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CHARACTERISTICS OF THE GINGIVAL EPITHELIUM OF CHILDREN'S THIRD MOLARS AT DIFFERENT STAGES OF ROOT FORMATION

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Summary

Extraction of impacted and dystopic third molars is a rather traumatic manipulation accompanied by a large bone defect and wound infection. Therefore, it is advisable to perform hermectomy, which has a number of advantages over typical and atypical methods of tooth extraction. An important stage of any surgical intervention is the postoperative period, which is characterized by a certain duration and the presence of complications, which is due, among other things, to the regenerative potential of tissues, especially epithelial tissues.

Aim of the study is to evaluate the condition of the gingival epithelium in the area of third molars in children who have to have these teeth extracted for orthodontic reasons, taking into account the stage of tooth formation, i. e. its root.

Material and Methods. *Third molar extraction for orthodontic indications was performed in 95 children aged 11 to 18 years. Clinical and radiologic examinations were performed in three groups: Group I (n=30) – children aged 11-13 years; Group II (n=35) – children aged 13-16 years, and Group III (n=30) – children aged 16-18 years. During surgery, gingival tissue was taken from the adjacent areas for examination. The material was fixed, dehydrated, and paraffin-embedded for histologic processing. Immunohistochemical techniques were performed according to the manufacturer's protocols. Specifically, immunohistochemical detection of Ki-67 antigen was performed with primary antibodies against it. Digital copies of the images were analyzed using the specialized computer program ImageJ v1.52 (freeware, USA). The obtained digital data were statistically processed.*

*The digital data obtained were statistically processed. A preliminary test for normality of the distribution was carried out using the Shapiro-Wilks method. According to this criterion, the hypothesis of normality of the distribution was not rejected for all the statistical samples studied (at the significance level of $p=0.05$), so we used mainly parametric methods of statistical analysis: calculation of the arithmetic mean and its error, unpaired two-sided Student's *t*-test. However, because the statistical samples were small, the non-parametric Mann-Whitney test was used in addition to the Student's *t*-test.*

The study was conducted as part of the complex research work of the Department of Pediatric Dentistry of the Bukovinian State Medical University on the topic «Development of methods of prevention and treatment of the main dental diseases in children, taking into account the risk factors of their development» (state registration number: 0121U110122). The conducted research complied with bioethical norms, as stated in the conclusions of the Bioethics Commission of the Bukovinian State Medical University (№ 1 dated 17.09.2020).

Conclusions. *Thus, the histological and immunohistochemical study of the gingiva in the area of the third molar allows us to conclude that during the formation of the root of this tooth, a number of changes occur in the epithelial cover of the gingiva, namely: an increase in the number of epithelial layers with a decrease in their proliferative activity. The obtained data indicate a decrease in the regenerative potential of the multilayered squamous epithelium with increasing age of the child and the degree of third molar formation, which is reflected in the clinical picture of the course of surgical intervention.*

Key words: *Children; Tooth Extraction Surgery; Third Molars: Gingival Epithelium.*

Introduction

Third molar retention and dystopia occurs in 35-50 % of the population and is one of the prerequisites for the development of a number of orthodontic problems. Extraction of these teeth has a preventive effect on the development of dentoalveolar anomalies and deformities and is a prerequisite for the stability of orthodontic treatment [1, 2]. However, the extraction of retained and dystopic third molars is a rather traumatic manipulation associated with a large bone defect and wound infection [3-6]. Therefore, according to many scientists [7-8], it is advisable to perform a hermectomy, which has a number of advantages over typical and atypical methods of tooth extraction. In addition, the third molar follicular tissue removed during surgery is a valuable source of stem tissue, which is currently widely used in experimental and practical medicine [9-20].

An important phase of any surgical procedure is the post-operative period, which is characterized by a certain duration and the presence of complications, due in part to the regenerative potential of tissues, especially epithelial tissues.

Aim of the study is to evaluate the state of gingival epithelial coverage in the area of third molars in children who

have had these teeth extracted for orthodontic reasons, taking into account the stage of tooth formation, namely the root.

