



MACEDONIAN DENTAL REVIEW

I S S N | 4 45
2545-4757 | 2022

Macedonian Dental Review is publishing by the Faculty of Dentistry, University „Ss. Cyril and Methodius“,
Skopje, Republic of North Macedonia and Macedonian Dental Society

<http://stomfak.ukim.edu.mk/msp/>

Cherubism - A case report. <i>Bolis-Gjurikj T., Bogdanovska B., Dimovska R., Bajram M.</i>	115
Microbiologic evaluation of 2 methods of archwire ligation. <i>Cana A., Bajraktarova Mishevskaja C., Mitikj K., Alimani Jakupi J., Polozani G., Simonovikj M., Emini A.</i>	122
Hygienic - dietary regimen and occurrence of dental caries in 10-year-old children in the area of Bitola Municipality. <i>Milevska E., Milevski M., Gjorgievska Jovanovska S., Pachanoska V., Gjorgievska E.</i>	127
Expression of IL6 in periodontal affected sites with different attachment lost. <i>Pandilova M., Trajkov D., Georgieva L., Ugrinska A.</i>	135
Defining the comprehension level of the teaching staff in primary schools regarding first aid procedures for dental trauma. <i>Talimdzioska K., Sotirovska-Ivkovska A., Najdenoska-Bojchinoska S., Arsova Apostolovska M., Alili Sh.</i>	139
Evaluation of the efficiency of botox therapy in a patient with unilateral idiopathic neuralgia on the trigeminal nerve. <i>Shushak Z., Popovski V., Mitikj J., Baftijari D.</i>	147

Macedonian Dental Review is publishing by the Faculty of Dentistry, University „Ss. Cyril and Methodius“, Skopje, Republic of North Macedonia and Macedonian dental society.

Editor in chief - Prof. **Kjiro Ivanovski**, PhD, e-mail: kiroivanovski@stomfak.ukim.edu.mk

Associate editor - Prof. **Elizabeta Gjeorgievska** PhD, Department of Pediatric and Preventive Dentistry, e-mail: egjorgievska@stomfak.ukim.edu.mk

Secretary - **Vlatko Kokalanski**, PhD, e-mail: vkokolanski@stomfak.ukim.edu.mk

Adress - Macedonian Dental Review, str. Majka Tereza br. 43 Skopje, Republic of North Macedonia, <http://stomfak.ukim.edu.mk/msp/>

Editorial Board

Mira Jankulovska, Skopje, North Macedonia
 Sonja Apostolska, Skopje, North Macedonia
 Silvana Georgieva, Skopje, North Macedonia
 Aleksandar Grchev, Skopje, North Macedonia
 Lidija Kanurkova, Skopje, North Macedonia
 Maja Pandilova, Skopje, North Macedonia
 Vesna Stevkovska, Skopje, North Macedonia
 Boris Velichkovski, Skopje, North Macedonia
 Vasilka Rendzova, Skopje, North Macedonia
 Ivan Alajbeg, Zagreb, Croatia
 Ivan Anastasov, Sophia, Bulgaria
 Nikola Angelov, Houston, USA
 Gurhan Caglayan, Hacettepe, Turkey
 Domagoj Glavina, Zagreb, Croatia
 Peter Jevnikar, Ljubljana, Slovenia
 Hristo Kisov, Plovdiv, Bulgaria
 Darko Macan, Zagreb, Croatia
 Ljubo Marion, Ljubljana, Slovenia
 John Nicholson, London, UK
 Darije Planchak, Zagreb, Croatia
 Kristina Popova, Sophia, Bulgaria
 Sasha Stankovikj, Nish, Serbia
 Dragoslav Stamenkovikj, Belgrade, Serbia
 Zrinka Tarle, Zagreb, Croatia
 Ljiljana Tihachek, Belgrade, Serbia
 Georgi Tomov, Plovdiv, Bulgaria
 Radomir Ugrinov, Sophia, Bulgaria
 Ana Angelova-Volponi, London, UK
 Vaska Vandevska, Oslo, Norway
 Radosveta Vasileva, Sophia, Bulgaria
 Miroslav Vukadinovikj, Belgrade, Serbia
 Nikola Petrichevikj, Zagreb, Croatia

Advisory board

Alberto Benedeti, Skopje, North Macedonia
 Aleksandar Dimkov, Skopje, North Macedonia
 Ana Sotirovska Ivkovska, Skopje, North Macedonia
 Aneta Atanasovska Stojanovska, Skopje, North Macedonia
 Antonio Kirkov, Skopje, North Macedonia

Biljana Kapusevska, Skopje, North Macedonia
 Biljana Dzipunova, Skopje, North Macedonia
 Vera Radojkova Nikolovska, Skopje, North Macedonia
 Vladimir Popovski, Skopje, North Macedonia
 Gabriela Kurchieva Cuckova, Skopje, North Macedonia
 Goran Pancevski, Skopje, North Macedonia
 Gordana Kovacevska, Skopje, North Macedonia
 Daniela Veleska Stevkoska, Skopje, North Macedonia
 Danica Popovikj Monevska, Skopje, North Macedonia
 Evdokija Jankulovska, Skopje, North Macedonia
 Edvard Janev, Skopje, North Macedonia
 Emilija Bajraktarova Valjakova, Skopje, North Macedonia
 Emilija Stefanovska, Skopje, North Macedonia
 Zlatko Georgiev, Skopje, North Macedonia
 Ilijana Muratovska, Skopje, North Macedonia
 Jadranka Bundevska Josifovska, Skopje, North Macedonia
 Julijana Nikolovska, Skopje, North Macedonia
 Katarina Dirjanska, Skopje, North Macedonia
 Kristina Mitikj, Skopje, North Macedonia
 Lidija Popovska, Skopje, North Macedonia
 Ljuben Guguvcevski, Skopje, North Macedonia
 Marija Peeva Petreska, Skopje, North Macedonia
 Marija Stevanovikj, Skopje, North Macedonia
 Marina Eftimoska, Skopje, North Macedonia
 Marina Kacarska, Skopje, North Macedonia
 Meri Pavleska, Skopje, North Macedonia
 Mira Jankulovska, Skopje, North Macedonia
 Mirjana Popovska, Skopje, North Macedonia
 Natasa Toseska Spasova, Skopje, North Macedonia
 Nikola Gigovski, Skopje, North Macedonia
 Oliver Dimitrovski, Skopje, North Macedonia
 Sanja Pancevska, Skopje, North Macedonia
 Saso Elencevski, Skopje, North Macedonia
 Snezana Pesevska, Skopje, North Macedonia
 Stevica Ristoska, Skopje, North Macedonia
 Suzana Dvojakovska, Skopje, North Macedonia
 Cvetanka Bajraktarova Mishevskaja, Skopje, North Macedonia

Употреба на Carriere Motion III апарат. <i>Болис-Ѓуриќ Т., Боѓдановска Б., Димовска Р., Бајрам М.</i>	115
Микробиолошка евалуација на 2 метода на лигирање на ортодонтските брекетети. <i>Цана А., Бајраќиќарова Мишевска Ц., Мийќиќ К., Алимани Јакуќи Ј., Положани Г., Симоновиќ М., Емини А.</i>	122
Хигиено-диететски режим и појавата на дентален карлес кај деца од 10 годишна возраст на подрачјето на општина Битола. <i>Милевска Е., Милевски М., Ѓорѓиевска Јовановска С., Пачаноска В., Ѓорѓиевска Е.</i>	127
Експресија на IL6 во регии со различно изразен губиток на припој. <i>Пандилова М., Трајков Д., Георѓиева Л., Уѓринска А.</i>	135
Одредување на степенот на познавање на постапките при давање прва помош кај дентални траума на наставниот кадар во основни училишта. <i>Талимџиоска К., Соќировска-Ивковска А., Најденоска-Бојчиноска С., Арсова Ајосќоловска М., Алили Сх.</i>	139
Евалуација на ефикасноста на ботокс терапијата кај пациент со идиопатска, унилатерална тригеминална неуралгија која ги напаѓа трите дивизии на тригеминалниот нерв. <i>Шушак З., Појовски В., Мийќиќ Ј., Бафќиќари Д.</i>	147

Македонски стоматолошки преглед го издава Стоматолошкиот факултет при Универзитетот „Св. Кирил и Методиј“ Скопје, Република Северна Македонија и Македонското стоматолошко друштво.

Одговорен уредник: Проф. д-р **Ќиро Ивановски**, e-mail: kiroivanovski@stomfak.ukim.edu.mk
 Заменик одговорен уредник: Проф. д-р **Елизабета Ѓорѓиевска**, e-mail: egjorgievska@stomfak.ukim.edu.mk
 Секретар на списанието: д-р **Влатко Коколански**, e-mail: vkokolanski@stomfak.ukim.edu.mk

Адреса - Македонски стоматолошки преглед, ул. Мајка Тереза бр. 43 Скопје, Република Северна Македонија
<http://stomfak.ukim.edu.mk/msp/>

Уредувачки одбор

Мира Јанкуловска, Скопје, Северна Македонија
 Соња Апостолска, Скопје, Северна Македонија
 Силвана Георѓиева, Скопје, Северна Македонија
 Александар Ѓрчев, Скопје, Северна Македонија
 Лидија Кануркова, Скопје, Северна Македонија
 Маја Пандилова, Скопје, Северна Македонија
 Весна Стевковска, Скопје, Северна Македонија
 Борис Величковиќ, Скопје, Северна Македонија
 Василка Ренцова, Скопје, Северна Македонија
 Иван Алаќбеќ, Загреб, Хрватска
 Иван Анастасов, Софија, Бугарија
 Никола Ангелов, Хјустон, САД
 Гурхан Цаглајан, Хачетене, Турција
 Домаѓој Главина, Загреб, Хрватска
 Петер Јевниќар, Љубљана, Словенија
 Христо Кисов, Пловдив, Бугарија
 Дарко Мачан, Загреб, Хрватска
 Љубо Марион, Љубљана, Словенија
 Џон Николсон, Лондон, В. Британија
 Дарије Планчак, Загреб, Хрватска
 Кристина Попова, Софија, Бугарија
 Саша Станковиќ, Ниш, Србија
 Драгослав Стаменковиќ, Белград, Србија
 Зринка Тарле, Загреб, Хрватска
 Љљана Тихачек, Белград, Србија
 Георѓи Томов, Пловдив, Бугарија
 Радомир Угринов, Софија, Бугарија
 Ана Ангелова Валпони, Лондон, В. Британија
 Васка Вандевска, Осло, Норвешка
 Радосвета Василева, Софија, Бугарија
 Мирослав Вукадиновиќ, Белград, Србија
 Никола Петричевиќ, Загреб, Хрватска

Советодавен одбор

Алберто Бенедети, Скопје, Северна Македонија
 Александар Димков, Скопје, Северна Македонија
 Ана Сотировска Ивковска, Скопје, Северна Македонија

Анета Атанасовска Стојановска, Скопје, Северна Македонија
 Антонио Ќирков, Скопје, Северна Македонија
 Билјана Капушевска, Скопје, Северна Македонија
 Билјана Цицунова, Скопје, Северна Македонија
 Вера Радојќова Николовска, Скопје, Северна Македонија
 Владимир Поповски, Скопје, Северна Македонија
 Габриела Курчиева Чучкова, Скопје, Северна Македонија
 Горан Панчевски, Скопје, Северна Македонија
 Гордана Ковачевска, Скопје, Северна Македонија
 Даниела Велеска Стевковска, Скопје, Северна Македонија
 Даница Поповиќ Моневска, Скопје, Северна Македонија
 Евдокија Јанкуловска, Скопје, Северна Македонија
 Едвард Јанев, Скопје, Северна Македонија
 Емилија Бајраќтарова Ваљакова, Скопје, Северна Македонија
 Емилија Стефановска, Скопје, Северна Македонија
 Златко Георѓиев, Скопје, Северна Македонија
 Илијана Муратовска, Скопје, Северна Македонија
 Јадранка Бундевска Јосифовска, Скопје, Северна Македонија
 Јулијана Николовска, Скопје, Северна Македонија
 Катарина Дирјанска, Скопје, Северна Македонија
 Кристина Митиќ, Скопје, Северна Македонија
 Лидија Поповска, Скопје, Северна Македонија
 Љубен Гугувчевски, Скопје, Северна Македонија
 Марија Пеева Петреска, Скопје, Северна Македонија
 Марија Стевановиќ, Скопје, Северна Македонија
 Марина Ефтимоска, Скопје, Северна Македонија
 Марина Кацарска, Скопје, Северна Македонија
 Мери Павлеска, Скопје, Северна Македонија
 Мира Јанкуловска, Скопје, Северна Македонија
 Мирјана Поповска, Скопје, Северна Македонија
 Наташа Тошеска Спасова, Скопје, Северна Македонија
 Никола Гиговски, Скопје, Северна Македонија
 Оливер Димитровски, Скопје, Северна Македонија
 Сања Панчевска, Скопје, Северна Македонија
 Сашо Еленчевски, Скопје, Северна Македонија
 Снежана Пешевска, Скопје, Северна Македонија
 Стевица Ристоска, Скопје, Северна Македонија
 Сузана Двојакоска Божовиќ, Скопје, Северна Македонија
 Цветанка Бајраќтарова Мишевска, Скопје, Северна Македонија

CHERUBISM- A CASE REPORT

УПОТРЕБА НА CARRIERE MOTION III АППАРАТ

Bolis-Gjurikj T.¹, Bogdanovska B.², Dimovska R.³, Bajram M.⁴

¹Private Dental Office „Luka dent“ - Skopje, ²Faculty of dental medicine, University „St. Cyril and Methodius“-Skopje, ³Private Dental Polyclinic „Kruna MS“-Skopje, ⁴Private Dental Office „Dental Designe“

Abstract

Introduction: Cherubism is a rare hereditary benign fibro-osseous disorder characterized by bilateral enlargement of the mandible and/or the maxilla, with varying degrees of involvement and a tendency for spontaneous remission. On radiographs, cherubic lesions appear as cystic multilocular radiolucencies that limited to the jaw bones. **Case report:** A 4-year-old girl came to our dental office with deformations of the lower and middle section of her face and missing teeth. After taking her history, a panoramic radiograph and a computed tomography revealed missing teeth and caries in her remaining teeth. Based on the cystic formations and overall appearance, the patient was suspected of having Cherubism and was referred to the University Clinic for Face, Jaws and Neck Surgery - Maxillofacial surgery Clinic in Skopje for a confirmatory diagnosis. **Conclusions:** The radiologic features of cherubism are not pathognomonic, but the bilateral, relatively symmetrical jaw involvement limited to the jaw bones, combined with clinical and histopathological findings, strongly suggest the diagnosis. It should be noted that genotypic characterization confirms the diagnosis. **Key words:** cherubism, clinical signs, diagnosis, hereditary syndrome.

Апстракт

Цел: Черубизмот е невообичаено наследно бенигно фибро-коскено нарушување кое се карактеризира со билатерално проширување на мандибулата и максилата што се манифестира со различни степени на зафатеност и склоност кон спонтанa ремисија. На ртг, черубичните лезии се појавуваат како цистична мултилокуларна радиолуценција ограничена на коските на вилицата. **Приказ на случај:** 4-годишно девојче прв пат дојде во ЛУКА ДЕНТ поради деформација на долниот и средниот дел на лицето и отсуство на заби. Панорамската снимка и компјутерската томографија открија отсуство на заби и кариес лезии на постојаните заби. Поради формирањето на цисти и целокупниот изглед, пациентката беше суспектна на черубизам, и беше препратена на Универзитетската клиника за максилофацијална клиника во Скопје каде е потврдена дијагнозата черубизам. **Заклучок:** Радиолошките карактеристики на черубизмот не се патогномонични, но дијагнозата е силно сугерирана со билатерално релативно симетрично зафаќање на вилицата што е ограничено на коските на вилицата и, заедно со клиничките и хистопатолошките наоди, го потврдува черубизам. Генотипската карактеристика ја потврдува дијагнозата. **Клучни зборови:** Черубизам, фибро-коскено нарушување, вилици.

Introduction

Cherubism is a skeletal dysplasia characterized by the development of bilateral symmetrical fibro-osseous lesions on the mandible and/or maxilla. It is described for the first time in 1933 by William A. Jones⁷⁻¹¹.

The name of cherubism is correlated with the specific facial appearance of the patients: rounder cheeks and upper eyelids, giving them so-called „angelic appearance“^{4,2,3}. It is one of the rarest disorders in medicine with approximately 350 documented instances worldwide. Due to the rarity of this condition, it is difficult to determine the frequency (incidence) of this disorder. From an epidemiological standpoint, the female and male populations of all races and ethnicities are equally affected by it.

Cherubism is a genetic disorder with an autosomal dominant type of inheritance, and the cause of its appearance is a mutation of the SH3BP2 gene⁴.

The radiological definition of this disorder is a symmetrical expansive radiolucent lesion of the mandible and/or maxilla. Children are born with a normal appearance, and the maxilla and mandible usually swell between the ages of 2 and 7, until adolescence. The lesion then begins to spontaneously resolve and bone remodeling continues until the age of 30⁵. As cherubism is a syndrome with a brief duration, surgical intervention is not necessary, unless patients or their parents seek it for cosmetic reasons.

Cherubism lesions can be classified according to their severity: grade I, bilateral involvement of the ascending ramus of mandible; grade II, bilateral involvement of the ascending ramus of mandible and maxillary tuberosity; grade III, complete involvement of the maxilla and mandible compromising the coronoid processes and condyles⁶.

Albert Durer, a German painter, printmaker, and theorist of the German Renaissance who lived from 1471 to



Picture 1. Head of weeping cherub

1528, kept the look of cherubs in his paintings. This is an interesting fact. Head of Weeping cherub is the name of the picture (Picture 1)

Case report

Four-year old female patient came to our dental office for the first time for clinical examination in August 2021



Picture 2. Extraoral status (*Status localis extraoralis*)

with a specific appearance. During the extraoral examination, swollen cheeks were observed bilaterally, submandibular and sublingual glands were enlarged, mobile and painless on palpation. The face was asymmetrical with a strong swelling in the area of the cheeks (Picture 2).

Swelling was discovered during the intraoral examination in the area of the tuberosity of maxilla and in the area of the ramus of the mandible (*ramus mandibulae*). The local tissue enlargements were painless on palpation. Carious lesions were also observed in the area of deciduous molars. Polypomatous formation (*pulpitis chronica*



Figure 3. Intraoral status (*status localis intraoralis*)

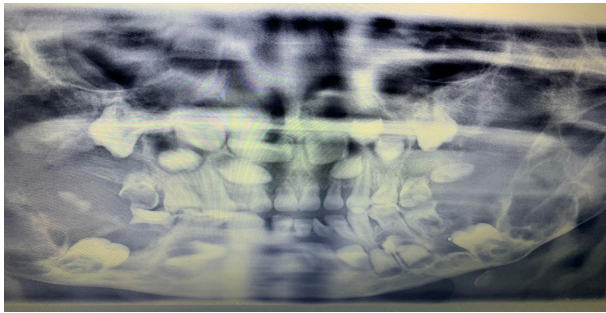
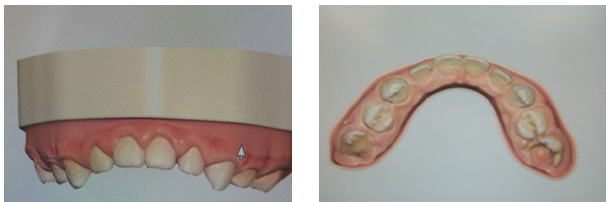


Figure 4. Panoramic x-ray



Picture 5. Scans from upper jaw



Picture 6. Scans from lower jaw

aperta hyperplastica) and luxation of the teeth were observed on all four second deciduous molars (Picture 3).

