ORIGINAL ARTICLE

IMPREGNATION OF ORAL MUCOSA OVER IMPACTED TEETH BY SUBPOPULATIONS OF MACROPHAGES M1 AND M2

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ABSTRACT

The aim: Of the study is to research quantitative parameters of mucous membrane macrophages populations M1 (CD68+) and M2 (CD163+) over vestibularly and palatally impacted teeth.

Materials and methods: A group of 21 people aged from 10 to 16 years was formed to conduct the research. Clinical situation according to diagnostic criteria was identical in all the patients. The group was divided into two groups - control and experimental, which in their turn were fragmented into two subgroups. Immunohistochemical studies of mucosal biopsies were performed in accordance with the recommendations for selection.

Results: Study of ratio of CD68+/CD163+ cells revealed imbalance in individuals with vestibularly impacted teeth due to higher infiltration density of CD163+ ($p<0,05$), compared to CD68+ of control group. In individuals with palatally impacted teeth, ratio of CD68+/CD163+ increased 3,6 times, as well as compared with control group, but due increased infiltration density of CD68+.

Conclusions: In the epithelium of oral mucosa located over impacted teeth, both on vestibular and palatal surface, number of CD 68+ and CD163+ cells had no significant differences compared to control group. In biopsies of the lamina propria of mucosa over vestibularly impacted teeth, the ratio M1/M2=0,91±0,11 ($p<0,05$) decreases, with predominance of macrophages CD163+ subpopulation activity, and over palatally impacted teeth balance of M1/M2 macrophages elevated (M1/M2= 2,10 ± 0,32, $p<0,05$), due to increased infiltration density of CD68+.

KEY WORDS: children, macrophages, impacted teeth, surgical and orthodontic treatment

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INTRODUCTION

Impacted teeth are the result of abnormality of teeth eruption, when the formed tooth has not come in and stay in a jaw for two years after a period of physiological eruption. Despite of advanced orthopedic and implant techniques used to replace defects in dentition, natural teeth are still the most valuable [1-4].

The effectiveness of comprehensive orthodontic treatment of impacted teeth directly depends on communication and interaction with an oral surgeon, because a choice of the best method of «bringing out» of impacted teeth, taking into account all possible risk factors, is not always predictable, due to the fact that impaction is polyetiological dental anomaly. Methods of surgical opening of access to impacted teeth crowns to optimize the tactics of their orthodontic movement also need constant improvement. At the stage of planning fenestration technique of the mucous membrane over the crowns of impacted teeth, it is necessary to take into account all the features of their location and follow indications in each individual case to avoid mistakes, complications and get the desired orthodontic result [5-10].

Electron microscopic studies of mucous membrane over impacted teeth [7] indicate that they have almost complete absence of nerve elements and a significant reduction in the number of microvessels and this can lead to neurotrophic disorders at the local level. Reduction of nerve impulses is one of the components of impaction and at the same time there is violation of autolytic processes in connective tissue. That gave the authors reason to assume that such changes in connective tissue of papillary layer of lamina propria prevent gum epithelium and enamel epithelium from converging [7].

Impacted teeth, depending on vestibular or palatal location, respond differently to orthodontic interventions. In our previous studies [8], a comprehensive analysis of mucosal micropreparations in patients with palatally and vestibularly impacted maxilla canines confirmed presence of disorders in microcirculatory bed with decreased vascular density and dyscirculatory manifestations. This may contribute to the formation of zones of ischemia in this area, an onset of foci of necrobiotic changes, initiation of sclerosis. In addition, we have shown that dystrophic and sclerotic processes with insignificant activity of adaptive – compensatory mechanisms prevailed in mucous membrane over vestibularly impacted canines [8, 9].

