ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Serum Marker Ctx (Crosslaps) and Bone Healing of Oral Surgical Deffects in Patients with Diabetes Mellitus.

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ABSTRACT

The bones are complex, dynamic connective tissue with a triple function: structural support, organs protection and maintenance of mineral homeostasis. The bone remodeling is a lifelong active metabolic process involving osteoclasts and osteoblasts. The balance between bone resorption and bone formation can be detected by tracing the circulating proteins (biological markers) divided in two groups of formative and resorptive markers. Diabetes is mentioned as one of the cause factors of metabolic bone disorder. The aim of the study was to determine the value of serum marker CTX (resorptive bone marker) and its possible influence on bone mineralization of oral surgical defects in patients with diabetes. Prospective clinical study was made including 100 subjects divided into two groups, patients with diabetes and control group. The serum values of CTX and the percent of relative bone healing in oral surgical defects were followed for over a 12 months period. Serum values of CTX in diabetic patients suggested reduced activity of bone metabolism with no influence on bone mineralization process followed by bone density increase in oral surgical defects.
Keywords: bone metabolism, CTX, spontaneous bone healing, diabetes mellitus

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ISSN: 0975-8585

INTRODUCTION

The bones are complex, dynamic and supporting mineralized tissue of an organism with a triple function: structural support, organs protection and maintenance of mineral homeostasis. Bone tissue is composed of several cell types, proteins, blood vessels, nerve elements and bone mineral matrix that allow an operation called metabolism, regulated by many factors. The bone remodeling is a lifelong active metabolic process involving osteoclasts and osteoblasts on the same place. While osteoclasts absorb the old bone, osteoblasts form a new one. Imbalance between these activities, for any reason, results in metabolic bone disorder [1-2].

The balance between bone resorption and bone formation can be detected by tracing the circulating proteins (biological markers) in serum and urine. Bone markers are divided in two groups of formative and resorptive markers [3].

The most analysed marker of resorptive markers group is β crossLaps(CTX), high sensitive marker of bone resorption, stable in serum and urine. Collagen cross links are released during bone matrix resorption (early breakdown of collagen type 1) and can be detected by specific tests. [4-5].

Hossein-Nezhad A. [6] show different bone resorptive markers including CrossLaps. It is one of the most sensitive markers formed directly with the onset of collagen type 1 degradation. In bone physiology, C-terminal telopeptide (carboxy-terminal collagen crosslinks-CTX) can be used as bone turnover serum biomarker. The detecting test of CTX marker -serum CrossLaps is used more often than any other currently available test for bone resorption process determination [7].

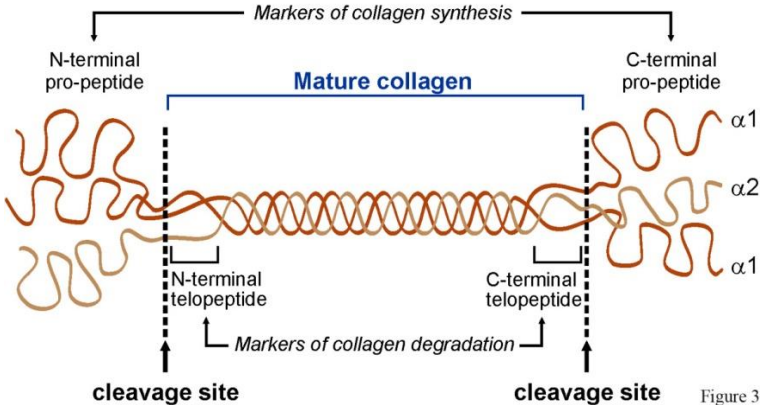

Figure 1. Structure of collagen molecule

This specific protein sequence is separated from the osteoclasts during bone resorption[Figure1]. Therefore the serum CTX levels are proportional to the osteoclastic activity in the time when blood sample was taken [8-9].

Reference intervals of CTX in menopausal women are well studied, but in other age groups there is much less information (healthy children show variations associated with the age). Despite these shortcomings, serum CTX, fully or partially meets all the criteria for a reference bone resorption marker [10].

The values of bone turnover markers obtain clinically usable informations about either normal or pathological processes affecting bone cells activity [11].