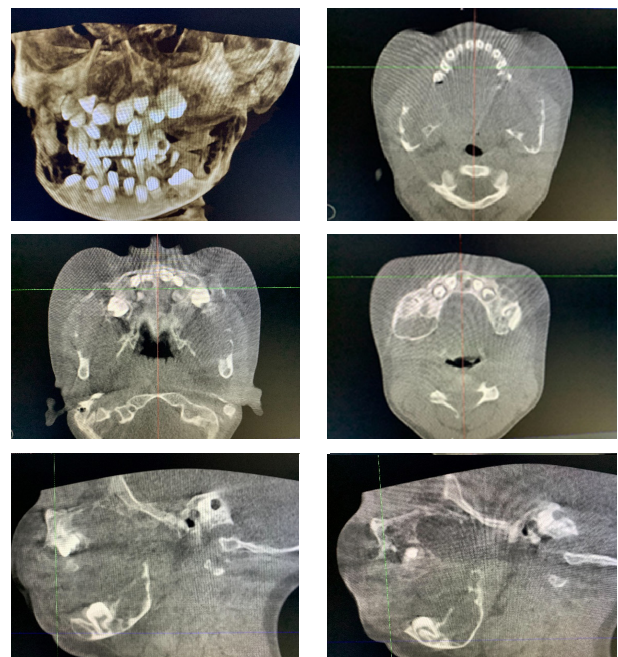
From the orthodontic point of view, the patient has a High arched palate (Gothic palate), displaced middle, an open bite and crowding in the mandible.

Family history for cherubism was positive. The father and several cousins from his family have already been diagnosed with this disorder.

Based on thorough medical and dental history and an extensive physical examination, we suspected cherubism and referred the patient to the University Clinic for Face, Jaws and Neck Surgery - Maxillofacial Surgery for further study and confirmation of the diagnosis (Picture 4)

Meanwhile, all carious lesions were cured. Due to the luxation of the lower left incisor, it was extracted.

A digital impression of the upper and lower jaws was obtained at the private polyclinic KRUNA MS – Skopje, without a bite determination because of the severity of the case and the patient's age. The scanning was per-



Picture 8. CBCT scans

Центар за мутни, молекуларна генетска истражувања (ЦММГ), Институт за мутнобиолошка и хумана генетика, Медицински факултет, Универзитет "Св. Кирил и Методиј", 1109 Скопје, Република Македонија. Контакт: +389-2-3118-998. E-mail: info@cmg.ukim.edu.mk

Center for Mutations, Molecular and Genetic Investigations (CMGI), Institute of Biotechnology and Human Genetics, Faculty of Medicine, University "St. Cyril & Methodij", 1109 Skopje, Republic of Macedonia. Contact: +389-2-3118-998. E-mail: info@cmg.ukim.edu.mk

ИЗВЕШТАЈ ОД ГЕНЕТСКО ИСПИТУВАЊЕ			
Презиме и име: [Redacted]	Испитан од: Д-р Тереза Болис-Гуриќ	Амбул. бр: ГенС 033/2022	Мат. бр: [Redacted]
Датум на раѓање: [Redacted]	Примен примерок: ДНК од плуќка	Упатна клиничка дијагноза:	
Примерокот е земен на: 05.04.2022	Тест: Анализа на генот SH3BP2		
Клиничка повест: Черубизам, со позитивна семејна историја.			
РЕЗУЛТАТ:			
<ul style="list-style-type: none"> Позитивен резултат Во испитуваниот примерок е откриена патогена варијанта во генот SH3BP2 асоциран со автозомно доминантен черубизам. 			
Ген	Варијанта	Генотип	Класификација
SH3BP2	c.1258G>A (p.Gly420Arg)	Хетерозигот	Патогена
* Со наредноста на автозомно доминантен начин.			
Интерпретација: Во испитуваниот примерок е откриена патогена варијанта c.1258G>A (p.Gly420Arg), во генот SH3BP2. Генот SH3BP2 е асоциран со автозомно доминантен черубизам (MedGen UID: 40219). Черубизамот се карактеризира со прогресивно, билатерално зголемување на максилата и мандибулата предизвикани од брза косвена дегенерација со формирање на мултикуларни цисти (PMID: 12907058, 11381256, 11113824). Овие цисти се безболни, но доведуваат до карактеристично лицце со отекнување и често водат до нормалној развој на забите. Денталните абнормалности вклучуваат конгенитен недостаток на заби, прематурна ексфолијација на децидуалните (млечни) заби, и поместување на трајните заби. Пациентите на оваа болест обично започнуваат во раното детство и напредуваат побрзо до пубертетот. По пубертетот цистите постепено се заменуваат со нормална коска, а абнормалностите на лицето честопати се повлекуваат т.е. не се забележуваат до возраст од 30 години. Многу ретко се забележани и другиот коска. Таквата на клоничката слива е варијабилна, од субвенечни манифестации (PMID: 21045962) до сериозни промени кои го афектираат димњето, видот, говорот и голтањето (PMID: 10716121, 10740181). Откриената варијанта c.1258G>A во генот SH3BP2 доведува до замена на глицин со аргинин во кодонт 420 од протеинот SH3BP2 (p.Gly420Arg). Оваа варијанта не е присутна во популациите бази на податоци (нема фреквенција во популацијата). Оваа генска промена е забележана на индивидуи со черубизам (PMID: 12900899, 23298620). Откриената варијанта е присутна во ClinVar базата на податоци (Variation ID: 372620). Алгоритмите за предвидување на ефектот на промената врз протеинската структура и функција се со контрадикторни резултати (БФТ: "Phenotype", PolyPhen-2: "Probably Damaging", Algn-GVGD: "Class C19"). Експерименталните студии покажуваат дека варијантата c.1258G>A во генот SH3BP2 ја зајакнува функцијата на SH3BP2 протеинот (PMID: 22153076). Поради наведените промени, откриената варијанта е класифицирана како патогена.			
Препорака: Се препорачува генетско советување.			
Забелешка: Листата на леќи и резултатите во апликацијата, како и дополнителните информации поврзани со резултатот се наведени во приложеното оригинално резултат.			
Асист. д-р Горѓи Милашовски	Проф. д-р Тодор Арсов Генетски советник 04.05.2022	Проф. д-р Александар Петликовски Расширител на ИФЖ	

Picture 7. Results from the genetic examination



Figure 9. Panoramic x-ray after 8 months

formed on a MEDIT brand scanner I 1500 owned by the above private polyclinic (Picture 5, 6).

At the same time, a karyotype genetic examination was conducted. The diagnosis of cherubism was confirmed with this genetic examination. Presented is the result of the genetic test (Picture 7).

CBCT scan was performed six months later. (Picture 8)

New panoramic X-ray was performed eight months following the first one. (Picture 9).

Discussion

In addition to the presented clinical case, the following section of the article describes the diagnostic, clinical, and therapeutic aspects of this unusual condition.

• *Clinical description*

In the majority of instances, there is a symmetrical expansive lesion affecting the mandible and/or maxilla, accompanied by enlargement of the submandibular and cervical lymph nodes. The severity of the disease phenotype is highly variable even within a family, with one member having a mild form of the disease and another member having a more severe form of the disease.

The first changes seen on X-ray are usually presented in the angle of the mandible (angulus mandibulae). These lesions are asymptomatic, although they may influence the development or eruption of the first permanent molars.

Cherubism is usually limited to both jaws, and very rarely the condyle of mandible and the zygomatic arch are affected⁷⁻¹¹. Due to the fenestration in the cortical bone, a more aggressive variety can lead to the creation of fibrous tissue, and if located in the orbit, can cause eye problems¹². Less frequently, respiratory changes might lead to obstruction of the upper airways. If they occur, they can cause mouth breathing, snoring, chronic nasal infection and obstructive sleep apnea¹³.

Cherubism can have an impact on the development of deciduous and permanent teeth. Eruption of permanent dentition can result in missing teeth (usually the first molars), rudimentary, abnormally shaped and ectopically erupted teeth¹⁴⁻¹⁶.

According to contemporary scientific data, radiographic manifestations of Cherubism typically include multilocular, bilateral radiolucent areas within the mandible, mostly located at the angles and rami. The coronoid processes are most commonly involved, whereas the condyles are rarely affected. Lesions on the mandible are symmetric, whereas maxillary lesions may be asymmetric. Radiographic images typically reveal expansive remodeling of the involved alveolar bones, thinning of the cortices, and multilocular radiolucencies with a coarse trabecular pattern.

• *Biochemical markers*

In patients with cherubism, the serum levels of calcium, parathyroid hormone, calcitonin and alkaline phosphatase are normal. The urine markers for bone remodeling, pyridinium and deoxypyridinium hydroxyproline and calcium-creatinine, are elevated. The serum value of alkaline phosphatase in active phase increases¹⁶⁻¹⁸.

• *Histological characteristics*

A considerable number of multinucleated giant cells resembling osteoclasts are observed in all patients. Three different phases are distinguished in the lesions of cherubism¹⁹. In the first osteolytic phase, round, fusiform and multinucleated giant cells resembling osteoclasts are observed. The tissue is extensively vascularized, and hemosiderin, a breakdown product of hemoglobin can be observed as a sign of hemorrhage²⁰. The second phase is characterized by the proliferation of spindle-shaped cells that are associated with a reparative phase. The third phase is the formation of bone with cells that are positive for alkaline phosphatase (probably osteoblasts) and a high level of ATP associated with a presence of mineralizing matrix.

• *Diagnose*

The diagnosis is based on the family history, age of the patient, the clinical examination, X-ray investigations, CBCT, genetic and biochemical examinations, biopsy and FNAC (fine needle aspiration cytology).

Family history is usually positive for this disease.

Clinically, cherubism should be distinguished from

1. Craniofacial fibrous dysplasia - this disease is usually unilateral
2. Masseteric hypertrophy-rarely observed in children and bone structures are not affected.
3. Gigantiform cementoma - is more prevalent in adults and manifests as a widespread swelling.
4. Gigantocellular granuloma-swelling in the area of the first molar
5. Brown tumor- is presented unilaterally and accompanied by systemic manifestations.

With the help of roentgenography, this disease should be separated from

1. Ameloblastoma
2. Odontogenic myxoma
3. Aneurysmic bone cyst
4. Craniofacial fibrous dysplasia
5. Gigantocellular granuloma
6. Brown tumor

- *Age*

The typical clinical presentation for this disease is characterized by bilateral cheek enlargement, enlarged lymph nodes, high “gothic” palate and early loss of primary teeth in children aged 2 to 7 years. There is a possibility of tongue enlargement, which can cause problems with breathing, speech, mastication and swallowing. Fastest progression of the disease is between 2-4 years until 7-8 years, when it begins to spontaneously withdraw until the puberty. At the age of 20, new bone tissue is already being formed, and at the age of 41, the bone structures of the affected parts have returned to normal. In the mandible, the ramus mandibulae, angulus mandibulae and processus coronoideus mandibulae are more frequently affected than the condyles.

Genetic testing is required to prove SH3BP2 mutation (21)

Sequence analysis of SH3BP2 detects small intragenic deletions, insertions and missense, nonsense and splice site variants. Typically, exon or whole-gene deletions or duplications are not detected. If pathogenic genetic variant cannot be identified, gene-targeted deletion and duplication analysis could be considered; however, because cherubism occurs through a gain-of-function mechanism and large intragenic deletion or duplication have not been reported, testing for intragenic deletions or duplication is unlikely to identify a disease-causing variant.

As previously stated, to confirm the cherubism, a panoramic image and a CBCT (cone-beam computed tomography) image are required. FNAC (fine needle aspiration cytology) aspiration to demonstrate the presence of abnormal cells. If the soft tissues are also involved, an MRI (magnetic resonance imaging) should also be performed.

Complications of cherubism

Many complications are associated with cherubism. First, severe malformation of the jaws may influence the physical functions such as chewing and swallowing, and may affect the social and psychological well-being of the individual, thereby necessitating surgical intervention. However, it should be noted that the recurrence of the jaw lesions after surgery is possible. Although the cherubic appearance of patients is expected to be reduced due to involution of the bubble-like distensions of the jaws in early adulthood, this may not be the case for all patients. The facial disfigurement associated with cherubism can affect an individual's sense of self-worth and lead to bullying. A recent Scandinavian study, however, reported that persons with cherubism were psychosocially well adapted and enjoyed a good quality of life²². Difficulties with pronunciation have not been reported as a significant problem, although they exist in a smaller number of cases of cherubism.

Patients with cherubism frequently complain of oral pain and discomfort when consuming food. The most affected persons have misplaced, unerupted, unformed, or absent, teeth or teeth that may appear to float in cyst like spaces. Malocclusion, premature exfoliation of deciduous teeth and root resorption may be the most common occurrences.

Some of the patients suffer from respiratory problems. These can include obstructive sleep apnea and upper airway obstruction caused by backward displacement of the tongue.

Prior reports of patients with maxillary involvement leading to orbital mass effect have varied in their ophthalmic sequelae and age of occurrence, with occurrence ranging from age 7 to 27 years^{23,24}.

Treatment

Due to the rarity of cherubism, treatment protocols for its complications are not well established and are evolving as a result of recent advances in our understanding of the autoinflammatory nature of this bone disease. Given that cherubism is considered to be a self-limited condition that improves over time, treatment should be tailored to the individual's presentation and the evolution of the disease. Depending on the severity, surgery may be needed for functional and esthetic concerns.

Children with cherubism should be referred to a maxillofacial clinic with pediatric experience for ongoing management. A craniofacial clinic associated with a major pediatric medical center usually includes a surgical team, general dentists, orthodontic specialists, ophthalmologists and child psychologist or social worker.

Surgical interventions in these patients include curettage with or without bone grafting. Liposuction has also been used successfully to re-contour the jaws. Surgical interventions are likely to occur between ages 5 and 15 years in individuals with disfiguring enlargement of jaws or locally aggressive lesions associated with complications such as impaired swallowing, respiratory issues, nasal airway obstruction, or tongue displacement. Surgical therapy needs to be individually tailored and not create unrealistic expectations since recurrence of the lesions is possible and surgery may not halt disease progression.

Some individuals with orbital manifestations such as lower lid retraction, ptosis, diplopia, eyeball displacement and visual loss caused by optic atrophy may require ophthalmologic treatment.

Speech and language therapy may be necessary in rare cases where physical obstruction to the production of speech or swallowing is present.

And perhaps the most significant is orthodontic treatment. It is commonly required because the jaw distortion

leads to permanent dental abnormalities including a mal-occlusive bite, premature loss of deciduous teeth and widely spaced, misplaced, unerupted or absent permanent teeth.

Further approach in treatment

Current contemporary researches on a mouse model indicate a high level of tumor necrosis factor^{25,26}. The presence of TNF in the circulatory system contributes to the regression of the disease. This holds true for a number of autoimmune-inflammatory diseases. It leads to a decrease in pro-inflammatory production of cytokines and a reduced effect of osteoclasts.

Additionally, mechanisms that will act on the SH3BP2 gene are being investigated. However, this type of therapy still requires proper development.

Forecast

In most cases, the lesions spontaneously regress by the age of 12. The X-ray examination reveals filling of the lesions with normal bone tissue. In some cases, the lesions may be filled with sclerotic bone and in more severe cases, they may remain unfilled with bone. However, it should be noted that spontaneous fractures are not observed²⁷.

Conclusion

Cherubism is a rare disease that has a significant impact on children and their families. In this particular case, the girl inherited from her father. Surgery is usually not required as the lesions resolve spontaneously by the age of 12. This autonomous disease is caused by a genetic change of the SH3BP2 gene in the chromosomal part 4q16.3.5 which codes for the protein. When the coding gene is mutated, the protein changes and cannot perform the function or performs it inadequately.

Mild forms of cherubism without facial deformities do not need to be treated. It is sufficient to monitor the patients with annual panoramic images and after regression of the disease, the images should be taken every 2 to 5 years. Surgical intervention is required if there are functional or aesthetic problems. Lytic changes occur in the bone with resorption of the roots and a floating appearance of the teeth, the girl already loose teeth 81,71 and 75. It leads to the premature loss of deciduous teeth and the more difficult eruption of permanent teeth. In this situation, a possible solution would be to install space brackets. As a choice of therapy, calcitonin is administered, in the form of tablets or nasal spray, with a note that if there are rapidly growing lesions, its use is contraindicated^{8,27}.

Interferon is still in experimental use.

Reference

1. Jones WA. Familial multilocular cystic disease of the jaws. The American Journal of Cancer. 1933 Apr; 17(4):946-50.
2. Jones WA. Further observations regarding familial multilocular cystic disease of the jaws. The British Journal of Radiology. 1938 Apr; 11(124):227-41.
3. Jones WA, Gerrie J, Pritchard J. Cherubism—a familial fibrous dysplasia of the jaws. Oral Surgery, Oral Medicine, Oral Pathology. 1952 Mar 1; 5(3):292-305.
4. Preda L, Dinca O, Bucur A, Dragomir C, Severin E. Identical mutation in SH3BP2 gene causes clinical phenotypes with different severity in mother and daughter—case report. Molecular Syndromology. 2010; 1(2):87-90.
5. Wajel J, Luczak K, Hendrich B, Guziński M, Szaśadek M. Clinical and radiological features of nonfamilial cherubism: A case report. Pol J Radiol. 2012; 77(3):53-57. doi:10.12659/pjr.8833756
6. Lima Gde M, Almeida JD, Cabral LA. Cherubism: clinicoradiographic features and treatment. J Oral Maxillofac Res. 2010; 1(2):e2. Published 2010 Jul 1. doi:10.5037/jomr.2010.1202
7. Motamedi MH. Treatment of cherubism with locally aggressive behavior presenting in adulthood: report of four cases and a proposed new grading system. Journal of oral and maxillofacial surgery. 1998 Nov 1; 56(11):1336-42.
8. Raposo-Amaral CE, de Campos Guidi M, Warren SM, Almeida AB, Amstalden EM, Tiziane V, Raposo-Amaral CM. Two-stage surgical treatment of severe cherubism. Annals of plastic surgery. 2007 Jun 1; 58(6):645-51.
9. Anderson DE. Cherubism: hereditary fibrous dysplasia of the jaws. 1 Genetic considerations. Oral Surg.. 1962;15(2):5.
10. Reichenberger EJ, Levine MA, Olsen BR, Papadaki ME, Lietman SA. The role of SH3BP2 in the pathophysiology of cherubism. Orphanet Journal of Rare Diseases. 2012 Jun;7(1):1-2.
11. Thompson N. Cherubism: familial fibrous dysplasia of the jaws. British Journal of Plastic Surgery. 1959 Jan 1;12:89-103.
12. Timoșca GC, Găleşanu RM, Cotutiu C, Grigoraș M. Aggressive form of cherubism: report of a case. Journal of oral and maxillofacial surgery. 2000 Mar 1;58(3):336-44.
13. Battaglia A, Merati AL, Magit A. Cherubism and upper airway obstruction. Otolaryngology—Head and Neck Surgery. 2000 Apr;122(4):573-4.
14. Faircloth Jr WJ, Edwards RC, Farhood VW. Cherubism involving a mother and daughter: case reports and review of the literature. Journal of oral and maxillofacial surgery. 1991 May 1;49(5):535-42.
15. Pontes FS, Ferreira AC, Kato AM, Pontes HA, Almeida DS, Rodini CO, Pinto Jr DS. Aggressive case of cherubism: 17-year follow-up. International journal of pediatric otorhinolaryngology. 2007 May 1;71(5):831-5.
16. Ireland AJ, Eveson JW. Cherubism: a report of a case with an unusual post-extraction complication. British Dental Journal. 1988 Feb 20;164(4):116-7.
17. Özkan Y, Varol A, Turker N, Aksakalli N, Basa S. Clinical and radiological evaluation of cherubism: a sporadic case report and review of the literature. International journal of pediatric otorhinolaryngology. 2003 Sep 1;67(9):1005-12.
18. Shah N, Handa KK, Sharma MC. Malignant mesenchymal tumor arising from cherubism: a case report. Journal of oral and maxillofacial surgery. 2004 Jun 1;62(6):744-9.
19. Roginsky VV, Ivanov AL, Ovtchinnikov IA, Khonsari RH. Familial cherubism: the experience of the Moscow central Institute for Stomatology and Maxillo-Facial Surgery. International journal of oral and maxillofacial surgery. 2009 Mar 1;38(3):218-23.
20. Chomette G, Auriol M, Guilbert F, Vaillant JM. Cherubism: Histo-enzymological and ultrastructural study. International journal of oral and maxillofacial surgery. 1988 Aug 1;17(4):219-23.
21. Ueki Y, Tiziani V, Santanna C, Fukai N, Maulik C, Garfinkle J, Ninomiya C, doAmaral C, Peters H, Habal M, Rhee-Morris L. Mutations in the gene encoding c-Abl-binding protein SH3BP2

-
- cause cherubism. *Nature genetics*. 2001 Jun;28(2):125-6.
22. Prescott T, Redfors M, Rustad CF, Eiklid KL, Geirdal AØ, Storhaug K, Jensen JL. Characterization of a Norwegian cherubism cohort; molecular genetic findings, oral manifestations and quality of life. *Eur J Med Genet*. 2013;56:131–7.
23. Colombo F, Cursiefen C, Neukam FW, Holbach LM. Orbital involvement in cherubism. *Ophthalmology*. 2001;108:1884–8.
24. Ahmadi AJ, Pirinjian GE, Sires BS. Optic neuropathy and macular chorioretinal folds caused by orbital cherubism. *Arch Ophthalmol*. 2003;121:570–3.
25. Peñarrocha M, Bonet J, Mínguez JM, Bagán JV, Vera F, Mínguez I. Cherubism: a clinical, radiographic, and histopathologic comparison of 7 cases. *Journal of oral and maxillofacial surgery*. 2006 Jun 1;64(6):924-30.
26. Ueki Y, Tiziani V, Santanna C, Fukai N, Maulik C, Garfinkle J, Ninomiya C, doAmaral C, Peters H, Habal M, Rhee-Morris L. Mutations in the gene encoding c-Abl-binding protein SH3BP2 cause cherubism. *Nature genetics*. 2001 Jun;28(2):125-6.
27. Wong M, Ziring D, Korin Y, Desai S, Kim S, Lin J, Gjertson D, Braun J, Reed E, Singh RR. TNF α blockade in human diseases: mechanisms and future directions. *Clinical immunology*. 2008 Feb 1;126(2):121-36.