Apparently, to increase the effectiveness of treatment of impacted teeth, it is important to establish by immunohistochemical method the role of macrophages populations of proinflammatory M1 (CD68+) and anti-inflammatory M2

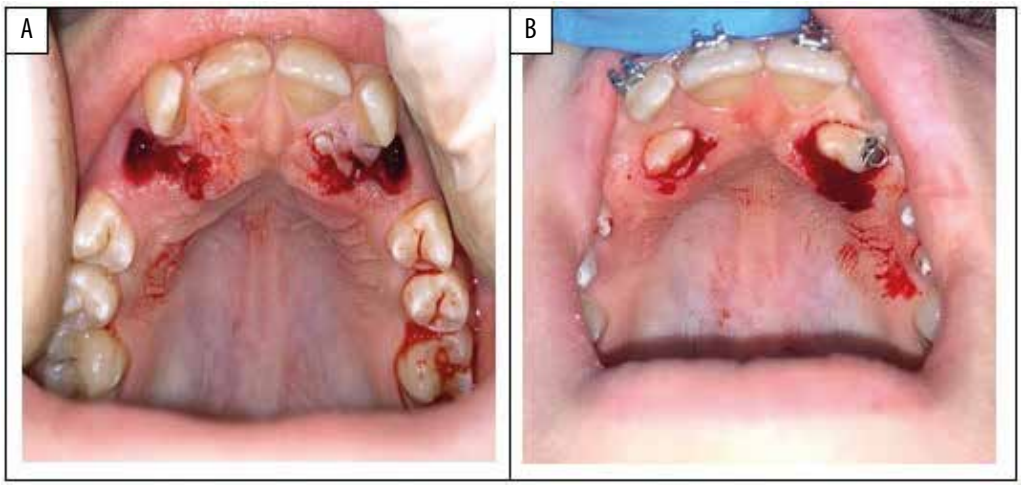

Fig. 1. Photo of operative field on palate after removal of 53, 63 teeth (A) and ectomy of muco-periosteal flap over impacted 13, 23 and formation of open window (B). Patient Z. 14-year-old, outpatient chart № 21.

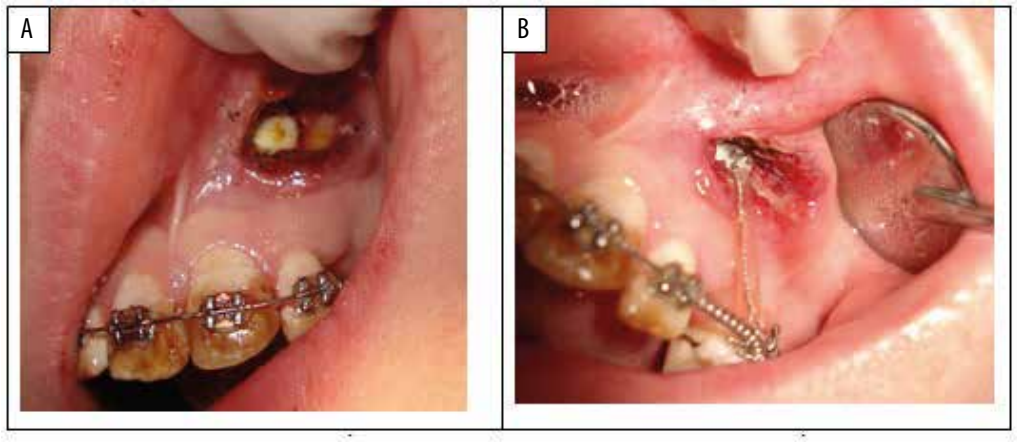

Fig. 2. Photo of operative field of vestibular surface of alveolar process (A) after separation of muco-periosteal flap over impacted 23 tooth and clamping of fixed orthodontic appliance (B). Patient H. 15-year-old, outpatient chart №19.

(CD163+) in mucous membrane of the alveolar process. However, to date there is no reliable information about the fact of establishing their biological role in the formation of prerequisites for impaction of teeth. In this connection, we have focused on the study of macrophages populations such as M1 and M2 in mucosa located in the area of impacted teeth and it can optimize surgical procedures and reduce duration of orthodontic treatment.

THE AIM

The aim is to study quantitative parameters ofmacrophages populations M1 (CD68+) and M2 (CD163+) of mucous membrane over vestibularly and palatally impacted teeth.

MATERIALS AND METHODS

The study was conducted on clinical basis of the Department of Pediatric Surgical Dentistry, scientific and educational therapeutic and prophylactic «Dental Center», Research Institute of Genetic and Immunological Basis of Pathology and Pharmacogenetics of UMSA, Poltava, municipal institution «Poltava Regional Center of Dentistry - Dental Clinic». An approval of the UMSA Bioethics Commission was obtained prior to the start of the study.