Material and Methods

Third molar extraction for orthodontic indications was performed in 95 children aged 11 to 18 years. The clinical examination of the children was carried out using conventional methods. Radiographic assessment of the condition of the third molar ridges was performed according to the Demirjian method [21], which is currently widely used internationally [22, 23]. Clinically and radiographically, three observation groups were identified: I (n=30) – children aged 11-13 years whose third molar was at the stage of weakly mineralized rudiment (Demirjian stage D); II (n=35) – children aged 13-16 years whose third molar was at the stage of mineralized rudiment and the initial stages of root formation (Demirjian stages E and F) and III (n=30) – children aged 16-18 years whose third molar was at the final stages of root formation (Demirjian stages G and H).

During surgery, gingival tissue was taken from adjacent areas for examination. The material was fixed in neutral pH buffered 10 % formalin solution for 20-22 hours. After fixation, the gingiva was dehydrated in an isopropyl alcohol system and embedded in paraffin at 560C for

further histological processing. Standard serial histological sections of 5 µm thickness were prepared using a rotary microtome. After deparaffinization, haematoxylin and eosin staining was performed on histological sections [24], and immunohistochemical techniques were performed on other serial sections according to the manufacturer's protocols. In particular, immunohistochemical detection of Ki-67 antigen was performed using primary antibodies against this antigen. Visualization of the primary antibodies was performed using a polymeric imaging system with diaminobenzidine dye, which gives a brown color to the sites of the studied antigens. Digital copies of the image were analyzed using a specialized computer program ImageJ v1.52 (freeware, USA) [25].

The digital data obtained were statistically processed. A preliminary test for normality of the distribution was carried out using the Shapiro-Wilks method. According to this criterion, the hypothesis of normality of the distribution was not rejected for all the statistical samples studied (at the significance level of $p=0.05$), so we used mainly parametric methods of statistical analysis: calculation

of the arithmetic mean and its error, unpaired two-sided Student's t-test. However, because the statistical samples were small, the non-parametric Mann-Whitney test was used in addition to the Student's t-test [26].

The study was conducted as part of the complex research work of the Department of Pediatric Dentistry of the Bukovinian State Medical University on the topic «Development of methods of prevention and treatment of the main dental diseases in children, taking into account the risk factors of their development» (state registration number: 0121U110122). The conducted research complied with bioethical norms, as stated in the conclusions of the Bioethics Commission of the Bukovinian State Medical University (№ 1 dated 17.09.2020).

Results and discussion

The table shows the digital data of the thickness of the squamous epithelium and the proliferative activity of the gingival epithelium, especially the basal layer of the epithelial surface, according to the age of the patients.

Table

Characteristics of the gingival epithelium in the area of the third molars at different stages of root formation

Observation Groups	Group I		Group II		Group III	
The stage of embryo formation according to Demirjian	D	E	F	G	H	
Gingival epithelial thickness (µm)	102±2,6	128±2,7	159±3,8	201±4,0	289±4,3	
Percentage of Ki-67 positive cells in the basal layer of the gingival epithelium (%)	52±0,9	44±0,7	33±0,6	27±0,6	19±0,4	

In general, it should be noted that the thickness of the epithelial layer varied considerably from patient to patient, but the table shows the averages obtained from several dozen measurements in each histological section, which reflect the average trends well.

The table clearly shows that the thickness of the epithelium in the stages of root formation gradually increases from stage D to stage H. At the same time, it should be noted that the thickness of the gingival epithelial layer in children of group I is on average lower ($p<0.05$ according to both the non-parametric Mann-Whitney test and the parametric Student's t-test) than in children of group II and III, and in children of group II it is lower than in children of group III ($p<0.05$ according to both

the non-parametric Mann-Whitney test and the parametric Student's t-test) than in children of groups II and III, and in children of group II it is lower than in children of group III ($p<0.05$ according to the above-mentioned criteria for statistical evaluation of differences in mean trends).