MICROBIOLOGIC EVALUATION OF 2 METHODS OF ARCHWIRE LIGATION

МИКРОБИОЛОШКА ЕВАЛУАЦИЈА НА 2 МЕТОДА НА ЛИГИРАЊЕ НА ОРТОДОНТСКИТЕ БРЕКЕТИ

Cana A.¹, Bajraktarova Misevska C.², Mitic K.³, Alimani Jakupi J.⁴, Polozani G.², Simonovic M.¹, Emini A.¹

¹Department of Orthodontics, PHO University Dental Clinical Centre "St.Pantelejmon"- Skopje, Republic of North Macedonia, ²Department of Orthodontics, Faculty of Dentistry, Ss. "Cyril and Methodius" University in Skopje, Republic of North Macedonia, ³Department of Oral and Periodontal Diseases, Faculty of Dentistry, "Ss. Cyril and Methodius" University in Skopje, Republic of North Macedonia, ⁴Faculty of Medical Sciences, State University of Tetovo, Republic of North Macedonia

Abstract

Aim: The aim of this study was to determine the changes in microbial flora after orthodontic bonding and whether two different archwire ligation techniques affect these changes. **Material and method:** twenty-four orthodontic patients undergoing treatment with fixed appliances took part in the present study. Each orthodontic arch was fixed with elastomeric rings on one side of the midline, and on the other side with steel ligatures. The BANA test was used to detect *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* from the bottom of the periodontal pockets of the maxillary premolars and the mandibular incisors on both sides of the jaws. Microbial records were collected before bonding (T1), one week later (T2), and three months after the bonding (T3). ANOVA Chi Sq test and Cochran Q test were used to statistically compare the groups. **Results:** Teeth ligated with elastomeric rings contained greater numbers of microorganisms one week and three months after the bonding than teeth ligated with steel ligature wires. A significant difference in the values of BANA test was also determined between all three examined periods in the teeth ligated with elastomeric rings, while an insignificant difference in relation to T1, T2, and T3 was observed in the teeth ligated with wire ligatures. **Conclusion:** The method of archwire ligation has a significant impact on the microbiological flora of patients with fixed orthodontic appliances. Elastomeric rings stimulate the growth of periodontopathogenic bacteria which is a predisposing factor for the occurrence of caries and inflammation of the gingiva. **Key words:** archwire ligation, microbial flora, elastomeric rings, periodontopathogens, fixed orthodontic appliances.

Апстракт

Цел: Цел на оваа студија беше да се утврдат промените во микробиолошката флора кај пациенти со фиксни ортодонотски апарати, како и да се утврди влијанието на различниот метод на лигирање на брекетите врз овие промени. **Материјал и метод:** Испитувањата беа спроведени кај дваесет и четири пациенти со фиксни ортодонотски апарати. Кај сите испитаници, на едната половина од вилицата брекетети беа лигирани со еластични лигатури, а на другата половина со жичени лигатури. За детекција на *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* од дното на пародонталниот џеб во предел на максиларните премолари и мандибуларните инцизиви, од двете страни на вилиците, беше применет BANA тестот. Микробиолошките наоди беа одредувани пред започнувањето на ортодонотската терапија (T1), една недела по поставувањето на фиксниот апарат (T2) и три месеци по поставувањето (T3). Разликите меѓу групите беа анализирани со ANOVA Chi Sq тестот и Cochran Q тестот. **Резултати:** Резултатите покажаа сигнификантно зголемување на бројот на микроорганизми една недела и три месеци по поставувањето на фиксниот апарат кај забите лигирани со еластични лигатури, во споредба со забите лигирани со жичени лигатури. Статистички сигнификантна разлика во вредностите на BANA тестот беше утврдена и меѓу сите три испитувани периоди кај забите лигирани со еластични лигатури, додека незначајна разлика во релација T1, T2 и T3 беше забележана кај забите лигирани со жичени лигатури. **Заклучок:** Методот на лигирање на ортодонотските брекетети има значајно влијание врз микробиолошката флора кај пациентите со фиксни апарати. Еластичните лигатури го стимулираат растот на пародонтопатогените бактерии кои се предиспонирачки фактор за појава на кариес и инфламација на гингивата. **Клучни зборови:** метод на лигирање на брекетите, микробиолошка флора, еластични лигатури, пародонтопатогени бактерии, фиксни ортодонотски апарати.

Introduction

During orthodontic therapy with fixed appliances, the risk of periodontal inflammatory lesions and enamel demineralization increases as a result of the reduction of physiological self-cleaning mechanisms and increased retention of plaque on the surface of the component elements of the fixed appliances. These elements as plaque

retention sites lead to bacterial colonization and an increased number of microorganisms^{1,2}.

Orthodontic therapy followed by poor oral hygiene can cause severe damage to the periodontium³⁻⁵. Several clinical and microbiological studies have shown that in the absence of good oral hygiene, the placement of orthodontic bands in children results in periodontal pocket formation. In addition, there is a quantitative

increase in the microbial composition of subgingival plaque formation, which resembles the plaque typically found in periodontal disease where *Tannerella forsythia*, *Porphyromonas gingivalis*, and *Treponema denticola* are present⁶⁻¹².

Several authors have investigated the impact of fixed orthodontic appliances on the microbiological flora and periodontal status^{5,13-17}. Their findings showed that therapy with fixed appliances increases the values of all periodontal indices and stimulates the growth of periodontopathogenic bacteria. However, despite causing periodontal changes, it has no destructive effects on the deeper periodontal tissues.

Although several studies have examined the influence of fixed orthodontic therapy on the microbial colonization of the periodontium and the condition of the periodontal tissues, few authors have examined the method of ligation of brackets as an additional factor influencing the occurrence of these changes^{1,2,18,19}. Most of these authors agree on the harmful influence of elastomeric rings in the accumulation of dental plaque and periodontal changes in patients with fixed orthodontic appliances.

The aim of this study was to determine the changes in microbial flora after orthodontic bonding and to determine whether two different archwire ligation techniques affect these changes.

Materials and method

The study population was comprised of twenty-four healthy patients, aged 13 to 18 years, who were patients at the Department of Orthodontics, PHO University Dental Clinical Centre "St.Pantelejmon"- Skopje. All patients included in this study had healthy periodontium and no previous orthodontic treatment with fixed appliances. Subjects who had taken antibiotics within three months before the study and those with a systemic disorder were excluded. The study was approved by the Teaching and Science Research Council of Ss. Cyril and Methodius University - Skopje.

All subjects had fixed orthodontic appliances, which include Master Series® metal brackets, LP® metal tubes, wires, and ligatures from American Orthodontics, USA. Brackets on the right side of the patient were ligated with elastomeric rings, and brackets on the left side were ligated with conventional stainless steel ligature wires. To determine the microbial complexes, the BANA test (BANA-Zymetm - Hexagon International (GB) Ltd) was used in all subjects at the Department of Oral and Periodontal Diseases, and the microbial swab was taken from the bottom of the periodontal pocket of the maxillary premolars and the mandibular incisors on both sides of the jaws. The presence of *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* was determined. To collect the swab, a graduated periodontal probe and a Gracy curette were used to penetrate the deepest part of the subgingival space to take a sufficient amount of material for examination of the microbial complexes. Microbial records were collected before bonding (T1), one week later (T2), and three months after the bonding (T3).

Statistical analysis was conducted using the computer program Statistica 7.1 for Windows and SPSS 23.0. The presence of *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* in the relations T1, T2, and T3 were analyzed using the Cochran Q test (Q/p). An analysis of variance (ANOVA) Chi Sqr and McNemar's test (p) were used to compare the presence of *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* in relation to T1 and T3.

Results

The differences between the values of the Bana test between the examined teeth on both sides of the jaws before starting the orthodontic therapy, one week and three months after the placement of the fixed appliances are shown in table 1-5.

The results showed a significant increase in values (p<0.001) one week and three months after the place-

Table 1. BANA test differences between elastomeric rings and wire ligatures.

Variable	Rank Sum Elastomeric	Rank Sum Wire	U	Z adjusted	p-level	N Elastomeric	N Wire
Bana test T1	612,00	564,00	264,00	0,70	0,48	24	24
Bana test T2	764,50	411,50	111,50	4,18	0,000	24	24
Bana test T3	843,50	332,50	32,50	5,64	0,000	24	24

Table 2. BANA test differences in elastomeric rings in relation T1 & T2 & T3.

Variable	Average Rank	Sum of Ranks	Mean	Std.Dev.
Bana test T1	1.21	29.00	0.25	0.44
Bana test T2	2.15	51.50	1.04	0.36
Bana test T3	2.65	63.50	1.46	0.51

ANOVA Chi Sqr. = 35,57 and $p < 0,001$

Table 3. BANA test differences in elastomeric rings between T1 & T2 & T3.

Pair of variables	Valid	T	Z	p-level
Bana test T1 & Bana test T2	24	0.00	3.62	0.000
Bana test T2 & Bana test T3	24	0.00	2.80	0.005
Bana test T1 & Bana test T3	24	0.00	4.01	0.000

Table 4. BANA test differences in wire ligatures in relation T1 & T2 & T3.

Variable	Average Rank	Sum of Ranks	Mean	Std.Dev.
Bana test T1	1.90	45.50	0.17	0.38
Bana test T2	2.15	51.50	0.38	0.58
Bana test T3	1.96	47.00	0.21	0.41

ANOVA Chi Sqr. = 2,17 and $p > 0,05$

Table 5. test differences in wire ligatures between T1 & T2 & T3.

Pair of variables	Valid	T	Z	p-level
Bana test T1 & Bana test T2	24	15.00	1.27	0.20
Bana test T2 & Bana test T3	24	7.00	1.18	0.24
Bana test T1 & Bana test T3	24	12.00	0.34	0.74

ment of fixed appliances in teeth ligated with elastomeric rings compared to values in wire ligatures (Table 1).

A statistically significant difference in the values of the BANA test was also determined between all three examined periods in the teeth ligated with elastomeric rings (Table 2).

Seven days after the placement of elastomeric rings (T2), the value of the BANA test for $Z = 3.62$ and $p < 0.001$ is significantly higher than the value of the BANA test before orthodontic therapy (T1), and three months after the placement (T3) the value of the BANA test for $Z = 2.80$ and $p < 0.01$ is significantly higher than the value of the BANA test seven days after the placement of these ligatures (T2) (Table 3).

An insignificant difference in the values of this test was observed in the teeth ligated with wire ligatures in relation T1, T2, and T3 (Table 4).

Seven days after the placement of the wire ligatures (T2), the BANA test value for $Z = 1.27$ and $p > 0.05$ is insignificantly higher than the value of the BANA test before orthodontic therapy (T1), while three months after the placement (T3) the value of the BANA test for $Z = 1.18$ and $p > 0.05$ is insignificantly lower than the value of the BANA test seven days after the placement of these ligatures (T2) (Table 5).

Discussion

The analysis of the effect of different types of ligatures on the amount of periodontopathogenic bacteria from the red microbial complex revealed that the type of ligatures has a significant impact on the microbial colonization of the periodontium.

The results showed a progressive increase in the values of *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* seven days and three months after the placement of the appliance on the side of the jaw where elastomeric rings were placed. On the side where the teeth were ligated with wire ligatures, an insignificant increase in values was observed seven days after the placement and an insignificant decrease in the same three months after the application of the fixed appliance. This finding is consistent with the results of several studies^{1,18-23}. Souza et al. revealed a significant increase in gram-species with the use of elastomeric rings¹⁹.

The degree of bacterial colonization associated with orthodontic appliances is affected by the energy and roughness of the appliance surfaces, as well as their design and dimensions²⁴. This appears to be a key factor in the efficient performance of hygiene procedures²⁵. Even though changes in the microbial system involve all types of orthodontic appliance, more rapid modifications occur during fixed orthodontic treatment. Perinetti et al. stated

that the role of subgingival bacteria in periodontal disease modifications need to be evaluated alongside the action of enzymes activated in response to the stimuli of orthodontic forces²⁶.

Fixed orthodontic appliances significantly increase the colonization of *Streptococcus mutans* and *Lactobacilli*^{1-3,16}. Periodontal pathogenic bacteria such as *Actinobacillus actinomycetemcomitans* and *Tannerella forsythia* are also significantly associated with gingival inflammation during orthodontic therapy^{3,27}. Among the various types of bacteria found in individuals with periodontal disease are *Porphyromonas gingivalis*, *Prevotella intermedia*, *P. nigrescens*, *Bacteroides forsythus*, *A. actinomycetemcomitans*, *Fusobacterium nucleatum* and *Treponema denticola*¹¹. The chemical and physical characteristics of these bacteria make them the most important members of the periodontopathogenic microbial flora²⁸. However, not all patients with periodontal disease harbor all putative periodontal pathogenic species⁸. *Porphyromonas gingivalis* and *Prevotella intermedia* are frequently found in prepubescent patients with periodontitis²⁹. Lyons et al.³⁰ consider that irreversible damage to the host tissue occurs only when the level of bacteria reaches a critical level.

The analysis of the differences in the number of microorganisms from the red complex between the examined teeth ligated with elastomeric rings and wire ligatures indicated a significant level of values in T2 and T3 on the side of elastomeric rings, which is in agreement with the findings of Forsberg et al., Sukontapatipark et al., and Souza et al.^{1,18,19} An increased number of microorganisms on the side of the elastomeric rings was also found by Türkahraman et al.², but unlike previous authors, this difference was not statistically significant.

The differences in microbiological findings found in the literature are likely due to differences in the microorganisms analyzed, the type of microbial test, the period of evaluation, and host resistance factors¹⁹. The time necessary for gingival inflammation to develop when oral hygiene is poor varies from person to person and depends on the rate of biofilm formation. The results obtained within this research showed a strong correlation between elastomeric rings and the quantity of periodontopathogenic bacteria in patients with fixed orthodontic appliances. In patients with inadequate oral hygiene, elastomeric rings significantly increase the microbial accumulation on the surface of the teeth around the braces, which is a predisposing factor for the development of caries and inflammation of the gingiva. Therefore, the obtained results indicate the importance of the ligation of orthodontic brackets with wire ligatures as one of the necessary conditions for the preservation of periodontal health in patients undergoing fixed orthodontic therapy.

Conclusion

The archwire ligation technique has a significant impact on the microbiological flora in patients with fixed orthodontic appliances. The values of *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* progressively increased seven days and three months after the placement of the appliance on the jaw side with elastomeric rings. On the side of the jaw with wire ligatures, the amount of periodontopathogenic bacteria of the red complex insignificantly increased seven days after the placement and an insignificantly decreased three months after orthodontic treatment. The analysis of the differences in the number of microorganisms from the red complex between the examined teeth ligated with elastomeric rings and wire ligatures indicated significantly higher values in the T2 and T3 periods on the side of the jaw where elastic ligatures were placed.

Reference

1. Forsberg C M, Brattström V, Malmberg E, Nord C E. Ligation Wires and Elastomeric Rings: Two Methods of Ligation, and Their Association With Microbial Colonization of *Streptococcus Mutans* and *Lactobacilli*. *Eur J Orthod*. 1991;13(5):416-20.
2. Türkahraman H, Sayin M O, Yeşim Bozkurt F, Yetkin Z, Kaya S, Onal S. Archwire Ligation Techniques, Microbial Colonization, and Periodontal Status in Orthodontically Treated Patients. *Angle Orthod*. 2005;75 (2):231-6.
3. Lundström F, Hamp SE, Nyman S. Systematic plaque control in children undergoing long-term orthodontic treatment. *Eur J Orthod* 1980;2:27-39.
4. Huber SJ, Vernino AR, Nanda RS. Professional prophylaxis and its effect on the periodontium of full-banded orthodontic patients. *Am J Orthod Dentofacial Orthop* 1987;91:321-7.
5. Huser MC, Baehni PC, Lang R. Effects of orthodontic bands on microbiologic and clinical parameters. *Am J Orthod Dentofacial Orthop* 1990;97:213-8.
6. Bue Lo A M, Marco Di R, Milazzo I, Nicolosi D, Cali G, Rossetti B, Blandino G. Microbiological and clinical periodontal effects of fixed orthodontic appliances in pediatric patients. *New Microbiologica*. 2008;31:299-302.
7. Blandino G, Lo Bue A.M, Milazzo I, Nicolosi D, Cali G, Cannavò V, Rossetti B. Comparison of systemic flurithromycin therapy and clinical producers in the treatment of periodontal diseases. *J. Chemother*. 2004;16:151-155.
8. Haffajee A.D, Teles R.P, Socransky S.S. Association of *Eubacterium nodatum* and *Treponema denticola* with human periodontitis lesions. *Oral Microbiol Immunol*. 2006; 21:269-282.
9. Huserm C, Baehni P.C, Lang R. Effects of orthodontic bands on microbiologic and clinical parameters. *AmJ Orthod Dentofacial Orthop*. 1990; 213-218.
10. Latronico M, Segantini A, Cavallini F, Mascolo A, Garbarino F, Bondanza S, Debbia E, Blasi G. Periodontal disease and coronary heart disease: an epidemiological and microbiological study. *New Microbiol*. 2007;30:221-228.
11. Socransky S.S, Haffajee A.D. Dental biofilm: difficult therapeutic targets. *Periodontol*. 2002; 28:15-55.
12. Ximenez-Fyvie L.A, Haffajee A.D, Socransky S.S. Microbial composition of supra- and subgingival plaque in subjects with adult periodontitis. *J. Clin. Periodontol*. 2000;27:722-732.

