NEUTROPHIL BACTERICIDAL ACTIVITY THROUGH THE STAGES OF PLACEMENT OF DIFFERENT DENTAL IMPLANTS DEPENDING ON THEIR CHEMICAL COMPOSITION

AKTYWNOŚĆ PRZECIWBAKTERYJNA NEUTROFILÓW W ODPOWIEDZI NA RÓŻNEGO RODZAJU IMPLANTY DENTYSTYCZNE W ZALEŻNOŚCI OD ICH SKŁADU CHEMICZNEGO

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ABSTRACT

Introduction: The analysis of data provided by implant system manufacturers has demonstrated that implants, i.e. parts screwed into the bone, are of different chemical composition of the implant. Sometimes they have little amount of metal contaminants,which are not biologically passive.

The aim: To explore the effects produced by dental titanium implants containing metal contaminants on the stimulation of antimicrobial properties of neutrophils.

Material and Methods: A total of 24 patients who had from 1 to 4 titanium implants with different chemical compositions were subjected to the comprehensive check-up to this end. The functional activity of neutrophils was evaluated by nitroblue tetrazolium (NBT) reduction test. It was dynamically in 5-7 days after the implant fitting into the bone, and in 3 months after the procedure of implant placement.

Results: On the 5-7th day following the placement of implants with weight percentage of titanium (Ti) in the composition from 25 to 50%, the share of active neutrophils significantly increased compared with share of active neutrophils prior the surgical procedure. However, after 3 months, this parameter in patients with implants, whose titanium content was low, remained significantly high.

Conclusions: The placement of dental implants systems led to an increase in the share of active neutrophils in the peripheral blood of the patients in 5-7 days following the procedure of implant insertion. However, this indicator for implant systems with a higher content of Ti in the remote period returned to its original value, which indicates their higher biocompatibility with the tissues of the human body.

KEY WORDS: implantation, neutrophil activity, osыeointegration.

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INTRODUCTION

Nowadays complete or partial anodontia has been considered as quite prevalent dental disorder that according to the WHO reports affects nearly 75% of the population worldwide [1,2]. This poses the necessity of replacing dentition defects with various prosthetic devices, among which the procedure of dental implant placement is gaining *popularity* for replacing missing *teeth* [3]. *D*ental implant placement is known to provide a high level restoration of dentition anatomical and functional integrity and maximal aesthetic effect that promotes social rehabilitation of patients during and after the treatment [4]. In more than 90% of cases the implant osseointegration in the alveolar bone is reported to be successful, but implant placement may also be accompanied by some complications that can occur during the procedure of dental implant placement as well as in the postoperative period [5, 6]. Therefore, dental surgeons should plan thoroughly all surgical stages of the procedure of dental implant placement and rehabilitation measures taking into account the biological characteristics of the dentofacial system and its functioning. Last, and by no means least, is the choice of plants that would meet most of demand relating to biocompatibility, because the number of dental implant systems available on the market is constantly growing [7].

In order to achieve adequate osseointegration and to prevent peri-implant bone loss the implants meet some demands including biological indifference, corrosion resistance, mechanical durability and stability, which enables to obtain proper shape quality and surface characteristics of physiological relevance. The analysis of data provided by national and international implant system manufacturers has demonstrated that implants, i.e. parts screwed into the bone, are of different chemical composition of the implant. Some implant materials have little amount of metal contaminants, e. g. aluminium, vanadium, which are not biologically passive and can cause sensitization, contributing to the development of peri-implantitis, and, consequently, to rejection of implants [8, 9]. Placement of implant systems with a high content of aluminium and

Table I. Chemical composition of surfaces of α-dent and NOVA dental implants, %.

Dental implant system	α-dent		NOVA	
Element \ Share	Weight	Atomic	Weight	Atomic
Oxigen	50.47	69.96	11.99	19.45
Sodium	0.67	0.65	1.27	1.43
Magnesium	4.66	4.25	-	-
Aluminum	6.48	5.33	2.18	2.09
Silicium	6.98	5.51	0.32	0.30
Sulphur	-	-	0.26	0.21
Chlorine	-	-	1.25	0.91
Potassium	1.88	1.07	0.36	0.24
Calcium	0.37	0.20	1.44	0.93
Titanium	25.26	11.69	59.26	32.11
Vanadium	1.43	0.62	2.74	1.40
Iron	1.80	0.71	-	-
Total	**100.00**	**100.00**	**100.00**	**100.00**

vanadium increases the amount of these elements detected in the peri-implant tissues and slows down the process of osseointegration [10].

The process of osseointegration is accompanied by inflammatory and immune responses that result is separating the body tissue from foreign material of the implant placed into the alveolar bone tissue. This process is kept by the adsorption of surface proteins, by activation of the complement system and enhancement of fibrin matrix with subsequent activation of granulocytes, monocytes and macrophages that control the amplitude and duration of the immune response [11]. A key role in the immune defence of oral cavity belongs to nonspecific factors that act as a powerful barrier and are the first to respond to foreign agents entering the mouth [12, 13, 14]. Therefore, studying the effects produced by prosthetic appliances and dental implants on the state of nonspecific body defence is of great clinical importance [13]. There are diverse approaches available to predict the peculiarities of immune response of oral tissues to dental manipulations. The damaging effect produced by immune cells and their releasing of a significant amount of neurotransmitters influence the duration and intensity of immune responses and inflammation. As a diagnostic aid, *nitroblue tetrazolium* (*NBT*) *reduction* by *neutrophils* has been widely applied for assessing the level of non-specific immune response. This test is used for evaluating the condition of bactericidal peroxidase systems of phagocytic cells. NBT test enables to assess the level of antigen sensitivity of inactive granulocytes. The production of reactive oxygen species by neutrophils due to respiratory explosion occurs in the process of phagocytosis that plays an important role in providing non-specific body defence. Bactericidal activity of neutrophils in the body response to dental implant systems made of different chemical compounds is still little studied [15].

