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INDICATORS OF PROTEINS AND PEPTIDES AFTER BONE GRAFTING WITH OSTEOREPLACEMENT MATERIAL

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Abstract
Tooth loss accelerates the physiological age-related atrophy of the alveolar processes and jaws in general. Tooth extraction quite often remains a traumatic procedure, leading to direct destruction and loss of bone tissue of the alveolar process and surrounding soft tissue. The morphological changes that occur after the removal of a tooth or tooth root can be reduced by using techniques aimed at preventing these changes. The effectiveness of osteoplastic materials is largely due to the presence of minerals and soft collagen fibers in their composition. In biochemical blood tests, the study of indicators reflecting metabolic processes in the bone tissue of the jaws revealed differences in the content of water-soluble proteins and peptides, as well as enzyme activity, depending on gender.

Introduction

Relevance of the topic A biochemical blood test examines the presence and quantity of substances, in which changes in the functioning of organs are detected almost immediately, before the development of an obvious clinical picture. Human blood contains various substances, the study of which improves the diagnosis of diseases, because of this, the study of biochemical blood analysis is considered one of the main tools of a modern doctor and specialists in other specialties [2,4,6].

Determining these indicators helps to accurately identify diseases of the kidneys, endocrine and musculoskeletal systems, and diseases of the gastrointestinal tract. However, for the correct interpretation of such a study, it is not enough just to know the norms for the amount of certain blood substances.

The problem of tooth loss lies much deeper than superficial ideas about it and has important medical and social significance.

Tooth extraction quite often remains a traumatic procedure, leading to direct destruction and loss of bone tissue of the alveolar process and surrounding soft tissues.

In the subsequent period, especially during wound healing, a complex cascade of biochemical and histological reactions occurs, which ultimately leads to physiological changes in the alveolar bone and soft tissue architecture [1,2,3].

The effectiveness of osteoplastic materials is largely due to the presence of minerals in their composition, as well as soft collagen fibers [6,7]. However, natural substances do not have predictable and significantly expressed osteoplastic properties, especially in patients with anomalies of reparative osteogenesis, due to hereditary or acquired qualities and as a result of various unfavorable factors [3,4].

However, the current lack of an optimal set of bone replacement materials that provide targeted bone tissue regeneration determines the need for further research in this area [15,16].

At the same time, the current lack of a set of optimal osteoreplacement materials that ensure targeted regeneration of bone tissue determines the need for further research in this direction" [18].

In the practice of dentistry and maxillofacial surgery, there is often a need to fill bone defects of the jaws resulting from injuries, removal of cysts, sequestrectomy, bone grafting, etc.

The use of osteotropic material when replacing a cavity bone defect optimizes regeneration, which is confirmed by a significant number of studies in which the results are presented [11,12,20]. A large number of studies have been devoted to the study of the properties and methods of producing mineral composites from collagen with hydroxyapatite and tricalcium phosphate [19]. When choosing an osteoplastic material, surgeons give preference to materials that have both osteoconductive and osteoinductive properties, which not all materials have.

In addition, most osteoplastic materials are available in powder form, which is very inconvenient to introduce into the defect cavity.

Thus, the development of artificial biomaterials that imitate the composition and properties of natural bone, possessing both osteoconductive and osteoinductive properties, as well as a comfortable design during use, remains one of the most important.

Materials and Methods

To fulfill the task set in our study to study the process of osseointegration of the A-Oss osteoreplacement material, 20 patients aged 23 to 30 years who did not have chronic diseases were selected**.**

We used the method of D.S. Young. (1997) to determine ALP activity in biopsies by measuring the rate of hydrolysis of p-nitrophenyl phosphate to release p-nitrophenol, which produces a yellow color in an alkaline environment.

The method of Hillmann G. (1971) was used to determine the activity of AP in the assays, which is studied by the rate of hydrolysis of 1-naphthylphosphate with the formation of 1-naphthol, since in the presence of diazonium salt 1-naphthol is colored, the intensity of the color was determined spectrophotometrically at a wavelength of 405 nm.

"To determine the activity of lactate dehydrogenase (LDH), standard reagent kits from Human (Germany) were used.

"The method for determining the activity of lactate dehydrogenase is based on its ability to catalyze the reduction of pyruvate to lactate with the simultaneous oxidation of NADH to NAD+. The

decrease in the optical density of the solution is directly proportional to the LDH activity in the sample and is measured spectrophotometrically at a wavelength of 340 nm" [5].

Research results and discussion

In the process of bone tissue processing, cells of the immune complex and signals are actively involved, including enzyme activity, changes in those cytokines and many other regulatory substances. The repair process begins with resorption by osteoclasts [1,3,6].