-
13. Scheie AA, Arneberg P, Krogstad O. Effect of orthodontic treatment on prevalence of *Streptococcus mutans* in plaque and saliva. *Scand J Dent Res.* 1984;92:211–217.
 14. Sinclair PM, Berry CW, Bennett CL, Israelson H. Changes in gingiva and gingival flora with bonding and banding. *Angle Orthod.* 1987;57:271–278.
 15. Rosenbloom RG, Tinanoff N. Salivary *Streptococcus mutans* levels in patients before, during, and after orthodontic treatment. *Am J Orthod Dentofacial Orthop.* 1991;100:35–37.
 16. Chang HS, Walsh LJ, Freer TJ. The effect of orthodontic treatment on salivary flow, pH, buffer capacity, and levels of mutans streptococci and lactobacilli. *Aust Orthod J.* 1999;15:229–234.
 17. Glans R, Larsson E, Ogaard B. Longitudinal changes in gingival condition in crowded and noncrowded dentitions subjected to fixed orthodontic treatment. *Am J Orthod Dentofacial Orthop.* 2003;124:679–682.
 18. Sukontapatipark W, el-Agroudi MA, Selliseth NJ, Thunold K, Selvig KA. Bacterial colonization associated with fixed orthodontic appliances. A scanning electron microscopy study. *Eur J Orthod.* 2001;23:475–484.
 19. Souza R A, Araújo Magnani M B B, Nouer D F, Silva C O, Klein M I, Sallum E A, Gonçalves R B. Periodontal and Microbiologic Evaluation of 2 Methods of Archwire Ligation: Ligature Wires and Elastomeric Rings. *Am J Orthod Dentofacial Orthop.* 2008;134(4):506-12.
 20. Thornberg M J, Riolo C S, Bayirli B, Riolo M L, Van Tubergen E A, Kulbersh R. Periodontal pathogen levels in adolescents before, during, and after fixed orthodontic appliance therapy. *Am J Orthod Dentofacial Orthop.* 2009;135(1):95-8.
 21. Liu H, Sun J, Dong Y, Lu H, Zhou H, Hansen F B, Song X. Periodontal health and relative quantity of subgingival porphyromonas gingivalis during orthodontic treatment. *Angle Orthod.* 2011;81(4):609-15.
 22. Kim SH, Choi DS, Jang I, Cha BK, Jost-Brinkmann PG, Song JS. Microbiologic changes in subgingival plaque before and during the early period of orthodontic treatment. *Angle Orthod.* 2012;82(2):254-60.
 23. Nağacı R, Özat Y, Çokakoğlu S, Türkkahraman H, Önal S, Kaya S. Effect of bracket type on halitosis, periodontal status, and microbial colonization. *Angle Orthod.* 2014;84(3):479-85.
 24. Lucchese A, Bondemark L, Marcolina M, Manuelli M. Changes in oral microbiota due to orthodontic appliances: a systematic review. *J Oral Microbiol* 2018;10(1):1-22
 25. Øilo M, Bakken V. Biofilm and dental biomaterials. *Materials.* 2015;8:2887–2890.
 26. Perinetti G, Paolantonio M, Serra E, et al. Longitudinal monitoring of subgingival colonization by *Actinobacillus actinomycetemcomitans*, and crevicular alkaline phosphatase and aspartate aminotransferase activities around orthodontically treated teeth. *J Clin Periodontol.* 2004;31(1):60-67.
 27. Sallum EJ, Nouer DF, Klein MI, Gonçalves RB, Machion L, Sallum AW, et al. Clinical and microbiologic changes after removal of orthodontic appliances. *Am J Orthod Dentofacial Orthop* 2004;126:363-6.
 28. Fives-Taylor PM, Meyer DH, Mintz KP, Brissette C. Virulence factors of *Actinobacillus actinomycetemcomitans*. *Periodontol* 2000, 1999;20:136-67.
 29. Watanabe K. Prepubertal periodontitis: a review of diagnostic criteria, pathogenesis, and differential diagnosis. *J Periodontal Res* 1990;25:31-48.
 30. Lyons SR, Griffen AL, Leys EJ. Quantitative real-time PCR for *Porphyromonas gingivalis* and total bacteria. *J Clin Microbiol* 2000;38:2362-5.

HYGIENIC-DIETARY REGIMEN AND OCCURRENCE OF DENTAL CARIES IN 10-YEAR-OLD CHILDREN IN THE AREA OF BITOLA MUNICIPALITY

ХИГИЕНО-ДИЕТЕТСКИ РЕЖИМ И ПОЈАВАТА НА ДЕНТАЛЕН КАРИЕС КАЈ ДЕЦА ОД 10 ГОДИШНА ВОЗРАСТ НА ПОДРАЧЈЕТО НА ОПШТИНА БИТОЛА

Milevska E.¹, Milevski M.², Gjorgievska Jovanovska S.³, Pacanoska V.³, Gjorgievska E.⁴

¹PHI Health care Center „Dr. Haim Abravanel“ - Bitola; ²PHO „Dr. Milevski - ORTHODONTICS“ - Bitola; ³PHI General Hospital - Prilep;

⁴Department of Pediatric and Preventive Dentistry, Faculty of Dentistry, Ss. „Cyril and Methodius“ University in Skopje

Abstract

Aim: The main goal of this study is to evaluate the hygienic-dietary regimen and the state of oral health of children 10-year-old children from the Municipality of Bitola from urban and rural environments. **Material and methods:** 300 respondents who were examined at PHI „Dr. Haim Abravanel“ - Bitola were included in the study, including 164 males and 136 females, students in the fourth grade of elementary school. In addition to basic personal and general anamnestic data, data related to the hygienic-dietary regimen of the participants were included, i.e. data on the consumption of different types of food and drinks, as well as frequency and duration of tooth brushing. In the study, the structure of the DMFT index was evaluated, and, the condition of the teeth that had undergone a preventive procedure of fissure sealing was recorded. **Results:** The prevalence of carious, extracted and/or filled teeth was only 20%, among the respondents who brushed their teeth 3 or more times a day. There was a 47.98% incidence of decayed, extracted, and/or restored teeth among children who consumed sugar. We observed carious, extracted and/or filled teeth in 71.43% of the subjects with discarded sealants, and in only 35.44% of the subjects with sealed fissures. **Conclusion:** Regular oral hygiene, controlled intake of sugars, fissure sealing, application of fluorides, health education and promotion of regular preventive dental examinations have an important role in the prevention of dental caries and its complications. **Key words:** dental caries, oral hygiene, nutrition, fissure sealing.

Апстракт

Цел на трудот: Основната цел на овој труд е евалуација на хигиено-диететскиот режим и состојбата на оралното здравје кај деца на 10годишна возраст од општина Битола од градска и рурална средина. **Материјал и методи:** Во студијата беа вклучени 300 испитаници кои што биле на преглед во Службата за заштита на деца до 14 години при ЈЗУ Здравствен дом „Д-р Хаим Абраванел“ - Битола. Беа опфатени 164 машки и 136 женски испитаници, ученици од IV одделение. Покрај основните лични и општи анамnestички податоци беа опфатени податоци кои се однесуваат на хигиено-диететскиот режим на испитаниците, односно податоци за консумација на различни видови на храна и пијалоци, фреквенција и времетраење на четкање на забите. Во истражувањето беше направена и евалуацијана структурата на КЕП индексот, а беше евидентирана и состојбата на забите кај кои претходно било извршено превентивно залевање на фисурите. **Резултати:** Кај испитаниците кои што ги четкале забите 3 или повеќе пати на ден, застапеноста на кариозни, екстрахирани и/или пломбирани заби е само 20%. Кај децата кои внесувале шеќери евидентирани се кариозни, екстрахирани и/или реставрирани заби кај 47.98%. Забележуваме присуство на кариозни, екстрахирани и/или пломбирани заби кај дури 71.43% од испитаниците соодпаднати залевачи, а кај само 35.44% од испитаниците со залевачи. **Заклучок:** Правилната и редовна орална хигиена, контролираниот внес на шеќери, залевањето на фисурите и јамичките на забите, употребата на флуориди, здравствена едукација и промовирањето на редовни превентивни стоматолошки прегледи имаат клучички улога во превенцијата на денталниот кариес и неговите компликации. **Клучни зборови:** дентален кариес, орална хигиена, исхрана, залевање на фисури.

Introduction

Researching the etiology of dental caries is one of the most important tasks in dental science for its successful prevention. Caries is a multifactorial disease caused by complex interactions between the host - the tooth, dental plaque, diet, and time. One of the best definitions of caries is provided by Loesch, who defines it as a chron-

ic, complex, bacterial infectious disease that results in milligram mineral losses in dental structures¹.

The goal of preventive dentistry, as an essential segment of dental medicine, is to recommend and ensure the application of appropriate efficient and effective measures and means for the prevention of dental caries and other oral diseases by applying modern scientific and clinical knowledge in the field of etiopathogenesis.

Despite significant scientific achievements and the fact that caries can be prevented, it continues to be a global public health problem that negatively impacts the quality of life. According to the World Health Organization, 60-90% of school-age children around the world have dental caries, with the highest prevalence among Asian and Latin American children².

In recent decades, there has been a significant decrease in the occurrence of caries in children in highly developed countries^{3,4}. This condition is due to the increased health culture, the application of fluorides and the sealing of fissures and pits, the use of better and more advanced oral hygiene products, improved access to dental health care, as well as preventive campaigns and programs aimed at increasing awareness among the population for the preservation and promotion of oral health. On the other hand, an increased frequency of dental caries has been registered in developing countries, especially in those countries in which preventive dental programs have not yet been established and implemented^{5,6,7}.

Cooperation with the dentist and adherence to his recommendations are the key element for the prevention and early treatment of caries in children. Additional education, motivation, and regular dental check-ups increase the likelihood of interceptive treatment of caries while it is still in a reversible phase. In fact, preventive therapy in dentistry is of exceptional importance and includes lifetime oral health care and maintenance of optimal oral hygiene and a healthy lifestyle^{8,9}.

The primary objective of this paper is to evaluate the hygienic-dietary regimen and the state of oral health in 10-year-old children from the Municipality of Bitola, both in urban and rural settings, in light of current scientific knowledge. Special emphasis was placed on toothbrushing as a basic oral hygiene measure, the types of food that children usually consume and fissure sealing as a key preventive measure in childhood, as well as their impact on the structure of DMFT index and overall oral health.

Materials and methods

300 respondents were included in the study - children aged 10 from urban and rural areas of the Municipality of Bitola, who were examined at the Healthcare Service for children under the age of 14 at PHI Health care Center „Dr. Haim Abravanel“- Bitola. 164 male and 136 female respondents, students in the IV grade from two elementary schools in the city of Bitola, as well as two elementary schools in the rural areas of the municipality of Bitola were used as a representative sample.

The data were collected in a questionnaire for each respondent separately. In addition to the basic personal

and general anamnestic data, data related to the hygienic-dietary regimen of the respondents were included, i.e. data on the consumption of different types of food and drinks which were classified into several categories (milk and dairy products, meat and fish, fruit and vegetables, sweets, cakes and sugary drinks), the degree of oral hygiene expressed through the frequency and duration of tooth brushing, replacement of the toothbrush, etc. The research also included data on the dental status of the respondents through the evaluation of the structure of the DMFT index, regarding the prevalence of permanent teeth with caries, extracted permanent teeth, and permanent teeth with fillings (filled teeth). Also, the condition of the teeth that had previously undergone the preventive procedure of fissure sealing with Fuji TRIAGE[®] glass ionomer, the time of their sealing, the need for their resealing and the time spent since resealing, and that of the first premolars and the first permanent molars were recorded.

All data were collected with the consent of the parents of the respondents, and the examinations were conducted as part of the routine activities carried out by the Department of Pediatrics and Preventive Dentistry in the Municipality of Bitola as part of the action plan for the National Strategy for the Prevention of Oral diseases in children aged 0 to 14 years.

All collected data were processed using appropriate statistical methods. Statistical analysis was performed in the program Statistica 10.0 (Data Analysis Software System).

Results

150 children from the city of Bitola and 150 children from the rural areas of the municipality of Bitola, all at the age of 10, participated in this study and they were classified according to gender and place of residence (city/village). Out of a total of 300 respondents, 164 respondents or 54.66% were male, and 136 or 45.33% were female (Table 1).

Table 1. Distribution of respondents according to gender and place of residence

	male	female	Total
city	86 (57.33)%	64 (42.66)%	150
village	78 (52.00)%	72 (48.00)%	150
Total	164 (54.66)%	136 (45.33)%	150

As the results of the evaluation of the oral hygiene status of the studied groups demonstrate, special emphasis was placed on the use of oral hygiene products, namely the frequency and duration of tooth brushing as the main parameters, as well as their association with the occurrence of dental caries. According to the processed answers, it was determined that only a small percentage of children brush their teeth 3 times a day (10%). Most of the respondents (75%) stated that they brush twice a day, 9% only brush once a day, and 6% of the respondents brush very rarely, which is less than once a day (Table 2 and Graph 1).

Table 2. Tooth brushing frequency

	number of respondents
less than once a day	18 (6 %)
once a day	27 (9 %)
twice a day	225 (75 %)
3 or more times a day	30 (10 %)

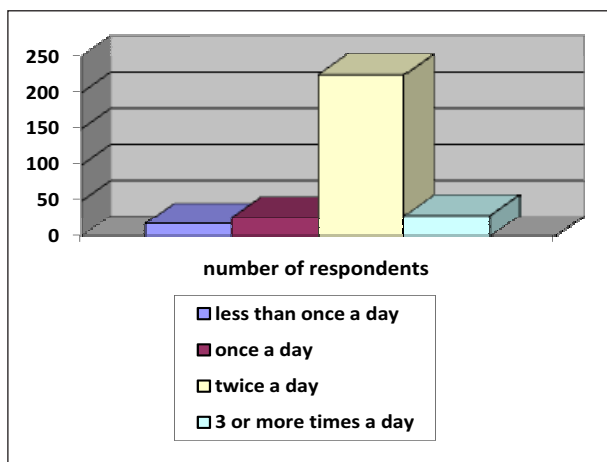


Chart 1. Tooth brushing frequency

A great percentage of the respondents (65%) answered that they brush their teeth for 1-2 minutes, 20% brush their teeth for less than 1 minute, and only 15% brush for 3 or more minutes (Table 3 and Graph 2). 70% of the respondents (210 children) replace their toothbrush twice a year, 20% (60 children) every 3 months, and 10% (30 children) once a year.

The studied population has an age-appropriate diet that abounds in sugars and products from the modern confectionery industry. An evaluation of the dietary regime included the different types of food and drinks that are most often preferred, as well as their connection with the

Table 3. Duration of tooth brushing

	number of respondents
less than a minute	60 (20 %)
1-2 minutes	195 (65 %)
3 or more minutes	45 (15 %)

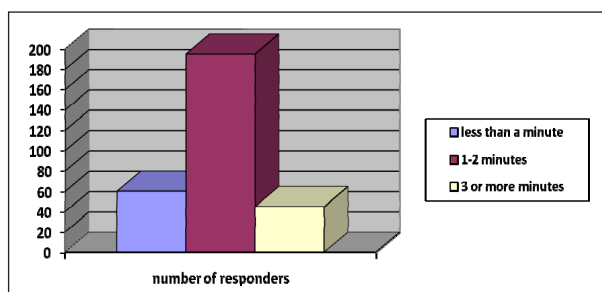


Chart 2. Tooth brushing frequency

occurrence and prevalence of caries. A huge percentage of the respondents answered that they consume a variety of nutritious products that include milk and dairy products, meat and fish, and, fruits and vegetables. However, a significantly higher number consumed foods and drinks rich in sugars, in contrast to those who consumed healthy foods such as fruits and vegetables (Chart 3).

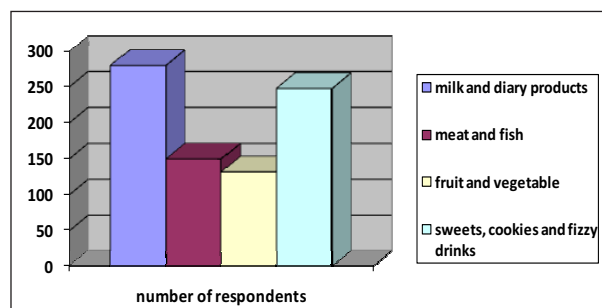


Chart 3. Consumption of types of food and drinks

Concerning the sealing of the fissures and pits of the teeth as an extremely important preventive measure, the condition of the sealing of the teeth which was performed on the first premolars and the first permanent molars, as well as the need for their possible resealing, as well as the connection of this preventive measure with the prevalence of caries, was monitored. In 237 out of 300 children, the teeth had sealed fissures, and in 63 children, the sealants were completely or partially discarded, necessitating resealing.

The oral status was evaluated by noting the structure of the DMFT index among children aged 10 from urban and rural areas in the area of the municipality of Bitola.

Table 4 and Table 5 showcase the results obtained from the structure of the DMFT index, expressed through the number of respondents without or with carious, extracted, and/or filled (restored) permanent teeth in relation to gender and place of residence.

The following tables show the results obtained from the statistical analysis regarding the correlation of the number of respondents with and without carious, extract-

ed and/or filled (restored) permanent teeth and several previously considered parameters, namely: the frequency of tooth brushing (Table 6), the duration of tooth brushing (Table 7), the consumption of food and drinks rich in sugar (Table 8) and the condition of the fissure sealants (Table 9).

Among the respondents who brushed their teeth 3 or more times a day, the prevalence of carious, extracted

Table 4. Distribution of carious, extracted and/or restored permanent teeth among respondents in relation to gender

	number of respondents	
	Male (%)	Female (%)
D-decayed(carious) permanent teeth		
don't have	140 (85.37)%	120 (88.24)%
have	24 (14.63)%	16 (11.76)%
M-missing (extracted) permanent teeth		
don't have	162 (98.78)%	136 (100)%
have	2 (1.22)%	0
F-filled (restored) permanent teeth		
don't have	125 (76.22)%	88 (64.71)%
have	39 (23.78)%	48 (35.29)%

Table 5. Distribution of carious, extracted and/or restored permanent teeth among respondents in relation to the place of residence (urban/rural environment)

	number of respondents by place of residence	
	City (%)	Village (%)
D- decayed (carious) permanent teeth		
don't have	132 (88.00)%	128 (85.33)%
have	18 (12.00)%	22 (14.67)%
M- missing (extracted) permanent teeth		
don't have	148 (98.67)%	155 (100)%
have	2 (1.33)%	0
F- filled (restored) permanent teeth		
don't have	109 (72.67)%	104 (69.33)%
have	41 (27.33)%	46 (30.67)%

Table 6. Correlation between the brushing frequency and the prevalence of carious, extracted and/or filled teeth

	number of respondents N	number of respondents without/with carious, extracted and/or filled teeth	
		don't have	have
Less than once a day	18	6	12
Once a day	27	14	13
Twice a day	225	127	80
Three times a day	30	24	6
Total	300	171	129

and/or filled teeth was only 20%. In contrast, among respondents who brushed less than once a day, the presence of caries, extracted, or filled teeth was 66.67%. This leads to the conclusion that there is a statistically significant difference in the prevalence of carious, extracted and/or filled teeth between the four groups regarding the frequency of brushing (Pearson Chi-square= 12.48, df=3, p=0.05).

Concerning the duration of tooth brushing, we can conclude that there is also a statistically significant difference regarding the prevalence of carious, extracted and/or

filled teeth between the three groups (Pearson Chi-square=20.08, df=2, p= 0.05).

Among the children who consumed sugar, the presence of carious, extracted, and/or filled teeth was observed in 47.98%. On the other hand, among the children who did not consume such food, the prevalence of carious, extracted and/or filled teeth is only 19.23%.