When analyzing the digital data in the table, it was clear that the data on the proliferative activity of the epithelium (based on immunohistochemical examination of the Ki-67 antigen in cell nuclei) were opposite to the data on the thickness of the epithelium. In particular, the proliferative activity of the epithelium, which was observed only in the basal layer, decreased with increasing stage of tooth formation. This is illustrated in Figures 1-5. It was highest in children in group I and lowest in group III.

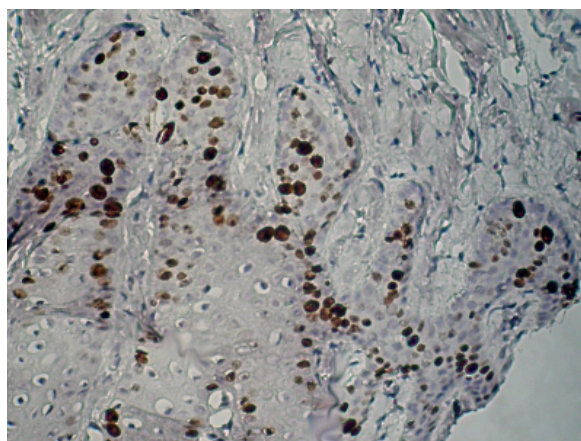


Fig. 1. Gingival epithelium of a 12-year-old patient at the stage of root formation «D». Ki-67-positive epithelial cell nuclei are brown. Immunohistochemical technique using primary antibodies against Ki-67, polymer detection system and diaminobenzidine visualization with nuclei stained with haematoxylin. Optical magnification 200x (approx. 20x, Ob.10x)

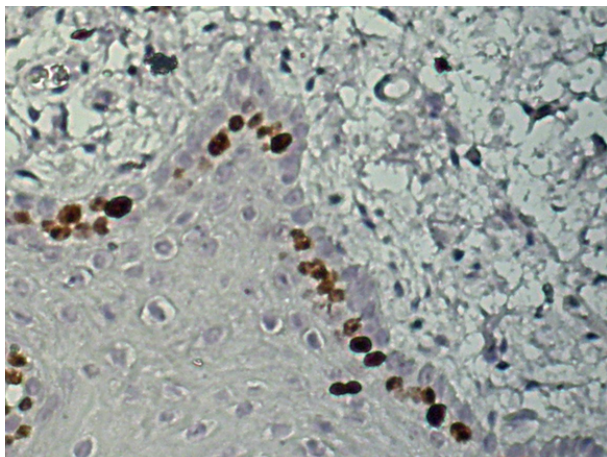


Fig. 2. Gingival epithelium of a 13-year-old patient at the stage of root formation «E». Ki-67-positive epithelial cell nuclei are brown. Immunohistochemical technique using primary antibodies against Ki-67, polymer detection system and diaminobenzidine visualization with nuclei stained with haematoxylin. Optical magnification 200x (approx. 20x, Ob.10x)

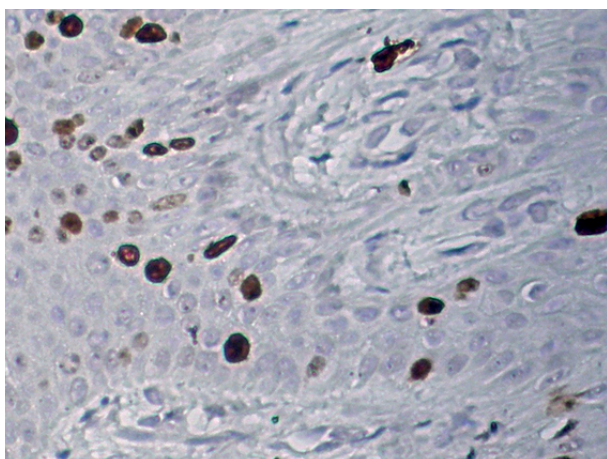


Fig. 3. Gingival epithelium of a 15-year-old patient at the stage of root formation «F». Ki-67-positive epithelial cell nuclei are brown. Immunohistochemical technique using primary antibodies against Ki-67, polymer detection system and diaminobenzidine visualization with nuclei stained with haematoxylin. Optical magnification 200x (approx. 20x, Ob.10x)