Simultaneously, after resorption, bone tissue formation occurs, characterized by the secretion by osteoblasts of the main component of the intercellular matrix of bone - collagen, as well as alkaline phosphatase, osteocalcin and other proteins involved in bone mineralization [10,13]. The result is the creation of a mineralized bone matrix through multiphase deposition of secreted proteins and precipitation of calcium phosphate salts from the blood plasma. At the end of the mineralization phase, structured collagen of bone tissue forms layers of bone tissue of varying degrees of maturity [8,9].

Thus, osteoreparation is a dynamic biological process that includes all phases and stages of bone formation.

The patients' blood was obtained after surgery and at 3 and 6 months. Both male and female patients were included in the study. In the blood of patients, the amount of total protein, osteogenic cytokine - TNF-a, as well as the activity of alkaline phosphatase and LDH were determined (Table 1).

According to the data obtained, the amount of total protein in the blood of male patients is significantly higher ($p < 0.05$) compared to the data obtained from female patients. There is also a tendency ($p > 0.05$) to an increase in the amount of alkaline phosphatase activity in the blood of men.

It should also be noted that there are significant differences in the content of the studied indicators depending on the gender of the patients. Thus, in women, unlike men, a higher activity of alkaline phosphatase in the blood was determined.

Thus, the study of indicators reflecting metabolic processes in the bone tissue of the alveolar processes of human jaws revealed differences in the content of water-soluble proteins and peptides, as well as enzyme activity, depending on gender.

Table 1Content of proteins and peptides in the blood of patients before osteoplasty (M±w)

Proteins and peptides		
	Men	Women
total protein (mg/g tissue)	3,01±0,37	3,36±0,37
Alkaline phosphatase (IU/mg tissue)	6544±0,76	9,94±1,07
lactate dehydrogenase (IU/mg tissue)	322±4,10	319±2,19
Tumor necrosis factor (pg/mg tissue)TNF	4,24±0,50	4,90±0,26

Studying the blood of patients after implantation of the material "A-Oss"

The “A-Oss” material, used to fill defects in the bone tissue of the alveolar process of the lower jaw, changes the content of a number of water-soluble proteins and enzymatic activity (Tables 2 and 3).

Thus, in men after bone grafting, a significant increase ($p<0.05$) in alkaline phosphatase activity and total protein content was detected in the blood.

Lactate dehydrogenase (LDH) activity did not change significantly. In men, a significant decrease in the amount of TNF-a was determined.

In the blood of women after osteoplasty with A-Oss material, a significant increase ($p<0.05$) in alkaline phosphatase activity was also observed in relation to the data before surgery. When studying the amount of total protein and LDH activity, their increase was revealed, which, however, was unreliable. The content of TNF-a in women is unreliably reduced; these processes occur against the background of pronounced formation of osteoclast-activating factor.

The formation of fibrous tissue appears to be more active in women, and mineralization processes occur more quickly in men.

At the same time, a decrease in the amount of TNF-a can be considered as a possible desire to suppress the synthesis of proteins involved in the destruction of protein and mineral phases in the area of the bone defect.

There is a significant twofold increase in total protein in men after implantation of the A-Oss material in the area of defects in the alveolar process of the upper jaw.

Alkaline phosphatase activity increased 7 times compared to the activity detected before surgery. The identified changes undoubtedly indicate active osteogenesis occurring in the upper jaw of men after implantation of the A-Oss osteoplastic material.

In the blood of patients after implantation of the “A-Oss” material in women, a significant ($p<0.05$) increase in the content of total protein and alkaline phosphatase activity was also determined compared with the values before surgery.

Noteworthy is the fact that in blood taken from women, LDH activity significantly increases ($p<0.05$) than in men.

Statistical analysis of differences in metabolic parameters between jaws after osteoplasty with A-Oss material showed that in men and women there are statistically significant differences ($p<0.05$) in the content of TNF-a, and in women also in the activity of alkaline phosphatase. Implantation of the A-Oss material led to a decrease in TNF-a content.

Table 2 Content of proteins and peptides in the blood of patients 3 months after bone grafting with A-Oss material

Proteins and peptides	Men		Women	
	before osteoplasty	3 months after osteoplasty	before osteoplasty	3 months after osteoplasty
total protein (mg/g tissue)	5,34±0,36	7,42±0,26	5,45±0,25	7,41±0,43
Alkaline phosphatase (IU/mg tissue)	8,08±0,60	47,6±4,33	12,6±2,48	29,2±1,79
Tumor necrosis factor (pg/mg tissue)	2,37±0,42	0,81±0,11	1,45±0,65	0,39±0,12

Table 3 Content of proteins and peptides in the blood of patients 6 months after osteoplasty with A-Oss material.

