone, there would occur less bone loss, but if the interface was crestal or sub-crestal there were greater amounts of bone resorption present. (10-12)

Nickenig *et al.* compared the surfaces of implants of macro and microstructure at the marginal bone level during the period of healing without stress and under functional loading. Radiographic assessment of the marginal bone level with placed implants with micro threaded neck design or rough surfaces showed that implants with a micro threaded design have caused minimal changes in the level of crestal bone during healing (without stress) and under functional loading. (13)

The surface structure of the implant affects the process of osseointegration. The degree of surface roughness depends on the system of implant. The roughness of the surface is achieved by machine processing: sandblasting and etching, laser processing or applying of a layer of a special coating. The purpose is to increase the surface which, with such processing, can be increased about 6 to 10 times. Thus, the process of osseointegration itself is speeded up and an additional micromechanical connection with the surrounding bone is achieved. With smooth implant surfaces, osseointegration may not be present, and rougher surfaces are more prone

to ion emission and corrosion. The ideal implant surface is still being researched, and numerous manufacturers claim that the surface of their implants are optimal. Some surfaces behave better in specific conditions. The surface structure of the implant consists of nano-, micro- and macrostructure. Nanostructure refers to the chemical and biochemical properties of the implant surface and it may exert influence on the function and orientation of cells. Microstructure refers to the chemical, mechanical or physical structuring of the surface. Macrostructure refers to elements of design including threads, voids or pores. Studies have shown that physical properties of surfaces at the beginning accelerate tissue reactions and affect the processes such as adhesion and differentiation of cells in the tissue surrounding the implant. (14)

The aim of this research is to show the overall level of crestal bone loss around the Ritter spiral implant after one year.

Material and methods:

Within research, it was accessed to the database of CBCT images taken at the Faculty of Dentistry of the University of Sarajevo in the period from 2020 to 2023, for various dental purposes, where out of examined 83 CBCT images, 73 CBCT images were analyzed meeting the inclusion criteria of the study.

Inclusion criteria were:

1. acceptable quality of images
2. patients to whom were placed Ritter spiral implants length 8 and 10 mm.
3. plan of placement of implants performed exclusively by radiological measurement
4. in the database, there is a CBCT image before placement and 10 to 12 months after the placement of the implant.

The exclusion criteria were:

1. irregular bone volume and the presence of pathological changes in the region of measurement such as cysts, tumors, periapical lesions
2. peri-implantitis
3. patients under 18 years of age

According to age, patients were divided in 3 groups: patients aged 20 to 37 years, patients aged 37 to 47 and patients aged 47 to 65 years. According to dental status, the patients were divided into dentate, partially edentulous and totally edentulous patients.

CBCT images were taken by using an ORTHOPHOS SLX device. The nominal output power of this device is 2kW at 90 kV/16mA, nominal frequency 50Hz/60Hz. The tube voltage is 60-90kV (for 90kV max. 12mA), and the power in the tube is 3-16mA (for 16mA max. 69kV). The frequency of high voltage generation is 40-120kHz. The exposure time of the image is max. 14.9 seconds. Overall filtering of X-ray tube is > 2.5 mm Al/90 IEC 60522 0.3 mm Cu. The size of the focal point according to IEC 60336, measured in the central X-ray beam, is 0.5 mm.

The images were analyzed in the GALILEOS program, where the measurements were made in an axial cross-section, and the reference points for the measurement were from one side of the implant to the furthest point of bone loss on that side. Measurements were made on the images 12 months after implant placement on all 4 sides: vestibular, oral, mesial and distal.

Results:

In this research, we included 73 images of patients, out of which 37 were men with average age of 41.5 years (range: 23 to 60), and 36 were women with average age of 41 (range: 19 to 63). The most of the patients were in the age group 37 to 47 years (48%), then patients in the age group 47 to 65 (38.35%) and the least number of patients was in the age group 20 to 37 years (13.69%).

In the age group of 20-37 years, all patients had dentition; the group of patients aged between 37 and 47 had 3 dentate patients (11.53%), 23 partially edentulous patients (88.46%). The group of patients under the age of 65 has the largest number of edentulous patients (77.80%) and 2 patients were partially edentulous (22.2%).

The average value of the quantity of resorption measured with all patients and on all four sides around the implant was 0.8 mm.

Measurements were conducted on all four sides of the implants of all 282 implants and by arithmetic mean, the reference value of the loss of crestal bone on an annual level was obtained.

Table 1. shows the average value of resorption depending on the type of intervention.

Table 2 shows the quantity of bone loss according to the sex of patients, where we found that resorption of bone is slightly higher in women than in men.

The greatest loss of crestal bone is on the distal side of the implant (0.93 mm) and the smallest on the vestibular side (0.63mm) of the implant.

The greatest loss of crestal bone on an annual level had implants in the area of molars in the upper jaw at positions 16, 17, 26, 27. The least resorption was at implants at positions 31, 32, 35 and 41 in the lower jaw.

Type of intervention	Number	Mean value (mm)	Standard deviation	Percentage
Implantation	73	0.81	0.083	100,00%
Sinus lift with augmentation	8	1.23	0.25	10,95%
Augmentation	14	0.93	0.07	19,17%

Table 1. Quantity of resorption according to type of intervention

Sex of patients	Number of percentage	Number of implants	Mean value (mm)	Standard deviation
Male	37	165	0.72	0.05
Female	36	117	0.91	0.03

Table 2. Quantity of resorption according to gender

Mean value	Number of implants	Mean value (mm)	Standard deviation
M - mesial	282	0.75	0.09
D - distal	282	0.93	0.09
O - oral	282	0.89	0.08
V - vestibular	282	0.63	0.05

Table 3. Quantity of resorption according to sides of implants

Position of implants	Number of implants	Mean value (mm)	Standard deviation	Percentage
11	17	0.72	0,05	6.02%
12	8	0.73	0,06	2.83%
13	13	0.82	0,04	4.60%
15	14	0.78	0,05	4.96%
16	22	0.95	0,03	7.80%
17	10	0.91	0,03	3.54%
21	14	0.71	0,06	4.97%
22	7	0.70	0,06	2.48%
23	13	0.83	0,05	4.70%
25	12	0.79	0,05	4,24%
26	14	0.95	0,02	4.97%
27	9	0.89	0,06	3.19%
31	12	0.70	0,06	4.24%
32	9	0.71	0,04	3.19%
33	8	0.79	0,04	2.83%
35	17	0.70	0,08	6.02%
36	16	0.79	0,05	5.67%
37	11	0.81	0,06	3.90%
41	11	0.71	0,06	3.90%
42	9	0.74	0,22	3.19%
43	9	0.80	0,04	3.19%
45	13	0.79	0,02	4.60%
46	14	0.81	0,05	4.96%

Table 4. Loss of crestal bone according to position of implant marked by FDI

Discussion

In this research, the main goal was to determine the amount of crestal bone loss on an annual level and to show the success rate of implants by radiological measurement. In this research we used the implants of Ritter Dental Implant System, made out of grade 5 titanium, with self-tapping threads and a nano surface for quick osseointegration. They are available in 3 specific models, in different lengths and diameters and suitable for all types of bones and surgical applications. Ritter Dental Implant System is intended for the replacement of one or more missing teeth in the upper or lower jaw in order to restore a patient's lost function of chewing. (15, 16)

The change in the level of the marginal bone in the first year after implant placement should not be higher than 1 to 1.5 mm. (17) The measured arithmetic mean of bone loss around the implant from the mesial, distal, vestibular and oral sides was less than the specified critical values. The mean value of crestal bone loss after one year was 0.80 mm, specifically 0.88 mm for 170 implants placed in the maxilla, and 0.75 mm for 112 implants placed in the mandible. These results to some extent match with the research of Ajanović *et al*, who obtained average values of resorption of 0.47 mm to 0.67 mm in the maxilla, and 0.48 to 0.96 mm in the mandible. They did not find any statistically significant difference in bone loss between maxillary and mandibular implants. They conducted measurements around implants Bredent blueSKY® with a diameter of 4.0 x 8 mm and 3.5 x 10 mm. The measured mean level of resorption around dental implants with a diameter of 4.0 x 8 mm was lower than around those with a diameter of 3.5 x 10 mm, but the difference was not statistically significant. (18)

In this research, measurements were conducted for implants with a replaceable platform. Numerous authors find lower values of crestal bone loss in implants with a replaceable platform than in implants with a conventional platform. (19-21) On the other hand, Pan *et al* obtained higher values of crestal bone loss with implants having a replaceable platform than with a conventional platform at 6, 12, and 36 months after the implant placement. The value of the vertical loss of marginal bone after one year was: 0.99 ± 0.95 for implants with a replaceable platform and 0.89 ± 0.58 mm for implants with a conventional platform. The horizontal bone loss for implants with a replaceable platform and conventional platform were 1.16 ± 0.59 and 1.00 ± 0.51 mm, respectively. (22) Therefore, the type of platform can be an additional significant factor influencing the remodeling of the crestal bone.

Rasouli *et al* in their research conducted on Nobel Biocera implants reached the result that the total resorption was 0.935 mm, therefore slightly higher resorption than in our research. For the distal crestal resorption, they obtained a value of 0.688 mm, and the mesial resorption was 0.665 mm. However, there is no statistically significant difference between those values. In addition, they did not find a statistically significant difference in relation to the implant position, bone quality, region of implantation, implant design and technique of bone augmentation that was used. (23) Other authors also do not find a difference between mesial and distal resorption. (18, 24, 25) The values of distal resorption in our research totaled 0.93 mm, while the smallest resorption was on the vestibular side of the implant (0.63 mm). With regards to augmentation techniques, the resorption following the sinus lift with augmentation was the highest (1.23 mm), while the lowest was with implantation without augmentation. Kim *et al* state that local procedures of bone grafting may provide an adequate quantity of bone for the implant placement, but it should be taken into consideration that the pattern of resorption, especially in the front part of the maxilla, is unpredictable and individual. In their research, sinus lift surgery with augmentation did not demonstrate a connection with an increased risk factor for implant cancellation, unlike other risk factors, such as for example smoking habits. (26)

Conclusion

Based on the conducted measurements, a conclusion was reached that the annual loss of the crestal bone is 0.81 mm. Thereby, each of those

implants is successfully osseo-integrated and functionally loaded. Regarding the bone loss towards the sides of the implant, the results show that the greatest bone loss is on the distal side of the implant. Measurements of the bone loss were conducted according to the position of the implant in jaws. The greatest loss of crestal bone is in the positions where molars are located, both in the upper and lower jaw. A greater quantity of resorption is in the upper jaw than in the lower jaw.

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REFERENCE GENE SELECTION FOR REAL-TIME PCR STUDIES OF PERIAPICAL INFLAMMATORY LESIONS

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ABSTRACT

Objective: The appropriate normalization strategy is crucial for accurate real-time PCR data analysis. The aim of this study was to identify the most stable reference genes for expression analyses by real-time PCR in human periapical inflammatory lesions.

Methods: We compared the expression profiles of nine candidate reference genes, B2M, GAPDH, SDHA, YWHAZ, EIF4A2, TOP1, CYC1, ACTB and 18S, using two algorithms, geNorm and NormFinder, on 20 samples categorized as: an experimental group consisting of chronic periapical inflammatory lesions (periapical granulomas (n=6) and radicular cysts (n=7)), and a control group consisting of healthy gingiva tissue (n=7).

Results: According to geNorm algorithm, B2M, SDHA and TOP1 were the three most stable genes and the most optimal combination for normalization in periapical inflammatory lesions. To note, a commonly used reference gene GAPDH was the least stable gene. According to NormFinder algorithm, SDHA, CYC1 and YWHAZ were the most stable genes. 18S was among the least stable genes indicated both by geNorm and Normfinder.

Conclusion: SDHA was the most optimal reference gene, while commonly used GAPDH and 18s showed unstable expression in periapical inflammatory lesions.

Keywords: House-keeping gene, qPCR, periapical granuloma, periapical cyst, SDHA

Introduction

Real-time quantitative PCR (qPCR), known as quantitative polymerase chain reaction, is a widely employed methodology in numerous scientific investigations for the precise and quantitative assessment of gene expression levels. This technique proves to be important in various domains of research, such as molecular biology, genetics and diagnostics, as it offers researchers the ability to scrutinize gene expression patterns, identify indicators of diseases and explore the influence of experimental factors on gene expression. The efficacy of this technique is reliant upon the utilization of reference genes, also referred to as housekeeping genes, which are genes presumed to possess consistent levels of expression across diverse conditions and experimental factors (1).

qPCR is a fast, simple and efficient method for simultaneous measurement of mRNA transcription levels in different types of samples (2). Accurate normalization of data in expression profiles is a crucial step in avoiding variations resulting from differences in quality and amount of starting material, enzymatic efficiencies and transcriptional activities of different types of tissues and cells (3). One of the most widely applied normalization strategies is the usage of reference genes, also known as 'housekeeping genes. An appropriate housekeeping gene shows minimal variability in expression between samples, has great amplification efficiency and is unaffected by the experimental treatment (4, 5). The reference gene serves as the foundation for proper analysis and interpretation, since inappropriate reference gene can result in misleading associations and obscure the statistical significance of alterations in gene expression.

As for now, a small number of studies have been conducted using more than one gene as internal control (6). Moreover, earlier studies have proven that normalization to only one reference gene was unsuitable for a sample of human origin (7, 8). Quantification of several endogenous reference genes simultaneously has been widely accepted as the most reliable method for real time PCR data normalization (9). By employing multiple reference genes, one can discern and accommodate any deviations or irregularities in the levels of expression of each individual reference gene, ultimately resulting in a more dependable standardization of the target gene's expression. This approach aids in mitigating the influence of experimental variables or circumstances that may impinge upon the expression of a solitary reference gene, thereby furnishing a more resilient normalization strategy (1).

The use of multiple reference genes also increases the confidence in the results and reduces the risk of misinterpretation or bias in the analysis. To date, there is no consensus on a single gene/combination of multiple genes that can be used as a reference gene/s for many different types of samples since the expression varies across different tissues, development stages and experimental conditions (1). Thus, prior to use every reference gene is supposed to be validated for particular cell/tissue and experimental conditions.

Human periapical inflammatory lesions, histopathologicallly classified as periapical granulomas and radicular cysts, are lesions of human jaw bone resulting from continuous inflammatory stimuli from infected root canals (10, 11). Accurate differentiation between periapical granuloma and radicular cyst is difficult since it requires surgical removal of lesion tissue. Nowadays, many studies are being carried out on selection of a genetic marker for differentiation between types of human periapical inflammatory lesions which is crucial for proper treatment choice (12). However, neither suitable reference gene nor combination of multiple genes has been selected for performing qPCR analyses in periapical inflammatory lesions.

A number of applets regarding selection of reference genes (9, 13) have been developed recently supporting correct analysis of data in lots of experiments (14-16). Despite obvious differences between possible algorithms to be used for proper reference gene selection, the aim of each algorithm is to present gene expression stability as a numeric value by comparing the expression across a sample set and to rank genes according to their expression stability where the best-ranked ones are taken as the most stable ones (6). The aim of our study was to identify a combination of the most stable reference genes required for correct real-time PCR data normalization in periapical inflammatory lesions. In this study, we assessed a panel of 9 candidate

reference genes on a biologically diverse set of 20 human periapical inflammatory lesions and healthy gingiva samples by employing two algorithms, geNorm and NormFinder. The objective of the study was to compare the expression patterns of 9 common reference genes in order to identify the most stable reference gene for the use in real-time PCR experiments on human periapical inflammatory lesions.

Materials and methods

Samples

This study examined a total of 20 different samples. Six samples from this group were determined to be granulomas, seven samples were determined to be radicular cysts and the remaining seven samples came from healthy gingiva. Immediately after collection, all samples were flash frozen in liquid nitrogen and deposited in a -80°C refrigerator for long-term storage. All procedures performed in study were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. As the research was done on human samples, all patients gave their written consent to participate in the study.

RNA isolation was carried out on homogeni-zed samples with *RNeasy Mini Kit* (Qiagen, Germantown, MD, USA) according to the manufacturer's instructions. The concentration of isolated RNA was quantified by measuring absorbance at 260 nm in ng/µl *(BioSpec nano-UV-VIS Spectrophotometer, Shimadzu Scientific Instruments).* To assess the RNA purity, absorbance at 280 nm and 230 nm was measured and A260/280nm and A260/230nm ratios were calculated. Only high-quality RNA was used for further analysis. cDNA synthesis was performed using *High Capacity cDNA Reverse Transcription Kit* (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions.

Selection of candidate genes and qPCR amplification

Nine candidate genes were chosen from the geNorm system for identification of best reference gene (*Reference gene assays with PerfectProbe by PrimerDesign, UK*) including: beta-2-microglobulin (*B2M*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), succinate dehydrogenase complex flavoprotein subunit A (*SDHA*), tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (*YWHAZ*), eukaryotic translation initiation factor 4A2 (*EIF4A2*), DNA topoisomerase I (*TOP1*), cytochrome c1 (*CYC1*), actin beta (*ACTB*) and 18S ribosomal RNAs (*18S*). Probe-based qPCR was performed for each sample with two replicates using Stratagene Mx3005p qPCR (Agilent Technologies, Santa Clara, USA). The reaction volume was 20 µl and consisted of 10 µl PCR master mix, 1 µl of primer probe mix, 7 µl of RNase/DNase free water and 2 µl cDNA. qPCR amplification was done in following conditions: 95°C for 10 minutes, then 40 cycles of 95°C (15 seconds) and 60°C (1 minute).

Determination of reference gene stability

The Ct (cycle threshold) value in PCR represents the number of cycles required to replicate enough DNA or RNA to be detected (cross a threshold line). Initially, the sample had a higher DNA/RNA content, as indicated by the lower Ct value.

Ct value for each gene was derived by calculating the average value from two replicates. geNorm algorithm-based qbase+ software v. 3.1 and Normfinder algorithm v. 20 were used to determine expression stabilities of candidate reference genes. geNorm algorithm was also used to determine the optimal number of reference genes required for successful normalization.

Statistical analysis

Ct values were tested for normality using Kolmogorv-Smirnov test prior to analysis. The significance level was defined at $P<0.05$. Data was represented as mean and standard deviation. Statistical analysis was performed using SPSS v. 20.2. (Armonk, NY: IBM Corp).

Results

qPCR results

Real-time PCR was used to determine the mRNA levels of nine candidate reference genes across three examined sample groups: periapical granuloma, radicular cyst and healthy gingiva. Derived Ct values were used for determination of gene expression and their stability. Nine candidate reference genes showed a wide expression range (Fig. 1).

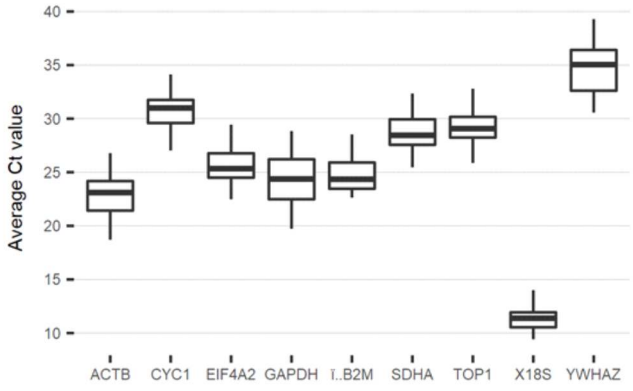

Fig. 1
Expression levels of nine candidate reference genes detected by real-time PCR are depicted on Y-axis as Ct values. Each reaction is done in duplicate. Lower Ct values represent higher expression of the corresponding gene

Ct values ranged from 9.28 to 39.29 and the mean Ct values ranged from 11.57 to 34.67. According to the Kolmogorov-Smirnov test, all genes, except *EIF4A2* and *18S* genes, showed normal distribution in Ct values and they fit both normality criteria (P>0.1, α=0.05). *YWHAZ* was the least expressed gene with Ct values ranging from 30.56 to 39.29. *18S* gene showed highest expression with Ct values ranging from 9.42 to 16.40. Interestingly, *GAPDH* displayed highest variation in mRNA expression compared to the other examined genes, which directly indicated its low expression stability in examined sample groups.