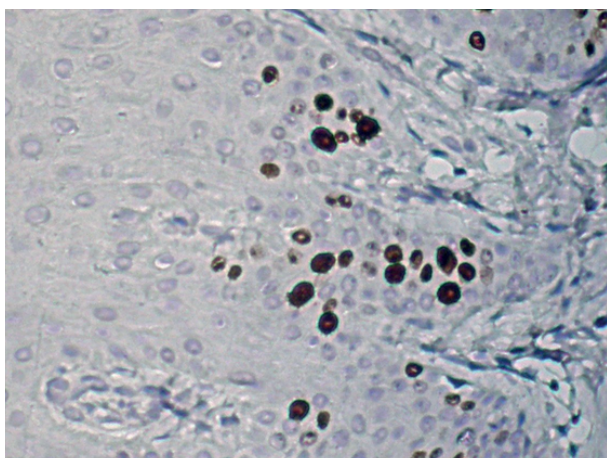


Fig. 4. Gingival epithelium of a 16-year-old patient at the stage of root formation «G». Ki-67-positive epithelial cell nuclei are brown. Immunohistochemical technique using primary antibodies against Ki-67, polymer detection system and diaminobenzidine visualization with nuclei stained with haematoxylin. Optical magnification 200x (approx. 20x, Ob.10x)

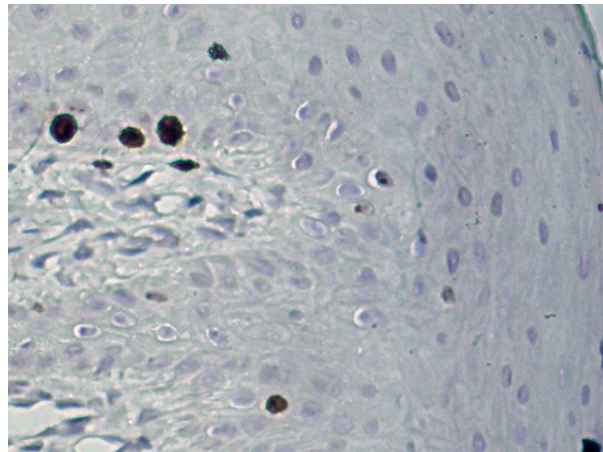


Fig. 5. Gingival epithelium of an 18-year-old patient at the stage of root formation «H». Ki-67-positive epithelial cell nuclei are brown. Immunohistochemical technique using primary antibodies against Ki-67, polymer detection system and diaminobenzidine visualization with nuclei stained with hematoxylin. Optical magnification 200X (approx. 20X, Ob.10X)

Conclusions. Thus, the histological and immunohistochemical study of the gum in the area of the third molar allows us to conclude that during the formation of the root of this tooth, a number of changes occur in the epithelial cover of the gum, namely: an increase in the number of epithelial layers against the background of a decrease in their proliferative activity. The established data indicate a decrease in the regenerative potential of the squamous epithelium with increasing age of the child and the degree

of formation of the third molar, which is reflected in the clinic.

Prospects for further research. To study the connective tissue structures underlying the squamous epithelium, including multipotent mesenchymal cells.

Conflict of interest: none.

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ХАРАКТЕРИСТИКА ЕПТЕЛІЮ ЯСЕН У ДІТЕЙ У ДІЛЯНЦІ ТРЕТІХ МОЛЯРІВ НА РІЗНИХ ЕТАПАХ ФОРМУВАННЯ КОРЕНЯ ЗУБА

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Резюме.

Екстракція ретинуваних та дистопованих третіх молярів є достатньо травматичною маніпуляцією, яка супроводжується великим дефектом кісткової тканини та інфікуванням рани. Тому доцільним є проведення герметомії, яка має низку переваг перед типовим і атипичним методами видалення зубів. Важливим етапом будь-якого оперативного втручання є післяопераційний період, котрий характеризується певною тривалістю та наявністю ускладнень, що серед іншого обумовлено регенераторним потенціалом тканин, насамперед епітеліальних.