THE AIM

This study was aimed at exploring the effects produced by dental titanium implants containing metal contaminants on the stimulation of antimicrobial properties of neutrophils by using NBT tes.

MATERIALS AND METHODS

A total of 24 patients of average age group by WHO (46-59 years) were subjected to the comprehensive check-up. The test group enrolled 16 people who had from 1 to 4 titanium implants (8 patients had *α - dent* dental implants (Israel), 8 patients had NOVA dental implants (Israel)). 8 people of the same age without severe oral and maxillofacial pathology made up the control group. According to Kamalov P. H. etc. weight percentage of titanium (Ti) in the composition of α - dent dental implants constitutes 25, 26%, and 59, 26% in NOVA implants [8]. The chemical composition of the surfaces of α-dent and NOVA implants according to Kamalov R. H. etc. is presented in the Table I.

As it was essential to trace dynamic changes, the study was designed to have three stages: prior the procedure of implant placement, in 5-7 days after the implant fitting into the bone, and in 3 months after the procedure of implant placement before the placement of a healing cap. Samples of capillary blood were taken into sterile vials containing heparin and then delivered to the laboratory.

The functional activity of neutrophils was evaluated by *nitroblue tetrazolium* (*NBT*) *reduction* test (spontaneous

Table II. Dynamic changes in the number of active neutrophils in peripheral blood of patients through the stages of α-dent and NOVA dental implants, % (M ± m)

	Control group, n=8,	Test group of the patients with α-dent implants, n = 8,	Test group of the patients with NOVA implants n=8,
Before the surgical procedure	33,6±2,72	35,9±1,13	35,6±1,85
In 5-7 days	34,0±4,28	60,6±2,20*	55,4±4,21
In 3 months	33,8±2,66	41,1±1,36*	36,0±1,93

Note: *significant differences in values of the group of patients with α-dent implants to value of neutrophil activity before the implant placement, $p < 0.05$ reliability.

NBT by Wixman M. E., Mayansky A.N.) [16]. Among the 100 cells we counted the share of active neutrophils (AN), containing dark-violet formazane (*diformazane*) *granules and presented their percentage. N*eutrophils containing clearly visible *diformazane deposits were regarded as active cells, and netrophils with residual granules were considered as inactive.* Statistical data processing was performed using Microsoft Excel 2010; statistical significance was determined by Student's test. Data were statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

According to our data, on the 5-7th day following the placement of α-dent implants, the share of active neutrophils significantly increased by 1.7 fold ($p < 0.05$) compared with the previous values obtained before the implant fitting (Table 2). In 5-7 days after the placement of NOVA implants we found 1.6 fold increase in the share of active neutrophils ($p < 0.05$) compared with the values obtained before the operation. The results obtained can be explained by body response to the surgical intervention, as the procedure of implant insertion typically develops aseptic inflammation. Tissue damage causes necrosis and cell degeneration that in turn result in the increase of capillary permeability, oedema, and increase in the number of polymorphonuclear leukocytes at the site of inflammation. Then we observed the stimulation of macrophage production and the transition to the macrophage stage of inflammation. Only when the macrophage-fibroblastic barrier around the implant is formed, fibroblastic last stage of inflammation starts developing and is completed by the formation of connective tissue capsule. Functional reserve of neutrophils, readiness of phagocytes to engulf and *digest* cellular components grew during the stimulation of macrophage production and the transition to the macrophage, reaching the peak values.

This can indicate the activation of cellular innate immunity level triggered by dental implant insertion, namely the stimulation of oxygen- dependent bactericidal function of neutrophils.

In remote period following the implant insertion (3 months), the values of neutrophil activity decreased in both test groups and differed depending on the dental implant system. Thus, the share of active neutrophil in the patients with NOVA implants almost returned to the initial level that was determined before the surgical procedure of implant insertion. The share of active neutrophils in the patients with α-dent implants decreased as well, but was significantly higher compared with share of active neutrophils prior the surgical procedure that may suggest the development of sensitization of the patient's body and a lack of biocompatibility between the host tissues and implant material compared with the NOVA dental implants. This is likely due to low Ti content in the composition of these two dental implants and the significant amount of concomitants.

The control group showed no significant changes in the functional activity of neutrophils through the study.

CONCLUSION

Thus, the placement of dental implants of both α-dent and NOVA systems led to an increase in the share of active neutrophils in the peripheral blood of the patients in 5-7 days following the procedure of implant insertion that corresponds to the active stage of inflammation caused by surgical intervention. But the level of neutrophil activity observed in cases of fitting α-dent implants, which contain low weight percentage of titanium was significantly higher even in remote period after the surgical procedure. The intensity of activation of oxygen-dependent mechanisms of neutrophil killing in the patients with NOVA implants decreased to baseline in three months that is an evidence of their higher biocompatibility with human tissue.

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