Evaluation of reference gene stability

Two statistical applets (geNorm and NormFinder) were employed in determination of expression stability of nine examined reference genes. In each analysis genes were ranked from most stable to least stable.

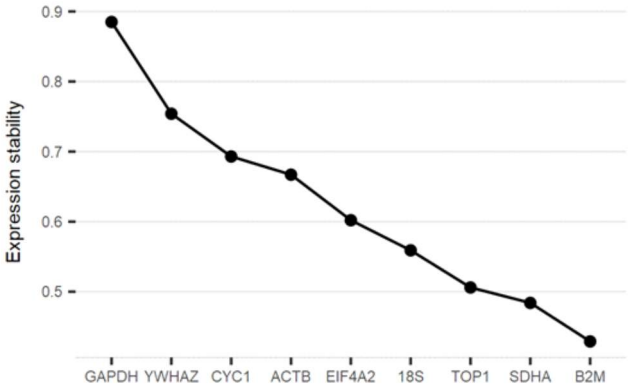

Fig. 2
Average expression stability (M) of nine candidate reference genes by geNorm analysis. The least stable reference gene with highest M value is positioned on the left and the most stable gene with lowest M value is positioned on the right side of the plot. Most stable reference genes were deduced by stepwise exclusion of the least stable genes

geNorm Analysis Nine reference genes were ranked from most stable to least stable based on their stability value (M value), as shown in Fig 2. All tested candidate reference genes demonstrated limited variance in expression, M values being lower that 1.5, which is set as default limit (M<1.5). This indicates a high stability of mRNA expression in experimental conditions. *B2M, SDHA* and *TOP1* were the three most stable genes in examined samples. Surprisingly, *GAPDH* was the least stable gene.

geNorm was also used to determine the optimal number of reference genes required for accurate normalization of qPCR data (Fig 3). Pairwise variations (V) derived by geNorm represent standard

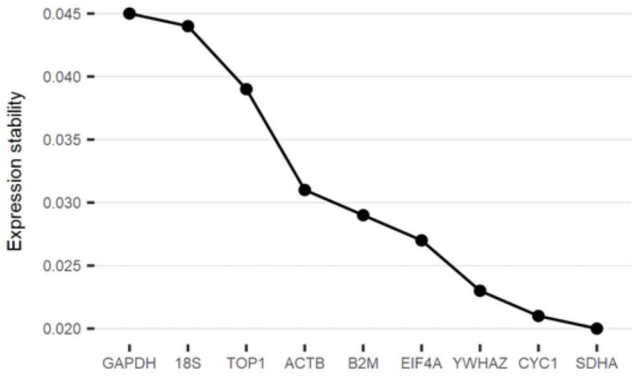

Fig. 3
Pairwise variation analysis of nine candidate reference genes. Optimal number of genes was determined by comparison of Vn/n+1 value (default threshold of variation=0.15)

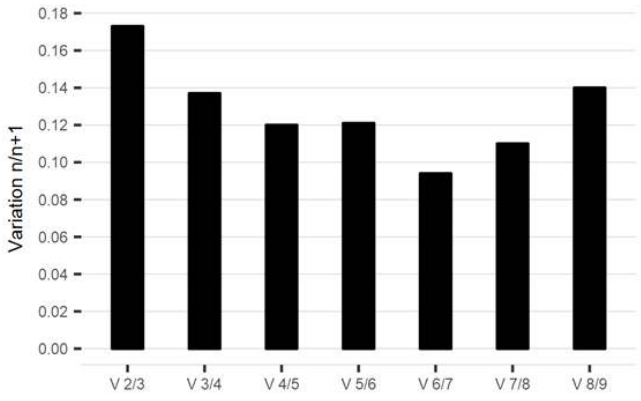

Fig. 4
Expression stability (M) of nine candidate reference genes by NormFinder analysis. The least stable reference gene with highest M value is positioned on the left and the most stable gene with lowest M value is positioned on the right side of the plot.

deviation of log_2-transformed expression ratios of any pair of reference genes. geNorm analysis revealed that the optimal number of genes for normalization in given sample group is three, given that the pairwise variation value V3/4 was lower than threshold value (0.15).

Normfinder Tested reference genes were ranked according to their stability values in given experimental conditions using Normfinder (Fig 4). *SDHA* was listed as the most stable gene with *CYC1* and *YWHAZ* as the second and third most stable genes. Unlike the results generated by geNorm where *YWHAZ* was among two least stable genes, it is found in top three genes in Normfinder. *GAPDH* was ranked as the least stable gene by Normfinder confirming the results generated by geNorm. *18S* was the least stable gene indicated both by geNorm and Normfinder.

Discussion

One of the most appropriate methods for mRNA quantification studies is qPCR analysis)17). However, normalization of expression data requires a suitable gene with stable expression across samples taken from different time points or under different experimental conditions (4). Nowadays, there is a vast number of software and algorithms developed for identification and validation of best reference genes for normalization (18). The choice of combination of reference genes in studies of gene expression in human periapical inflammatory lesions was not previously determined. Moreover, a single reference gene was used for normalization in most gene expression studies in periapical granuloma and radicular cysts without previous determination of the most stable one (19, 20). Even though this problem has been previously addressed, researchers still tend to randomly choose reference genes and, thereby, increase chance of statistical error (8).

By using single gene SD-based approach, scientists assumed that gene expression showed normal distribution across the sample (21). But normality of distribution was rarely assessed during the expression studies. Nowadays, there are algorithms that do not require the reference gene to be normally distributed and the idea of using several reference genes for normalization has been introduced (22). We have previously seen that using only one gene as reference, *SDHA*, gives statistically significant results for studied sample group (periapical granuloma, radicular cyst and healthy gingiva) (23).

Literature review on reference genes used in qPCR analysis in several studies in dental research revealed that the most frequently used housekeeping gene was *β-actin*, which was not among the 3 most stable reference genes in our analysis. Several studies (19, 20, 24, 25) used *β-actin* as the reference of choice in qPCR analyses in odontogenic keratocysts, human gingival fibroblasts, periapical inflammatory lesions (granulomas and cysts), periodontal ligament and similar tissues. Bildt et al. (26) and Kusumi et al. (27) used *G3PDA* as housekeeping gene. Heikinheimo et al. chose *PL2A* as reference gene in their study done on dental follicles and odontogenic keratocysts (28). *GAPDH* was used as a reference gene in the study of radicular cysts and keratocysts done by Hayashi et al. (29).

Different approaches in choosing the best reference genes and to estimate the stability were described previously (18). In our study, we used two free algorithms: geNorm and NormFinder to evaluate expression stability of investigated candidate reference genes. Our study is the first to evaluate the combination of best reference genes for application

in qPCR analysis of periapical granuloma, radicular cysts and healthy gingiva samples. Out of 9 candidate reference genes, following three were among the most stable ones according to geNorm: *B2M*, *SDHA and TOP1. GAPDH* was ranked as the least stable reference gene by geNorm. Even though G*APDH* gave good results in many studies, it is not recommended to be used as reference gene since its expression can vary in different conditions (30, 31).

Unlike geNorm, following genes were found to be the most stable in NormFinder: *SDHA, CYC1* and *YWHAZ. YWHAZ* was considered a poor normalizer in geNorm. This difference can be due to types of algorithms geNorm and NormFinder use, since geNorm excludes poorly expressed genes and NormFinder uses model-gene approach and ranks all tested reference genes independently from their expression level. It is important to mention that according to both, geNorm and NormFinder results, *GAPDH* was among two least stable genes, even though it is among the most used reference gene in previous dental studies in human periapical inflammatory lesions (28).

geNorm algorithm also calculates pairwise-variation (V) value to estimate the adequate number of reference genes to be used. V analysis showed that the V3/4 (0.137) value was just underneath the proposed cut-off value (0.15) indicating that three genes are needed for accurate normalization of expression data (*TOP1, SDHA* and *B2M*).

CONCLUSION

As normalization of qPCR expression data is crucial for analysis of gene expression, proposed genes might help scientists in choosing the best reference gene or combination of multiple genes to use in their studies. Researchers in the field of dentistry must possess knowledge regarding the significance of employing reference genes in order to ensure the reliability of the acquired research findings, as qPCR studies are widely utilized in this particular domain. The study aimed to identify stable reference genes for real-time PCR analysis in human periapical inflammatory lesions by examining the expression profiles of nine candidate reference genes using geNorm and NormFinder algorithms. According to geNorm, B2M, SDHA, and TOP1 were the most stable genes, while GAPDH was the least stable. According to NormFinder, SDHA, CYC1, and YWHAZ were the most stable genes. Thus, SDHA was identified as the most optimal reference gene, while GAPDH and 18S showed unstable expression in periapical inflammatory lesions.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards**.**

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Informed consent Informed consent was obtained from all individual participants included in the study.

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SELF-REPORTED SLEEP AND AWAKE BRUXISM AMONG UNIVERSITY STUDENTS AND THEIR ASSOCIATION WITH PERCEIVED STRESS

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ABSTRACT

Introduction: Bruxism represents an oral condition of excessive teeth grinding or jaw clenching. It is important to make distinction between sleep bruxism (SB) and awake bruxism (AB) due to different manifestations and consequences for the stomatognathic system. Awake bruxism mainly consists of teeth clenching, while teeth grinding is more common in sleep bruxism.

Objectives: This study determined the frequency of awake and sleep bruxism in relation to gender, faculty type and year of study and investigate the association between awake and sleep bruxism and the level of stress in the participants.

Methodology: A total of 756 participants anonymously completed questionnaires consisting of two parts. The first part of the questionnaire contained basic information about the participant, the presence/absence of bruxism and its symptoms. The second part of the survey questionnaire was related to the perceived stress scale.

Results: Statistically significant higher frequency of teeth clenching compared to teeth grinding has been proven, indicating a higher prevalence of "awake" bruxism. Men had significantly more teeth clenching during the day, while women had it during the night. Participants in the first three years of the study had bruxism more frequently during the day, while in the fourth, fifth and sixth year, they had it more frequently during the night. The association between AB and SB with stress levels has not been proven.

Conclusion: The high prevalence of self-reported AB and SB bruxism among the student population emphasizes the importance of a definitive diagnosis through questionnaires, medical history, clinical examination and instrumental assessment. A multidimensional evaluation of the presence of bruxism, associated comorbidities, etiology and consequences of bruxism opens up the possibility of its prevention and appropriate therapy.

Keywords: Student's population, awake and sleep bruxism, stress.

Introduction

Bruxism represents an oral condition falling within the scope of interest for various disciplines, including dentistry, neurology, psychology and sleep medicine. Each of these disciplines has proposed several definitions for this condition necessitating the search for a common definition. (1)

In 2013, the International Expert Group on Bruxism proposed a consensus based on the concept that bruxism is "repetitive jaw activity" that can occur during sleep (SB) or wakefulness (AB). The experts provided separate definitions for sleep bruxism (SB) and awake bruxism (AB):

"Sleep bruxism is the activity of the masticatory muscles during sleep, characterized as rhythmic (phasic) or non-rhythmic (tonic), and it is not a movement or sleep disorder in otherwise healthy individuals."

"Awake bruxism is the activity of the masticatory muscles during wakefulness, characterized by repeated or sustained teeth contact and/or jaw clenching or thrusting, and is not a movement disorder in otherwise healthy individuals (2,3)."

Awake bruxism is commonly known as 'bruxomania' and primarily involves teeth clenching, with teeth grinding being less frequently observed. In sleep bruxism, teeth grinding is more commonly reported during various types of motor activity (4, 5).

It is estimated that bruxism occurs in 8-31% of the population with no significant gender differences (2,3,6). The prevalence of "sleep bruxism" (SB) varies from 9.3% to 15.9%, while the prevalence of "awake bruxism" (AB) ranges between 22.1% and 31% among adults (7-11).

The etiology of bruxism involves a multifactorial model with central factors considered to play a key role. In addition to central factors, psychosocial factors and peripheral factors are involved in the etiology. It is currently believed that peripheral factors play a minor role in the development of bruxism (12,13).

For SB, it is considered to be centrally mediated, with a complex interaction of all factors affecting the functioning of the autonomic system during sleep. AB is equally centrally mediated but is mainly related to psychosocial factors and behavioral changes associated with lifestyle modifications. Ultimately, this means that the alleged etiological factors can vary depending on the circadian manifestations of bruxism (14-16).

Numerous studies have shown a connection between bruxism and specific personality traits (e.g., aggression or emotional suppression) (3, 17-21), psychosocial factors (22, 23), and psychological stress (16, 22-25).

Students represent a unique population experiencing elevated levels of stress, frustration or depression. For students, in addition to exam situations, stressors can include factors such as independence, transitioning to a more mature age group and building a new social life (26).

Stress arises due to a lack of time, the misalignment between academic and personal activities and often financial problems (27,28). Studying during the COVID-19 pandemic entailed strict adherence to epidemiological measures that significantly altered people's lives, including students. Studying shifted to online classes made students found themselves completing their academic responsibilities from the confines of their own rooms. All these changes led to shifts in lifestyle, creating additional stress and various studies have confirmed an increase in the incidence of self-reported bruxism during the pandemic (29, 30).

Some studies suggest that the influence of psychosocial factors is significant for awake bruxism, while their impact on sleep bruxism is less clear (31-33). Given the limited epidemiological data on the prevalence of AB and SB among the student population and the association of AB and SB with stress, this study was conducted.

Research Objectives:

1. Examine the frequency of awake and sleep bruxism.

2. Determine the prevalence of awake and sleep bruxism in relation to gender, faculty type and year of study.

3. Investigate the correlation between awake and sleep bruxism and the level of stress among the participants.

Methodology

In order to gather information on the frequency of awake and sleep bruxism among students from the Faculty of Dentistry with a Dental Clinical Center and the Faculty of Pharmacy at the University of Sarajevo, a cross-sectional study was conducted.

The study was carried out in April 2020 and was approved by the Ethics Committee of the Faculty of Dentistry with a Dental Clinical Center in Sarajevo (reference number: 02-3-4-19-1-7/2022).

The students participated in the survey anonymously and voluntarily, providing informed consent. The first section of the questionnaire included basic information about the participants, such as age, gender, the faculty they were attending, information about the presence/absence of bruxism and its symptoms and, as well as, the influence of specific situations on the manifestation of bruxism (34).

The second section of the questionnaire consisted of questions related to the Perceived Stress Scale (PSS), which is the most commonly used psychological instrument for measuring the perception of stress. Individual PSS scores can range from 0 to 40, with higher scores indicating a higher perceived level of stress (35).

The results were statistically analyzed and presented in tables and graphs.

Statistical data analysis methods

Statistical data analysis was conducted using IBM SPSS Statistics v.21 software. Arithmetic means and standard deviations of quotative variables, then absolute and relative frequencies of nominal variables were calculated from the descriptive statistical analysis. In the study the parametric Pearson linear correlation was used, and the chi-square test was used as a non-parametric statistical method. The research hypotheses were tested at a 95% confidence level, which equates to a 5% risk level.

Results

A total of 756 respondents participated in the study, of which 170 (22.5%) were male, and 586 (77.5%) were female. At the Faculty of Dentistry with a Dental Clinical Center, 439 respondents were surveyed, including 125 males and 314 females, while at the Faculty of Pharmacy, 317 respondents were surveyed, with 45 males and 272 females.

Students from all years of both faculties were surveyed and the distribution of participants according to their year of study at the Faculty of Dentistry and the Faculty of Pharmacy is shown in Figure 1.

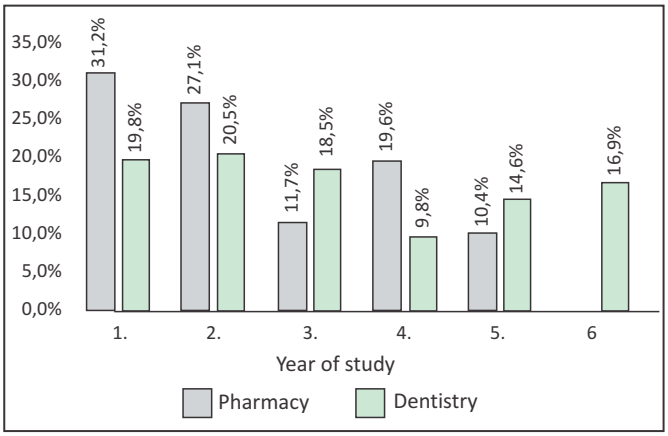

Figure 1
Distribution of participants according to their year of study at the Faculty of Dentistry and the Faculty of Pharmacy

Regarding the frequency of bruxism among the surveyed students, 53.2% of respondents did not report bruxism, while a slightly smaller percentage, 46.8%, indicated the presence of bruxism (Figure 2).

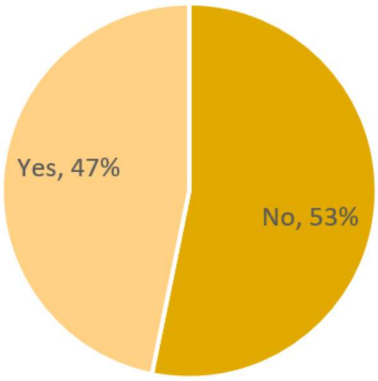

Figure 2
The presence of bruxism in student population

Regarding the frequency of awake and sleep bruxism, the largest percentage (26.6%) of surveyed students from both faculties who reported bruxism had symptoms of teeth clenching, while 4.0% of students reported symptoms of teeth grinding. A statistically significantly higher frequency of teeth clenching (p<0.000) was confirmed compared to the frequency of teeth grinding. Given that teeth clenching is a characteristic of "awake" bruxism, this research demonstrated a higher prevalence of "awake" bruxism compared to "sleep" bruxism.

A comparison of the differences in the frequency of awake and sleep bruxism by gender is presented in Table 1. A statistically significant difference in frequency was only observed in response to the question "Have you noticed clenching your teeth?" where men experienced this occurrence significantly more often during the day, while women had it more frequently during the night.

		Gender				Total	
		Male		Female			
		n	%	n	%	n	%
Have you noticed teeth clenching?	Yes, during the day	54	72.0%	147	60,5%	147	60,5%
	Yes, during the night	21	28,0%	96	39,5%	96	39,5%
	Total	75	100%	243	100%	243	100%

X^2 = 2,787, df = 1, p <0,095

Table 1. The differences in the frequency of awake and sleep bruxism by gender

By comparing the differences in the frequency of awake and sleep bruxism across different years of study, the Chi-square test revealed a statistically significant difference in the distribution of frequency between categories for the question "Have you noticed clenching your teeth?" The results suggest that in the first three years of study, respondents experienced this type of bruxism more frequently during the day, while in the fourth, fifth and sixth years, it was more frequent at night. For other questions regarding bruxism and years of study, no statistical significance was found. The results are presented in Table 2.