It can be stated that there is a statistically significant relationship between the condition of fissure sealants and the prevalence of carious, extracted and/or filled teeth (Pearson Chi-square=26.30, df=1, p=0.05). Thus, we

Table 7. Correlation between the brushing frequency and the prevalence of carious, extracted and/or filled teeth

	number of respondents N	number of respondents without/with carious, extracted and/or filled teeth	
		don't have	have
Less than a minute	60	21	39
1-2 minutes	195	115	80
3 or more minutes	45	35	10
Total	300	171	129

Table 8. Correlation between the consumption of sugary foods and the incidence of carious, extracted and/or filled teeth

	number of respondents N	number of respondents with carious, extracted and/or filled teeth
consume sugary foods	248	119 (47.98%)
don't consume foods rich in sugars	52	10 (19.23%)
Total	300	129

Table 9. Correlation between the condition of fissure sealants and the prevalence of carious, extracted and/or filled teeth

	number of respondents N	number of respondents without/with carious, extracted, and/or filled teeth	
		don't have	have
respondents with fused fissures	237	153	84
respondents with fallen waterers	63	18	45
Total	300	171	129

observe the presence of carious, extracted and/or filled teeth in as many as 71.43% of the subjects with discarded sealants, but in only 35.44% of the subjects with sealed fissures, indicating the significant preventive role of fissure sealing when performed correctly and promptly.

Discussion

The recent research results from several epidemiological studies in pediatric population indicate that the prevalence of caries has decreased significantly in developed countries such as the countries of Western Europe and the USA¹⁰. This is due to the implementation of systematic school preventive programs, health education programs, intensive and continuous application of fluorides, improved oral hygiene, and changes in lifestyle and living conditions^{11,12}.

The main task of oral hygiene is to reduce the amount of dental plaque, and consequently, to reduce the total number of bacteria in the oral cavity and indirectly, the number of acidic products created and their demineralization potential. Inadequate and irregular oral hygiene is not a direct cause of caries, but it significantly promotes the growth and reproduction of microorganisms in dental plaque, which increases the acidity in the oral cavity, thereby creating suitable conditions for the manifestation of the conditionally pathogenic properties of some cariogenic microorganisms¹³.

According to our study, the majority of children brush their teeth twice a day, in the morning and the evening. However, a large part of the respondents still uses only the basic means for oral hygiene, a toothbrush and a toothpaste, and 6% of them brush their teeth less than once a day, which is obviously insufficient to prevent the accumulation of dental plaque and occurrence of caries. The use of dental floss and rinsing liquids is still relatively uncommon, despite the increasing awareness of children and their parents about their benefits. The duration of toothbrushing in most children in our study is 1 to 2 min-

utes, which is significantly less than the recommended time.

The clarification for the mechanism of caries occurrence given by Miller (1889) a long time ago, presents caries as a pathological process caused by the action of oral bacteria on a substrate (fermentable food), with the decomposition of which organic acids are created that cause enamel demineralization and the appearance of the initial carious lesion¹⁴. Even though the respondents stated that they consume a variety of foods, the representation of sweets, cakes, and fizzy drinks in the daily base of nutrition, was present in 82.67%. The frequent intake of carbohydrates, especially between meals, significantly prolongs the harmful demineralizing effect, which means it shortens the time required for remineralization of the demineralized enamel. At the same time, the pH value in the plaque decreases, and the ionic reserves found in the plaque are depleted, preventing the restoration of the demineralized surface of the enamel.

It is well known that the occlusal surfaces of the teeth have the highest incidence of dental caries in children and young people, and pits and fissures represent caries susceptible parts because the occlusal surfaces of the teeth have a rather complex morphology¹⁵. Namely, fissures can penetrate deeply into the occlusal surface, exhibit localized narrowing, and have different depths. Fissures are classified according to their depth (shallow, deep and intermediate fissures), as well as based on their morphological characteristics, whereby we distinguish two main fissure types:

- a) extensive and shallow U- and V-shaped fissures from which dental deposits and food residues are easily removed and are caries-resistant and
- b) narrow and deep Y-shaped fissures, from which it is much more difficult to remove deposits, they are more susceptible to caries and with cracks towards the enamel-dentine connection¹⁶.

The rate at which dental caries develops in fissures is directly related to the depth of the fissure, that is, the deep-

er it is and the closer it is to the enamel-dentine junction, the more susceptible it is to the development of dental caries¹⁷. The sealing of fissures and pits is one of the most common and effective modern prophylactic measures and is aimed at protecting the occlusal surfaces of teeth from the influence of cariogenic factors¹⁸.

As per our analysis, it was determined that the sealing of the first permanent molars and first premolars is carried out continuously every year based on the action plan for the National strategy for the prevention of oral diseases of children aged 0-14 years. According to the records, it was established that the percentage of durability of sealants in teeth with a fissure system is satisfactory, that is, a high percentage (79%) of the respondents, while in 21%, full or partial resealing of the fissures was performed. The reason for the long clinical success after sealing treatment lies in the ability of the sealant to form a micromechanical bond with the inorganic enamel substrate. On the other hand, the marginal micropermeability, that can be observed following the application of fissure sealant, facilitates the penetration of bacteria under the sealant, initiating the process of caries formation^{19,20,21}.

From the obtained data on the structure of the DMFT index, it can be noted that the number of carious, extracted and/or filled teeth is somewhat higher among children from the male population compared to children from the female population. It can be concluded that the results presented in this research coincide with the results given by Kumar et al.⁸, Gauba et al.²², and Jose et al.²³, which indicate that children from the male population have poorer oral hygiene compared to the children from the female population and are in contradiction with the results presented by Saha and Sarkar²⁴, and Retnakumari²⁵ who stated that there is no difference between the DMFT index in children from the male and female populations. The values of the structure of the DMFT index of the children from the rural environment of the municipality of Bitola are greater than the values from the city of Bitola.

Oral health is largely conditioned by the habits, attitudes and behavior of the population. Thus, decades ago, priority was given to oral health care in the Scandinavian countries, with prevention organized at the state level, available to all children, and financed by state funds, which led to a significant reduction in the prevalence of caries. Children were invited at certain terms, those who did not come regularly were also monitored, and all levels of oral health care were included^{26,27,28}.

However, regardless of the good knowledge of its nature and measures for its prevention, dental caries remains one of the most common diseases in the world, affecting 90% of the total population, according to the report of the World Health Organization^{29,30}. It can be concluded that dental caries is the most prevalent chronic dis-

ease in the oral medium, especially among children and adolescents³¹.

Conclusion

Based on the obtained results of the research conducted for this paper, it can be concluded that the oral hygiene habits and dietary regimen of 10-year-old children from the area of the Municipality of Bitola have a significant impact on the state of oral health, especially the prevalence of dental caries and its complications. The practice of correct and regular oral hygiene, controlled intake of sugars, sealing fissures and pits of the teeth, application of fluorides, health education and promotion of regular preventive dental examinations play a vital role in the prevention of dental caries, as well as the maintenance and promotion of overall oral health.

Reference

1. Loesch WJ. Clinical and microbiological aspects of chemotherapeutic agents used according to the specific plaque hypothesis. *J Dent Res.* 1979;58:2404-2412.
2. Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S. The global burden of oral diseases and risks to oral health. *Bull World Health Organ.* 2005 Sep;83(9):661-9.
3. Marthaler TM, O'Mullane D, Vbric V. The prevalence of dental caries in Europe 1990-95. *Caries Res* 1996 30:237-255.
4. Burt BA. Trends in caries prevalence in North American children. *Int Dent J* 1994 44: 403-413.
5. Nithila A, Bourgeois D, Barmes DE et al. WHO Global Oral Data Bank, 1986-96: an overview of oral health surveys at 12 years of age. *Bulletin of the World Health Organization* 1998 76:237-244.
6. Petersen PE, Razanamihaja N. Oral health status of children and adults in Madagascar. *Int Dent J* 1996 46:41-47.
7. Petersen PE, Kaka M. Oral health status of children and adults in the Republic of Niger, Africa. *Int Dent J* 1999 49:159-164.
8. Kumar MP, Joseph T, Varma RB, Jayanthi M. Oral health status of 5 years and 12 years school going children in Chennai city - an epidemiological study. *J Indian Soc Pedod Prev Dent.* 2005, 23(1), pp. 17-22.
9. Burt BA. Prevention policies in the light of the changed distribution of dental caries. *Acta Odontol Scand.* 1998, 56 (3), pp. 179-86.
10. Centers for Disease Control and Prevention, 2001, Recommendations for using fluoride to prevent and control dental caries in the United States. *Morbidity and mortality weekly report*, 50 (RR-14), pp.1-42.
11. Action Programme For Improving Oral Health In Europe, WHO, Geneva, 1993.
12. Klein H. The family and dental disease IV. Dental disease (DMF) experience in parents and offspring. *J Am Dent Assoc.* 1946, 33, pp. 735-4.
13. Gibson S, Williams S. Dental caries on per-school Children: Associations with Social class, tooth brushing habit and consumption of sugar-containing foods, National Diet and Nutrition Survey of children aged 1.5-4.5 years. *Caries Research.* 1999, 33 (2), pp. 101-113.
14. Miller WD. *The microorganisms of the human mouth*, Philadelphia, SSWhite Dental Manufacturing Company, 1890. Reprinted Basel, Switzerland. Karger, 1973.
15. Marthaler T, Steiner M, Menghini G, Brandi A. Caries prevalence

-
- among schoolchildren in the Canton Zurich. Results of the period 1963-1987. *Schweiz Monatschr Zahnmed* 1988;98, 1309-1315.
16. Gwinnett AJ, Caputo L, Ripa LW, Disney JA. Micromorphology of the fitting surface of failed sealants. *Pediatr Dent* 1982;4:237-239.
 17. *Pediatric dentistry Infancy Through Adolescence*. Pinkham, Casamassimo, Fields, McTigue, Nowak. 2005, 1999,1994,1988 Elsevier Inc. p.525-539.
 18. Beiruti N, Frencken JE, van 't Hof MA, van Palenstein Helderma WH. Caries preventive effect of resin-based and glass ionomer sealants over time: a systematic review. *Community Dent Oral Epidemiol* 2006; 34(6): 403-9.
 19. Simonsen RJ. Pit and fissure sealant: Review of the literature. *Pediatr Dent* 2002; 24:393-414.
 20. Feigal RJ. The use of pit and fissure sealants. *Pediatr Dent* 2002; 24:415-422.
 21. Duangthip D, Lussi A. Variables contributing to the quality of fissure sealants used by general dental practitioners. *Oper Dent* 2003; 28:756-764.
 22. Gauba K, Tewari A, Chawla HS. the Frequency distribution of children according to dental caries status in rural areas of northern India. *J Indian Dent Assoc.* 1986;58:505-12.
 23. Jose A, Joseph MR. Prevalence of dental health problems among school going children in rural Kerala, *J Indian Soc Ped Prev Dent* 2003;21(4), pp. 147-151.
 24. Saha S., Prevalence and severity of dental caries and oral hygiene status in rural and urban areas of Calcutta. *J Indian Soc Ped Prev Dent.* 1996;23 (1), pp. 17-22.
 25. Retnakumari N. Prevalence of dental caries and risk assessment among primary school children of 6-12 years in the Varkala municipal area of Kerala. *J Indian Soc Ped Prev Dent.* 1996;5(1), pp. 146-151.
 26. Hale K, Heller K. Fluorides: getting the benefits, avoiding the risks. *Contemp Pediatr.* 2000; 2, pp. 121.
 27. Iljovska S, Pavlevska M, Jankulovska M, Fildisevski A, Dimkov A, Sovremeni aspekti na prevencijata na karies kaj decata. *Makedonski stomatoloski pregled* 2006;30 (1), pp. 38-41.
 28. Jacobi A, The dentition and its derangements. Course lectures delivered in New York Medical College, New York: New York Medical College, 1986; pp. 1830-1919.
 29. Ravindran S, George A. Biomimetic extracellular matrix - mediated somatic stem cell differentiation: applications in dental pulp tissue regeneration. *Front Physiol* 2015; 6:118. 10.3389/fphys.2015.00118.
 30. Federationden denaire Internationale Technical report No.20: The Prevention of Dental Caries and Periodontal Disease. *Inter Dent J.* 1986; 34 (2), pp. 141-158.
 31. Bauman LJ, Silver EJ, Stein RE. Cumulative social disadvantage and child health. *Pediatrics*, 2006; 117 (4), pp. 1321-1328.

EXPRESSION OF IL6 IN PERIODONTAL AFFECTED SITES WITH DIFFERENT ATTACHMENT LOST.

ЕКСПРЕСИЈА НА IL6 ВО РЕГИИ СО РАЗЛИЧНО ИЗРАЗЕН ГУБИТОК НА ПРИПОЈ

Pandilova M.¹, Trajkov D.², Georgieva L.³, Ugrinska A.⁴

¹Department of periodontology and oral pathology, Faculty of Dentistry, "Ss. Cyril and Methodius" University, Skopje, Republic of North Macedonia, ²Institute of Immunobiology and Human Genetics, Faculty of Medicine, "Ss. Cyril and Methodius" University, Skopje, Republic of North Macedonia, ³PHI Doc. Dragan Lukic, ⁴Institute of Positron Emission Tomography Faculty of Medicine "Ss. Cyril and Methodius" University, Skopje, Republic of North Macedonia

Abstract

Introduction: Adult periodontal disease is characterized by a chronic inflammatory process that destroys the supporting tissues of the teeth. It has been proposed that periodontal pathogens stimulate the inflammatory cytokine expression. Some cytokines and their role during periodontal disease have been thoroughly investigated, but the expression of IL6 in our population has never been investigated. **Objective:** Our objective was to study the levels of IL6 in gingival tissue from sites with different attachment loss. **Material and methods:** Gingival tissue biopsies were obtained from 30 patients. Patients with different degrees of attachment loss and with no gingival recession were selected. All examined patients were between 20 and 40 years old and they all had regions with healthy periodontal tissue. Gingival tissue biopsies were taken from the examined region and the healthy region of each patient. Obtained samples were frozen on -80 °C. Using ELISA method, the levels of the examined cytokine was determined in frozen tissue sections. **Results:** Analysis of the values of the examined cytokine led to the conclusion that IL6 levels exhibited no statistical difference concerning attachment loss or gingival inflammation. Statistical significances were noted only using ANOVA for the groups. **Conclusion:** Regarding the role of IL6 in periodontal disease, our results pointed out that for bone destruction, gingival inflammation, attachment loss during periodontal disease are not associated with elevated levels of IL6 in our population. **Key words:** periodontal disease, cytokines, IL6, inflammation, attachment loss.

Апстракт

Вовед: Пародонтитот се вбројува во инфламаторните процеси, која покрај разрушувањата на ткивата на пародонтот, се карактеризира и со експресија на за неа карактеристични цитокини. И покрај тоа што одредени цитокини се карактеристични за пародонталната афекција, сепак нивното присуство кај нашата популација досега не е испитувано. **Цел:** Целта на нашето испитување беше да се докаже присуството на IL6 во регии со различен губиток на припој. **Материјал и метод:** Испитувањето е спроведено кај 30 пациенти со различен степен на пародонтална деструкција, без гингивална рецесија, на возраст помеѓу 20-40 години. Пациентите покрај здравствената состојба беа селектирани и така да секој има барем една здрава регија. По земената согласност и запазените етички принципи кај пациентите беше земен ткивен исечок од засегнатата и здравата регија. Исечоците беа замрзнати на -80 °C. По хомогенизацијата со помош на ELISA method system Biotrak™ од Amersham Pharmacia Biotech беа одредени количествата на IL6 следејќи ги упатствата на производителот. **Резултати:** По анализата на добиените резултати не добивме статистички сигнификантна разлика на вредностите на IL6 кај регионите ниту со различен губиток на припој, ниту со различно изразена гингивална инфламација. **Заклучок:** Резултатите не наведуваат на заклучок дека гингивалната инфламација и различната пародонтална деструкција кај нашата популација не е поврзана со зголемени количества на IL6. **Клучни зборови:** пародонтална деструкција, цитокини, IL6, инфламација.

Introduction

The periodontium is a complex tissue structure comprised of resident cells, including epithelial cells, fibroblasts and bone, as well as various types of inflammatory cells, which emigrate from the microvasculature of the gingiva in response to plaque accumulation. In response to initial stimulation, resident cells in the gingival tissue release various cell communication signals in form of chemical cytokines. Individually or collectively, these molecules participate in the resolution or destruction of periodontal tissues.

Large amounts of evidence indicate that cytokines released during periodontal affection are primarily responsible for the course of the disease^{1,2}, due to their mediating role during inflammation. Among the various types of cytokines, interleukins play a significant role during chronic inflammatory responses by transmitting messages between different types of leukocytes^{3,4,5}.

Cytokines released in the earliest stages of disease determine the type of immune response (6)

Since the first cytokines discovered originated from leucocytes, they were referred to as lymphocytes or interleukins. Different types of cytokines belong to this

group, such as, growth factors responsible for anabolic events in the tissue, chemokines responsible for tissue cell migration, interferons responsible for lymphocyte activation, and tumor necrosis factor with pleotropic effects.

The profile of cytokine secretion depends on numerous circumstances, including local and individual genetic factors.

Numerous cytokines are closely associated with chronic periodontal disease. Elevated levels of IL1 are considered to be a risk factor for disease severity. IL6 is traditionally believed to be associated with gingival tissue inflammation and progressive bone resorption, but recent data contradicts the conventional belief. Elevated levels of IL6 in some studies have been proven to have protective effects on periodontal tissues.

Lack of information regarding IL6 expression in our population during periodontal disease prompted us to conduct the present study, the primary objective of which was to:

Determine the expression of IL6 in gingival tissue in our population at different stages of attachment loss during periodontal disease.

Material and methods

Examinations were conducted on 30 patients with diagnosed periodontal disease at the clinic of Periodontology and oral disease in Skopje. All of the patients met the following criteria:

- Age between 20 and 40 years old
- Absence of any kind of systemic disease
- Clinically diagnosed adult periodontal disease according to the criteria proposed from the American academy of Periodontology (7)
- Presence of regions with inflammation - free tissue
- Gingival margin positioned on or above the cement enamel junction.

According to attachment loss, the patients were divided into 3 groups

- **First group** - patients with attachment loss not greater than 3mm
- **Second group** - patient with attachment loss between 3 and 6 mm
- **Third group** - patient with attachment loss equal or greater than 6mm

According to the criteria proposed by Silness-Loe, gingival inflammation and gingival bleeding were also recorded for every patient.

Tissue samples were obtained from the affected region, as well as from the healthy ones for each patient.

Tissue samples were frozen at -80 °C. After tissue homogenization IL6 was detected by using ELISA method system Biotrak™ from Amersham Pharmacia Biotech.

All assays were conducted in accordance with the manufacturer's instructions. All investigations regarding tissue samples homogenization, safe keeping and detection of IL6 were performed at the Institute of Immunobiology and Human Genetics, Faculty of Medicine, "Ss. Cyril and Methodius" University, Skopje, Republic of North Macedonia. Results were expressed as picograms of cytokine per milligram of tissue.

Statistical evaluation of data was performed with computer program Statistic 6, 0 using Student t Test in order to establish differences between healthy and diseased tissue samples and ANOVA in order to establish differences within the groups.

Results

The results obtained for IL6 in tissue samples were as follows:

- For healthy gingiva $-0,286 \pm 0,54$ picograms of cytokine per milligram of tissue
- For attachment loss of 3mm $-0,163 \pm 0,2659$ picograms of cytokine per milligram of tissue
- For attachment loss between 3-6mm $-0,142 \pm 0,14$ picograms of cytokine per milligram of tissue
- For attachment loss over 6mm $-0,204 \pm 0,21$ picograms of cytokine per milligram of tissue

No statistical significance was established between the healthy and diseased tissue samples (Figure 1.)

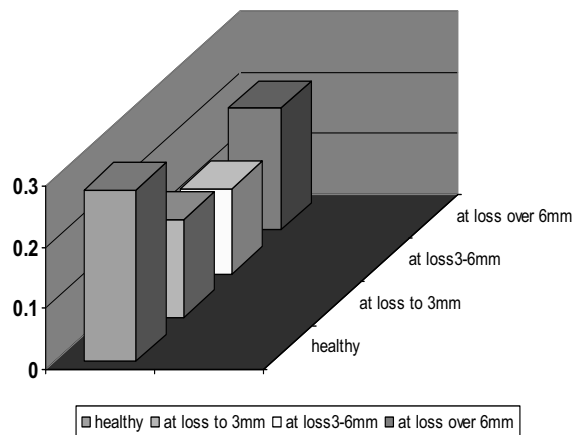


Figure 1. Average level of IL6 in tissue samples from periodontally affected patients adherent to sites with different attachment loss

Lack of statistical differences, imposed the need to regroup the obtained data according to the level of tissue inflammation recorded. There were no statistically significant differences between the different degrees of inflammation nor between the healthy and the diseased (Table 1).