Biochemical markers are used in medicine for osteoporosis treatment initiation. The serum marker of osteoclastic activity (CTX) is used to evaluate bone resorption degree [12-13]. In oral and maxillofacial surgery CTX is used for risk prediction of jaws osteonecrosis development after oral surgical procedures in patients receiving oral bisphosphonate therapy and evaluation of bone mineral density in elderly patients.

In the early years of the 21st century, the relationship between bisphosphonate usage and impaired bone physiology was observed. The strong inhibition of the osteoclast function caused by bisphosphonate therapy leads to bone metabolism inhibition with impaired wound healing after trauma (such as dental surgery) or jaw bone fractures. Because bisphosphonates are primarily deposited in bones with a high rate of metabolism, it is possible to have increased levels of bisphosphonates in the jaw bones [14].

Diabetes is mentioned as one of the cause factors of metabolic bone disorder. Diabetic alterations in mineral and bone metabolism result in bone mass decrease [15-16].

Diabetes affects bones through diabetic microangiopathy leading to blood supply reduction and reduced formation of collagen fibers due to osteoblast inhibition. The reduced amount of bone collagen matrix is followed by reduced deposition of mineral salts resulting in prolonged bone healing [17].

AIM

Based on the presented literature we set the aim of this study:

- To determine the serum values of CTX (as a direct indicator of bone resorption process) and its possible impact on bone mineralization followed up by bone density increase in oral surgical defects in patients with diabetes
- To determine the correlation between serum markers representative (CTX) with type of diabetes, duration of diabetes and diabetes regulation degree.

MATERIALS AND METHODS

Prospective clinical study was done at the Clinic of Oral Surgery, University Dental Clinical Center and the Department of Oral Surgery at the Faculty of Stomatology in cooperation with University Clinic of Endocrinology, diabetes and metabolic disorders in Skopje.

The survey included 100 respondents divided into two groups: participants with diabetes (60) and a control group without diabetes (40). For each patient detailed medical history, clinical examination and radiographic analysis was performed, leading to indication for oral surgical intervention.

Each patient was voluntarily involved in the research confirmed by a signature on the prepared consent form.

For biochemical analysis (HbA1C, CTX) blood sample from the antecubital vein was taken from each patient. Biochemical analyzes were conducted at the University Clinic of clinical biochemistry in Skopje.

Oral surgical interventions were performed at the Clinic for Oral Surgery respecting the specified operative protocol.

After 24 hours x-ray verification of the oral surgical defect was made (panoramic radiography). Panoramic radiographs were repeated after 6 and 12 months. The subsequent radiographs were digitized through professional photo scanner, Epson perfection V 600, and analyzed by Adobe Photoshop 7.0 for Windows 7 using the gray scale histogram where illumination intensity of the measured field is converted and expressed in pixels.

Converting the area of interest in gray scale enables accurate estimation of increased or decreased bone density which is equivalent to the degree of bone defect mineralization.

The percentage was calculated using the formula presented in Ihan Hren, Milijavec [18] analysis:

Relative bone healing = {NB (new bone formation density)/ SB (surrounding bone density)} X 100

The statistical programs STATISTICA 7.1 and SPSS 17.0 were used for the statistical analysis.

ISSN: 0975-8585

RESULTS AND DISSCUSION

By abandoning the concept of bone tissue inertness as the mainstay of an organism, rich metabolic activity of bone tissue is frequently mentioned, provided by the the composition of tissue involving multiple cell types and collagen matrix.

Bone metabolism is regulated by bone cells. The bone cells activity and bone metabolism indirectly can be followed through the values of serum bone formation and resorption markers (osteocalcin and ßcrossLaps-CTX).

Serum marker of bone resorption ßcrossLaps-CTX (Collagen cross links), released from the bone matrix after resorption (early breakdown of collagen type 1), is one of the most sensitive markers of this group.Collagen type 1makes about 90% of the bone organic matrix . It is helical protein associated with short bridges of N-amino and C-carboxy molecule terminals. Osteoclasts secrete a mixture of acidic and neutral protease during bone resorption that degrades the mature collagen in molecular fragments including C-terminal telopeptide (CTX). These CTX fragments, released into the bloodstream during bone resorption, serve as a specific marker for mature collagen type 1 degradation. Elevated serum concentrations of CTX has been reported in patients with increased bone resorption [19].

The results obtained from the research analysis showed that the average value of bone resorption marker (CTX) in the control group was 0.3 ± 0.1ng/ml (minimum 0.115ng /ml, maximum 0.657ng/ml), while in experimental group was lower at 0.2 ± 0.09ng/ml (minimum 0.113ng/ml, maximum 0.645ng/ml) [Table1]. The difference between the values of CTX is statistically significant for $p < 0.05$. [Table2].