		Have you noticed teeth clenching ?				Total	
		Yes, during the day		Yes, during the night			
		n	%	n	%	n	%
Year of study	First year	46	22,9%	23	19,7%	69	21,7%
	Second year	51	25,4%	20	17,1%	71	22,3%
	Third year	27	13,4%	10	8,5%	37	11,6%
	Fourth year	34	16,9%	20	17,1%	54	17,0%
	Fifth year	29	14,4%	21	17,9%	50	15,7%
	Sixth year	14	7,0%	23	19,7%	37	11,6%
	Total	201	100%	117	100%	318	100%

X^2 = 14,967, df = 5, p <0,011

Table 2. The differences in the frequency of awake and sleep bruxism by different years of study

		Have you noticed teeth grinding? (during the day=0, during the night= 1)	Have you noticed teeth clenching? (during the day=0, during the night= 1)	Has anyone told you that you grind your teeth? (during the day=0, during the night= 1)	Has anyone told you that you clench your teeth? (during the day=0, during the night= 1)
Individual score for PSS	r	-0,039	-0,015	-0,118	-0,108
	p	0,638	0,784	0,285	0,372
	n	146	318	84	71

r- Pearson linear correlation, p- probability of rejecting the null hypothesis with a 5% risk., n-sample size

Table 3. The influence of stress level on awake/sleep bruxism

		Presence of bruxism					
		No		Yes			
		n	%	n	%	X^2	P
Is opening your mouth painful or difficult when you wake up in the morning?	Yes	3	0,8%	47	14,1%	55,58	**0,000**
	No	390	99,2%	286	85,9%		
Do you experience pain in your facial muscles?	Yes	25	6,4%	92	27,1%	58,50	**0,000**
	No	363	93,6%	248	72,9%		
Do you experience pain in your jaw joints?	Yes	29	7,4%	99	29,1%	4,79	**0,000**
	No	365	92,6%	241	70,9%		
Do you have frequent headaches?	Yes	117	30,2%	130	38,1%	32,08	**0,029**
	No	271	69,8%	211	61,9%		
Have you noticed any teeth wear or changes in appearance?	Yes	56	14,5%	108	32,5%	13,44	**0,000**
	No	331	85,5%	224	67,5%		
Do you have sensitive or painful teeth when exposed to cold?	Yes	110	28,2%	140	41,4%	16,81	**0,000**
	No	280	71,8%	198	58,6%		
Does anyone in your family grind or clench their teeth?	Yes	81	23,4%	112	38,8%	4,62	**0,000**
	No	265	76,6%	177	61,2%		
Does toothache bother you at night?	Yes	3	0,7%	11	3,1%	38,49	**0,032**
	No	399	99,3%	340	96,9%		
Have you ever been told by a dentist that you have bruxism?	Yes	0	0,0%	34	9,9%	48,04	**0,000**
	No	394	100%	311	90,1%		

– sample size (number of respondents), X^2 - chi square test value, p-- probability of rejecting the null hypothesis with a 5% risk.

Table 4. The frequency of symptoms and signs of bruxism among students in relation to the presence of bruxism.

In the examination of the correlation between sleep and awake bruxism and the level of stress among the participants, the Pearson correlation coefficients are low and not statistically significant, leading to the conclusion that this type of relationship was not established (Table 3).

The frequency of symptoms and signs of bruxism among students who reported bruxism and those who did not is presented in . As observed in the table, respondents who reported bruxism significantly more often indicated the presence of various symptoms in response to all questions.

Discussion

The high prevalence of bruxism among the student population (46.8%) obtained in this study is consistent with another researches (31,32,33). Self-assessment by the participants regarding the presence of bruxism showed a higher frequency in teeth clenching (26.6%), while teeth grinding was present in a small percentage and mostly during the night (4%), which was also statistically significant. Considering that teeth clenching was identified during the day, and teeth grinding during the night, there is a higher prevalence of "awake" bruxism compared to sleep bruxism, consistent with the results of studies at the Medical Faculty in Split (34) and the University of Salerno in Italy (36).

Many studies presented a higher prevalence of awake bruxism (AB) compared to sleep bruxism (SB), with this prevalence being higher in a younger population including our respondents (7,37,38,39).

Contrary to our study, research conducted by Osses-Anguita and colleagues among dental medicine students at the University of Madrid demonstrated a higher prevalence of sleep bruxism (SB) during the pandemic (40). Students at the Faculty of Dentistry in Sarajevo mostly attended classes and practical exercises during the pandemic, which could result in an acute experience of stress arising from daily personal and social interactions, potentially increasing anxiety levels and prompting a higher level of AB. Awake bruxism has been induced in experimental situations by acute stress activities (41) or anxiety (42). It is assumed that SB among students at the University of Madrid was higher because the lockdown caused a sense of helplessness, a lack of activity, increased levels of depression or passive coping.

A higher prevalence of sleep bruxism (SB) compared to awake bruxism (AB) has been demonstrated in studies conducted in Vietnam and the Netherlands (43, 44).

The most common method used in studies on self-assessment of bruxism is a survey or interview due to its simplicity. However, this method is effective only for diagnosing symptomatic bruxism or awareness of its existence. SB is typically noiseless and unconscious in about 80% of cases. The distinction between AB and SB is difficult to discern during a clinical examination due to numerous similar signs and symptoms. For this reason, SB can be easily overlooked or inaccurately reported (45).

In the study on bruxism among Israeli adolescents (46), there is a high concordance between SB and AB, meaning that reporting SB increases the odds of reporting AB fivefold, and vice versa.

The literature has been unable to definitively establish a difference in the frequency of types of bruxism between different genders (47).

In this study, when exploring the difference in the frequency of SB and AB in relation to gender, a significant difference was observed only for the question "Have you noticed clenching your teeth?" where men experienced this occurrence significantly more often during the day, while women had it more frequently during the night.

Gałczyńska-Rusinat at all., who examined the biomechanical behavior of the masseter muscle in patients with self-reported bruxism, found increased tension and stiffness in the contracted masseter in the male gender (48). Considering its primary role in elevating the mandible and exerting force during chewing where these movements dominate during teeth clenching, this could explain the higher prevalence of AB in men in our study (45).

Findings from certain studies indicate potential disparities in response to stressful situations, with men displaying a tendency toward direct action, while women tend to favor emotion-focused coping strategies when confronted with stress-inducing circumstances (49).

Kato at all. demonstrated a higher prevalence of AB in women (50), while in other studies, differences

in the type of bruxism between genders were not found (51,52).

In this study, a statistically significant difference in the occurrence of AB and SB in relation to the type of faculty was not established. However, in relation to the year of study, it was demonstrated that in the first three years of study, respondents more frequently experienced teeth clenching during the day, while in the fourth, fifth and sixth years, it was more frequent at night. These results are not surprising, considering that student life represents an introduction to independence, financial matters, success, adherence to schedules and obligations and choosing a social environment in which an individual can comfortably integrate. The daily stressors that this population faces (psychological, emotional, and social) have negatively impact on their learning and academic achievements (53). Exposure to a range of psychosocial stressors in the initial years of study has led to a higher prevalence of awake compared to sleep bruxism.

In a study at Tel Aviv University's School of Dental Medicine, a higher frequency of awake bruxism during the 3rd and 4th years of study was reported (32). The higher prevalence of sleep bruxism among senior students in our study could be explained by their greater maturity and different ways of coping with stress. Coping modalities could include emotion-focused coping or avoidance coping, which might be present in older adolescents who try to cognitively, emotionally or behaviorally detach from stressors or their own psychological and physical stress reactions. This emotional suppression could lead to sleep problems or sleep bruxism (54).

The association between AB and SB and the level of stress was not statistically significant. However, it should be noted that a high level of stress was detected according to the PSS-10 scale (the average stress score for students at the Dental Faculty was 20, while for the Pharmacy Faculty, it was 21.3), which resulted in a high prevalence of AB. Many researchers suggest that psychological factors, stress and anxiety influence awake bruxism (12), while Carra et all. believe that anxiety and stress are risk factors for bruxism in sleep (55).

Hilgenberg-Sydney at all. in their study also did not confirm a connection between the level of anxiety obtained through a questionnaire and AB (52).

A study by Câmara-Souza at all. demonstrated a positive and significant correlation between psychological factors and AB among students (15). Consistent with this research, many studies have not found an association between the intensity of SB and perceived stress according to self-assessment by respondents (2, 3, 32).

The belief that stress may be correlated with SB has been supported by studies that explored the relationship between sleep bruxism, perceived stress and coping strategies. Klara at all. demonstrated that a higher score of perceived stress is associated with a higher bruxism index (54). Similar findings have been shown in other studies on the relationship between stress and SB (56,57). A study that examined bruxism among students in Netherlands supports the suggestion that psychosocial stress is associated with both types of bruxism. However, this observation could also be due to the respondents' belief that they usually report an increase in bruxism symptoms during stressful life periods (58). The explanation for different claims regarding the relationship between stress and types of bruxism is likely due to the fact that it is difficult to clinically differentiate between AB and SB, especially when the diagnosis is made through questionnaires (50).

Respondents who reported the presence of bruxism in a statistically significant percentage also mentioned various associated symptoms. They experienced frequent headaches, facial muscle pain, temporomandibular joint pain, sensitivity to hot or cold, changes in tooth appearance and difficulty or painful mouth opening. We can conclude that these results are due to a significantly higher presence of parafunctional habits that have exceeded the specific structural tolerance of each component of the masticatory system, and when it is exceeded, damage occurs. All of this leads to the presence of symptoms and clinical signs that disrupt the functioning of the stomatognathic system (59).

Baad-Hansen at all. conducted a systematic review of the literature and concluded that bruxism is, to some extent, associated with musculoskeletal symptoms, although the evidence was contradictory and depended on various factors, such as age, type of bruxism and the quality of diagnosis. The literature does not support a direct linear causal relationship between bruxism and such symptoms (60,61).

The limitation of this study is that the diagnosis of bruxism was made through questionnaires without

personal history and clinical examination representing a lower level of diagnosis of "possible" bruxism according to the International Expert Group on Bruxism. The diagnosis of definitive bruxism is established with a positive self-assessment, positive clinical examination and based on instrumental assessment, i.e., information obtained through technical devices (2, 62).

Future research should consider using the standardized bruxism assessment tool (STAB), which allows multidimensional evaluation of the presence of bruxism, associated comorbidities, etiology and consequences of bruxism (63).

Conclusion

The high prevalence of self-reported AB and SB bruxism among the student population highlights the importance of a definitive diagnosis through questionnaires, anamnesis, clinical examination and, if necessary, the use of technical devices. With a detailed multidimensional evaluation of the etiology and clinical manifestations of bruxism, it is possible to access the prevention and adequate therapy of bruxism.

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Conflict of interest

The authors declare no conflict of interest related to this study of any kind.

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ANALYSIS OF UniViSS CLINICAL PERFORMANCE IN OCCLUSAL CARIES DETECTION

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ABSTRACT

There is a large number of visual systems for describing carious process propagation on dental surfaces. Despite numerous advantages, all these systems show some deficiencies. Wishing to overcome the deficiencies of visual systems for the detection and description of caries, a group of authors created Universal Visual Scoring System (UniViSS). This system is designed to compensate for the disadvantages of existing visual diagnostic systems, to meet the contemporary requirements set for caries detection / diagnosis and to be flexible.

The aim of this study was to evaluate diagnostic performances of UniViSS on occlusal surfaces in clinical conditions, through sensitivity, specificity, positive and negative predictive values.

Material and methods: Study sample consisted of 140 permanent molars with established occlusal caries lesion. During clinical examination, each lesion was classified according to UniViSS criteria, and laser fluorescence and digital radiography examination were performed. For ethical reasons, if two of three diagnostic methods confirmed existence of caries lesion, cavity was opened, as validation method. The depth of the lesion propagation was recorded using World Health Organization's graduated probe, as the distance between the deepest point of the cavity and enamel surface.

Results of study are presented tabularly and compared to similar investigations through comparing sensitivity, specificity, positive and negative predictive values of registered UniViss scores.

Key words: occlusal caries detection, occlusal caries diagnosis, UniViSS.

Introduction

Diagnosis is a clinical procedure preceding a decision on treatment. Caries diagnosis implies detection of a carious lesion, determination of its depth and degree of demineralization followed by decision making regarding its activity. It can be seen as a procedure that involves three steps - identification/detection of the lesion, assessment of the severity of the lesion and assessment of its activity. Active lesions require active treatment. It implies preventive, non-operative, but also operative treatment in order to restore the integrity of the dental surface in a way of anabling patient to clean those surfaces [1, 2].

An ideal diagnostic method should be safe for both the patient and the therapist and enable the detection of the lesion at an early stage. It should also be objective and quantitative, non-invasive and inexpensive [3].

Visual-tactile examination is the basis for the identification of carious lesions that require biologically based treatment demanding an assessment of lesion activity and surface integrity. Each surface of each tooth in the dental arch is examined using a standardized sequence of procedures that establish the existence of deviations from the average regularity, in terms of changes in color, luster, texture of the surface of the dental crown and surface integrity. The process of carious lesion detection involves distinguishing lesions with cavitation, active lesions without cavitation and stopped lesions (with or without cavitation), in relation to the average regular appearance of the surface of the tooth crown [4].

Visual inspection is widely used in clinical practice for the detection of carious lesions on the occlusal surfaces of teeth [5]]. A clinical examination with the use of a dental mirror and probe is usually used for the diagnosis of caries. However, the value of probing has been criticized as it does not improve diagnostic accuracy. Due to the variation in the morphology of the fissure system, the sharpness of the probe and the pressure produced by the examiner, can give different results. The use of a dental probe in the area of white spot or cloudy enamel can lead to irreversible damage in the area of occlusal fissures, leading to irreversible enamel defects in demineralized occlusal fissures by converting subsurface lesions into cavitations. The probe also enables the transmission of cariogenic bacteria from one infected site to another, damages the integrity of the fissure, facilitates the progression of the lesion and may be less accurate in establishing a diagnosis than the visual examination itself. [6, 7, 8, 9, 10].

Ekstrand et al. (in papers published in 1997, 1998, 2001), and Ricketts et al. (in a paper published in 2002) created and evaluated a scoring system for the detection of occlusal lesions, prediction of their depth, assessment of activity and prediction of whether the lesion is infected. Nyvad et al (in a paper published in 1999) developed a system for the diagnosis of caries which differentiates early carious lesions from lesions with cavitation being able to differentiate ten scores/values in suboptimal clinical conditions. However, this system was not exclusively visual, but visual-tactile, because a dental probe was used to determine the characteristics of the surface of the examined lesion as smooth, rough or with fractures. Fyffe et al created a visual caries diagnosis system based on diagnostic thresholds: D1 (enamel and dentin, including initial lesions), D2 (enamel and dentin, but excluding initial lesions), and D3 (dentin). The system describes eight categories of lesions to which the appropriate codes are assigned [11, 12].

The visual appearance of the initial carious lesions is described and evaluated in numerous studies using the International Caries Detection and Assessment System (ICDAS) [13]. It is designed to detect six stages of the carious process, ranging from early clinically visible changes in enamel caused by demineralization to extensive cavitation. The system is divided into sections including coronary caries (caries of fissures and pits, mesial/distal and buccal/oral surfaces), root caries and caries associated with restorations and fissure sealants.

In 2021, Bulbuk et al. [14] presented the LOV/DD cavity classification. The classification includes two divisions of cavities. The first division was made according to the Depth of Destruction (DD), and divides cavities into 5 categories (0 – 4). The second division of cavities divides according to Localization of the defect, Occlusal load and Volume of the defect (LOV), and according to it, all cavities are divided into four groups.

The diagnostic systems of Ekstrand et al., Nyvad et

al., and the ICDAS working group included carious lesions without cavitation, but classified carious lesions only on the basis of several criteria. Due to the fact that the clinical appearance of a carious lesion is complex, it is likely that a limited set of criteria will not be able to describe the clinical appearance of the lesion accurately enough. The ICDAS criteria, for example, do not distinguish between white and brown discolorations of a carious lesion, which, in fact, provide important information about the activity of the lesion. Classification of lesions characterized by both white opacities and brown discolorations is difficult as precise differentiation of brown discolorations and microcavitations. Such clinical experiences, as well as the fact that some auxiliary diagnostic methods (eg laser fluorescence, or qualitative light-induced fluorescence) did not give satisfactory results in the diagnosis of lesions without cavitation, were the main impetus for the development of the Universal Visual Scoring System (UniViSS) for lesions on occlusal and smooth surfaces. This system is designed to compensate for the shortcomings of existing visual diagnostic systems, to meet the modern requirements for caries detection/diagnosis methods, as well as to be adaptable. System can be used without restrictions in patients of all age groups, in the conditions of field, clinical and laboratory research [15, 16].

UniViSS uses a three-step diagnostic procedure for a detailed classification of the complex clinical appearance of the caries lesion:

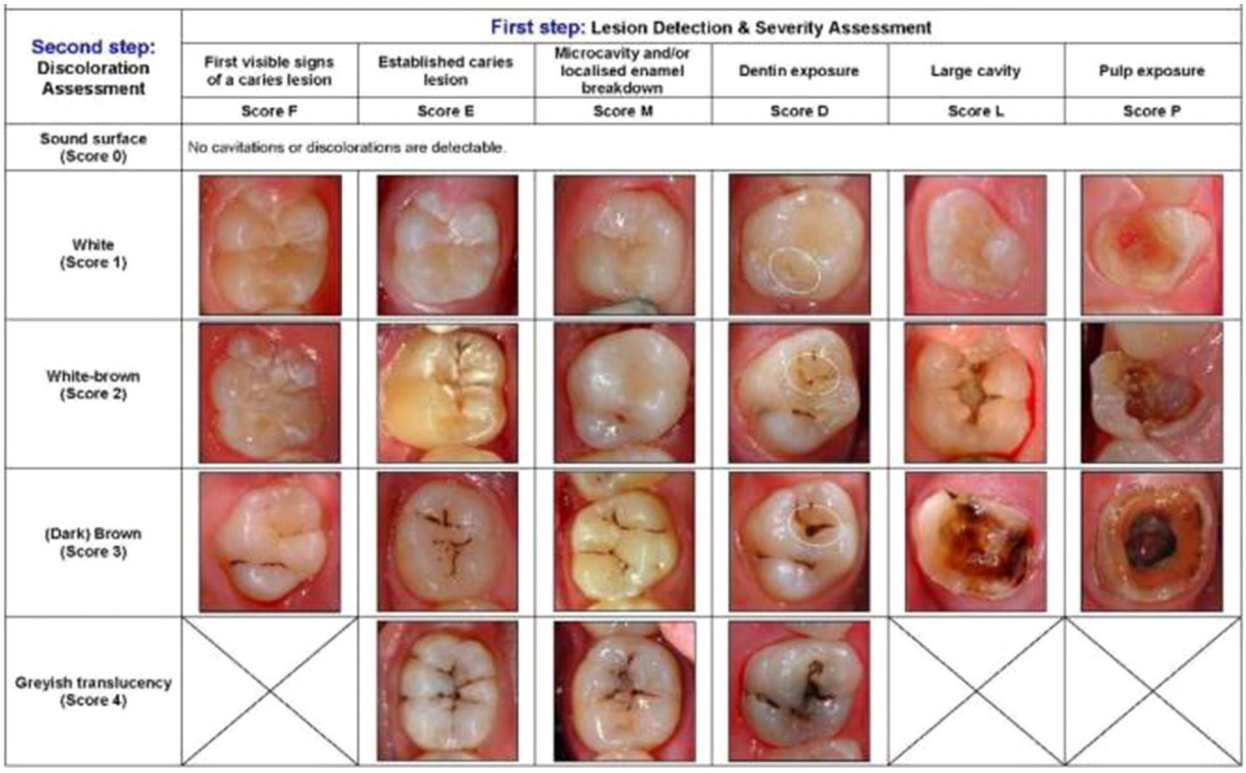
1. assess the severity of the lesion
2. estimate discoloration
3. assessment of the activity.

The standard equipment required for the examination consists of a CPI probe and a dental mirror. The CPI probe is used as an auxiliary instrument in the metric differentiation between established lesions and microcavitations, as well as for tactile examination of dental surfaces without pressure [15].

Material and methods

The study sample consisted of 140 permanent molars. Each of the tooth involved in the study had to have occlusal surface meeting the following criteria:

1. Patient older than 16 years, considering post eruptive maturation of enamel.
2. Occlusal fissure system with visible discoloured brown or black area without cavitation.



Picture 1. Universal Visual Scoring System for pits and fissures (UniViSS occlusal) (15)

3. Gray discoloration derived from carious dentine underneath the non-cavitated enamel.

4. Obvious occlusal cavitation.

The appearance of the fissure system on the occlusal surface is classified according to the UniViSS scoring system (Picture 1.). All teeth were also examined using laser fluorescence (DIAGNOdent 2095, KaVo, Biberach, Germany), and digital radiography (De Götzen xgenus® digital device, De Götzen S. r. l. Via Roma, 45-21057 Olgate Olona (VA)-Italy, software version 1.30.113). Validation of the collected data was performed by cavity opening. For ethical reasons, the decision on operative treatment was made if the results of two diagnostic methods were in favour of the presence of caries in the vicinity of the enamel-dentinal border, or dentinal caries. The depth of the lesion propagation was recorded using World Health Organization's graduated probe, as the distance between the deepest point of the cavity and enamel surface. Diagnostic procedure is described in details in previously published papers of the same group of authors [17, 18, 19].