Table 1. Average level of IL6 in tissue samples from periodontally affected patients with different gingival inflammation (according to Sillnes-Loe index(S.L) for gingival inflammation)

	X	SD	df	t	r	F	R
healthy	0.16	0.26					
S.L 1	0.28	0.76	29	0.52	0.37	7.41	0.09
S.L 2	0.49	0.22	27	0.91	0.44	18.1	0.08
S.L 3	0.23	0.11	24	0.77	0.60	1.38	0.54

Table 2. post hoc Scheffe's test.

	SS	df	MS	F	p
Between groups	0,509791	2	0,254895	12,62974	0,004759
Into the group	0,141275	7	0,020182		
total	0,651066				

Statistical differences was established for ANOVA between the three groups for $p=0,004$. Results for ANOVA test and post hoc Scheffe's test are presented in Table 2.

Discussion

Interleukin 6 is a product of lymphocytes, fibroblast and monocytes. IL6 realise can be elicited by LPS, IL1, and TNFalpha. Female hormones such as progesterone and estrogens can diminish IL6 synthesis. Takashaki et al.⁸ have reported elevated levels of m RNA IL6 in diseased tissue principally associated with endothelial cells, fibroblasts and macrophages. In vitro, IL6 is a potent inducer of monocytic differentiation into multi-nucleated giant cells which are capable of resorbing bone. Yamazaki et al.⁵ have reported an elevation of IL6 secreting cells in periodontitis compared to gingivitis.

Studies by Reinhardt¹⁰ have demonstrated that IL6 is elevated in gingival cervical fluid of refractory patients as compared to stable adult periodontitis. The authors have also shown enhanced levels of IL6, IL1 and IL8 in estrogen-deficient women compared to estrogens supplemented women pointing out the association of these cytokines, estrogens and bone resorption.

IL6 is traditionally associated with periodontal disease. Ellis¹⁰ indicates that elevated levels of IL6 in periodontal-affected tissue contribute to chronic inflammation by protecting the macrophages from apoptotic cell death. Elevated levels of IL6 during periodontal disease have been found in the studies of Yamazaki⁵, Reinhardt⁹ Balta¹¹ Shengnan Z¹².

Our results revealed no statistically significant differences between the healthy tissue samples and the samples

from inflamed regions with different attachment loss. Lack of statistically significant differences may be due to variety of values of IL6 obtained from our patients and the higher values of standard deviation.

The possible explanation of our results may be found in the studies of Yumoto¹³. He points out that the cytokine profile of periodontal cells is generally the same, but varies based on the type of bacterial colonization, interaction between them and the host immune system.

Different microorganisms of dental plaque induce varying levels of IL6 production in fibroblasts. Studies conducted in vitro confirm that a lower concentration of *Treponema denticola* increases production of IL6, while a higher concentration inhibits the production of IL6¹⁴. Micro organisms which have the ability to produce trypsin like enzymes such as *Porphyromonas gingivalis* are capable of degrading IL6¹⁵. It is possible that such process had occurred in our material since we had no data regarding the microbial colonization of plaque. Furthermore, the detection of various components in tissue samples represents a frozen moment in different stages of a very dynamic process, such as periodontal inflammation with no specific evidence of past or future events.

IL-6 has traditionally been regarded as a proinflammatory mediator, because it is induced by IL-1 and TNF- α , early in the inflammatory cascade, and because it stimulates the expression of acute-phase proteins. However, recent data demonstrate that IL-6 lacks many typical proinflammatory properties and also exerts a number of anti-inflammatory activities. Balto¹⁶ in his study emphasizes that the anti-inflammatory properties of IL-6 predominate in inflammatory responses. Although the mechanisms of action have yet to be defined, they may involve either the

direct suppression of IL-1 or the induction of endogenous antagonists or inhibitors of IL-1. Individual host response, which some reports consider to be a characteristic of parts or entire populations, remains to be considered. Cytokines production is genetically determined, allowing for a similar response to different types of microbial challenge in chronic disease such as periodontal disease. Studies conducted on large group of our population showed no significant differences for allele, genotype, and haplotype distributions of the IL-6 (174, kt 565) between healthy and periodontally affected individuals, nor between periodontally affected individuals with different attachment loss¹⁷.

Conclusion

Our investigation revealed no statistically significant difference between tissue levels of IL 6 between samples obtained of periodontally affected tissue adherent to sites with different attachment loss. No statistical significance was found in tissue samples from different stages of gingival inflammation.

Reference

- Gemmell E., Marshall R. I., Seymour G. I. Cytokines and prostaglandines in immune homeostasis and tissue destruction in periodontal disease, *Periodontology* 2000 1997: 14 112-43.
- Weber R. L., Iakono V. J. The cytokines: a review of interleukins *Periodont. Clin. Invest.* 1997 19: 17-22.
- Hillmann G., Hillmann B., Gartsen W. Immunohistological determination IL1 beta in inflamed human gingival epithelium. *Arch. Oral Biol.* 1995 40: 353-9.
- Hou L. T., Lui C. M., Rossomondo E. F. Cervical interleukin-1 beta in moderate and severe preiodontitis patients and the effect phase I periodontal treatment. *J. Clin. Periodontal* 1995 22: 162-7.
- Yamazaki K., Nakajima T., Gemmell E. IL4 and IL6 producing cells in human periodontal disease tissue. *J. Oral Pathol. Med.* 1994 :23: 347-53.
- Roit I, Brostoff J., Male D. *Immunology* Mosby 2020 13 th Edition 1.2- 1.6.
- American Academy of Periodontology. Proceedings of the World Workshop in Clinical Periodontics. Chicago the American Academy of Periodontology 1989: 1 123-1/24.
- Takahashi H, Takigawa M, Takashiba S. Role of cytokine in the induction of adhesion molecules on cultured human gingival fibroblasts. *J. Periodontol.* 1994 65:230-5.
- Reinhardt RA, Masada MP, Johnson GK. Gingival fluid levels of IL1 and IL6 in refractory periodontitis *J. Clin. Periodontal.* 1993;20:225-231.
- Ellis S.D., Tucci M.A., Serio F.G. Factors for progression of periodontal disease. *J. Oral. Pathol. Med.* 1998 27: 101-5.
- Balta MG, Papathanasiou E, Blix JJ, Van Dyke. Host Modulation and Treatment of Periodontal Disease. *Dent Res.* 2021 Jul;100(8):798-809.
- Shengnan Zhang , Yingjun Liu, Xuekui Wang, Na An Xiangying Ouyang. STAT1/SOCS1/3 Are Involved in the Inflammation-Regulating Effect of GAS6/AXL in Periodontal Ligament Cells Induced by Porphyromonas gingivalis Lipopolysaccharide In Vitro. *Immunol Res.* 2021 Oct ;2021:577-695.
- Yumoto H, Nakae N, Fujinaka K, Ebisu K, Matsuo T. Interleukin-6 (IL-6) and IL-8 Are Induced in Human Oral Epithelial Cells in Response to Exposure to Periodontopathic Eikenella corrodens. *Infection and Immunity,* 1999;67:384-394.
- Nixon C, Steffen M, Ebersole J. Cytokine response to Treponema denticola and Treponema pectinovorum in human gingival fibroblasts. *Infect and Immunity* 2000 68(9) :5284-5292.
- Steffen M.J, Holt S, Ebersole J. Porphyromonas gingivalis induction and cytokine secretion by human gingival fibroblasts. *Oral. Microbiol.Immunol* 2001 :67: 1450-1454.
- Balto K, Sasaki H, Stashenko F. Interleukin-6 Deficiency Increases Inflammatory Bone Destruction. *Infection and Immunity,* 2001;69: 744-750.
- Atanasovska-Stojanovska A. The association between cytokines gene polymorphisms and chronic periodontal disease with Macedonian population. Doctoral Thesis 2008 Faculty of dentistry "Ss. Cyril and Methodius" University, Skopje, Republic of Macedonia.

DEFINING THE COMPREHENSION LEVEL OF THE TEACHING STAFF IN PRIMARY SCHOOLS REGARDING FIRST AID PROCEDURES FOR DENTAL TRAUMA

ОДРЕДУВАЊЕ НА СТЕПЕНОТ НА ПОЗНАВАЊЕ НА ПОСТАПКИТЕ ПРИ ДАВАЊЕ ПРВА ПОМОШ КАЈ ДЕНТАЛНИ ТРАУМА НА НАСТАВНИОТ КАДАР ВО ОСНОВНИ УЧИЛИШТА

Talimdzioska K.¹, Sotirovska-Ivkovska A.², Najdenoska-Bojchinoska S.³, Arsova Apostolovska M., Alili Sh.⁴

¹PHI Health Center - Prilep, ²Department of Pediatric and Preventive Dentistry, Faculty of Dentistry, Ss. "Cyril and Methodius" University in Skopje, Republic of North Macedonia, ³PHI Health Center - Krushevo, ⁴University Dental Clinical Center "St. Panteleymon" Skopje, Republic of North Macedonia

Abstract

Introduction: Dental trauma is a significant functional and aesthetic issue. The prognosis of the traumatized tooth depends on the first aid provided to the child by those present at the moment of the injury. **The aim** of our research is to determine primary school teachers' capacity in administering first aid for dental trauma. **Material and method:** Our study included 61 teachers from two primary schools. The teachers have been chosen randomly. A questionnaire was used to collect data for the research. **The results:** According to their level of dental knowledge, 67.2% (n=41) of the teachers are aware that children have permanent incisors by the age of 10; this difference is statistically significant (p<0.001). When questioned whether the tooth fragments can be reattached, only 21.31% (n=13) of respondents are aware that the tooth fragments could be reused to repair damaged teeth, while 42.62% (n=26) responded that they cannot be reattached and 28.57% (n=22) do not know, with the difference between the groups being statistically significant (p<0.001). 62.8% (n=38) of respondents are unaware of the ideal storage conditions for tooth fragments. Only 6.56% (n=4) are aware that teeth should be preserved in moist conditions, whereas 31.14% (n=19) believe that dry environment is required, which is statistically significant. **Conclusion:** As a result of our research, we have concluded that primary school teachers lack the necessary degree of knowledge and expertise to provide adequate first aid to children who have sustained traumatic injuries. **Key words:** dental trauma, students, teachers, first aid, permanent dentition.

Апстракт

Вовед: Денталната траума претставува значаен функционален и естетски проблем. Во зависност од првата помош на детето од страна на присутните во моментот на повредата, зависи прогнозата на повредениот заб. **Цел** на нашето истражување беше да се утврди колкаво е познавањето на наставниците од основните училишта за давање прва помош при дентална траума. **Материјал и метод:** Во нашата студија беа вклучени 61 наставник од две основни училишта. Наставниците беа одбрани по случаен избор. Истражувањето беше спроведено со помош на прашалник. **Резултатите:** Според степенот на познавање на видот на забите 67,2% (n=41) од наставниците знаат дека на 10 годишна возраст децата имаат трајни инцизиви, а разликата е статистички сигнификантна (p<0.001). На прашањето дали скршените делови од забот можат да се искористат, само 21,31% (n=13) знаат дека деловите можат да се искористат за реставрирање на повредените заби, 42,62% (n=26) одговориле дека не можат да се искористат, а 28,57% (n=22) - не знаат, а разликата помеѓу групите е статистички сигнификантна (p<0.001). Во однос на познавањето во каква средина треба да се чуваат скршените делови на забите 62,8% (n=38) не знаат во каква средина, 31,14% (n=19) мислат дека треба сува средина, а само 6,56% (n=4) знаат дека забите треба да се чуваат во влажна средина, што е статистички сигнификантно. **Заклучок:** Од нашето истражување дојдовме до заклучок дека нивото на знаење и способност за пружање соодветна прва помош на деца кои имаат трауматска повреда од страна на наставниците во основните училишта е на незадоволително ниво. **Клучни зборови:** дентална траума, ученици, наставници, прва помош, трајна дентиција.

Introduction

Trauma can occur anywhere, at home, on the street, in the kindergarten, at school, and it disproportionately affects the younger population, children and students.

Injury-prone areas include places where children play, run, and engage in sports¹.

Although dental injuries can occur anywhere, young children and adolescents are most frequently injured at home and at schools². Young children frequently fall and

hurt themselves in the facial area, specifically the surrounding soft tissues and the hard dental tissues, as a result of gait instability and their lack of self-criticism regarding their abilities during the early stages of walking. Additionally, schoolchildren spend significant portion of their day at school participating in sports, play games, and run, which is a significant factor in orofacial injuries. Written sources indicate that 16% of dental injuries among children occur during school hours³.

All over the world, dental trauma is a significant functional and aesthetic issue. Dental trauma can range from a minor, barely perceptible cracks in the tooth enamel that do not affect the child's physical or mental well-being, to a crown fracture or avulsion that also affects the periodontal structures and the dentin-pulp complex in teeth. The prognosis of the injured tooth depends on the first aid provided to the child by those present at the time of the injury, including whether it will heal once more with the surrounding tissue or develop complications such as necrosis of the pulp tissue, ankylosis of the tooth's root, and finally resorption of the root and its expulsion from its position in the mouth occur^{4,5,7,9}.

Due to the aforementioned, children suffer psychological consequences while the parents and the health-care system become incur costs. The outcome of the therapy and the prognosis for the traumatized tooth would be improved by a suitable and prompt intervention by those present at the site of the incident, more specifically by the teachers. They should have sufficient theoretical and practical training for the procedure for various types of tooth injuries in order to respond quickly and appropriately following the injury.

Research has been conducted in various countries, revealing that teachers often lack fundamental knowl-

edge necessary to treat injuries to the surrounding soft tissues and hard dental tissues^{8,9,10,11}.

In light of this, the aim of our research was to determine the level of primary school teachers' knowledge regarding first aid for dental trauma.

Material and method

Our research included 61 teachers from two primary schools. The teachers have been chosen randomly. A questionnaire was used as an instrument of our research. The questionnaire was personally distributed to the project participants. The data were statistically processed using Microsoft Excel and the statistical program PSPP, and the results were as follows.

Results

The study involved 61 teachers from two primary schools, of which 22.95% (n=14) were male and 77.05% (n=47) were female (Chart No. 1). All of the teachers come from urban areas.

The majority of the teachers who responded to our questionnaire do not teach physical education, and 95%

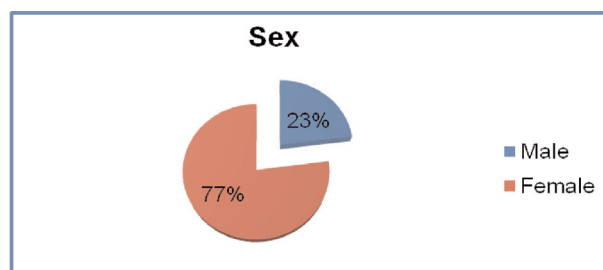


Figure 1. Figures by sex

Table 1. Figures by subjects taught

Subjects taught								
	P.E.		Other subjects		Total		p	X ²
	N	%	N	%	N	%		
Male	1	7.14	13	92.86	14.00	100	0.66	0.10329
Female	2	4.26	45	95.74	47.00	100		
Total	3	4.99	58	95.01	61.00	100		

Table 2. Figure of teachers who have/have not helped a student suffering a traumatic dental injury

Have you ever helped a student/child suffering a traumatic dental injury?								
	Yes		No		Total		p	X ²
	N	%	N	%	N	%		
Male	2	14.29	12	85.71	14.00	100	0.40566	0.52418
Female	4	8.51	43	91.49	47.00	100		
Total	6	9.84	55	90.16	61.00	100		

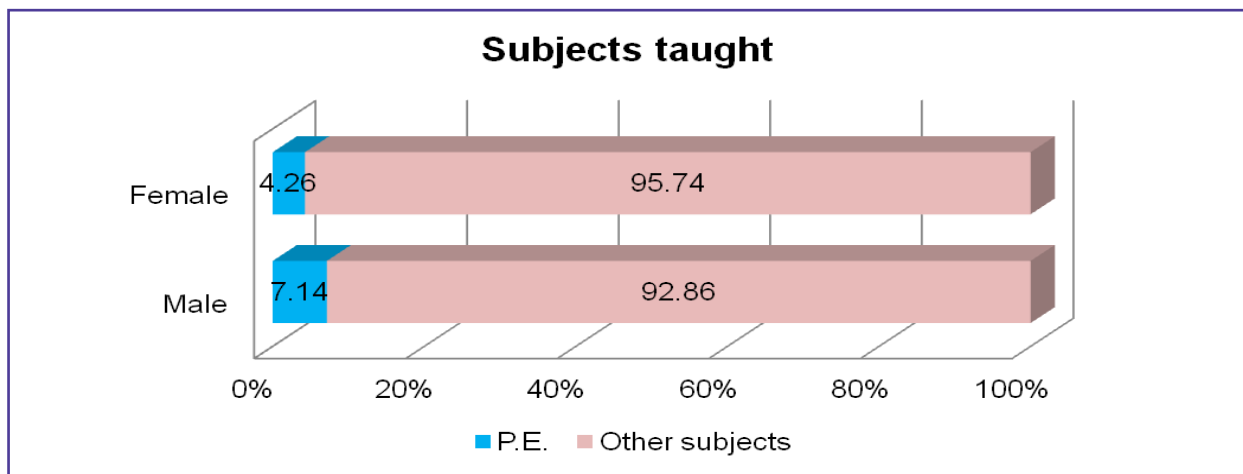


Figure 2. Figures by subjects taught

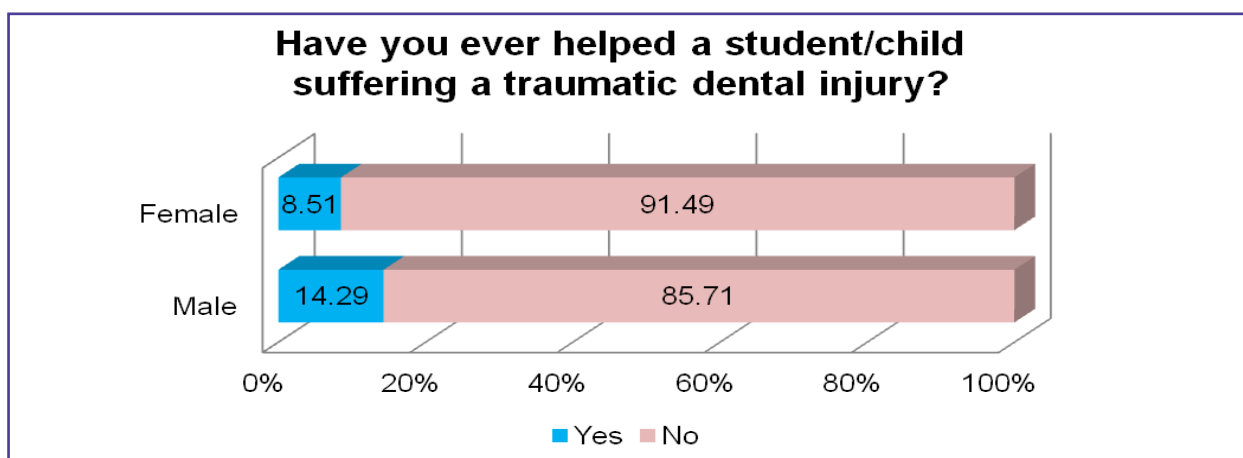


Figure 3. Figure of teachers who have/have not helped a student who has sustained a traumatic dental injury

(n=58) of them teach other subjects. Approximately 5% (n=3) of them are P.E.teachers. They are divided into 7.14% (n=1) males and 4.26% (n=2) females. While females make up 95.74% (n=45) and males who teach other subjects are 92.86% (n=13).