42 patients aged from 10 to 25 with delay in eruption of permanent teeth (female 27 – 64,3%, and male 15 – 35,7%) were selected for research to solve consistenly the tasks stipulated by the aim of the study. Among them, canine impaction was detected in 32 patients (76,2%), central incisors impaction in 8 (19,0%), lateral incisors impaction in 2 – (4,8%). A study protocol, which were developed by us, was filled in for each patient. It makes possible to analyze 24 risk factors of teeth impaction development.

To assess objectively the results of our study, an experimental group of 16 people aged from 10 to 16 years, with identical clinical situation according to diagnostic criteria presented in the previous research [8] was formed. In this study, experimental group was devided into two subgroups: 8 patients with vestibularly impacted canines and 8 patients with their palatal localization. Control group with physiological changes of canines consisted of 5 people aged from 10 to 12 according to diagnostic criteria specified by us [10].

Mucosal biopsies were fixed in 10% solution of neutral buffered formalin and embedded in paraffin. Expression of CD68+ and CD163+ macrophages was examined in all samples by immunohistochemical method of streptavidin peroxidase. Paraffin sections of mucosal tissue with thickness of 4 μm were incubated with murine monoclonal antibodies anti-CD68 (1:25, clone PG-M1, REF PD M065-S, Diagnostic BioSystems, USA) and anti-CD163 (1:100, clone 10d6, REF Mob460 -01, Diagnostic BioSystems, USA). Then the sections were treated in two steps using PolyVue ™ HRP / DAB Mouse /Rabbit detection