Indicators	Men		Women	
	before osteoplasty	6 months after osteoplasty	before osteoplasty	6 months after osteoplasty
total protein(mg/g tissue)	3,11±0,47	8,02±1,52	4,36±0,27	6,54±0,45
Alkaline phosphatase (IU/mg tissue)	6,41±0,56	41,0±2,58	10,94±1,17	42,3±2,80
Tumor necrosis factor (pg/mg tissue)	4,34±0,51	3,40±2,53	5,20±0,16	4582±0,76

Blood testing of patients after implantation of the "A-Oss" material

3 months after bone grafting with A-Oss material, the content of water-soluble proteins in the blood of men was 1.5 times higher.

An increase in alkaline phosphatase activity by 2.4 times was also noted. The increase in the number of these indicators was combined with a significant decrease in the content of TNF-a ($p < 0.05$). (Table 4.5)

Table 4 Content of proteins and peptides in the blood of patients 3 months after bone grafting with the Bio-Oss material (M ± m)

Proteins and peptides	Men		Women	
	before osteoplasty	3 months after osteoplasty	before osteoplasty	3 months after osteoplasty
total protein(mg/g tissue)	5,44±0,57	7,25±0,33	5,50±0,76	6,63±0,2
Alkaline phosphatase (IU/mg tissue)	8,12±0,61	29,0±2,94	11,6±2,36	25,7±1,42
TNF-a(pg/mg tissue)	2,07±0,82	0,06±0,01	1,35±0,5	0,02±0,015

Table 5 Content of proteins and peptides in the blood of patients 6 months after osteoplasty with Bio-Oss material (M±m)

Proteins and peptides	Men		Women	
	before osteoplasty	6 months after osteoplasty	before osteoplasty	3 months after osteoplasty
total protein(mg/g tissue)	3,01±0,36	6,74±0,35	3,36±0,32	6,06±0,27
Alkaline phosphatase (IU/mg tissue)	6,44±0,75	45,4±6,02	9,94±1,06	48,3±3,92
TNF-a(pg/mg tissue)	4,24±0,4	3,66±0,1	4,90±0,25	3,24±0,8

Similar changes in the amount of water-soluble proteins and the content of individual proteins in the defect area filled with the Bio-Oss osteoplastic material are also observed in women (Table 6). There is a continuous development of the retention base of the implanted osteoplastic material for diseases of the jaw bones, both in men and women equally.

There is a continuous development of the retention base of the implanted osteoplastic material for diseases of the jaw bones, both in men and women equally.

A different situation was observed after implantation of the Bio-Oss material when studying proteins and peptides in the blood of patients. Thus, in men after 6 months, the content of water-soluble proteins and alkaline phosphatase in the blood was significantly (2 times) increased ($p<0.05$). At the same time, the amount of TNF-a remained almost at the original level. In the blood obtained from women, similar changes in the content of proteins, peptides and alkaline phosphatase activity were detected, with increased LDH activity ($p <0.05$). Within six months, in the defect area with the Bio-Oss osteoplastic material, reactions of destruction of protein structures were reduced, which indicates bone regeneration.

Table 6 Indicators of reliability of differences in the studied parameters after osteoplasty with materials "A-Oss" and "Bio-Oss"

Indicators	Mann-Whitney U test for maxilla and mandible (P=)			
	total protein(mg/g tissue)	alkaline phosphatase	LDH	TNF-a
Women	0,07	0,03	0,18	0,39
Men	0,07	0,87	0,60	0,34
Women	0,89	0,04	0,07	0,07
Men	0,14	0,22	0,04	0,04

A comparative analysis was carried out between changes in protein content after implantation of A-Oss and Bio-Oss materials in both men and women. The study of the data showed that in women after implantation of osteoreplacement materials "A-Oss" and "Bio-Oss" there are significant differences in alkaline phosphatase activity ($p = 0.04$).

Thus, in women after implantation of the material, in addition to the above proteins, significant differences were found between and in the content of TNF-a.

We compared data from studies of bone regenerates after implantation of osteoreplacement materials "A-Oss" and "Bio-Oss" (Table 7).

Table 7 Indicators of reliability of differences in the studied parameters after osteoplasty with materials "A-Oss" and "Bio-Oss"

Indicators	Mann-Whitney U test for maxilla and mandible (P=)			
	total protein(mg/g tissue)	alkaline phosphatase	LDH	TNF-a
Women	0,04	0,08	0,68	0,04
Men	0,50	0,22	0,68	0,04
Women	0,50	0,07	0,89	0,22
Men	0,50	0,68	0,08	0,08

It follows from the table that significant differences in the studied parameters in the blood after implantation of the materials "A-Oss" and "Bio-Oss" were found in women in relation to the content of water-soluble protein, TNF-a. The blood of men contains TNF-a.