Results

For the purposes of statistical analysis, UniViSS scores were transformed into ordinal values:

0 – no caries

1 – opacity or barely visible discoloration on a wet surface, but clearly visible after air drying, the probe does not stick (F1, F2, F3)

2 - opacity or clearly visible discolouration and without drying, the probe gets stuck (E1, E2, E3, E4)

3 - localized enamel defect on discolored enamel and/or visible presence of discolored dentin through the enamel (M1, M2, M3, M4)

4 - cavity with exposed dentin (D1, D2, D3, D4, L1, L2, L3, P1, P2, P3).

Score values 1 and 2 were considered to represent caries in enamel, and values 3 and 4 were considered to represent caries in dentin.

The results are presented in Tables 1, 2, and 3.

		UniViSS				
		1 – opacity/ discoloration visible after drying	2 – opacity/ discoloration visible without drying	3 – localised enamel defect /microcavity	4 – dentine exposure	Total
Validation method	0 – no caries	3	0	0	0	3
	1 - enamel caries	11	6	0	2	19
	2 - caries in the outer half of dentin	20	35	18	25	98
	3 - caries affects more than half of dentinal depth	2	2	6	10	20
	Total	36	43	24	37	140

Table 1. Cross-tabulation of UNIViSS and validation method

			UniViSS	Diagno Dent	RVG	Validation method
Spearman's rho	UniViSS	Correlation Coefficient	1,000	,242(**)	,150	,409(**)
		Sig. (2-tailed)	.	,004	,077	,000
		N	140	140	140	140
	Diagno Dent	Correlation Coefficient	,242(**)	1,000	,200(**)	,277(**)
		Sig. (2-tailed)	,004	.	,018	,001
		N	140	140	140	140
	RVG	Correlation Coefficient	,150	,200	1,000	,457
		Sig. (2-tailed)	,077	,018	.	,000
		N	140	140	140	140
	Validation method	Correlation Coefficient	,409(**)	,277(**)	,457(**)	1,000
		Sig. (2-tailed)	,000	,001	,000	.
		N	140	140	140	140

12. Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed)

Table 2. Spearman's correlation coefficient

D2					
	SE (95% CI)	SP (95% CI)	PPv (95% CI)	NPV (95% CI)	AUC (95% CI)
UniViSS	**0.42** (0.20 – 0.67)	**1.00** (0.31 – 1.00)	**1.00** (0.63 – 1.00)	**0.21** (0.05 – 0.51)	**0.711** (0.480 – 0.881)
D3					
	SE (95% CI)	SP (95% CI)	PPv (95% CI)	NPV (95% CI)	AUC (95% CI)
UniViSS	**0.80** (0.70 – 0.87)	**0.64** (0.41 – 0.83)	**0.91** (0.83 – 0.96)	**0.41** (0.25 – 0.59)	**0.751** (0.664 – 0.826)

Table 3. Sensitivity values (SE), specificity values (SP), positive predictive values (PPV), negative predictive values (NPV) and values under ROC curve (AUC) for UniViSS

Discussion

Diagnostics of occlusal caries has become a big challenge for dental practitioners in recent years, due to the significant decrease in the prevalence of caries worldwide. The reason for this is a decrease in the incidence of deep dentine caries and an increase in the incidence of superficial caries of occlusal surfaces. The occlusal surfaces of the crowns are the surfaces most susceptible to caries in children, adolescents and adults. Caries diagnostics can be complicated by the possibility that occlusal caries of dentin occurs under the macroscopically intact surface of the enamel thus making diagnosis by conventional methods difficult. Conventional diagnostic procedure is based on a visual-tactile examination. The sensitivity of the visual-tactile examination does not meet the requirements of modern dentistry. False negative results of this diagnostic procedure can be a consequence of the morphology of the fissure system on the occlusal surface, but also of intense fluoridation. Initial lesions in fissures and pits of the occlusal surface often go undiagnosed because they start on their walls and are difficult to detect. That is why it is recommended to combine a visual or visual-tactile examination which is characterized by high specificity, but insufficient sensitivity, with one of the diagnostic methods characterized by high sensitivity. An ideal diagnostic method should be characterized by high sensitivity and high specificity [20, 21, 22].

Nelson et al. [23] point out that there is still no standard system for caries detection and evaluation that is universally accepted among researchers. There are several caries detection systems in use attempting to describe and diagnose the caries process. The evidence points to the poor results of these methods, considering the large number of false negative diagnoses in the case of the presence of a carious lesion, and the low to moderate number of false positive diagnoses in the absence of a carious lesion. During 2004, a total of 29 visual and visual-tactile caries detection methods that were in use from 1950 to 2000 were analyzed, and not a single method reached the desired validity standards. This lack of consistency in the detection, reporting, description and interpretation of caries among the mentioned systems limits the possibility of comparing the results of clinical studies.

Agnes et al. [24] state that visual inspection was considered an invaluable diagnostic tool during the past decades. Due to the high specificity values, it made possible to avoid unnecessary treatments, mainly in populations with a low caries prevalence. Recently, visual inspection has also shown low sensitivity values which is significant in order to prevent non-treatment. These authors believe that the aforementioned is a consequence of the introduction into practice of the visual diagnostic system of Ekstrand and associates.

The results of the research by Künisch et al. [10] support the view that visual inspection of a clean and dry occlusal surface is the method of first choice in everyday practice, but that the sharp probe should be replaced by a CPI probe with a spherical tip.

Künisch et al [15, 24] evaluated UniViSS performance on extracted third molars with histological validation. For enamel caries, recorded values of sensitivity and specificity were 1.00 and 0.58. For dentinal caries detection, sensitivity value was 0.63 and specificity 0.98. Taking into account the suggestion that the sum of sensitivity and specificity values should be approximately 1.6 in order for the diagnostic method to be considered a legitimate candidate for practical use, these values illustrate the potential of UniViSS. Considering the reproducibility UniViSS, the authors believe that the UniViSS score for discoloration is more difficult to reproduce in relation to the scores ICDAS II. In our sample, at diagnostic threshold D2, the sensitivity and specificity sum were 1.42 (SE = 0.42, SP = 1.00) and at diagnostic threshold D3 - 1.44 (SE = 0.80, SP = 0.64). These differences can be explained by the fact that this research was in vivo and that detailed histological evaluation of recorded scores was not possible for ethical reasons. The recorded results, regardless of the above-mentioned differences, are comparable to the results of Künisch and associates.

In an in vivo study of the effectiveness of diagnosing minimal carious lesions using a visual-tactile examination (according to the criteria of Ekstrand et al.), laser fluorescence, and histological validation as the gold standard, Živojinović and Marković [20] recorded a sensitivity value of 0.36 and

a specificity of 0.83, for the visual-tactile examination. The sample in this study consisted of 120 teeth of the transcanine sector that were determined for extraction for orthodontic, prosthetic or surgical reasons, and therefore histological validation of the results was possible. It was shown, based on the visual-tactile examination, that the correct therapeutic decision was made in 55% of cases. The authors point out that neither visual-tactile examination nor laser fluorescence are absolutely effective in diagnosing early carious lesions on occlusal surfaces.

Zaidi et al. [25] evaluated in vivo the effectiveness of laser fluorescence in the detection of occlusal caries compared to visual inspection and the use of an intraoral camera. For enamel caries, the sensitivity of the visual examination (Ekstrand criteria) was 0.65. For caries in dentin, the sensitivity of the visual examination was 0.57.

Künisch et al. [26] examined the detection of occlusal caries on permanent molars using the WHO basic method, ICDAS and laser fluorescence. In their research, 27.1% of fissures and 53.9% of pits were diagnosed as healthy using ICDAS and laser fluorescence. A noticeable difference occurred in obvious carious lesions needed restoration (ICDAS score $\geqslant$ 4, DIAGNOdent value >31). Both methods classified 5.2% of occlusal fissures and 3.4% pits in this category, 22.7% of fissures and 10.4% pits were assigned a lower ICDAS score (DIAGNOdent >31, ICDAS<4). Only 0.9% of fissures and 0.1% of pits were not in accordance with the expected DIAGNOdent values (DIAGNOdent <31, ICDAS $\geqslant$ 4). These facts once again highlight the difficulty in accurately diagnosing carious lesions without cavitation.

Dičak et al. [21] found that visual-tactile diagnostics and diagnostics with DIAGNOdent in 90.5% of cases showed a coincidence of no caries presence, in a research comparing the visual-tactile detection of caries and the application of laser fluorescence in 30 subjects on 960 teeth of the transcanine sector. In 5.94% of cases it is shown that caries exist. The ratio of actual agreement was 96.46%. The rate of coincidence was 85.73%. Absolute disagreement was recorded in 3.54% of cases.

Jablonski-Momeni et al. [27] also evaluated in vitro the use of ICDAS, laser fluorescence, fluorescence camera and digital retrocoronary radiography in the detection of occlusal caries. The validation method was cavity opening. For enamel caries (D1), sensitivity and specificity values for ICDAS were 1.00. For caries in dentin (D3), the sensitivity value for ICDAS was 0.70 and the specificity was 0.95. AUC value for ICDAS was 0.92. Spearman's correlation coefficient between all methods showed that all correlations were significant, both at the confidence level of 0.01 and 0.05. A strong positive correlation was found between ICDAS and the gold standard.

Conclusion

UniViSS is applicable in clinical practice, but considering the fact that ICDAS is more used in investigations and clinical research, we recommend more intensive promotion of UniViSS use in those conditions.

Declaration of interest

There is not any conflict of interest for this material in the manuscript for all authors, between the authors, or for any organization.

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EFFECT OF FLUORIDE ON PREVENTION OF DEMINERALIZATION ON ENAMEL DURING THE ORTHODONTIC TREATMENT

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ABSTRACT

The formation of white spot lesions, or enamel demineralization, around fixed orthodontic attachments is a common complication after the end of fixed orthodontic treatment. This article is a contemporary review of measures for the prevention of white spot lesions during orthodontic treatment with fixed appliances. Preventive programs must be included for all orthodontic patients by educating and oral hygiene motivation for using topical fluoride agents, casein phosphopeptide-amorphous calcium phosphate agents, high-fluoride toothpaste, fluoride mouthwashes, gels and varnishes during and after the orthodontic treatment so as antimicrobial agents and resin infiltration. Reducing the risk of WSL formation by using these methods and early treatment with the appropriate technique is recommended to obtain healthier and more aesthetic results.

Keywords: white spot lesions, fixed orthodontic appliance, topical fluorides

Introduction

After the orthodontic appliances are removed, it is common to see a regression appearance of WSLs due to natural remineralization by saliva and abrasion due to brushing in the presence of oral and food hygiene. This improvement depends on the severity of lesions and occurs in the period of 6 months after debonding process; however, it is not sufficient and these WSLs should be applied. As a result, Guzmán-Armstrong et al. recommends a delay of 6 months before treating these lesions.(1)

The prevalence of WSL on the enamel surface is caused by multiple factors in the period of fixed orthodontic treatment. The presence of bacterial plaque, fermentable carbohydrates, a susceptible tooth surface and a sufficient period of time are necessary for the formation of WSL. Many irregularities are present in the fixed appliance of orthodontic treatment which causes easy accumulation of food debris along with the cariogenic bacteria thus causing manual teeth cleansing more difficult as well as averts the self-cleansing action of tongue, lips and cheeks to remove accumulated food debris from the tooth surfaces. The gingival side of brackets are the area of great plaque deposition. (30)

Elastomeric rings ligated teeth have a greater number of cariogenic microorganism colonies which can't be seen clearly in stainless steel ligature wires ligated teeth. (31)

Prevention and management of WSLs

The prevention and management of white spot lesions can be achieved by applying multifunctional approaches during orthodontic treatment. Fixed orthodontic appliances create stagnation areas for plaque and make tooth cleaning difficult. Brackets, arch wires, ligatures and other orthodontic appliances complicate the use of conventional oral hygiene measures. This often results in significant plaque accumulation around the bracket bases. Demineralization of enamel around brackets can be an extremely rapid process and appears most frequently on the cervical and middle thirds of the buccal surfaces of the maxillary lateral incisors, the mandibular canines and the first premolars. (2) Demineralized enamel can remineralize after debonding under favorable conditions.(3, 4)

The first choice for the elimination of WSLs is remineralization which, apart from strict oral hygiene measures, involves repeated applications and the compliance of a motivated patient and may take some time. Several professional and home applied products are available in different forms for such a purpose: solutions, varnishes, creams, pastes and chewing gums. These products contain fluorides and/or casein phosphor-peptide-amorphous calcium phosphate. There is evidence with varying degrees of success in the dental literature. (5 - 8)

Fluoride is the most reactive element in the periodic table and its presence in the biofilm is important in limiting demineralization and stimulating remineralization of the hydroxyapatite crystal. There is convincing evidence that fluoride has a major effect on demineralization and remineralization of dental hard tissue. The source of this fluoride could either be fluorapatite (formed due to the incorporation of fluoride into enamel) or calcium fluoride-like precipitates, which are formed on the enamel and in the plaque after the application of topical fluoride. (9, 10)

The risk of enamel demineralization during fixed orthodontic treatment can be prevented by: improving patient's oral hygiene with mechanical plaque control methods, using bonding agents with fluoride, enhancing the enamel resistance to microbial acid by using topical fluoride and additional methods using different mechanisms.

Oral hygiene control

The most important prophylactic measure to prevent the occurrence of WSLs in orthodontic patients is implementing a good oral hygiene regimen. Good oral hygiene is thus more important in orthodontic patients treated with fixed appliances than in non-treated individuals. Mechanical plaque control by proper tooth brushing is of paramount importance. The use of a power toothbrush or daily water irrigation in combination with manual tooth brushing may be a more effective method in reducing plaque accumulation than manual tooth brushing

alone. (11) Zabokova (12) in her study concluded that improvement of oral hygiene was detected in the group where preventive treatment with Fluorogal was implemented and showed statistically significant difference between medium values of the simplified oral hygiene index (OHI-S) before and after orthodontic treatment, which was not the case with control group. This finding might be explained by the way the oral hygiene is maintained (adequate and not adequate oral hygiene). The subjects treated with dental cream (GC Tooth Mousse) had significantly decreased oral hygiene index at the end of orthodontic treatment (1.49) in comparison with the beginning of the treatment, where the average monthly value of the index of oral hygiene was 1.55. Besides oral hygiene at home, professional prophylactic cleaning is designed to reduce the bacterial load, enhance the efficacy of brushing and facilitate cleaning by the patient. Professional tooth cleaning two or three times a year maintains a healthy mouth and reduces the risk and number of teeth with caries. It allows proper cleaning of the areas that are hard for the patient to brush. The coronal surfaces can be polished using fluoridated pastes of progressively finer particle size, and elastomer polishing cups or brushes to impede the mechanical retention of bacteria. (13)

Usage of agents with fluoride

Fluoride application is commonly used method to reduce enamel tendency to demineralization. The concentration of fluoride in saliva and plaque is effective in prevention of demineralization and the formation of remineralization. The organic acids formed by cariogenic bacteria cause a decrease in pH level of plaque resulting in fluoride diffusion into enamel from plaque and saliva as response. Displacement of hydroxyl ions of enamel structure with fluoride causes existence of fluorapatite crystals. This new crystal form is more resistant to acids. Fluoride also affects the activities of cariogenic bacteria and prevents formation of caries. Laboratory studies have shown that low concentrations of fluoride make *Streptococcus mutans* to produce less amount of acid.

Different modes in which fluorides have been documented to prevent WSL are as follows: Fluoride Mouth Rinse, Fluoride Gel, Fluoride Toothpaste, Fluoride Varnish, Fluoride in Bonding Agents and Fluorides in Elastomers. The fluoride ion has a preventive effect against caries. It modifies bacterial metabolism in dental plaque by inhibiting some enzyme processes, inhibits the production of acids by acting on the composition of the bacterial flora and (or) on the metabolic activity of micro-organisms thus reduces demineralization and stimulates the remineralization of early carious lesions by exerting a remineralization effect, especially at low concentrations. (14) Fluoride enhances enamel remineralization following orthodontic treatment. The cariostatic effect of topical fluoride is primarily due to calcium fluoride (CaF_2) formation. It has been documented that a high fluoride concentration in the enamel is less important than a moderate increase in fluoride concentration in oral fluid. Proper oral hygiene maintenance, combined with daily use of topical fluoride, is found to significantly reduce enamel decalcification. When topical fluoride is applied on the tooth surface (enamel/dentin), a CaF_2 – like material builds up in plaque or in incipient lesions, which acts as a reservoir and releases fluoride ions when the pH is lowered during a caries attack. (15, 16)

Fluorinated toothpaste

The fluoride concentration of toothpaste (in the form of sodium fluoride, monofluorophosphate, and stannous fluoride) should be over 1000 ppm; toothpaste with higher fluoride concentrations is more effective. (17, 18) The use of a dentifrice with a high fluoride concentration (5000 ppm), twice daily, by patients at high risk for WSL is more effective than conventional formulations. (19, 20) However, such toothpaste (Duraphat) cannot be prescribed for patients under 16 years of age. Heymann and Grauer recommend this toothpaste for brushing in the evenings only. (21) Nonetheless, the use of fluoride toothpaste alone is not effective in preventing WSL in the majority of patients, even with good oral hygiene.(22) The regular use of fluoride toothpaste is a very common recommendation by the orthodontist, but it is shown to be inefficient in inhibiting white spot development around the orthodontic brackets. As orthodontic patients are at an increased caries risk, an appropriate level of fluoride ions is needed to provide an anticaries benefit by promoting enamel remineralization. Thus,

for orthodontic patients, fluoride concentration below 0.1% in dentifrices is not recommended. This is because when fluoride ions are incorporated into the enamel surface, a fluorapatite crystal structure is formed having lower solubility in the oral environment compared with hydroxyapatite.

Fluoridated mouthwashes

Daily use of fluoridated mouthwashes containing sodium fluoride has been shown to result in a significant decrease in the development of carious lesion around and beneath bands. Antibacterial agents have been incorporated into these mouthwashes, including chlorhexidine, triclosan or zinc to promote their cariostatic effects. Benson carried out a systematic review and recommended the daily use of 0.05% NaF mouthwash to prevent enamel demineralization during fixed orthodontic treatment. (23) A daily mouthwash containing NaF (0.05% or 0.2%) and/or weekly rinse containing alpha-1-fetoprotein (1.2%) have been demonstrated to decrease the incidence of enamel demineralization during fixed orthodontic treatment.