With $p > 0.05$ ($p = 0.66$, $X^2 = 0.10329$), the difference between male and female teachers who teach P.E. and other subjects is statistically insignificant. (Table 1 and Figure 2).

9.84% (n=6) of the teachers have been helpful compared to 90.16% (n=55) who have not provided any help. 14.29% (n=2) of the male teachers have helped compared to 85.71% (n=12) who haven't. With the female teachers the percentage is the following: 91.49% (n=43) haven't helped, 8.51% (n=4) helped the student in need of treatment.

The differ05 ($p = 0.40566$, $X^2 = 0.52418$) (Table 2 and Figure 3).

According to this categorization, 67.2% (n=41) of the teachers know that at the age of 10, children have perma-

nent front incisors. 16.4% (n=10) of respondents state they had primary teeth, and the same number state they don't know what kind of teeth children had at that age. 71.43% (n=10) of the male teachers think they are permanent teeth, 14.29% (n=2) believe they are primary teeth, and the same number do not know what kind of teeth 10-year-old children have. While 65.96% (n=31) of the female teachers know that the students' teeth are permanent, 17.02% (n=8) believe they are deciduous, and the same number 14.29% (n=8) do not know the type of teeth in 10-year-old children.

With $p < 0.001$ ($p < 0.001$, $X^2 = 155.92204$), there is a statistically significant difference between the groups in terms of their knowledge of the kind of teeth 10-year-old children have (Table 3 and Figure 4).

Only 21.31% (n=13) know that the fragments can be reattached to the fractured tooth. 28.57% (n=22) have responded that they do not have the knowledge, while 42.62% (n=26) state that fractured parts cannot be reattached. Male teachers' responses range from 42.86%

Table 3. Level of teacher comprehension of tooth type in children

Injured front teeth in a 10-year-old student are as follows:										
	Primary teeth		Permanent teeth		You Do not know		Total		p	X ²
	N	%	N	%	N	%	N	%		
Male	2	14.29	10	71.43	2	14.29	14	100	p<0.001	155.92204
Female	8	17.02	31	65.96	8	17.02	47	100		
Total	10	16.40	41	67.20	10	16.40	61	100		

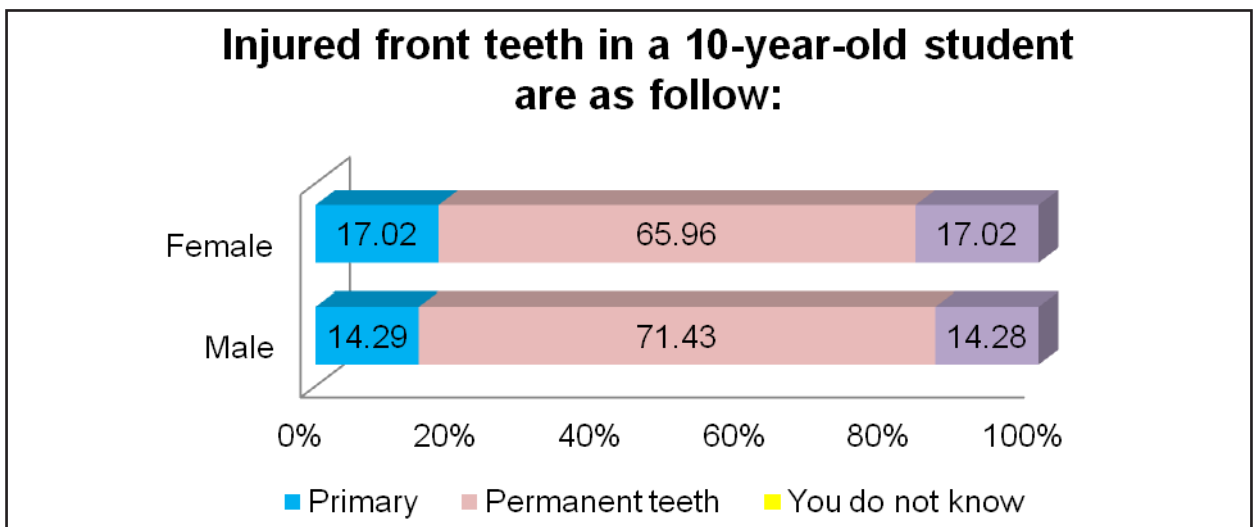


Figure 4. Level of teacher comprehension of tooth type in children

(n=6) saying the fractured fragments cannot be used again to 28.57% (n=4) saying they can. The same is the number of those lacking the knowledge in reference to the aforementioned. 42.55% (n=20) of the female teachers have responded that the fractured fragments of the tooth cannot be reattached. Only 19.5% (n=9) of respondents (n=18) state the opposite, compared to 38.30% (n=18) who don't know whether the fractured tooth can be used.

With p<0.001 (p<0.001, X²=74.0233), the difference between the groups is statistically significant (Table 4 and Fig. 5).

Regarding this issue, 62.8% of the respondents (n=38) cannot provide the answer. Only 6.56% (n=4) of the respondents are aware that moist environment is required for the fractured fragment until professional dental treatment is provided, compared to 31.14% (n=19) who believe that a dry environment is required.

Table 4. Teachers' opinion if fractured teeth fragments can be reattached

Found fractured teeth fragments:										
	Can be reused		Cannot be reused		You Do not know whether they can be used or not		Total		p	X ²
	N	%	N	%	N	%	N	%		
Male	4	28.57	6	42.86	4	28.57	14	100	p<0.001	74.0233
Female	9	19.15	20	42.55	18	38.30	47	100		
Total	13	21.31	26	42.62	22	36.07	61	100		

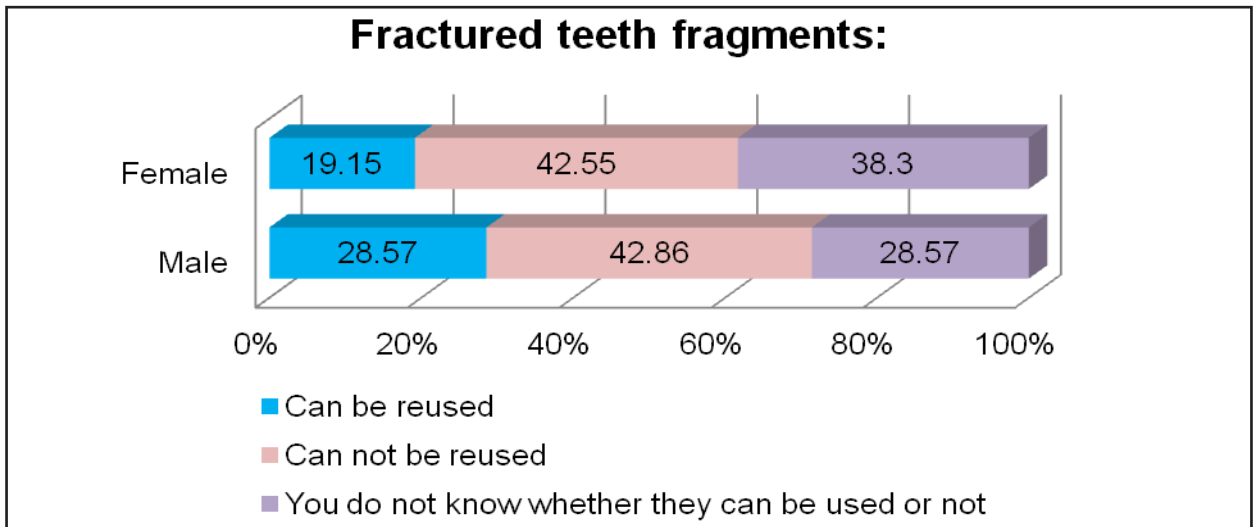


Figure 5. Teachers' opinion if fractured teeth fragments can be reattached

50% (n=7) of the male teachers lack knowledge on the issue, 28.57% (n=4) selected dry environment, 21.43% (n=3) voted for moist environment, compared to 65.96% (n=31), 31.91% (n=15) and 2.13% (n=1) of the female teachers, respectively.

With a p-value of 0.05 ($p=0.036742$, $X^2=6.607683$), the percentage difference between the groups is statistically significant (Table 5 and Fig. 6).

The child must be subject to additional medical procedures following the traumatic dental injury. 3.28 per-

Table 5. Environment in which the fractured tooth after the trauma should be kept before treated by a dentist

Storage environment of fractured tooth fragments										
	Dry environment		Moist environment		You Do not know		Total		p	X ²
	N	%	N	%	N	%	N	%		
Male	4	28.57	3	21.43	7	50.00	14	100	0.036742	6.607683
Female	15	31.91	1	2.13	31	65.96	47	100		
Total	19	31.14	4	6.56	38	62.30	61	100		

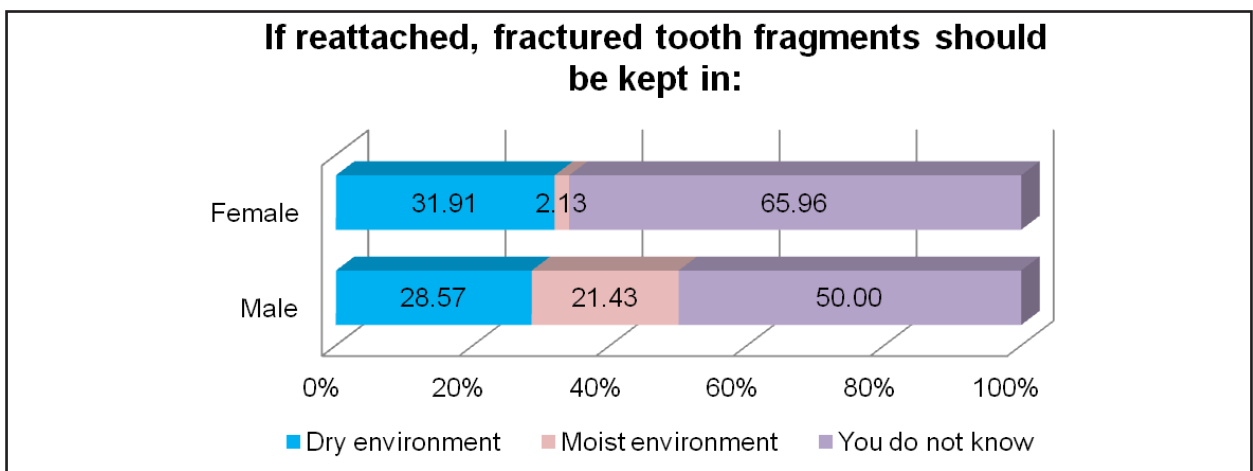


Figure 6. Environment in which the fractured tooth after the trauma should be kept before treated by a dentist

Table 6. Type of medical care teachers will seek following tooth injury in a child

Type of medical care required following dental trauma:																
	General practitioner		Hospital		Primary care physician		The nearest dentist		Faculty of dentistry		Pedodontist		Total		p	X ²
	N	%	N	%	N	%	N	%	N	%	N	%	N	%		
Male	1	7.14	1	7.14	1	7.14	5	35.72	0	0	6	42.86	14	100	p<0.001	119.103
Female	1	2.13	2	4.25	1	2.13	29	61.70	0	0	14	29.79	47	100		
Total	2	3.28	3	4.92	2	3.28	34	55.74	0	0	20	32.78	61	100		

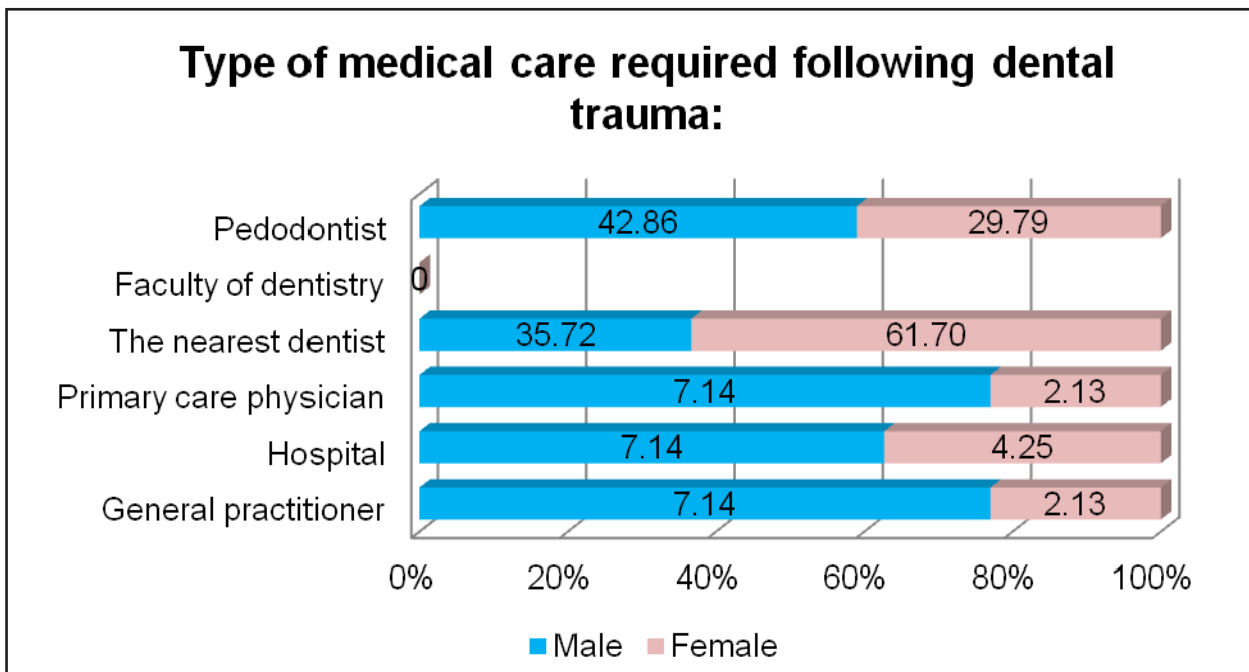


Figure 7. Type of medical care teachers will seek following tooth injury in a child

cent (n=2) of the teachers will send the injured child to the general dentist, 4.92% (n=3) will take him/her to hospital, 3.28% (n=2) will take him/her to the primary care dentist, 55.74% (n=34) of the respondents will take the child to the nearest dentist and 32.78% (n=20) will take the child to a pedodontist. None of the respondents would take the child to the Faculty of Dentistry.

7.14% (n=1) of male teachers will take the child to a general practitioner, 7.14% (n=1) will take the child to a hospital, 7.14% (n=1) to a primary care physician, 35.72% (n= 5) will take the child to the nearest dentist, 42.86 % (n=6) will take the child to a pedodontist.

2.13% (n=1) of the female teachers will take the child to a general practitioner, 4.25% (n=2) will take the child to a hospital, 2.13% (n=1) will take the child to a primary care physician, 61.70% (n=29) will take the

child to the nearest dentist, 29.79% (n=14) will take the child to a pedodontist.

With p<0.001 (p<0.001, X²=119.1026144) the difference between the groups is statistically significant (Table 6 and Fig.7).

Discussion

Dental trauma is a major global health issue and a common occurrence among the young population. Data on the prevalence of dental trauma varies by country and ranges from 4.9% to 59%. It is estimated that at least half of children have suffered some form of trauma. Dental trauma is more common in young children and adolescents overall, and more frequently in boys than in girls. Girls tend to sustain injuries during their second and third

years of life, while boys typically sustain injuries between the ages of 2-4 and 9-10.

Students spend the majority of their time in class, but they occasionally run, play, or take part in some sports. It is possible for any type of trauma to happen during those activities, especially trauma to the orofacial area. Therefore, it is imperative that all members of the teaching staff, especially those who teach physical education, are trained and prepared to administer first aid in the event of any injury, including dental trauma. The majority of injuries happen in these classes.

Written materials have shown that numerous countries lack this type of training for their teaching staff. A small percentage of teachers have minimal, but unsatisfactory knowledge of dental trauma. These teachers haven't received proper training; instead, they have accidentally learned about dental trauma procedure from brochures and the Internet. Others lack any knowledge thus their comprehension of dental trauma is insufficient^{1,2,11,13,14,15,16,17}.

Out of a total of 61 teachers, only 6 (9.84%) have attended and provided aid to an injured student. In contrast to our findings, obtained data show that 20–60% of teachers have witnessed dental trauma and have been asked to provide first aid on the spot^{2,6,8,11,12,13,14,15}. Due to the large number of students in schools, there are numerous traumatic cases and teacher interventions. As a result, there are proportionally more dental injuries as there are more children.

67.2% of the respondents are aware that teeth in the front are permanent in 10-year-old children, 16.4% believe they are primary teeth, and the same number are unaware of the dentition type present at that age. This indicates that people lack general awareness of tooth physiology as a result of the low health culture. Consequently, little attention is paid to dental health, which in turn increases the risk of caries in our population. According to Fares S Al-Sehaibani et al.¹¹, 66.3% of their respondents are unaware that 10-year-old children have permanent front teeth. Costa Fabio Wildstone et al.¹⁵, has found results comparable to ours, showing that 58.7% of teachers are aware that an 8-year-old child's teeth in the front are permanent, while 29.4% believe they are primary teeth.

Only 21.31% of teachers are aware that the fractured fragment found following a traumatic injury can be reattached. The remaining 36.07% are not aware about its usability, while 42.62% believe it cannot be used. Our findings correlate with other written data, where 31% of mothers would take the fractured fragment, 46.1% would not use it, and 22.9% do not know what to do with it^{8,17}. This information is crucial because providing the fractured tooth fragment not only saves time but also gives a second chance to the fractured tooth to attain perfect aesthetics.

Only 6.56% of respondents have correctly identified a moist environment as the one in which the fractured tooth fragment should be transported to a dentist; 31.14% responded that it should be in dry environment; and 62.3% don't know the answer. Our data correlate with those obtained from other sources. According to research by Fajlinda Baharin et al, 2.5% of the teachers who were questioned would take the fractured tooth in moist environment, in some liquid^{6,10,15}.

In case of a dental injury when the child feels fine apart from the orofacial tissues, 55.74% of the teachers have correctly responded to the question about the type of medical assistance they would seek if a dental injury occurred without other symptoms, choosing the nearest dentist. 32.78% would refer him/her to a pedodontist. The remaining 11% would take the child to a general practitioner or hospital. Obtained data from written sources correlate to ours in relation to the type of medical aid provided after dental trauma¹⁴. Most teachers will also seek assistance from a dentist, whether it be the nearest dentist, a primary care dentist, or a pedodontist.

Conclusion

Traumatic injuries to the orofacial area and dental traumas are prevalent all around the world, particularly among the young population who have a low level of self-criticism regarding their physical and dynamic capabilities which consequently results in high incidence of traumatic injuries, including to their teeth.

Our research has led us to the conclusion that primary school teachers lack the necessary knowledge or capability to adequately provide first-aid to children who have sustained traumatic dental injuries.

It is imperative that all participants in the educational process receive sufficient instruction in first aid for traumatic dental injuries from professionals due to the existence of a real possibility that any child attending the school could suffer from a traumatic injury, whether it occurs in physical education class or over the holidays. This would prevent complications caused by inappropriate and untimely reaction to an injury in its earliest stages.

Reference

1. Kaul R. et. all. Evaluation of knowledge, and attitude toward emergency dental trauma management among the schoolteachers of Kolkata. *Indian J Dent. Res.* 2017;28(6): 595-603.
2. Kumar A. et all. Knowledge, attitude, and practice of elementary school teachers toward emergency management of dental trauma in Sirmaur District, Himachal Pradesh: A questionnaire study. *Indian J Dental Res.* 2017;9(3): 194-197.
3. Raouf M. et all. Elementary school staff knowledge and attitude with regard to first-aid management of dental trauma in Iran: a basic premise for developing future intervention, *Dental traumatology*, 2012;28 (6):441-447.