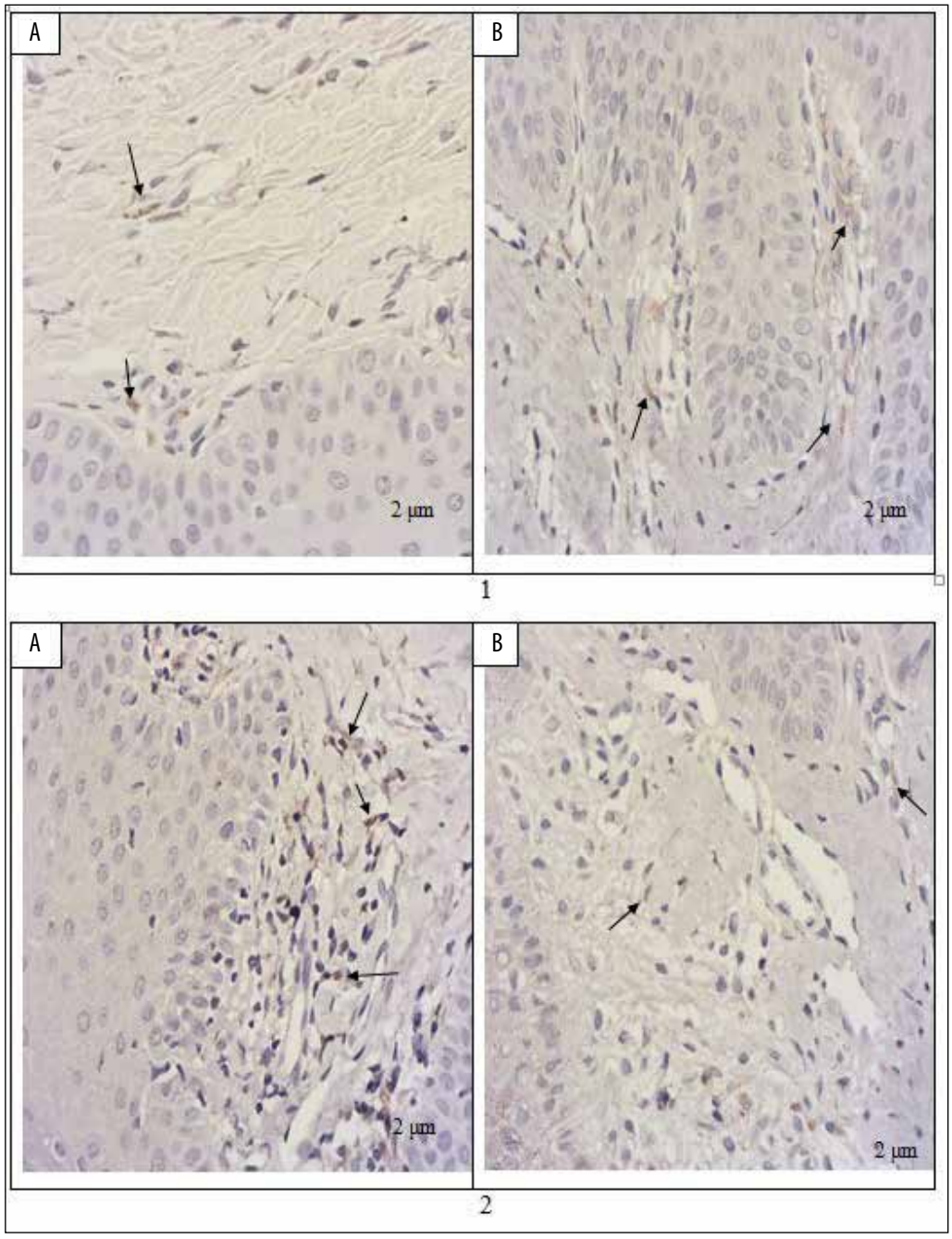

Fig. 3. Expression of CD68+ (1A, 1B) and CD163+ (2A, 2B) macrophages in lamina propria of oral mucosa, hematoxylin staining, mg. ×400.

system (Diagnostic BioSystems, USA) with visualization by DAB chromogen ; the nuclei were counterstained with Mayer's haemalaun. Quantitative parameters were obtained by counting immunopositive CD68+ (M1) and CD163+ (M2) cells throughout a field of view (x 40) and evaluated the index of their ratio. Sections were examined under a microscope and followed by photography (×200, ×400; Axio Lab.A1, Zeiss, Germany).

Mean sample values and mean errors (M±m) were calculated. The statistical significance of differences was evaluated by the Mann-Whitney U test, and Spearman's correlation coefficients R were determined. Indicators were analyzed, taking into account generally accepted in medical and biological studies probability of error $p<0,05$ [11].

RESULTS

Data of complex examination (photometry of face, analysis of diagnostic models of the jaws, orthopantomograms and 3D computed tomography) showed the presence of 20 canines impaction in 15 patients. Topographic diagnostic criteria of impacted teeth in patients of experimental group were identical: depth of impaction of medium level (II-III according to Y.I. Zhigurt, 1996) [5], completely formed root, the angle of inclination of longitudinal axes from 35 to 85º with displacement of more than 2 mm palatally (Fig. 1) – 10 teeth and also 10 teeth with vestibular displacement (Fig. 2).

Patients gave and signed voluntary informed consent to participate in the study. The clinical examination of all patients was performed according to a scheme of static and

Table I. Number of subpopulations of macrophages of proinflammatory CD68+ and anti-inflammatory phenotype CD16+ in lamina propria of mucous membrane (M±m).

Index	Control			Experimental		
	Vestibular biopsy, I subgroup (n=5)	Palatal biopsy, II subgroup (n=5)	Total	Vestibular fenestration, I subgroup (n=10)	Palatal fenestration, II subgroup (n=10)	Total
Lamina propria of oral mucosa CD68+	9,20±1,45	8,95±2,37	9,08±1,29	15,98±1,45 p_1<0,01	13,88±0,97 p_1>0,05 p_2>0,05	14,93±0,88 p_3<0,001
Lamina propria of oral mucosa CD163+	18,00±1,19	15,95±0,85	16,98±0,78	18,54±1,39 p_1>0,05	7,44±0,77 p_1<0,002 p_2<0,001	12,99±1,49 p_3>0,05
Ratio CD68+/CD163+	0,51±0,04	0,58±0,18	0,54±0,09	0,91±0,11 p_1<0,02	2,10±0,32 p_1<0,002 p_2<0,001	1,50±0,22 p_3<0,001

Significant difference value:
p_1 - between the same subgroups of control and experimental group;
p_2 - between I and II subgroups of experimental group;
p_3 - between totals of control and experimental group.

dynamic examination and recorded in outpatient charts of each individual.