Fluoride varnishes

For the prevention of white spot lesion, sodium fluoride is the agent that is mostly used, toothpaste is commonly used so as the fluoride varnish. For less motivated patients, fluoride varnish should be used. The use of a fluoride varnish gives a protective coating over the tooth surface reduces enamel solubility. These varnishes were produced to adhere to the enamel surface for longer period slowly releasing fluoride on the enamel surface. Usage of fluoride varnishes has proven to be a possible and protected technique of fluoride application. It has been reported that 44.3% reduction in enamel demineralization in orthodontic patients after application of fluoride varnish (24) Fluoride varnishes should be used in less-motivated patients in an intensive treatment schedule (three days running), repeated every three or four months, or at least twice or three times a year. The varnishes are thus used as a preventive measure to reduce demineralization of the enamel around the brackets, promote the remineralization of the carious lesions and avert further lesions. Fluoride varnishes (Fluor Protector* with 1% difluorosilane and 0.1% F, Duraphat* with 2.2% F, Bifluoride* with 5% F) are usually applied twice a year on specific areas with incipient lesions on smooth surfaces. These varnishes were developed to adhere to the enamel surface for longer periods (up to 12 hours or more) and release their fluoride slowly on the enamel surfaces. (25) The use of fluoride varnishes has proven to be a feasible and safe method of fluoride application. Advantages of the fluoride varnish over other topical fluoride regimens include providing fluoride protection of enamel despite patient noncompliance and delivering the fluoride in a sustained manner over a longer period of time. It has been reported that the application of a fluoride varnish resulted in a 44.3% reduction in enamel demineralization in orthodontic patients. Azarpazhooh et al. after a 3-year follow-up period, reported that application of fluoride varnishes every 6 months proved the most cost-effective technique for high- and medium-risk groups. (26) They also concluded that Durafluor and Duraphat released fluoride at a slow rate for up to 6 months, with the greatest release observed during the first 3 weeks, followed by a more gradual delivery. Therefore, they supported the recommendation of a biannual application of single-dose preparations. However, some studies have recommended an application every 90 days (every 3 months) to promote adequate protection. The application of a fluoride varnish every 6 weeks during orthodontic treatment has been shown effective in some other studies. (27) Recently, an in vivo study by Perrini et al. showed that periodic application of fluoride varnishes in patients undergoing fixed orthodontic treatment can provide some protection against WSLs, which might not be statistically significant if the patients exhibit excellent oral hygiene. (28) A one-time application of a fluoride varnish, just before the initiation of orthodontic treatment, did not provide any additional preventive advantage over good dental hygiene with the use of fluoride toothpastes in terms of the development of WSLs and gingivitis in patients at a low to moderate caries risk. Patients often undergo an application of fluoride varnish just before orthodontic treatment with fixed appliances. However, the efficacy of this technique is yet to be elucidated. (29) Demito et al. found there was an increase of 32% in demineralization in areas where varnish was not applied in comparison to a 30–50% reduction in WSLs in areas where Duraphat was

applied twice annually.(30) Zabokova (31) in her study concluded that the level of fluoride in enamel before and after bonding the brackets with composite resin (Dentaurum, Germany), and application of a fluoride varnish was significantly increased. Thus, the amount of fluoride in enamel before fixing the brackets was 614 ppm. After 30 days of fluoride varnish application the amount of fluoride in enamel was 844 ppm, which was statistically significantly higher than the initial coverage of fluoride in enamel. These results confirmed that fluoride varnish application is a simple and fast technique that could be useful in preventing enamel demineralization associated with orthodontic treatment.

Fluoride in bonding agents

Considering the low efficacy of patient-applied measures, there have been attempts to use the benefits of materials releasing fluoride over time, including continuous release of fluoride from the bonding system around the bracket base, which can be very advantageous. Fluoride-containing adhesives have not proved effective in decreasing demineralization, but compomers and glass-ionomer cements have been promising in this context. (33 - 35) Glass-ionomer cements are less strong than composite resins; therefore, there are more bracket failures when they are used for orthodontic bonding procedures. (36)

Generally, there is high risk of caries formation due to the longer duration of orthodontic treatment. Thus, continuous fluoride release would be highly beneficial around the bracket base. Glass ionomer cements (GICs) were at first initiated as orthodontic bonding adhesives exploiting some of their attractive attributes, to be specific, their capacity to chemically bond to the tooth structure, in addition to their continuous fluoride release following bonding. In addition to increasing the strength of the GICs bond, resin particles were added and these resin-modified GIC (RMGIC) bonding systems release fluoride like conventional GIC and have higher bond strength [26,27]. In the future, it has been suggested that RMGICs should play a greater role in orthodontic bracket bonding.

The release of fluoride from elastomeric ligatures might help decrease demineralization prevalence; however, incorporating fluoride into elastics might affect their physical properties, resulting in their faster deterioration in the oral cavity. (37) A recent study suggested that orthodontic cements with microcapsules release bioavailable fluoride, calcium and phosphate ions near the tooth surface, with the capacity to be recharged with fluoride and with no effect on the adhesion of the material to enamel. The incorporation of microcapsules into dental materials might promote remineralization. Various intraoral fluoride slow-release devices, including copolymer membrane device, glass device containing fluoride, hydroxyapatite-Eudragit RS 100 diffusion-controlled fluoride system and slow-release tablets for intrabuccal use have been introduced in recent years, with the capacity to release small amounts of fluoride over a long period of time, possibly for up to 6 months, before being replaced. (38)

It was reported that light-cured pit and fissure sealants placed on the buccal surfaces near bonded orthodontic brackets were very effective (80%) in preventing demineralization in vitro, requiring no patient compliance. (39) However, these sealants cannot be removed easily and require meticulous polishing after removal. Application of a fluoride-containing sealant to the buccal aspects of bovine incisors to prevent the development of carious lesions around orthodontic brackets showed that ProSeal sealant alone or in association with brushing and/or brushing and the use of a fluoride-containing mouthwash was more efficient in protecting enamel compared to brushing alone. (40)

Conclusion

White spot lesions are an iatrogenic effect of Orthodontic treatment. Their prevalence in Orthodontic populations is particularly high, and since they affect teeth in the aesthetic zone, they prove to be a concern to both patients and clinicians. While prevention is ideal, there are high-risk patients with these lesions and their management becomes increasingly important. While there are many different treatment modalities to manage these lesions, prevention is optimal and should constitute the first line of defense.

Oral hygiene motivation, topical fluoride agents, casein phosphopeptide-amorphous calcium phosphate agents, high-fluoride toothpaste, fluoride

mouthwashes, gels and varnishes during and after the orthodontic treatment, antimicrobial agents and resin infiltration are the current options for prevention and treatment of white enamel lesions. Reducing the risk of lesion formation by using these methods and early treatment with the appropriate technique is recommended to obtain healthier and more aesthetic results.

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IATROGENIC IN ORTHODONTICS – PART II

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ABSTRACT

The goal of this research was to investigate iatrogenic effects of orthodontic therapy through a systematic literature review. The systematic literature search was performed through computer research of several databases (Google Scholar, PubMed, ResearchGate, Scielo, SpringerLink, NCBI, American Dental Association ADA). 28 articles were included in the review. Iatrogenic effects of orthodontic treatment can be classified into two groups: local and systemic.

Introduction

Before starting every orthodontic treatment, as a long-term and complex procedure, it is very important to determine the correct therapy plan for each patient. It is important to take into account the risks that can be associated with the patient and thus ensure the success of the therapy. For the treatment to be considered successful the benefits offered by the treatment must outweigh any possible harm it may cause (1). Even though orthodontic therapy offers many benefits, still there is a possibility of side effects if certain oversights are made during the treatment. Side effects or disease caused by a dental treatment or by an oversight on the part of the therapist in the process of patient treatment is called iatrogenic effect or iatrogenic disease (2). Several factors are cited as the reason for their occurrence, among which these are the most common: orthodontist's clinical skill, application of orthodontic force, selection of an orthodontic appliance, orthodontic treatment procedures, length of therapy, but also the influence of the patients themselves (1). The orthodontist should control the entire course of the therapy, be aware of the risks and take appropriate steps to prevent complications. It is unacceptable to place an orthodontic appliance in the case of poor oral hygiene, dental plaque, periodontal disease or caries. Orthodontists' cooperation with doctors of other dental specialties, and above all with the patient, has a significant effect on achieving therapeutic success (3). Characteristics related to the patient that can influence the occurrence of side effects of orthodontic treatment refer to the patient's age, patient's sex, the environment in which the patient lives, genetic predisposition, psychological type of the patient, pathophysiological status, as well as the characteristics related to malocclusion (type, etiology, the severity of the case) and the existence of craniofacial anomalies (4). To reduce their effect and achieve the desired results it is important to process each case separately and plan treatment and conduct therapy based on that.

This research aims to explore which iatrogenic effects of orthodontic treatment there are through a systematic orthodontic review of the literature.

Materials and methods

Through computer research of several databases (Google Scholar, PubMed, ResearchGate, Scielo, SpringerLink, NCBI, American Dental Association ADA), we have found and analyzed various articles used in this research. Different combinations of keywords were used when searching the database: iatrogenic effects, risk factors and orthodontic treatment.

Inclusion criteria included papers in English language, available in their entirety.

A limitation of this search was that only articles that were fully available to the authors were used.

Results

185 articles were found searching various databases.

Within this literature review, 28 articles that met the given criteria were analyzed.

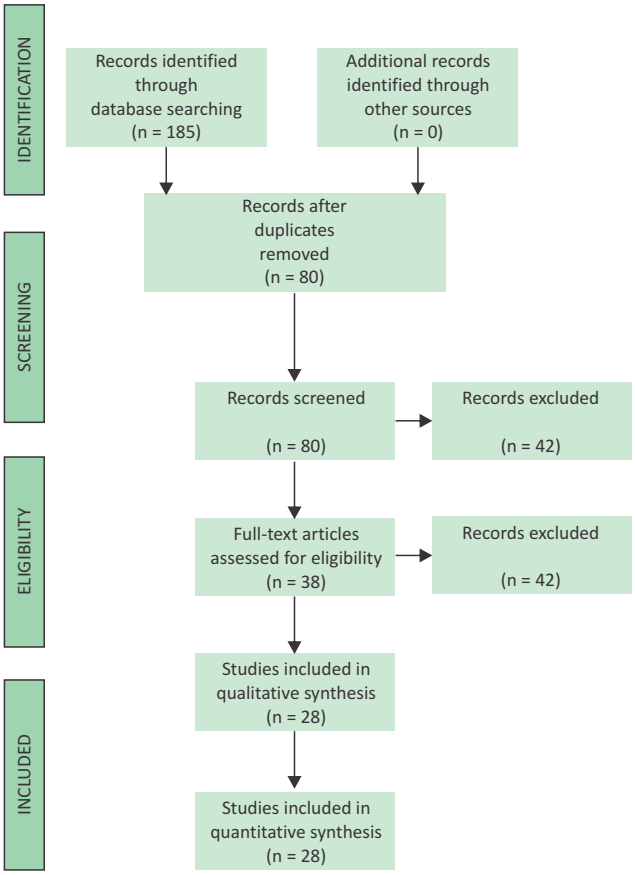

Figure 1. The flow of records trough the reviewing process

Discussion

Side effects associated with the orthodontic therapy itself are significantly lower than compared to other surgical and non-surgical interventions, but that does not mean that they should be ignored.

One of the challenges for most orthodontists is to complete the orthodontic therapy with the least possible effects on teeth and periodontium. Orthodontic therapy is usually contraindicated in patients with active periodontal disease or poor periodontal health because in this case, the chance for further deterioration of the condition becomes greater. Therefore, before starting orthodontic therapy, a thorough assessment of periodontal health and gingival level is recommended. It is especially important to emphasize the need to maintain good oral hygiene to achieve satisfactory results. Generally, the benefits of orthodontic therapy must outweigh any potential harm. The patient, as the main participant in making decisions about the treatment, has a right to be informed about the benefits and possible risks that may arise during and after the treatment (5).

Enamel damage during orthodontic treatment

Therapeutic procedures performed during orthodontic therapy can cause irreversible physical damage even to the deeper parts of the enamel (6). Phases of therapy associated with potential enamel damage include cleaning with abrasive agents before applying the acid, acid etching, removal of brackets, mechanical removal of composite residue with rotary instruments or brackets re-bonding (7). Additional enamel loss can occur as a result of intentional enamel reduction in cases when space is needed to straighten the teeth with high density or it is necessary to correct deviations in teeth size (8). One of the frequent complications that can occur during orthodontic treatment also includes demineralization of enamel and the formation of white spot lesions due to the accumulation of plaque on fixed orthodontic appliances, together with enamel discoloration or enamel abrasion due to the contact with brackets of antagonist tooth (9). Enamel damage during bracket placement is most often associated with the action of phosphoric acid. The effect of phosphoric acid etching involves the dissolution of enamel hydroxylapatite crystals leading to the demineralization of the surface layer of the enamel and exposure of the dentine collagen network. Phosphoric acid causes selective dissolution of the cores or boundaries of enamel prism and the formation of microporosity, with variations in depth from 10 to 80 μm (9). This creates a porous surface prone to retaining colored stains, although the porous surfaces are filled with precipitates over time (10). To control excessive enamel loss, maleic and polyacrylic acids were used as alternatives to phosphoric acid, but have resulted in weaker bond strength (11). Adhesive removal after bracket removal can lead to the loss of 14.3- 160 μm of enamel surface depending on the method used. The need to find an efficient and safe adhesive removal method after removing the brackets has resulted in the introduction of a wide range of instruments and procedures, including manual removal with scalers or pliers, the use of tungsten carbide burs with a configuration of 8 to 32 of low or high speed, Sof-Lex discs, special composite finishing systems with zirconium paste, as well as the application of ultrasound and laser (9).

Carious lesions associated with orthodontic treatment

Demineralization of the enamel surface or white spot is one of the main side effects of fixed orthodontic therapy with appliances, despite the progress of technology and materials in preventive dentistry and orthodontics. They appear during the treatment and sometimes persist even after it which can jeopardize the outcome of the treatment and result in early termination of the treatment (12). The presence of plaque retention sites in patients with malocclusion, and additionally placing fixed orthodontic appliances on the teeth, creates additional retention sites in those patients even on surfaces that are mostly not susceptible to caries. Oral hygiene is, therefore, harder to carry out indicating a much stronger connection between oral hygiene and caries frequency in orthodontic patients than in non-orthodontic patients (13). The presence of white spots is significantly higher in orthodontic patients than in those who were not treated with orthodontic appliances, and they can represent an

aesthetic problem years after the treatment. The literature review has shown that overall incidence varies widely between 2% and 96%, with the fact that the prevalence of white spots on at least one tooth was found to be 49.6% in patients who underwent orthodontic treatment compared to 24% in the control group of untreated patients (14). Generally, the prevalence of "white spots" in patients after orthodontic treatment varies from 15% to 85%, and most of the studies report 50% to 70% (15). Any tooth in the mouth can be affected, but most often those are molars, maxillary lateral incisors and canines, as well as mandibular premolars (14). Numerous studies have shown that the biggest responsibility for "white spots" lies with the patient. On the other hand, dentists and orthodontists play an important role in identifying high-risk patients guiding them toward an appropriate preventive regimen (16,17).

The effect of orthodontic treatment on the pulp

The force applied to the periodontium can cause mechanical damage and lead to inflammation of the pulp-dentine complex. Even though orthodontic tooth movement is relatively safe for pulp health, certain situations can lead to pulp necrosis. This usually happens in teeth with previously compromised pulp, e.g. teeth that suffered trauma, teeth with extensive restorations, etc (18). By applying orthodontic forces to the teeth molecular changes within the cells of the periodontium occur. The peripheral sensory nervous system contributes to the development of acute and chronic inflammatory processes through the local release of neuropeptides. However, there is still no accurate evidence of the relationship between orthodontic forces and pulp tissue in humans (19). Proffit et al claim that continuous mild force affects the PDL but has little or no effect on the pulp (20). Some authors believe that excessive intrusive/extrusive force can disrupt the circulation of the dental pulp and degenerate the odontoblastic layer (21). These factors potentially result in pulp necrosis. In their research, Wang et al find that the prevalence of tooth pulp damage in adolescence, which is a result of orthodontic treatment, was around 2%-17% for canal obliteration and 1%-14% for pulp necrosis (18). Although there are very few reports on the loss of tooth vitality due to excessive orthodontic force, it is still assumed that the application of higher orthodontic force can potentially cause changes in the pulp and the consequences are much more severe (18).

Temporomandibular dysfunction

There is a hypothesis that different orthodontic procedures (e.g. functional appliances, elastic tractions class II and III, chin cap, headgear, fixed or mobile appliances), and therapy plans can be considered etiological factors of temporomandibular dysfunctions (TMD) (22), and Henrikson and Nilner (23) have conducted research to compare the state and function of the joint in orthodontically treated and untreated patients with malocclusion II/1, aged 11 to 15, comparing both groups with patients having proper occlusion. All those patients were treated with a fixed appliance using a headgear or class II elastic tractions, or with extractions. Signs and symptoms of TMD were monitored for 2 years. Individual fluctuations of TMD symptoms have been reported in all three groups. In orthodontic group, the prevalence of TMD symptoms has been reducing during the two years period. The Class II group and normal occlusion group have shown less change during the two years. Clicking at the temporomandibular joint increased in all three groups over the two years. Therefore, orthodontic treatment did not increase the risk or worsen the signs of TMD from before the treatment. On the other hand, subjects with class II malocclusion and TMD signs of muscular origin appeared to have a functional benefit from the orthodontic treatment in the two-year perspective (23). Rey et al (24) compared class III patients treated with orthodontic cervical headgear, class I patients treated orthodontically without extractions and subjects that have not been previously treated for the presence or absence of TMD. Class III patients treated with cervical headgear and a fixed appliance for 2-3 years did not have a higher prevalence of TMD signs and symptoms than class I patients that were only treated with fixed appliances or were not treated at all. Therefore, modifications in the temporomandibular joint caused by treatment must be interpreted as changes in remodeling (24). Another topic of debate among orthodontists is, also,

the influence of orthognathic surgery on TMD. Some reports suggest that operation can relieve the signs and symptoms of TMD (25) while others indicate that surgery could initiate or worsen temporomandibular dysfunction (26). Scientific evidence was insufficient to evaluate the effects that orthognathic surgery had on TMD. According to current findings, the role of orthodontic treatment in the development of TMD has not been confirmed. Accordingly, a meta-analysis on the effects of orthodontic therapy on TMD reported that no study found that traditional orthodontic treatment, including appliances, class II elastic traction, bionator and headgear, face mask and chin cap, increased the prevalence of TMD (27). Although most of the studies consistently do not support the relation between orthodontic treatment and temporomandibular dysfunctions, it must be emphasized that definitive conclusions cannot be drawn due to the unknown causes of TMD, heterogeneity in methodology and study design and the lack of widely accepted classification schemes (22).

Accidentally ingested parts of the orthodontic appliance

Accidental ingestion of orthodontic appliance parts, although very rare, is a potential hazard that should not be taken as granted and all possible measures must be taken to prevent its occurrence. In practice, cases of ingesting the appliance and retainer wire are most often cited. Not only smaller components such as brackets, rings and tubes are swallowed, but even relatively larger devices such as quad helix and palatal expander. The palatal expander activation key is another component that can be accidentally swallowed (28). According to the above, procedures and measures can be found in literature to prevent this not-so-common, but still possible, orthodontic iatrogeny (2).

All orthodontic offices should take into account the possibility of such situations occurring and should be well-prepared to deal with any unforeseen circumstances. Every orthodontist must know how to provide first aid to a patient. Updating and improving these skills is recommended at least once every 2 years, as cardiopulmonary resuscitation (CPR) recommendations are updated every 5 years (2).

Conclusion

Orthodontic treatment offers numerous advantages, but there is still the possibility of unwanted effects. Iatrogenic consequences of orthodontic treatment can be classified into two groups: local and systemic. Fortunately, more serious damage is a rare occurrence. Before starting orthodontic treatment both the patient and the orthodontist should consider the benefits and the risks of the proposed treatment. Correct diagnosis, detailed therapy plan, regular check-ups and timely intervention, as well as good cooperation between the patient and the orthodontist, are the key to the success of any orthodontic therapy. Most of the harmful effects of orthodontic treatment can be avoided by taking certain precautions during the treatment. The orthodontist is obliged to familiarize the patient with all possible harmful effects of orthodontic therapy.

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MOLECULAR GENETIC IDENTIFICATION METHODS OF MICROORGANISMS IN ROOT CANALS

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ABSTRACT

Oral cavity is the habitat of a large number of microorganisms. In endodontic microbiology, research and identification of microorganisms was primarily based on cultivation methods. These methods have certain limitations, especially considering the diversity among endodontic microbiota, as well as the fact that certain percentage of bacteria found in root canals are uncultivable. With the introduction of molecular genetic technologies, many limitations of cultivation methods have been overcome. In molecular microbiology, 16S rRNA represents a gene, a part of DNA molecule that is used for determining the genetic diversity among bacteria species via sequencing methods. There are numerous advantages of these methods, especially emphasizing their speed, sensitivity and accuracy. By using these methods, a big step forward can be made in terms of evidence-based antimicrobial endodontic therapy. Through the identification and detailed studious analysis of obtained results, we can conduct further research and possibly create new therapeutic guidelines or revise existing therapeutic protocols, thereby increase the success of treatment and prevent the development of reinfections.

Keywords: microorganisms, root canal, next generation sequencing, Illumina, PCR.