Bone replacement drugs "A-Oss" and "Bio-Oss" were implanted in patients to fill defects in the upper and lower jaws for subsequent implantation and full orthopedic rehabilitation.

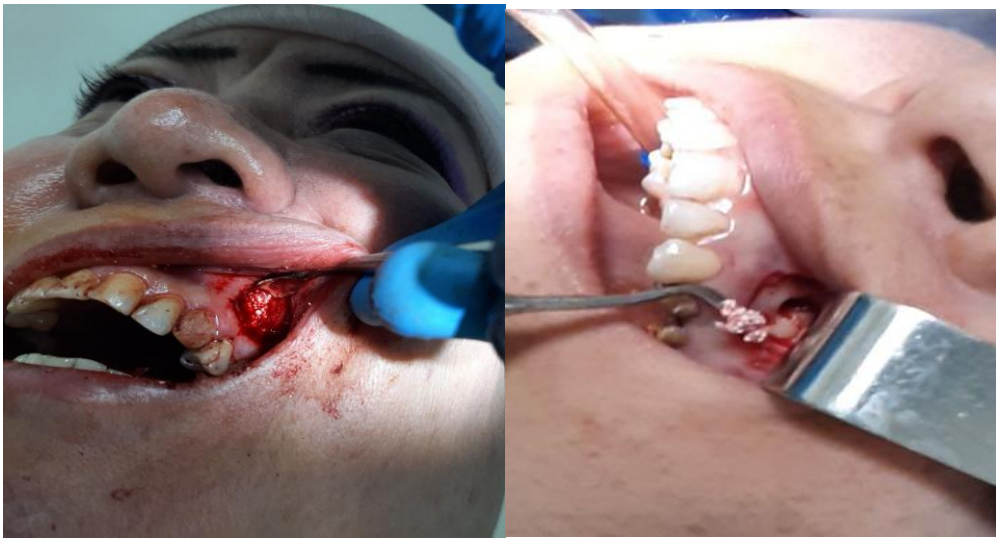

Fig. 1. Chronic osteitis of the upper jaw on the right. Creation of a window in the bone tissue and introduction of Bio-Oss + material.

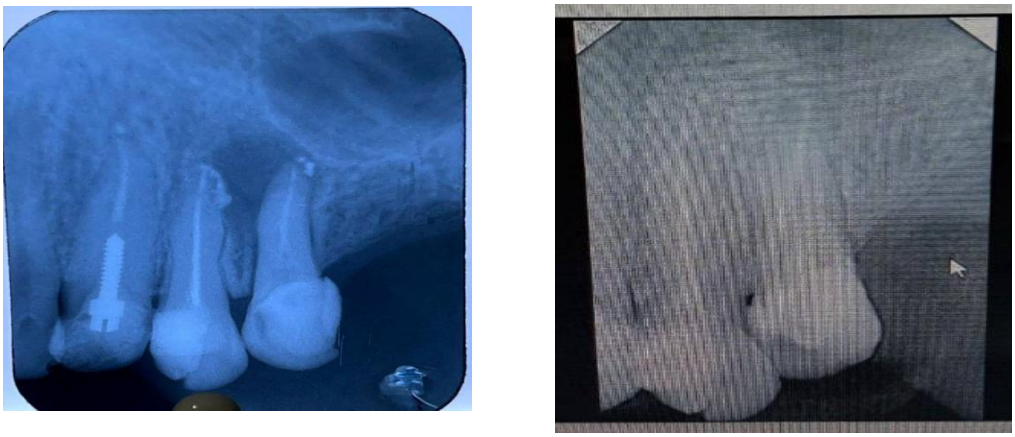

Fig.2 Patient M-va G., 1973

Diagnosis: radicular cyst of the upper jaw on the right in the area of 24.25 teeth

The results showed that a more intense reaction of the jaw bone tissue, associated with the activity of enzymes and the content of water-soluble proteins after implantation of osteoplastic materials, was in women on the upper jaw, and in men on the lower jaw. The use of "Bio-Oss" helps to obtain an optimal result with a decrease in the period of bone recovery after surgical interventions on the jaw bones, which prevents the development of atrophy of the alveolar process and preserves the supporting function of the prepared teeth.

CONCLUSIONS

1. The Bio-Oss material, used to fill defects in the bone tissue of the alveolar process of the lower jaw, changes the content of a number of water-soluble proteins and enzymatic activity.

Thus, in men after bone grafting, a significant increase ($p<0.05$) in alkaline phosphatase activity and total protein content was detected in the blood.

2. In biochemical blood tests, the study of indicators reflecting metabolic processes in the bone tissue of the jaws revealed differences in the content of water-soluble proteins and peptides, as well as enzyme activity, depending on gender.

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