1. CD68+ cells (arrows) over vestibularly impacted 13, Patient B., 15-year-old outpatient chart №5 (A), and palatily retained 13, Patient Z., 14-year-old, outpatient chart №16 (B).
2. CD163+ cells (arrows) over vestibularly impacted 13, Patient B., 15-year-old, outpatient chart №5 (A) and palatally impacted 13, Patient Z., 14-year-old, outpatient chart №16 (B).

Results of the analysis of quantitative parameters of CD68+ and CD163+ cells directly in epithelium of mucosa located over impacted teeth of both experimental and control groups did not reach significant values, but their values in lamina propria of mucosa are presented in table I.

According to the data shown in table 1, as to determination of quantitative distribution of CD68+ cells in lamina propria of mucosa over impacted teeth, there are significant differences with control group. The number of CD68+ (M1) cells in lamina propria of oral mucosa over impacted teeth predominated, compared to control group. In biopsies of lamina propria of oral mucosa located over vestibularly impacted teeth, quantity of CD68+ increased 1,7 times, and in the flaps of lamina propria of mucosa over palatally impacted teeth this number is 1,6 times higher than in control group.

Comparison of immunohystological statistic with respect to the quantity of CD163+ cells in lamina propria of mucosa over vestibularly and palatally impacted teeth also showed significant differences.There was decrease in the number of CD163+ (M2) over palatally impacted teeth compared to control group, and 2,5 times less compared to subgroup I (vestibularly impacted teeth).

The study of ratio of CD68+/CD163+ cells revealed an imbalance in individuals with vestibularly impacted teeth (subgroup I of experimental group) due to increased infiltration density of CD163+ ($p<0,05$), compared to CD68 + cells, which was 1,8 times different compared to control group.

In persons with palatally impacted teeth (subgroup II of experimental group), the ratio of CD68+/CD163+ increased 3,6 times compared to control group, due to increased infiltration density of CD68+ (M2), a significant difference between these quantitative indicators was confirmed.

Generalized results of carried out research are presented in the form of increase in balance of CD68+/CD163+ cells by the formula: Control group I subgroup (0,51± 0,04) < Control group II subgroup (0,58±0,18) < Experimental group I subgroup (0,91 ± 0,11) < Experimental group II subgroup (2,10 ± 0,32).

DISCUSSION

Scientific researches provides information about importance of macrophages in remodeling and maintaining of homeostasis of almost all tissues in the body, as well as their evident role in the first line of defense against many pathogens which gives them a leading part in the development of many diseases [12-15] .

Polarization of macrophages is an extremely meticulous and coordinated process as a result of which different variants of phenotypes can be differentiated. Each of phenotypes has potential to influence the development of pathological conditions in different ways depending on microenvironmental signals (classically activated M1, alternatively activated M2) [12].

A scientific review of literature on contribution of M1/M2 macrophages to pathogenesis of chronic periodontitis found that CD68+ (M1) macrophages may play a significant role in the initiation of its exacerbation by increasing the inflammatory response and destruction of underlying tissues, as well as cause suppression.

In modern experimental studies on rats, it was found that classically activated CD68+ (M1) macrophages promote resorption of alveolar bone due to the mechanical action of orthodontic appliances. Macrophages CD68+ (M1) act as precursors of odontoclasts, promote mechanisms of bone resorption and rapidity of tooth movement. Root resorption was enhanced by an increase in M1/M2 ratio and partially inhibited by a decrease in the M1/M2 ratio [14,15].

CONCLUSIONS

Thus, our study of the quantitative parameters of macrophages M1/M2 may be a new basis for the formation of a certain view and approach to surgical and orthodontic treatment and prevention of dental impaction. In the epithelium of oral mucosa located over impacted teeth, both on palatal and vestibular surfaces, the number of CD 68+(M1) and CD163+ (M2) cells had no significant differences. In the biopsies of lamina propia of mucous membrane over vestibular impacted teeth, the ratio M1/M2=0,91±0,11 ($p<0,05$) decreases, with the predominance of activity of macrophages CD163+ (M2) subpopulation. In lamina propria of mucous membrane over palatally impacted teeth, the balance of M1/M2 macrophages increases (M1/M2=2,10±0,32, $p<0,05$) due to increased infiltration density of CD68+ (M1).

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Conflict of interest:
The Authors declare no conflict of interest.

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