Introduction

Several hundred bacterial species inhabit the oral cavity and coexist in multispecies communities forming the unique ecosystem. However, dental pulp, the internal tooth tissue is physiologically sterile and any bacterial invasion to this connective tissue is a pathological sign (1). If the normal oral flora gets in touch with this sterile environment, bacteria would become an opportunistic pathogen causing damage to these tissues. They may reach dental pulp either through coronal leakage, caries exposure, faulty restorations, tooth cracks, traumatic injury, anachoresis or periodontal pathways (2). Bacteria that initially invades and colonizes dental pulp primarily causes intrapulpal infection. Some of these bacteria are involved in earlier stages of pulpal invasion to the further necrosis, or they may be latecomers invading canal after pulp necrosis by taking advantage of environmental condition in root canal. Bacterial consortium in primary intrapulpal infections contain 10^3 to 10^8 bacterial cells and 10 to 30 bacterial species per canal (3). Culture based methods and sophisticated molecular biology techniques have established the polymicrobial nature of primary endodontic infections where obligatory anaerobic bacterial species are dominant (4). The most frequent microorganisms in primary endodontic infections appeared to be anaerobic gram-negative bacteria whose species from several genera have been regularly found in infections associated with apical periodontitis and abscesses (3,5). These genera include *Fusobacterium* (e.g., *F.nucleatum),* assacharolytic species as *Dialister* (e.g., *D.pneumosintes, D.invisus*) and *Porphyromonas* (e.g., *P.gingivalis, P. endodontalis*), saccharolytic species- *Prevotella* (e.g., *P. nigrescens, P. intermedia, P. baroniae, P. tannerae*), spirochetes- *Treponema* (e.g., *T.socranski*, *T.denticola*) and *Tannerella* (e.g., *T. forsythia*). Even though gram-negative bacteria have been appeared the most common microorganisms in endodontic consortium, several gram-positive bacteria in prevalence are as frequent as gram-negative species. Gram-positive bacterial genera often found in primary endodontic infections include *Actinomyces* (e.g., *A.israelii*), *Olsenella* (e.g., *O.uli*), *Filifactor* (e.g., *F.alocis), Peptostreptococcus* (e.g., *P.stomatis, P. anaerobius), Streptococcus spp. (e.g., S. anginosus, S.sanguinis, S.mitisi), Enterococcus faecalis,Parvimonas* (e.g., *P.micra*), *Propionibacterium* (e.g., *P.acnes,P.propionicum*) and *Pseudoramibacter* (e.g., *P. alactolyticus*) (6,7,8).

Interactions between endodontic pathogens can be synergistic or antagonistic. Microbial synergism is phenomenon in which all microbe population support each other's proliferation and growth by providing nutrition to each other, inhibiting phagocytosis, secreting enzymes and growth factors, decreasing pH and oxygen concentration in the root canal (9). Antagonistic interaction between oral microbes implicates their competition for food sources and territory by suppressing or destructing bacterial species that are less dominant in endodontic infection (10).

Pioneer research in endodontic microbiology is based on cultivating root canal bacteria. As endodontic microbiota is diverse and 40% of bacteria found in root canal are uncultivable, different scientific studies establish that molecular diagnostic methods are more effective to discover new endodontic pathogens. Antagonistic interaction between bacteria can cause death of some species or they might be suppressed by dominant bacteria being not present in culture methods. Knowing that, development of molecular microbiology research has resulted with spectacular discovery in technology known as next generation sequencing methods. NGS technology use 16S ribosomal RNA (16S rRNA) gene to analyze bacterial diversity (11). Compared with other techniques, next generation sequencing is revolution in molecular technology in order to empower antimicrobial effectiveness and success of endodontic treatment.

Polymerase-chain reaction (PCR)

Culture procedures have been traditionally used as the reference in the assessment of the oral microbiota associated with infections of endodontic origin. However, there are certain limitations following this method (12).

First of all, the culture method routinely uses agar-plates as a medium in highest sample dilution causes an inadequate representation of the collected sample. In addition to the drawbacks of dilution, identification of microorganisms by their phenotypic traits has some other serious pitfalls, considering

that phenotypical traits are difficult to quantify, they are generally ambiguous and some species may even show a phenotypically convergent or divergent behavior. Furthermore, non-viable, uncultivable and in certain cases even fastidious or delicate microbes cannot be isolated using culture methods. The main reason for that is the difficulty in simulating the required environmental conditions for cultivation, for example the lack of the essential nutrients for bacterial growth or disruption of bacterial intercommunication systems. Production of substances inhibitory to the target microorganism by other present species might also be the reason for bacterial uncultivability (12, 13, 14, 15). Finally, the procedure itself lacks sensitivity, is costly and laborious and it can take several days to weeks for the cultivation and identification results to be completed (12, 14).

Because of all these reasons, this method has been recently questioned as the "gold standard" (the reference method) for bacterial identification (12, 16).

Many limitations of culture method have been successfully overcome by the induction of molecular technologies. Studies on DNA, RNA and proteins have introduced new methods of infectious agents' identification detecting microbial DNA rather than the microorganisms themselves in both research and clinical cases. These procedures have revolutionized our knowledge of infectious diseases, allowing us an effective and rapid diagnosis of many clinical conditions and they have definitely showed that the root canal microbial composition is much more complex than previously thought (12, 13, 17).

Molecular genetic diagnostics methods are more accurate, sensitive, specific and rapid than the culture technique. They do not demand carefully controlled anaerobic setting during sampling and transportation being a great advantage since some of the fragile microorganisms, especially anaerobic bacteria, can lose viability during transit. When higher number of samples is surveyed in epidemiological studies, multiple samples can be gathered and analyzed all at once (13,14).

There is a wide range of molecular genetic methods and the choice of a particular approach for the microbial identification depends on the question being addressed.

The most widespread advance in diagnostics technology has come from the application of polymerase-chain reaction (PCR) for detection of microbial pathogens. The PCR method was invented in 1983 and ever since has revolutionized the field of molecular diagnostics by being able to amplify as few as one copy of a particular DNA sequence into billions of copies of that sequence (13,14).

The PCR process relies on the in-vitro DNA replication via repetitive cycles of simple reactions. Basically, each ensuing cycle uses previously synthesized products as templates for the subsequent reactions. The outcome is the exponential amplification of DNA products. A typical PCR procedure includes 25–40 cycles of amplification (12, 14). One of the great benefits of the PCR method is that it does not require particularly pure or plentiful target DNA for the process to be completed (18). Amplification in theory is possible even if the sample contains a single molecule of DNA making this technique one of the most sensitive methods for detection of microbial DNA. Furthermore, under optimized amplification conditions, PCR shows no cross-reactivity (12, 19).

A single round of PCR is a standard and most often used method for microbial identification purposes. Nevertheless, since its inception, there have been developed numerous derivates of the method, such as reverse-transcriptase PCR (RT-PCR), Multiplex PCR, Broad-range PCR and Real-time PCR. These variations have also been used to directly survey clinical samples for the presence of bacteria (12,14).

Reverse Transcriptase PCR has been developed as a method through which a DNA can be generated from RNA, using the reverse transcriptase enzyme. Once the double-stranded DNA is synthesized, it can be used as a template for further amplification, just as in standard PCR (12, 14, 20).

Multiplex PCR enables the simultaneous detection of different microorganisms by using primers specific for different targets considering that more than one target sequences can be amplified at the same time. Practically, as multiplex PCR can detect a few different species at a time, this method minimizes time used for the analysis and the number of templates and reagents needed for detection. Careful design of the primers used in multiplex assays is an imperative, considering that similar annealing temperatures and no complementarity of the primers is required in order to avoid inaccuracy (12, 13, 14).

Broad-range PCR technology can be used to detect the entire microbial diversity in a given environment. This variation of standard PCR uses primers complementary to conserved regions. Conserved regions are parts of a particular gene shared by a group of microbes. What gives this method a certain superiority is the relative absence of selectivity, meaning that any kind of species present in a sample can be detected and later identified, even the unexpected ones. Broad-range primers bring a high risk for microbial contamination of the DNA and increased level of precautions is necessary (13, 14).

Real-time PCR enables monitoring of the amplification of a targeted DNA molecule during the PCR in real-time, using florescent dyes. What makes this method the exception from the most conventional PCR assays is the additional ability of quantification. In conclusion, real-time PCR allows the quantitation of target species as well as a total number of bacteria in clinical samples. This method saves time, since it can detect bacteria in a short period of time. The contamination of DNA is reduced because there is no postamplification manipulation (13, 14).

The use of PCR technology enabled us to detect some species of Gram-negative bacteria that are difficult to grow by culture method, such as the Fusobacterium spp., Porphyromonas spp., Treponema spp., Prevotella spp. and Tannerella spp. Additionally, their prevalence was much more emphasized with the use of PCR, differently from those studies based on culture techniques only. PCR has unrivaled sensitivity, being approximately 10 to 100 times more sensitive than any other identification method (12, 14, 21).

Although the PCR method has enabled us to detect many microorganisms in the root canal and identify them rapidly, there are still certain limitations. For example, a PCR cannot determine whether target DNA comes from live or dead bacteria (22).

In conclusion, PCR represents the basis for any other kind of analysis because of its ability to amplify target DNA extracted from the given sample. From there on, by sequencing, we collect information about the origin of the sequenced DNA as well as complete taxonomic data.

Next-generation sequencing

Sequencing is the process of determining the correct order of nucleotides in the exact DNA molecule. In molecular microbiology, 16S rRNA represents a gene, a part of DNA molecule that is used for determining the genetic diversity among bacteria species via sequencing methods. More than a few methods have been developed for this process. In 1977 was developed the first method of DNA sequencing named Sanger sequencing method (23). Since then, there have been quite a few innovations (such as development of fluorescent dyes and replacement of gel electrophoresis with capillary electrophoresis, as well as software improvements) which contributed to high success results rate of this methodology (24). In fact, this high rate of accuracy of the results leads to the main drawback of this method because it implies performing one reaction at a time. Also, because the price of this sequencing method is very high, Sangers sequencing method is mostly used for analyzing specific genes, for example, in human rare diseases (25). After the Sanger sequencing, the rise of technological development provided a next step in the DNA sequencing technology and has led to next generation of sequencing (NGS) (24). The main aim of using NGS in endodontic microbiology is to replace the characterization of pathogens according to their morphology, staining methods and metabolic characteristics with their genomic definition. The genomes of pathogens can give information about drug sensitivity, their relationship with other pathogens and also give more detailed information about infection out brakes which are in this case considered very important in expecting outcomes of endodontic treatment (26). A relatively new field named *Pharmacogenomics* uses NGS data to determine which drugs can result in the most successful outcomes in correlation to certain diseases, infections. In cooperation with this pharmaceutical branch, endodontists may be able to make significant strides in the improvement of endodontic therapy.

With NGS the disadvantages of Sanger sequencing were successfully eliminated, since in this case a large number, millions of sequencing reactions take place in parallel, simultaneously. Hence the name, massively parallel or deep sequencing. From endodontic point of view, this method of processing

root canal samples can represent a big step forward in terms of success of endodontic therapy. NGS identifies microorganisms (species) contained in the given sample. Based on the enormous amount of data received by NGS (the complete genome of certain isolated species) research on genetic diversity can be conducted. Consequently, with the addition of literature research on the sensitivity of certain species to specific drugs/chemicals, by comparing genomes, the existence of identical regions can be detected and therefore mutual characteristics defined, in this case sensitivity to drugs. Although these methods may seem expensive, the situation is different: an enormous number of reactions take place in parallel. This also overcomes disadvantages in terms of price, compared to the Sanger method (23).

If only a certain fragment is to be sequenced with a significantly smaller number of base pairs, Sanger sequencing is a method of choice due to a higher price rate of NGS. In general, this technology is able to produce enormous amount of data at very economic cost

Deficiency here is the obvious need for sophisticated software in order to analyze and interpret obtained results.

Illumina

The leading role in NGS represents *Illumina.* Illumina NGS platforms are capable of paired end sequencing (sequencing that occurs from both ends of a DNA fragment) generating high-quality sequence data with in depth coverage and high numbers of reads (27, 28).

There are several Illumina sequencing platforms, and as a matter of fact, Illumina is still producing newer and upgraded versions of already existing ones. The most popular, used and recognized Illumina platforms are: MiSeq, HiSeq, NextSeq series etc. Within these platforms there are also certain kits. The choice of appropriate Illumina platform and kit depends upon the subject of the research (29).

Users must be aware that there are some limitations and occurring errors when using each of these platforms. To avoid these possibilities, the application of different technologies is often used (23).

The attached Illumina results (Figure 1-4) are an example of laboratory analysis from the original research, performed using MiSeq platform and the 16s Metagenomics kit. It shows a complete taxonomic analysis of given samples, starting from the kingdom to the species classification of the identified bacteria. These results demonstrate the superiority and necessity of Illumina and NGS.

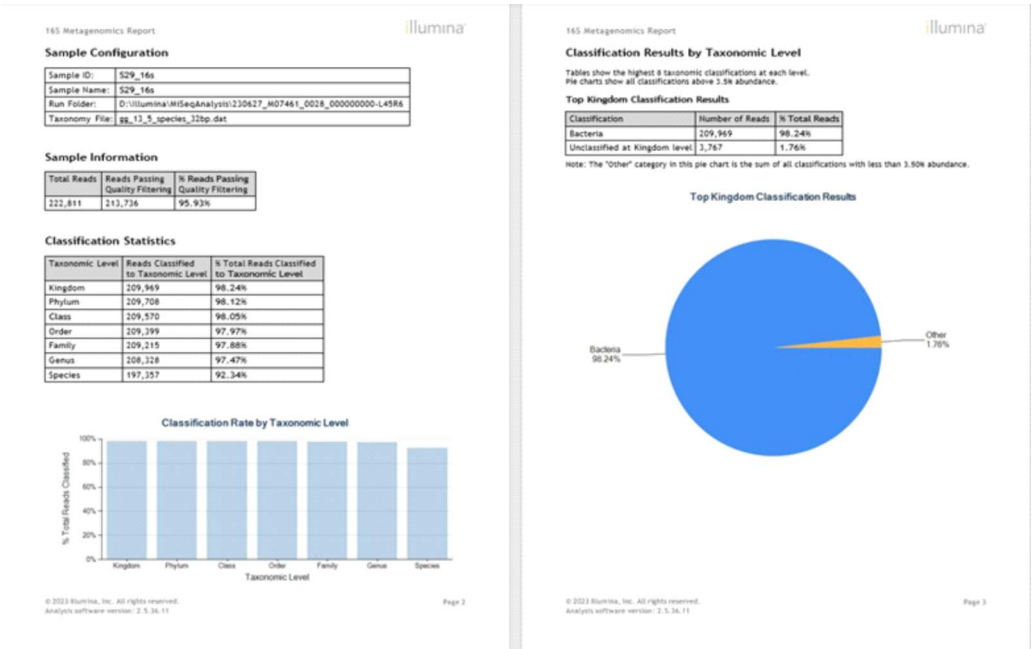
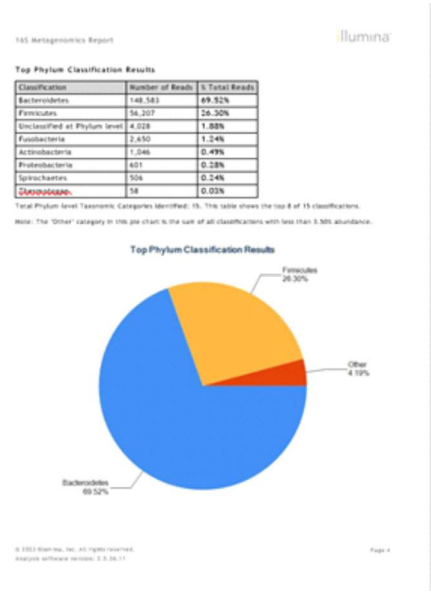


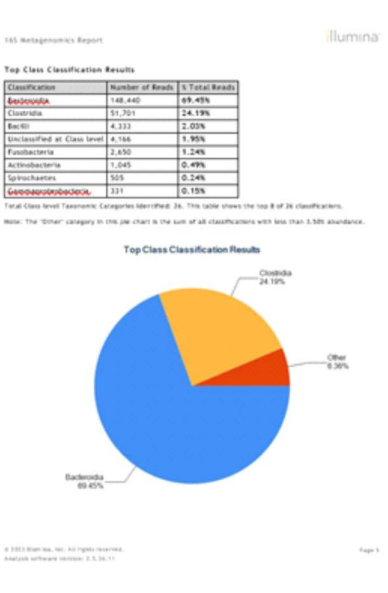
Figure 1.
Results of the original research on Molecular genetic identification methods of microorganisms in root canals



Top Phylum Classification Results

Classification	Number of Reads	% Total Reads
Bacteroidetes	148,583	69.52%
Firmicutes	56,207	26.30%
Unclassified at Phylum level	4,028	1.88%
Fusobacteria	2,650	1.24%
Actinobacteria	1,046	0.49%
Proteobacteria	601	0.28%
Spirochaetes	506	0.24%
[illegible]	58	0.03%

Top Phylum Classification Results

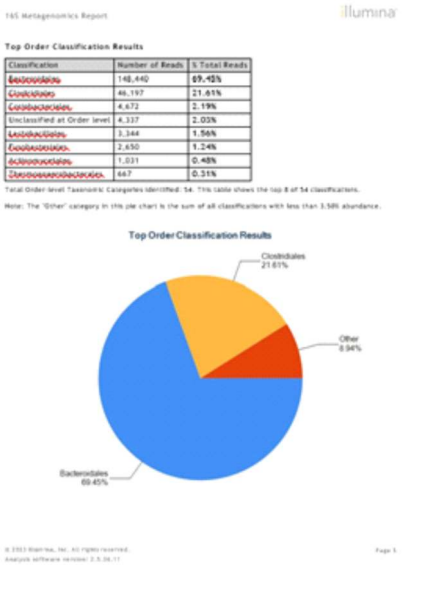


Top Class Classification Results

Classification	Number of Reads	% Total Reads
[illegible]	148,440	69.45%
Clostridia	51,701	24.19%
Bacilli	4,333	2.03%
Unclassified at Class level	4,166	1.95%
Fusobacteria	2,650	1.24%
Actinobacteria	1,045	0.49%
Spirochaetes	505	0.24%
[illegible]	331	0.15%

Top Class Classification Results

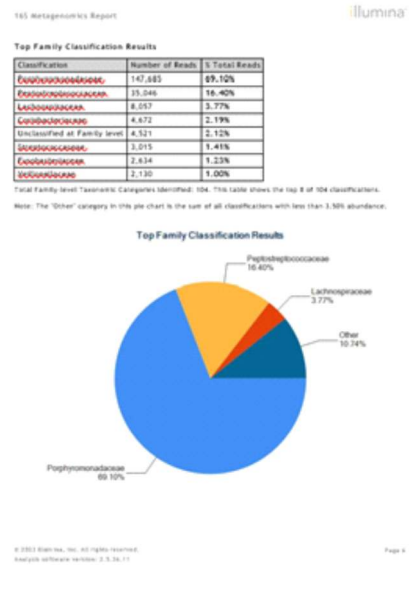
Figure 2.
Results of the original research on Molecular genetic identification methods of microorganisms in root canals. Phylum and class level taxonomic category.