Мета дослідження – оцінити стан епітеліального покриву ясен у ділянці третіх молярів у дітей, що потребують операції видалення цих зубів за ортодонтними показаннями, з урахуванням стадії формування зуба, а саме його кореня.

Матеріали та методи. Проведено операцію видалення третіх молярів за ортодонтними показаннями 95 дітям у віці від 11 до 18 років. Клініко-рентгенологічно виділено три групи спостереження: I (n=30) – діти віком 11-13 років; II (n=35) – діти віком 13-16 років, та III (n=30) – діти віком 16-18 років. Під час оперативного втручання відбувався забір ясен з прилеглих ділянок для дослідження. Матеріал фіксували, зневоднювали, парафінізували для подальшої гістологічної обробки. Застосовували імуногістохімічні методики у відповідності до протоколів, наданих виробником. Зокрема, було проведено імуногістохімічне визначення антигенів Ki-67 з первинними антитілами проти нього. Цифрові копії зображення аналізували за допомогою спеціалізованої комп'ютерної програми ImageJ v1.52 (freeware, США). Отримані цифрові дані оброблені статистично.

Отримані цифрові дані оброблені статистично. Застосовували попередню перевірку на нормальність розподілу за методом Шаріо-Вілкс. Для всіх вивчених статистичних вибірок згідно з цим критерієм гіпотеза про нормальність розподілу не була відхилена (на рівні значущості $p=0,05$), тому використовували переважно параметричні методи статистичного аналізу: обрахування середньої арифметичної та її похибки, непарний двобічний критерій Стьюдента. Однак, з причини, що статистичні вибірки були невеликими, то разом з критерієм Стьюдента застосовували й непараметричний критерій Mann-Whitney.

Дослідження проводилось як фрагмент комплексної науково-дослідної роботи кафедри стоматології дитячого віку Буковинського державного медичного університету на тему «Розробка методів профілактики та лікування основних стоматологічних захворювань у дітей з урахуванням факторів ризику їх розвитку» (державний реєстраційний номер: 0121U110122). Проведені дослідження відповідали біоетичним нормам, що зазначено у висновках комісії з біоетики Буковинського державного медичного університету № 1 від 17.09.2020 р.

Результати та їх обговорення. Результати досліджень показали, що товщина епітелію ясен у дітей у ділянці третіх молярів поступово зростає від $(102 \pm 2,6)$ мкм на стадії формування кореня «D» до $(289 \pm 4,3)$ мкм на стадії «H». Водночас, варто зазна-

чити, що у дітей віком 11-13 років товщина епітеліального покриття ясен на етапах формування кореня зуба є у середньому є меншою, аніж у дітей віком 13-16 та 16-18 років ($p < 0,05$), а в дітей 13-16 років менша, ніж у дітей 16-18 років ($p < 0,05$).

Встановлено, що проліферативна активність епітелію за даними імуногістохімічного дослідження антигену Ki-67 в клітинних ядрах має протилежну залежність від товщини епітеліального шару. Зокрема, найвищою вона є на стадії формування «D» – ($52 \pm 0,9$) %, а найнижчою – ($19 \pm 0,4$) %, – на стадії «H».

Висновки. Отже, проведене гістологічне та імуногістохімічне дослідження ясен в ділянці третього моляра дозволяє прийти до висновку, що в ході формування кореня цього зуба відбувається низка змін в епітеліальному покритті ясен, а саме: збільшення кількості шарів епітелію на тлі зменшення його проліферативної активності. Встановлені дані свідчать про зниження регенераторного потенціалу багатошарового плоского епітелію зі збільшенням віку дитини та ступеня сформованості третього моляра, що знаходить своє відображення в клінічній картині перебігу оперативного втручання.

Ключові слова: діти; операція видалення зуба, треті моляри, епітелій ясен.

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