-
4. Yassen G. H. et al. Knowledge and attitude of dental trauma among mothers in Iraq. *European Arch. of Pediatric Dentistry* 2013; 14:259-265.
 5. Kaur H. et al. Prehospital emergency management of avulsed permanent teeth: Knowledge and attitude of schoolteachers. *Indian J. Dent. Res.* 2012;23 (4):556.
 6. Al-Asfour A. et al. School teachers' knowledge of tooth avulsion and dental first aid before and after receiving information about avulsed teeth and replantation. *Dent Traumatol.* 2008;24(1):43-49.
 7. Haragushiku G. A. et al. Knowledge and attitudes toward dental avulsion of public and private elementary schoolteachers. *J Dent Child.* 2010;77(1):49-53.
 8. Qiu L. et al. Preschool teachers' knowledge on traumatic dental injuries first-aid in Shanghai, China. *Research Square*;2022.
 9. Ingle N.A. et al. Knowledge and attitudes of primary wing schoolteachers toward dental trauma and its management in Qassim, Kingdom of Saudi Arabia. *Journal of Oral Health and Community Dentistry.* 2020;14 (1):22-26.
 10. Baharin F. et al. Knowledge and attitude towards dental trauma management among primary school teachers. *Padjadjaran Journal of Dentistry.* 2019;31(3): 161-166.
 11. Al-Schaibany F.S. et al. Elementary school staff knowledge about management of traumatic dental injuries. *Clin Cosmet Investig Dent.* 2018;10:189-194.
 12. Sharma A. et al. Dental trauma management and its awareness in schoolteachers: A survey in Burhanpur, Madhya Pradesh, India. *International Journal of Applied Dental Sciences* 2018; 4(4): 371-375.
 13. Ivancic N. et al. Preschool teachers' knowledge and attitudes about dental trauma in Rijeka, Croatia: a cross-sectional study. *J Oral Res* 2021;10 (4):1-7.
 14. Tzimpoulas N. et al. A questionnaire-based survey for the evaluation of the knowledge level of primary school teachers on first-aid management of traumatic dental injuries in Athens, Greece. *Dent Traumatol.* 2020;36(1):41-50.
 15. Wildson Gurgel C.F. et al. Dental trauma: Knowledge and attitudes of Community Health Workers. *J Craniofac Surg.* 2014;25(5):490-495.
 16. Neshine N. et al. Comparison and Evaluation of Attitude and Knowledge Towards the Management of Dental Injury in School Teachers Before and After Oral Health Education. *Int J Clin Pediatr Dent.* 2018;11(5):425-429.
 17. Al-Schaibany F.S. et al. Knowledge on the management of traumatic dental injuries among Saudi Mothers. *Clin Cosmet Investig Dent.* 2018;10:123-128.

EVALUATION OF THE EFFICIENCY OF BOTOX THERAPY IN A PATIENT WITH UNILATERAL IDIOPATHIC NEURALGY ON THE TRIGEMINAL NERVE

ЕВАЛУАЦИЈА НА ЕФИКАСНОСТА НА БОТОКС ТЕРАПИЈАТА КАЈ ПАЦИЕНТ СО ИДИОПАТСКА, УНИЛАТЕРАЛНА ТРИГЕМИНАЛНА НЕУРАЛГИЈА КОЈА ГИ НАПАЃА ТРИТЕ ДИВИЗИИ НА ТРИГЕМИНАЛНИОТ НЕРВ

Shushak Z.¹, Popovski V.², Mitikj J.³, Baftijari D.⁴

¹PhD, DDM, DDS - Faculty of Medical Sciences, UGD – Stip, Private health institution „Dentoria-1 Specialist practice for oral surgery and implantology“ Ohrid, Republic of North Macedonia, ²PhD, MSD, MFS, DDM, MD – Faculty of Dentistry, „Ss. Cyril and Methodius“ University Skopje, Republic of North Macedonia, Clinic for maxillofacial surgery, ³PhD, DDM, MSD, trainee in periodontology - Private health institution „Gudadent“ Skopje, Republic of North Macedonia, ⁴PhD, MSD, DDM, MFS - Faculty of Medical Sciences, Tetovo University, Private health institution „Vita Dent - Implantology Center“ Tetovo, Republic of North Macedonia.

Abstract

Trigeminal neuralgia (TN) often known as „painful tick“ or „*tic douloureux*“ is a neurogenic disease that affects the facial segment. According to the intensity and clinical parameters of the pain, it is one of the most severe pain that the patient experiences on a daily basis. Botox therapy in trigeminal neuralgia is a relatively new strategy (*innovative therapy for the treatment of orofacial pain*), which is still in the experimental phase worldwide. Positive results have been reported in middle-aged and elderly patients who have been receiving conservative treatment (medications), despite skepticism regarding the side effects and complications of surgical treatment. The purpose of this case report is to describe the effect of botox therapy and improve the quality of life in a patient with *idiopathic, unilateral trigeminal neuralgia which affects all three branches of the trigeminal nerve* and is one of the most severe forms of this disease. **Key words:** Botox therapy, idiopathic trigeminal neuralgia, trigeminal nerve.

Апстракт

Тригеминалната неуралгија (ТН) позната уште како „болен тик“ т.е. („*tic douloureux*“) е нервно нарушување (патологија) која го засега лицевиот сегмент. По интензитет и клинички параметри на болката се смета дека е една од најтешките преживевани состојби со кои пациентот секојдневно се соочува. Ботокс терапијата при тригеминалната неуралгија е релативно нова стратегија, која во светски рамки се уште е во експериментална фаза, особени позитивни резултати се евидентираат кај средовечни и постари пациенти кои се долго време на конзервативен третман (лекарства), а се однесуваат со скепса кон несаканите ефекти и компликации на хирушкиот третман. Целта на овој приказ на случај е презентирање на ефектот на ботокс терапијата и подобрување на квалитетот на живот кај пациентка со *идиопатска, унилатерална тригеминална неуралгија која ги напаѓа трите дивизии на тригеминалниот нерв* и е една од најтешките форми на ова заболување. **Клучни зборови:** Ботокс терапија, идиопатска унилатерална тригеминална неуралгија, тригеминален нерв.

Introduction

Trigeminal neuralgia (TN) is defined by International Headache Society as „*unilateral disorder characterized by brief pain similar to an electric shock, sudden painful attacks and interruption of attacks limited to the distribution of one or more branches of the trigeminal nerve*“¹. According to International Association for the Study of Pain and International Headache Society, the latest classification

distinguishes 3 etiological categories: idiopathic TN (without neurovascular contact (WNC) or WNC without morphological changes of the trigeminal nerve); *classic TN* (due to neurovascular compression with morphological changes of the trigeminal nerve) and *secondary TN* (due to major neurological diseases such as tumors and multiple sclerosis)².

The treatment of TN remains a major challenge. The treatment principles remain basically the same, and widely

used medical treatment options include antiepileptic drugs, muscle relaxants and neuroleptic agents³. Botox therapy has been successfully applied as an adjunctive therapy for patients suffering from TN in the recent decades⁴.

Botulinum neurotoxin is a complex protein produced by the gram-positive anaerobic bacteria *Clostridium botulinum*. To date, seven serotypes of the toxin, i.e. A-G have been identified, according to their antigenic characteristics, however only A and B serotypes are commercially available. The serotypes range in size from 300 to 900 kDa and are composed of a neuroactive entity called botulinum neurotoxin (BoNT) which helps stabilize the complex protein. Each of the seven serotypes has a similar structure and molecular weight, consisting of heavy (H) chain and light (L) chain bonded by disulfide bond⁵.

Botulinum toxin type A, is regarded as the most powerful subtype. BoNT typ A is believed to be effective in the treatment of migraine and myofacial pain. Subcutaneous or mucosal (perineural) application of BoNT typ A is effective in adult TN patients. Numerous studies shown a decrease in average pain intensity and frequency with a maximal effect within 4 to 6 weeks⁶⁻⁸.

The purpose of this case report is to demonstrate the efficacy of botox therapy and improve the quality of life in patients with trigeminal neuralgia.

Case Report

A 57-year-old patient is being treated with a conservative method at the Maxillofacial Surgery Clinic in Skopje for a period of 7 years. Anamnestic patient describes unilateral pain localized in three divisions of trigeminal nerve. The pain occurs daily and might last anywhere from a few seconds to a several hours. Occurs spontaneously and with touch provocations and feeding. According to the patient's medical history we learn that the patient is treated with a conservative approach - Tegretol 400 mg twice a day with insignificant results in the last period over the past year.

The patient has suffered from hypertension for 7 years and is currently receiving antihypertensive therapy ENAP 10 mg 1 tablet daily. Her blood pressure ranges between 90 and 130 and between 120 and 180 mmHg. Extraoral examination revealed pain in the trigger points (figure 1.), However the intraoral examination reveals complete loss of teeth on the affected side (partial tooth loss in the upper and lower jaw, caries-non-resistant dentition, poor oral hygiene and presence of periodontal disease). In the previous period, paraclinical examinations including X-ray orthopantomography, computed tomography (CT), and laboratory blood tests were performed. A diagnostic block anesthetic was also administered to assist in the determination, i.e. confirmation of diagnosis.

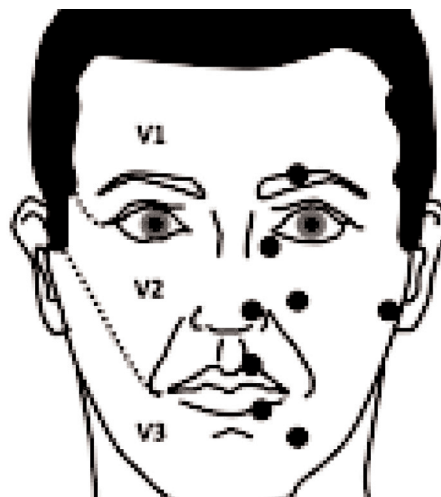


Figure 1. Regimens and application points for botulinum toxin.

We used Dysport (BoNT/A) 300 UI. The manufacturer is the company Ipses Biopharm Limited. The active ingredient of Dysport is *Clostridium botulinum* type A toxin – haemagglutinin complex (300 units). Before the administration, Dysport was dissolved in a physiological solution 0.9%. Our examination was carried out under in vivo conditions in order for the result to be a demonstration of the actual efficacy of botox therapy. The patient was examined by a therapist to exclude the subjective factors that would come from the work of several therapists. We gave the patient a questionnaire and she was asked to fill it out at zero, first, fifth and tenth week after the botulinum toxin application. **200 UI botulinum toxin type A. (DYSPORT Botox® - Ipsen Biopharm Limited)** (figure 2.), are administered in 3 regimens: **75 UI in the mental, 75 UI in the infraorbital и 50 UI in the frontal region.** The Botox solution was applied perineurally to the mental region using the intraoral method of the technique for anesthetizing n. mentalis. The patient's mouth was closed, and the lower lip and cheek were retracted. The needle puncture was in the alveolar mucosa in the area of the mesial root of the first molar and moved along the bone towards the mental foramen. The Botox solution was applied perineurally in the infraorbital region, following the intraoral method of the infraorbital anesthesia placement technique. The patient's mouth was closed or slightly open. The upper lip was retracted and the needle puncture was in the alveolar mucosa above the second incisor. The movement of the needle was upwards, backwards and outwards. The angle of the needle is such that if applied simultaneously in both foramens the needles would cut at the incisal point. A bone border was felt at a depth of 1.5 to 2 cm. This area encompasses the infraorbital canal. The appli-



Figure 2. UI botulinum toxin type A (DYSPORT Botox® - Ipsen Biopharm Limited) and application set of botulinum toxin.

cation of the solution was implemented within the given region. In the frontal region, the Botox solution was applied subcutaneously at 3 points. The central point was in the center of the glabella, and the lateral ones were 2 cm away, above the supraorbital foramen. Pain evaluation and evaluation for improved quality of life have utilized the following parameters: visual analogue scale (VAS), numerical scale for assessing pain (NRS), facial expression rating scale (FRS), Wong - Baker scale, Hamilton's Anxiety Scale (HAM-A) and the Hamilton Depression Scale (HDRS).

Results

Table 1. Mental Region - pain evaluation and evaluation for improved quality of life parameters.

Mental Region	Week zero	First week	Fifth week	Tenth week
VAS-1	(0)	(7)	(6)	(3)
VAS-2	(10)	(3)	(3)	(8)
NRS	(10)	(4)	(3)	(7)
FRS	(8)	(4)	(3)	(7)
HAM-A	(severe form of anxiety)	(severe form of anxiety)	(moderate depression)	(severe form of anxiety)
HDRS	(severe depression)	(moderate depression)	(moderate depression)	(moderate depression)

Table 2. Infraorbital region - pain evaluation and evaluation for improved quality of life parameters.

Mental Region	Week zero	First week	Fifth week	Tenth week
VAS-1	(0)	(5)	(6)	(3)
VAS-2	(10)	(5)	(4)	(7)
NRS	(10)	(5)	(4)	(7)
FRS	(10)	(5)	(4)	(7)
HAM-A	(severe form of anxiety)	(severe form of anxiety)	(moderate depression)	(severe form of anxiety)
HDRS	(severe depression)	(moderate depression)	(moderate depression)	(moderate depression)

Table 3. Frontal region - pain evaluation and evaluation for improved quality of life parameters.

Mental Region	Week zero	First week	Fifth week	Tenth week
VAS-1	(0)	(8)	(9)	(6)
VAS-2	(10)	(2)	(1)	(4)
NRS	(10)	(2)	(2)	(4)
FRS	(9)	(2)	(2)	(4)
HAM-A	(severe form of anxiety)	(severe form of anxiety)	(moderate depression)	(severe form of anxiety)
HDRS	(severe depression)	(moderate depression)	(moderate depression)	(moderate depression)

Discussion

Trigeminal neuralgia is a relatively rare pathological condition (1/8000), which dramatically reduces the quality of life of affected individuals, not only due to pain attacks, but also due to other accompanying conditions, such as anxiety and depression⁹. TN is often undiagnosed or untreated in practice, and more attention has been paid to this disease in the last decade. Treatment can be difficult and unsatisfactory. It consists mainly of patient education and pharmacotherapy with tricyclic antidepressants. Anticonvulsants, analgesics and surgery did not show significant results in TN therapy. Other pain relief strategies include hot and cold compresses, acupuncture, splints¹⁰.

Botulinum neurotoxin A is a powerful neurotoxin. It can inhibit the release of acetylcholine from neuromuscular junctions, resulting in muscle relaxation. It also inhibits vanilloid receptor expression TRPV1 on the surface of peripheral receptors responsible for inflammatory hyperalgesia. In addition, studies indicate that the analgesic effect of botulinum toxin is independent of its muscle relaxation.

Botulinum toxin has been used for over 20 years to treat various neurological diseases associated with pathologically increased muscle tone or impaired autonomic nerve regulation¹¹. Our case has shown a positive effect of botulinum toxin on pain management in a patient with severe form of TN, which is consistent with studies of Piovesan and cop.¹². A recently published meta-analysis concluded that botox therapy can be a safe and effective treatment option for patients with TN, with an average reduction in daily paroxysms to 29,8 %¹³. Jiangshan Wei and Xiangyu Zhu concluded in their meta-analysis that botox therapy is an effective and reliable method of treating TN. Due to the limited size and heterogeneity of the specimens, additional large and well-designed randomized controlled experiments are imperative in proving these results¹⁴. In the last decade, botulinum toxin has been used in numerous studies with the vast majority involving subcutaneous, intracutaneous, and perineural injections in the trigger zones of the painful facial region¹⁵⁻¹⁷.

In our case, the patient showed significant results in all parameters examined, with the greatest reduction in pain and other associated symptoms occurring in the fifth week, and the greatest improvement in quality of life occurring between the first to tenth weeks, accompanied by a period with a decrease in the degree of anxiety and depression severity.

Conclusion

This case report suggests that botox therapy for trigeminal neuralgia may be a useful method of pain management, improving the clinical picture and the quality of life in the most severe forms of the disease. Additional scientific studies are needed to confirm our results and further evaluate

doses when applied, type and techniques of application, as well as duration of treatment.

Reference

1. Headache Classification Committee of the International Headache Society. The International Classification of Headache Disorders. Cephalalgia. 2018; 38 (3rd edition): 1-211.
2. L. Bendtsena, J. M. Zakrzewskab,c, J. Abbottd, M. Braschinskye, G. Di Stefanof, A. Donnetg,P. K. Eideh,i, P. R. L. Lealj,k, S. Maarbjerga, A. Mayl, T. Nurmikkom, M. Obermannn, T. S. JensenoandG. Cruccu. European Academy of Neurology guideline on trigeminal neuralgia. European Journal of Neurology 2019,26:831–849.
3. Obermann M. Recent advances in understanding/managing trigeminal neuralgia. F1000Research2019,8: 505. doi.org/10.12688/f1000research.16092.1.
4. Jorns TP, Ieamsuwantada T, Puttakun P, Tiamkao S, Kitkhuandee A, Noisombut R. Single-Dose Botulinum Toxin as Adjunctive Treatment for Trigeminal Neuralgia: A Pilot Study. J Med Assoc Thai 2019; 102:68.
5. Poulain B, Longchamp E, Jover E, Popoff MR, Molgo J. Mécanismes d'action des toxines et neurotoxines botuliques. Ann Dermatol Venereol 2009 ; 136:S73-S76.
6. Wu S, Lian Y, Zhang H, et al. Botulinum Toxin Type A for refractory trigeminal neuralgia in older patients: a better therapeutic effect. J Pain Res. 2019;12:2177–2186. Published 2019 Jul 17. doi:10.2147/JPR.S205467.
7. Zhang H, Lian Y, Xie N, Cheng X, Chen C, Xu H, Zheng Y."Factors affecting the therapeutic effect of botulinum toxin A on trigeminal neuralgia: A follow-up retrospective study of 152 patients". Experimental and Therapeutic Medicine 18.5 (2019): 3375-3382.
8. Jing L, Ying-Ying X, Qi-Lin Z, Wei-Feng L. Efficacy and Safety of Botulinum Toxin Type A in Treating Patients of Advanced Age with Idiopathic Trigeminal Neuralgia. Hindawi Pain Research and Management. 2018, 7365148. doi.org/10.1155/2018/7365148.
9. Eder G, Juliana G. C, and Gerald W. Z. Trigeminal neuralgia: An overview from pathophysiology to pharmacological treatments. Molecular Pain. 2019; 16:1-18.
10. Agostoni E, Frigerio R, Santoro P. Atypical facial pain: clinical considerations and differential diagnosis. Neurol Sci (2005) 26:S71–S74DOI 10.1007/s10072-005-0412-y.
11. Himshweta D. Therapeutic effect of botulinum toxin in trigeminal neuralgia: Case Report. Ec Dental Science 18.5 (2019):992-996.
12. Piovesan E. An open study of botulinum -A toxin treatment of trigeminal neuralgia. Neurology 65.8 (2005):1306-1308.
13. M. E. Morra, A. Elgebaly, A. Elmaraezy et al., "Therapeutic efficacy and safety of botulinum toxin in a therapyintrigeminal neuralgia: a systematic review and meta-analysis of randomized controlled trials," Journal of Headache and Pain, vol. 17, no. 1, p. 63, 2016.
14. Wei J, Zhu X, Yang G, et al. The efficacy and safety of botulinum toxin type A in treatment of trigeminal neuralgia and peripheral neuropathic pain: A meta-analysis of randomized controlled trials. Brain Behav. 2019; 9:e01409. https://doi.org/10.1002/brb3.1409.
15. Ngeow WC, Nair R. Injection of botulinum toxin type A (BOTOX) into trigger zone of trigeminal neuralgia as a means to control pain. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:e47–55.
16. Xia JH, He CH, Zhang HF, et al. Botulinum toxin a in the treatment of trigeminal neuralgia. Int J Neurosci 2016;126:348–53.
17. Burmeister J, Holle D, Bock E, et al. Botulinum neurotoxin type A in the treatment of classical trigeminal neuralgia (BoTN): study protocol for a randomized controlled trial. Trials 2015;16:550.