Top Order Classification Results

Classification	Number of Reads	% Total Reads
[illegible]	148,440	69.45%
[illegible]	46,197	21.61%
[illegible]	4,672	2.19%
Unclassified at Order level	4,337	2.03%
[illegible]	3,344	1.56%
[illegible]	2,650	1.24%
[illegible]	1,031	0.48%
[illegible]	667	0.31%

Top Order Classification Results

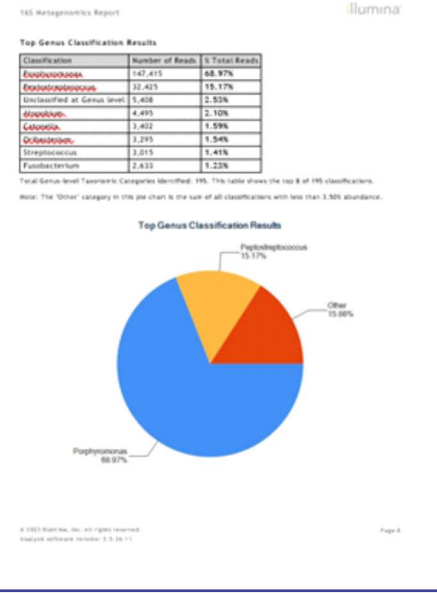


Top Family Classification Results

Classification	Number of Reads	% Total Reads
[illegible]	147,685	69.10%
[illegible]	35,046	16.40%
[illegible]	8,057	3.77%
[illegible]	4,672	2.19%
Unclassified at Family level	4,521	2.12%
[illegible]	3,015	1.41%
[illegible]	2,634	1.23%
[illegible]	2,130	1.00%

Top Family Classification Results

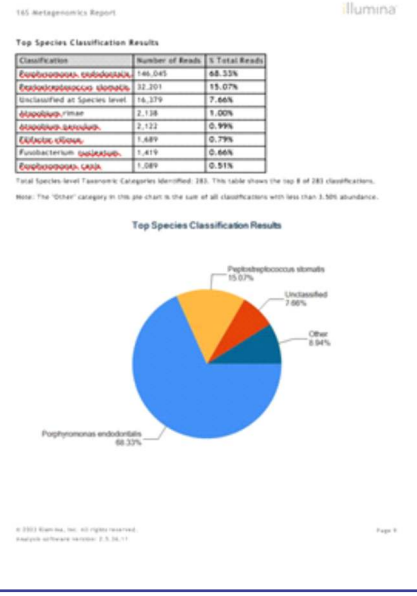
Figure 3.
Results of the original research on Molecular genetic identification methods of microorganisms in root canals. Order and family level taxonomic category.



Top Genus Classification Results

Classification	Number of Reads	% Total Reads
[illegible]	147,415	68.97%
[illegible]	32,425	15.17%
Unclassified at Genus level	5,408	2.53%
[illegible]	4,495	2.10%
[illegible]	3,402	1.59%
[illegible]	3,295	1.54%
Streptococcus	3,015	1.41%
Fusobacterium	2,633	1.23%

Top Genus Classification Results



Top Species Classification Results

Classification	Number of Reads	% Total Reads
[illegible]	146,045	68.33%
[illegible]	32,201	15.07%
Unclassified at Species level	16,379	7.66%
[illegible]	2,138	1.00%
[illegible]	2,122	0.99%
[illegible]	1,689	0.79%
Fusobacterium [illegible]	1,419	0.66%
[illegible]	1,089	0.51%

Top Species Classification Results

Figure 4.
Results of the original research on Molecular genetic identification methods of microorganisms in root canals. Genus and species level taxonomic category.

Conclusion

Oral cavity inhabits one of the highest accumulations of different microorganisms in the body, with bacteria being by far the most prevalent ones. Application of molecular genetic technologies in analyzing bacterial diversity has revealed broader and more diverse spectrum of oral bacteria compared to cultivation methods. Undoubtedly, with the well-directed use of molecular genetic methods, valuable information regarding the identification of the etiological factors associated with endodontic diseases will be provided. Moreover, these methods have the potential to make a diagnosis more rapid and to make directed evidence-based antimicrobial treatment a reality. Further efforts should be directed towards finding a connection of the predominant taxa with symptoms and other clinical conditions, unraveling the practical role of the detected species in the mixed endodontic consortium and disclosing their susceptibility to antimicrobial substances and therapeutic procedures.

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OVERCOMING OBSTACLES: A CASE STUDY OF SUCCESSFUL ENDODONTIC TREATMENT OF A TRAUMATIZED TOOTH WITH ADVANCED ROOT RESORPTION AND PULP OBLITERATION

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ABSTRACT

Background: This manuscript presents an interesting case report of a successful endodontic treatment of a traumatized permanent central incisor with advanced external apical root resorption, apical periodontitis and pulp obliteration. The main objective of this study was to shed light on the challenges faced in treating late complications of dental trauma and to emphasize the significance of careful diagnosis and management in such cases.

Methods: To assess the extent of the trauma and the condition of the tooth, the patient's dental history, clinical examination and radiographic evaluation were conducted. The treatment plan involved the use of ultrasonic tips and rotary endodontic files, along with calcium hydroxide paste and non-vital tooth bleaching. Regular clinical and radiographic evaluations were performed to monitor the healing progress of the periapical lesion.

Results: The endodontic treatment proved to be highly successful in preserving the traumatized tooth, despite its shortened root and extremely narrowed endodontic space. The use of appropriate techniques and materials played a crucial role in achieving this positive outcome as evidenced by the signs of healing observed during the regular follow-up evaluations.

Conclusion: This intriguing case report highlights the successful management of a complex endodontic case involving a traumatized tooth with advanced root resorption and pulp obliteration. The careful diagnosis, treatment planning and execution of the endodontic procedures were instrumental in achieving a favorable outcome. This study underscores the importance of personalized approaches in treating late complications of dental trauma to ensure the preservation of the natural dentition.

Keywords: dental trauma, incisor, root resorption, pulpal obliteration, discoloration, bleaching

Introduction

Following the dental injury, acute and follow-up dental care should be provided. The primary post-traumatic complications reported were pulp necrosis and infection, obliteration of the pulp space, different types of root resorption, marginal gingiva and bone breakdown (1,2).

Disruption of the neurovascular supply to the dental pulp may lead to sterile pulp necrosis, followed by infection. In mature teeth with severe traumatic displacement, pulp necrosis with infection of the root canal system accompanied by apical periodontitis is a common finding (3). Pulp necrosis occurred in 1/3 of traumatized teeth and mainly manifested years after the trauma occurred (2).

External inflammatory resorption (EIR) is a common sequela following traumatic dental injury due to damage to the cementum and /or periodontal ligament during avulsion or luxation (4). Moreover, EIR may result from inflammation of the periodontal tissues or orthodontic therapy. An infection-induced resorption process known as external apical inflammatory resorption usually responds favorably to routine endodontic treatment (5).

In addition, another type of late dental trauma sequela may be a pronounced deposition of tertiary dentine along the inner walls of the root canal, resulting in rapid narrowing of the endodontic space. Although a narrow root canal space always persists histologically, it does not frequently lead to complete obliteration due to the gradual narrowing of the entire root canal (6). Pulp canal obliteration (PCO) has high rates in cases of intrusion, extrusion and luxation injuries, especially in teeth with open apices indicating the presence of viable pulp tissue (1). The prevalence of PCO after dental trauma varies, ranging from 3.7% to 40% (6).

Traumatized teeth can exhibit color changes (3). Dental traumatic injuries often result in discoloration related to pulpal damage, ranging from pink to grey, as well as tooth crowns becoming yellow or yellow-brown due to the deposition of the irregular dentine in the pulp chamber.

The present case report describes the nonsurgical endodontic treatment and intra-coronal bleaching of the maxillary central incisor with advanced external apical root resorption, pulp obliteration and coronal discoloration, secondary to dental trauma.

Case report

A 23-year-old male visited the Department of Restorative Dentistry with Endodontics at the Faculty of Dentistry with Dental Clinical Center for a routine dental examination. The patient's medical and family history were unremarkable.

The patient reported that he accidentally fell while playing when he was a little boy, resulting in a fracture of the upper central incisor. Back then, he visited a nearby dental clinic, and a dental filling was placed. Years after the traumatic tooth injury, there were no clinical manifestations of that tooth.

An intraoral clinical examination revealed discoloration of tooth no. 21 with a huge dental filling (Fig. 1). Tooth #21 was not sensitive to palpation with sensitivity to percussion, as well as without gingival sulcular bleeding on probing, along with horizontal tooth mobility Grade 1. The response to the pulp sensitivity cold test was negative. A panoramic radiograph demonstrated a periapical lesion associated with tooth #21 (Fig. 2). Moreover, a

Figure 1. The crown discoloration #21.

Figure 2.
Initial panoramic of the periapical lesion of tooth #21.

Figure 3. Initial periapical radiography demonstrates a maxillary central incisor with periapical lesion, external apical root resorption, and pulpal obliteration in a maxillary left central incisor.

radiographic examination of #21 using a periapical radiograph showed apical radiolucency associated with extensive apical root resorption and the pulp canal showed signs of obliteration (Fig. 3).

Tooth discoloration, pulp necrosis with apical periodontitis and apical inflammatory resorption were diagnosed. Following the Treatment Decision Flowchart (7), depending on the observed signs and symptoms and with consultation with an oral surgeon, it was decided to perform non-surgical root canal treatment followed by non-vital bleaching and composite filling consecutively. An informed consent for the procedure was obtained from the patient.

Case management

In order to achieve preferred moisture control, a rubber dam was applied to all maxillary incisors (1). The access cavity was accomplished with a round diamond bur and an endo-access bur rotated parallel to the long axis of the tooth (7).

Calcification that covered the orifice of the root canal was identified in the center of the tooth and carefully removed using a diamond-coated ball ultrasound tip (P 14D, Satalec Acteon, France) under the control of the dental microscope (Carl Zeiss OPMI Pico, Germany). Afterward, with the operative

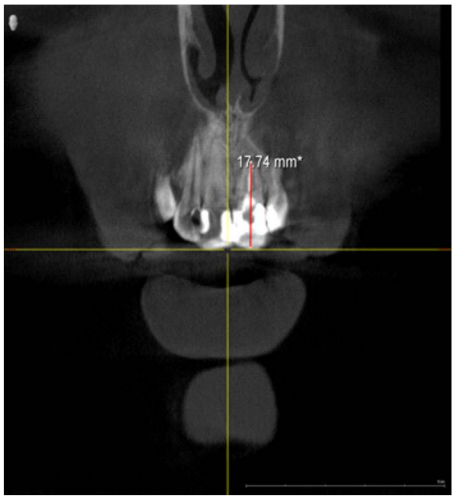

Figure 4. Working length confirmation on CBCT image

microscope's aid, a small orifice was identified. The identification of a canal orifice was based on the subtle differences in color between the tertiary dentine deposited in the pulp canal and the surrounding dentine (7). The calcified canal was negotiated by using a small hand file (0.08 K-file) (Dentsply Maillefer, Tulsa, OK) with watch-winding movements. The working length was confirmed by using an electronic apex locator Endo radar (Woodpecker Guilin) and confirmed with CBCT imaging (Figure 4). Subsequently, biomechanical instrumentation was performed using MTwo rotary endodontic files in the sequence (VDW GmbH, Munich, Germany) and copious irrigation with 1.5% sodium hypochlorite (NaOCl) combined with EDTA. In addition, the canal was dried using sterile absorbent paper points and an intracanal dressing of

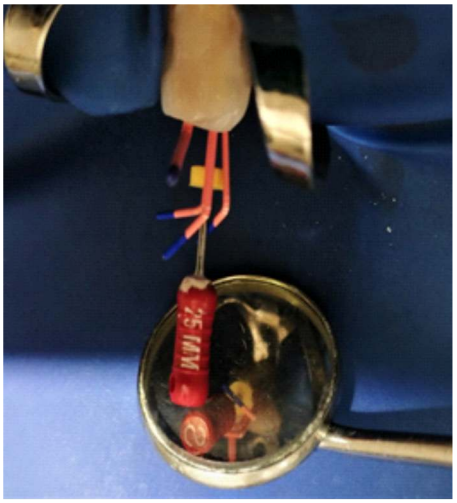

Figure 5. Endodontic filling using lateral compaction

Figure 6. Immediate post-obturation radiograph

calcium hydroxide (Calxyl, OCO-Praparate GmbH, Germany) was placed for four weeks and the access cavity was sealed using a temporary restorative cement (Cavit-G, 3M ESPE, St. Paul, MN, USA) (1).

The intracanal dressing was changed every 2 weeks. After that period, the canal was filled with gutta-percha and epoxy sealer (AH Plus, Dentsply Maillefer, Tulsa, OK, USA) using lateral compaction (Figure 5). The endodontic filling was sealed with temporary filling material (Cavit G, ESPE, Seefeld, Germany) and a postoperative radiograph was taken (Figure 6).

At the next visit, the defective composite restoration seen distally on the radiograph was replaced. At the subsequent visit, temporary materials and gutta-percha points at the orifice of the canal, 2 mm apically to the enamel-dentine junction, were removed. A thick layer of a protective glass ionomer was applied to cover the endodontic

Figure 7. Tooth #21 after restoration replacement and walking bleach therapy

Figure 8. Six-month follow-up periapical radiograph revealed periapical healing and stability of root resorption.

obturation for the walking bleach technique. The walking bleach paste was prepared as a mixture of 2g of sodium perborate tetrahydrate powder and 1g of distilled water. The mixture was applied, adapted by tamping with a cotton pellet and covered with thick temporary glass ionomer cement. The patient was evaluated five days later and the walking bleach paste was repeated. The procedure was repeated three times more until no progress in tooth shade was observed. After bleaching, we waited for one-week prior to bonded composite resin restoration application to improve the bond strength of the composite (Figure 7).

Radiographic and clinical controls of the involved tooth were performed after six months. The radiographic control suggested repairing of the periapical lesion (Figure 8); no subjective symptoms were reported.

Discussion

Trauma of teeth frequently occurs in primary and permanent dentition. The incidence of dental injuries affects no more than 5% of the world population, while the prevalence of dental trauma is high worldwide and presents a wide range from 6% to 59% (8). Approximately one-third of children and toddlers and one-fifth of adolescents and adults (permanent teeth) have sustained a traumatic dental

injury, mostly involving the maxillary central incisors, mainly from falls at home by toddlers and contact sports in adolescents (8). Dental trauma treatment is never the same and poses a challenge for the therapist.

Endodontic treatment was performed using advanced available diagnostic and therapeutic technologies, including cone beam computed tomography (CBCT), an operating microscope, endodontic ultrasonic tips and flexible rotary nickel-titanium files. An advanced 3D view using CBCT allows a detailed assessment of the extent of the periapical lesions compared to the periapical radiographs. Microscopic visualization and ultrasound instruments represent a safe and efficient combination for optimum treatment results (9). The dental operating microscope has been recognized as a useful tool in treating calcified canals and improving treatment outcomes in previous studies (7). When there is a high risk of perforation, it is crucial to use ultrasonic tips with high cutting efficiency that do not rotate (9).

Teeth with PCO provide an endodontic treatment challenge (7). Due to the partial obliteration of the canal, it was challenging to identify the remaining root canal orifice and to establish working length (3). It was decreased success endodontic treatment rate of the PCO in teeth with a preoperative lesion, as well as due to technical failures during calcified canal negotiation (3). The pulp chamber and coronal part of the canal are usually affected by partial PCO due to the corona-apical direction of obliteration, while the radicular part remains visible, albeit markedly narrowed (6). The vast majority of the root canals could be located and treated, regardless of the extent of canal obliteration visible on the radiograph (7).

The gray discoloration of the tooth crown in the traumatized teeth can be explained by the diffusion of blood disintegration products into dentinal tubules. Pulp breakdown by-products, derived from intrapulpal bleeding, as well as necrotic tissue remnants, may penetrate into tooth substance resulting in coronal discoloration (6). The tooth required post-endodontic intra-coronal bleaching to improve the appearance, and the walking bleach technique was the treatment of the choice. After three walking bleach treatments at a 1-week interval, the tooth color appeared stable. However, the clinician noticed the color was not similar to that of adjacent teeth. The patient was asked whether he was satisfied with the discoloration reversed. Whereas the patient had no complaints, the treatment was completed.

It is well known that a delayed release of the residual oxygen derived from the whitening agent affects the polymerization of resin-based materials (10). Therefore, the final restoration was placed one week after the removal of the bleaching gel.

From this case, it was considered that the patient with dental trauma must undergo a detailed examination along with regular monitoring at subsequent visits for post-traumatic complications. Providing patients with sufficient information on the importance of follow-up examinations after immediate management of traumatic injuries is essential. The success of the esthetic treatment by professional parameters may differ from subjective evaluation by the patient.

Conclusion

This case report presents a successful endodontic intervention for a permanently traumatized central incisor with advanced external apical root resorption, apical periodontitis and pulp obliteration.

The preservation of the tooth, despite having a shortened root and an extremely narrowed endodontic space, was accomplished. The successful outcome was attributed to the utilization of ultrasonic tips and rotary endodontic files, in conjunction with the application of calcium hydroxide paste and non-vital tooth bleaching. Regular clinical and radiographic assessments exhibited evidence of healing in the periapical lesion.

This case underscores the complexities confronted by dental practitioners when managing delayed complications resulting from dental trauma, thereby emphasizing the essentiality of meticulous diagnosis and treatment in such scenarios.

Declaration of Interest: Authors declare NO conflict of interest.

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ENDODONTIC MANAGEMENT OF MANDIBULAR THIRD MOLAR WITH RADIX ENTOMOLARIS

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ABSTRACT

This article provides two case reports representing endodontic management of mandibular third molar with radix entomolaris. Radix entomolaris is a supernumerary root located disto-lingually in mandibular molars. The endodontic treatment (ET) involves the removal of pulpal and necrotic tissue, preparing the root canals for sealing with inert filling material. The operator must have an in-depth knowledge of the morphology of root canal system and its variations that may affect the outcome and the success of the treatment. This study presents two cases of endodontic management of mandibular molar with radix entomolaris with a clinical diagnosis of irreversible pulpitis of one case and symptomatic apical periodontitis of another case. RC preparation in this case was performed using a new generation of rotary files with crown down technique. After following the standard irrigation protocol, the RCs were obturated using the warm vertical condensation technique. Knowledge of RC system variations, with their preparation and 3D obturation, is the appropriate qualification for ET.

Keywords: root canal, radix entomolaris, mandibular molar, crown down, vertical condensation, endodontic treatment.

Introduction

Mandibular molars usually present with two roots having two mesial and one distal canal. [1] One of the major variations in mandibular molars is the occurrence of this root presented lingually or buccally. Radix entomolaris was first described in 1844 by Carabelli as a supernumerary root located disto-lingually in mandibular molars. [2] Radix entomolaris is mostly shorter and curved compared to mesial and distal roots and can appear separately. The highest occurrence of radix entomolaris (RE) was found among the Mongolian race, including Chinese, Taiwanese and Koreans. In African, European and Asian demographics, the frequency ranges from 3% to 5%v, while in the Mongoloid populations it can reach a frequency of 40%. [3] The endodontic treatment involves the removal of pulpal and necrotic tissue, preparing the root canals with proper irrigation and sealing the prepared canals with inert filling material. The location of all root canals (RCs) and their adequate preparation, disinfection and obturation play an important role in successful endodontic treatment (ET) [4]. The operator must have an in-depth knowledge of the morphology of root canal system and its variations that may affect the outcome and the success of the treatment. Understanding the anatomical variations of root canal system is a must to achieve the main goals of the treatment. [5]

The root canal system is highly complex and its mandatory for the clinician to have thorough knowledge of varied types of root canal anatomies. The main aim of endodontic treatment is the elimination of microorganism from infected tissue from entire root canal system which is mainly achieved by thorough cleaning and shaping of root canals followed by tri-dimensional obturation with a coronal fluid tight seal. [6] The endodontic treatment needs an adequate chemical-mechanical preparation procedures and especially irrigation plays a crucial role in achieving effective disinfection and debris removal within the complex root canal anatomy. [7] An appreciate diagnosis, treatment plan, correct, debridement, disinfection and root canal system obturation are needed for successful treatment. [8] The root canal preparation must be done without procedural errors while respecting the root canals working length (WL) and maintaining its natural path. [9] This can be achieved with the correct selection of instruments in terms of their size and design. [10] Also, for a successful RC treatment the operator must have knowledge of internal RC morphology to locate all RCs and properly clean, shape and obturate RC space in three dimensions. [11] The radix entomolaris presents the major challenge to the clinician during the endodontic treatments. Despite all of this knowledge, RC preparation and obturation is still challenging due to the variety and complexity of RCs caused by different genetics, ethnicity, gender and age, as well as the existence of lateral or accessory canals and isthmus and curvatures.

Case Presentation

Case 1 (Figures 1 and 2):

A 35-year-old male patient came to the Private Dentistry Clinic "Dr. Berisha" in Pejë, Kosovo describing that he had a tooth ache, followed by a sharp pain upon thermal stimulus, with lingering pain, spontaneity and the referred pain frequency increasing during night. Percussion, palpation and thermal vitality test was negative. The treatment began after administering inferior alveolar nerve block anesthetic Septanest 1:100.000 (4% Articain hydrochloride with 1:100000 Epinephrine), (Septodont, Saint-Maur-des-Fosses Cedex, France) using Carpo syringe. The retro-alveolar radiography revealed an intact periapical tissue with radix entomolaris root canal variations. Tooth 38, lower left third molar, was with old amalgam filling and secondary caries.

The diagnosis of this tooth was symptomatic irreversible pulpitis (SIP).

Before the treatment, the patient was informed that his clinical information and radiographic images may be reported in the journal. So, the authors certify that they have obtained patient consent and the patient was informed that his name and initials will not be published.

Endodontic treatment was performed with a rubber dam.

After removing an old amalgam filling and caries, the access to the cavity was prepared and five root canal orifices were localized. Loupes with 3.0 magnification (Univet Loupes Spa, Rezzato, Italy) were used for canal localization. The root canals were explored with size 06, 08, and 10 K-files (Diadent, France). The patency was determined using k-file

size 06 and 08. Working length was set at 1 mm from the apical foramen with k-file size 10. The pulp tissue was removed, and the root canals were prepared with E-flex gold rotary files (Eighteeth, District, Changzhou City China) using crown down technique. During shaping and cleaning, the operators used 17% EDTA gel (Cerkamed, Stalowa Wola, Poland). After opening the pathway with hand files 06, 08 K-Files (Diadent, France) in these cases with curvature in middle third of mesial canal it is essential to continue with small k-file 06, 08 without applying apical pressure. When we notice the resistance at the tip of the file, we should not apply apical pressure. We go back cutting at the exit making a pull movement. We must repeat this process with k-file 06, 08 until reaching the working length and confirm it with apex locator. The operators used E-flex gold rotary file size 19/02 followed by size 20/04. The file should be inserted in the canal, connected to endomotor, but without rotation. After it reaches beyond the curvature, the rotation should be activated. Then, the shaping phase was finalized using E-flex gold rotary files size 25/04. The canals orifice was expanded with E-flex gold rotary file size 17/08 files and finished with E-flex gold rotary files size 25/04. Apical enlargement was performed with an E-flex gold rotary file size up to 25/04. During scouting, pre-flaring, glide-path and shaping the files must always be carefully observed to note if the flutes are intact or have been damaged due to stress. During the endodontic treatment, the operators used the endodontic hand piece E connect S (Eighteeth, District, Changzhou City, China) at a rotation 350 rpm, and torque 2.5 Ncm introducing the instrument passively into the root canal. The root canals were irrigated following Marcus Haapasalo protocol [12] and using Irriflex - flexible root canal irrigation needle (Produits Dentaires SA Vevey, Switzerland). During shaping and cleaning of each canal, the operators performed irrigation of 5.25 % sodium hypochlorite (Cerkamed, Stalowa Wola, Poland) and 17% EDTA (Cerkamed, Stalowa Wola, Poland) in each canal activated with ultrasonic device Ultra X. (Eighteet, District, Changzhou City, China). After shaping and cleaning, the operators continued with irrigation. The drying was performed with paper points. The root canal working length was verified with radiography followed by obturation of root canals with vertical gutta- percha condensation using Fast Pack and Fast Fill (Eighteeth, District, Changzhou City, China). Gutta-percha size was 25/04 (Diadent, France) in combination with Sealapex sealer (Kerr Corporation, Orange, CA, USA). Endodontic treatment was completed in one visit.

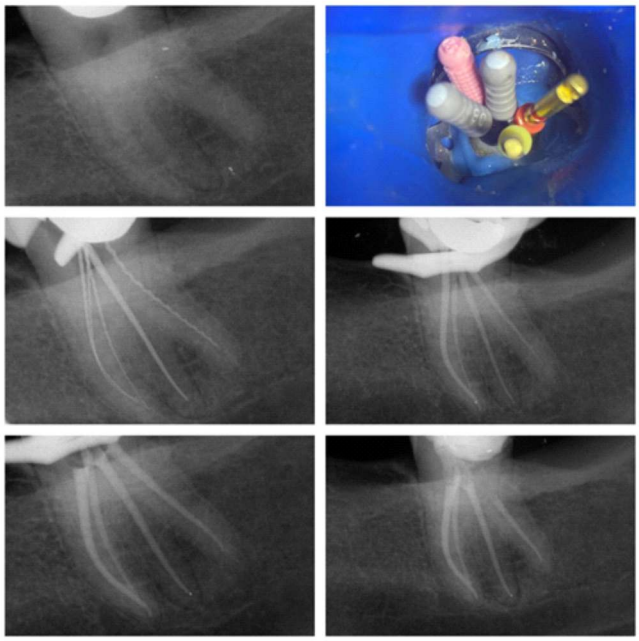

Figure 1. A. First diagnostic radiography; B. Working length determination under rubber dam; C. Verification of working length with rotary file; D. Verification of working length with gutta –percha; E. Radiographic image after obturation; F. Radiographic image after obturation without rubber dam.

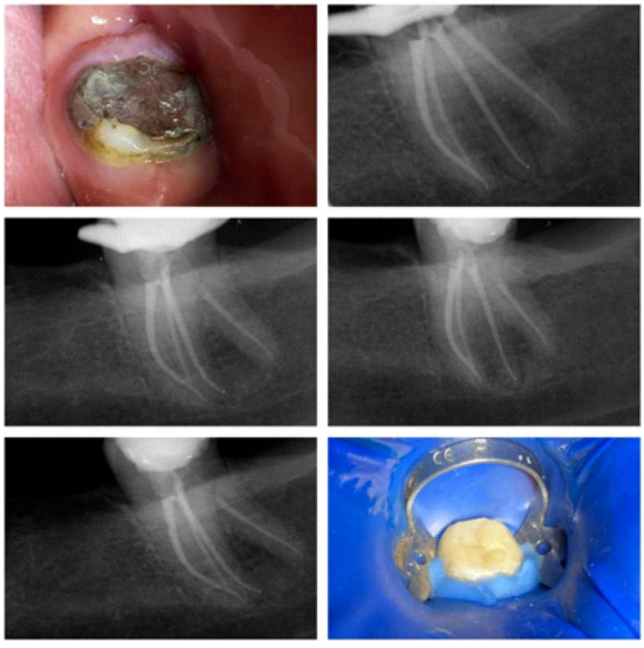

Figure 2. A. First diagnostic clinical photo from intra oral camera; B. Radiographic image after obturation; C. Radiographic image after obturation and fiber post under rubber dam; D. Radiographic image after obturation and fiber post image without rubber dam; E. Radiographic image after obturation and fiber post image without rubber dam from another view; F. Clinical photo with intra oral camera of composite filling.

Case Presentation

Case 2 (Figures 3,4,5):

A 23-year-old female patient came to the Private Dentistry Clinic "Dr. Berisha" in Pejë, Kosovo, describing that she had a tooth ache, followed by a painful response to biting and percussion. Percussion, palpation and thermal vitality test was positive. The treatment began after administering inferior alveolar nerve block anesthetic Septanest 1:100.000 (4% Articain hydrochloride with 1:100000 Epinephrine), (Septodont, Saint-Maur-des-Fosses Cedex, France) using Carpo syringe. The retro-alveolar radiography revealed a radiolucency of distal and mesial root of periapical tissue. Tooth 48, lower left third molar, was with old composite filling.

The diagnosis of this tooth was symptomatic apical periodontitis (SAP) with previously treated pulp.

Before the treatment, the patient was informed that her clinical information and radiographic images may be reported in the journal. So, the authors certify that they have obtained patient consent and the patient was informed that her name and initials will not be published.

Endodontic treatment was performed with a rubber dam.

After removing an old composite filling, a pulp residue was found in the cavity. After removing the pulp residue and the old root canal filling, the access to six root canal orifices were localized in the cavity. Loupes with 3.0 magnification (Univet Loupes Spa,Rezzato, Italy) were used for canal localization. The root canals were explored with size 10, 15 K-files (Diadent, France). Operator used eucalyptus oil (Cerkamed, Stalowa Wola, Poland) to disvolve the old gutta-percha. The patency was determined using k-file size 10 and 15. Working length was set at 1 mm from the apical foramen with k-file size 15. The residual pulp tissue was removed and old filling of the root canal and the root canals were prepared with E-flex gold rotary files (Eighteeth, District, Changzhou City China) using crown down technique. During shaping and cleaning, the operators used 17% EDTA gel (Cerkamed, Stalowa Wola, Poland). After opening the pathway with hand files 10, 15 K-Files (Diadent, France) in similar cases with curvature in apical third of distal canal, it is essential to continue with small k-file 10, 15 without applying apical pressure. When we notice the resistance at the tip of the file, we should not apply apical pressure. We go back cutting at the exit making a pull movement. We must repeat this process with k-file 15 until reaching the working length and confirmed with apex locator. The operators used E-flex gold rotary file size 19/02 followed by size 20/04. The file should be inserted in the canal, connected to endomotor, but without rotation. After it reaches beyond the curvature, the rotation should be activated. Then, the shaping phase was finalized using E-flex gold rotary files size 25/04. The canals orifice was expanded with E-flex gold rotary file size 17/08 files and finished with E-flex gold rotary files size 25/04. Apical enlargement was performed with an E-flex gold rotary file size up to 25/04. During scouting, pre-flaring, glide-path and shaping the files must always be carefully observed to note if the flutes are intact or have been damaged due to stress. During the endodontic treatment, the operators used the endodontic handpiece E connect S (Eighteeth, District, Changzhou City, China) at a rotation 350 rpm, and torque 2.5 Ncm introducing the instrument passively into the root canal. The root canals were irrigated following Marcus Haapasalo protocol 9 and using Irriflex - flexible root canal irrigation needle (Produits Dentaires SA Vevey, Switzerland). During shaping and cleaning of each canal, the operators performed irrigation of 5.25 % sodium hypochlorite (Cerkamed, Stalowa Wola, Poland) and 17% EDTA (Cerkamed, Stalowa Wola, Poland) in each canal activated with ultrasonic device Ultra X (Eighteet, District, Changzhou City, China). After shaping and cleaning, the operators continued with irrigation. The drying was performed with paper points. The root canal working length was verified with radiography followed by obturation of root canals with vertical gutta- percha condensation using Fast Pack and Fast Fill (Eighteeth, District, Changzhou City, China). Gutta-percha size was 25/04 (Diadent, France) in combination with Sealapex sealer (Kerr Corporation, Orange, CA, USA) were used. Endodontic retreatment was completed in one visit.

Figure 3. A. First diagnostic radiography; B. Working length determination under rubber dam with k-files; C. Verification of working length with gutta-percha; D. Radiographic image after obturation under rubber dam; E. Radiographic image from after obturation; F. Radiographic image from another view after obturation.

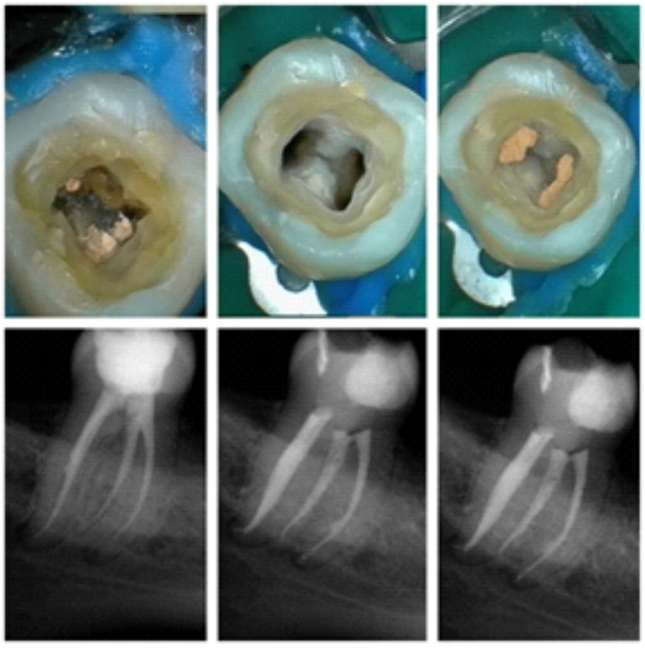

Figure 4. A. Photo from intraoral camera after removing the old composite shows us a pulp residue and old root canal filling; B. Photo from intraoral camera after cleaning and shaping; C. Photo from intraoral camera after obturation; D. First diagnostic radiography; E. Radiographic image after endodontic retreatment; F. Radiographic image from another angle after endodontic retreatment.

Figure 5. A. Photo from intraoral camera after removing the old composite shows us a pulp residue and old root canal filling; B. Photo from intraoral camera after cleaning and shaping; C. Photo from intraoral camera after obturation; D. D. Working length determination under rubber dam; E. Radiographic image after endodontic retreatment; F. Radiographic image from another angle after endodontic retreatment.

Discussion

The thorough knowledge of root canal anatomy is important for successful endodontic treatment. Variations in the root configurations can impose problems during endodontic treatments. The location of the canal orificiums may be difficult because of overhanging dentine. According to literature, the majority of radix entomolaris are curved. Possible variations in root canal anatomy need to be understood properly in order to prevent endodontic treatment failures. During the endodontic treatments, the use of magnification, such a dental loupes or microscope are useful in canal orifice identification. Radix entomolaris have from moderate to sharp curvature in buccolingual direction which causes technical difficulties during cleaning and shaping and has higher chances for instrument separation, furcal perforation, ledge formation, loss of working length, root canal transportation canal blockage. [13] A clinically

relevant method for cleaning, shaping and obturating a severe curvature root canal system has been presented utilizing a new generation of E-flex gold rotary file.

Conclusion

The clinical diagnosis of a radix entomolaris before root canal treatment is important to facilitate the endodontic procedure and to avoid missed canals. An endodontic treatment of lower third molar with radix entomolaris may be challenging. Radix entomolaris can be easily diagnosed by a careful evaluation of pre-operative radiographs when taken at different angulation. Thorough knowledge regarding the internal anatomy of root canal system and its most common variations of the teeth that are going to be treated, is essential to successfully finishing an endodontic treatment. The factors determining the success in negotiating the canals depends on the size and construction of the canal, degree of curvature, size and flexibility of file, along with the skills of operator.

Competing interests.

The author declare that they have no competing interests.

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RELATIONSHIP BETWEEN CHRONIC PERIODONTITIS AND VITAMIN D DEFICIENCY

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ABSTRACT

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms resulting in the progressive destruction of the periodontium caused by the inflammatory response of the host. Vitamin D plays an essential role in bone maintenance and immunity, and it is thought that vitamin D deficiency could negatively affect periodontal health. Previous research on vitamin D and tooth loss has been limited. The paper aims to present a possible connection between vitamin D deficiency and periodontal disease through a case report. In conclusion, it is stated that there is still conflicting evidence regarding the effects of 25(OH)D on the severity, progression and loss of teeth during periodontal disease. Limited evidence also supports a positive association between 1,25(OH)2D and periodontal health, as well as a trend towards better periodontal health with vitamin D supplementation being in agreement with our findings.

Keywords: periodontal disease, vitamin D, alveolar bone resorption, vitamin D supplementation

Introduction

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms resulting in progressive destruction of the periodontium caused by the inflammatory response of the host (1). Vitamin D plays an essential role in bone maintenance and immunity, and it is thought that vitamin D deficiency could negatively affect periodontal health. It has been hypothesized that vitamin D status could change the risk of periodontal disease (2,3,4).

In addition to its role in calcium homeostasis, the biologically active form of vitamin D, 1,25(OH)2D, has proven to be a powerful immunomodulator due to its anti-inflammatory effect through the inhibition of cytokine production in immune cells. It also stimulates monocytes or macrophages to secrete peptides with strong antibiotic activity. These are the reasons why they can be useful for the treatment of periodontal diseases. The diagnosis of vitamin D deficiency is made by analyzing the level of 25(OH)D in the serum. Normal range of serum 25(OH) D is 20–74ng/ml (5).

Previous research on vitamin D and tooth loss has been limited. Recent studies examining this relationship suggest that adequate vitamin D status may prevent tooth loss. Vitamin D is thought to reduce the risk of tooth loss through its effects on bone health, inflammation and the immune response (6).

The paper aims to present a possible connection between vitamin D deficiency and periodontal disease through a case report.

Case report

Patient S.Ć. 45 years old, came to the Clinic for Oral Medicine and Periodontology on the recommendation of her dentist. It is known from the medical history that she has a low level of vitamin D, but other etiological factors are not mentioned. Oral hygiene was satisfactory. Clinical examination revealed bleeding on probing and the presence of true periodontal pockets up to 4 mm deep, while the Plaque index was 0, as well as the Calculus Index.

After the clinical examination, an orthopantomogram was taken, where the loss of the alveolar bone is observed, according to the type of horizontal resorption indicating chronic periodontitis (figure 1.).

Figure 1. Orthopantomogram of the patient

Given that the patient is systemically healthy and without hormonal imbalance, after the clinical examination, X-ray analysis and vitamin D laboratory findings, periodontal therapy - subgingival curettage was recommended. The therapy is carried out under local anesthesia in all four quadrants. The patient is advised to supplement with 2000 IJ of Vitamin D once a day for 6 months.

At the follow-up examination 3 months after the periodontal treatment, the probing depth was reduced to no more than 2 mm, and the vitamin D level was within the normal range. A regular periodontological control examination in 3 months is suggested, as well as a vitamin D control.

Discussion

It is believed that vitamin D can influence the development of periodontal disease, caries and tooth loss. Vitamin D's essential role in calcium homeostasis, along with its anti-inflammatory and antimicrobial properties, may protect alveolar bone loss and subsequent tooth loss (7,8,9).

Searching through the literature, we did not find significant well-controlled clinical trials of the relationship between vitamin D, calcium and periodontitis where the periodontal disease status of patients was verified and monitored with parameters of vitamin D and calcium values over time (10).

The conclusion of the study conducted by Pavlesen S et al. in a sample of postmenopausal women shows that their data do not support an association between vitamin D status and tooth loss.

No statistically significant association was observed between the reduced value of 25(OH)D vitamin and the frequency of tooth loss due to periodontal disease being in agreement with our report (11).

A study by Antonoglou et al. included 55 subjects with chronic periodontitis and 30 periodontally healthy subjects whose serum levels of 25(OH)D, 1,25(OH)2D, C-reactive protein and high-density lipoprotein cholesterol were determined. The association between vitamin D and the periodontal condition of the study patients was determined using logistic regression analysis. The results showed that a low level of 1,25(OH)2 D in the serum was associated with periodontitis complying with previous knowledge regarding the relationship between the level of 1,25(OH)2 D in the serum and other inflammatory diseases. The authors emphasize that there is a dilemma as to whether this association is causal and it remains to be confirmed in future studies (12).

Conclusion

The available studies state that there is still conflicting evidence on the effects of 25(OH)D on the severity, progression and loss of teeth during periodontal disease. Some studies report beneficial effects of higher serum 25(OH)D concentrations on periodontal health and tooth retention, while others could not find such association. Limited evidence also supports a positive relationship between 1,25(OH)2D and periodontal health, as well as a trend towards better periodontal health with vitamin D supplementation being consistent with our findings.

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- Include a brief and self-explanatory title with explanations essential to the understanding of the table at the bottom of the table.
- Identify statistical measures of variations, such as standard deviation and standard error of the mean or other where appropriate.

Figures

- Provide each figure on a separate page of the manuscript after the references. Number the figures according to their sequence in the text. The text should include references to all figures.
- Graphs and figures should be in black/white or greyscale format. Colour illustrations are acceptable but not guaranteed. Minimal quality 300dpi, figures should be filed in suitable format (*.JPG, *.PNG, .*TIFF)
- If figures are not original provide source and permition.
- All figures should be 100% of a suitable final size and have the printing resolution of 300dpi and be cropped to include the figure only (no blank space).

Units of measurement

Measurements of length, height, weight, and volume should be reported in metric units (meter, kilogram, or liter) or their decimal multiples. All hematologic and clinical chemistry measurements should be reported in the metric system in terms of the International System of Units (SI).

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