Table 1: Average values of serum marker CTX in tested groups

group	average	Num.	St dev	min	max
KG	0.3	40	0.1	0.115	0.657
DG	0.2	60	0.09	0.113	0.645

Table 2: Mann-Whitney U test

	Rank Sum	Rank Sum	U	Z	p-level
CTX	2234.000	2816.000	404.0000	-5.60064	0.000000

Numerous studies have analysed the CTX marker and they all agree with the fact that in diabetic patients, CTX concentrations changes occur. These changes provide an information about the bone tissue condition in patients with this metabolic disorder.

Reduced values of bone resorption, indicated by significantly lower CTX values, indicate reduced general bone turnover, consistent fact with the findings of Linde [20].

On the contrary, histomorfometric studies from Brandi [5] did not show changes in the bone resorption in diabetic patients. Changes are reflected only in the process of bone formation, supported by the results of the bone markers values (osteocalcin and CTX).

Additional parameters for CTX values correlation (type of diabetes, the duration of diabetes and diabetes regulation degree) were included in the study.

The diabetes regulation degree was determined by the values of glycosylated hemoglobin-HbA1C. Glycosylated hemoglobin is formed in a reaction between hemoglobin and glucose in the blood. It is a direct indication of the glucose serum levels for a period of 2-3 months, as the half-life of hemoglobin in blood circulation. Because it is called “golden standard” in glicoregulation assessment [21].

In our research we set value of HbA1C at 7.0% as good glycemic control under research of Petrovski [22].

In studies of Thrailkill[23]; McCaeb[24]; Capoglu[25] and Winhofer[26], correlation with our data was shown, which means immutability of resorptive marker CTX depending on diabetes regulation degree [Table 3]. The analyzes of these studies indicate normal bone resorption, sometimes even reduced, while bone formation is significantly reduced, confirmed by decreased osteocalcin serum levels. Opposing views were found in the study of Achemlal [27], where values of CTX are in significant negative correlation with HbA1C values.

Table 3: Correlation between serum marker CTX versus duration of diabetes and HbA1C

CTX	duration of diabetes	HbA1C
DG	r=-0.2866	r=-0.0508
	p=0.026	p=0.700

The duration of diabetes in our study showed statistically significant negative correlation with the values of the serum marker CTX [Table 3]. Long duration of diabetes leads to clinical manifestation of some chronic complications. If microangiopathy occupies the renal tissue (diabetic nephropathy), renal hyperfunction and microalbuminuria will occure. Most of the complications occur after 15-25 years of having diabetes. This data is used in the study of Hossein-Nezhad et al [6] where increased urinary excretion of CTX is the reason for reduced values of the indicated tag.

The duration of diabetes in different studies shows variously interpreted influence on osteogenesis and concentrations of bone serum markers. Brandao [28] did not show correlation between bone serum markers and duration of the diabetes. Patients with short duration of diabetes (recently diagnosed diabetes) have affected bone formation due to the absence of insulin anabolic effect (in case of diabetes type 1). In case of diabetes type 2, long asymptomatic existence of hyperglycemia may lead to chronic vascular complications that have an impact on bone metabolism. Therefore, the duration of diabetes (in time of its diagnosis) may not be a relevant indicator for bone metabolism condition screened through analysis of serum markers. [29- 31]

In this study values of CTX between respondents with different types of diabetes did not show statistical significance The average value of CTX in type 2 diabetes group was 0.2 ± 0.1 ng/ml, while in type 1 diabetes was 0.2 ± 0.07 ng/ml [Table 4]. The difference is statistically insignificant for p> 0.05 [Table 5].

Table 4: Average serum marker CTX in different types of diabetes

Diabetes / CTX	average	number	St.dev	min	max
Diabetes type 2	0.2	31	0.1	0.123	0.645
Diabetes type 1	0.2	29	0.07	0.113	0.329

Table 5: Mann-Whitney U test

	Rank Sum	**Rank Sum**	**U**	**Z**	**p-level**
CTX	927.500	902.5000	431.5000	-0.266268	0.790033

Different type of diabetes affect bone metabolism by various mechanisms leading to different results in terms of serum bone markers, but we still need to keep attention on the different quality and quantity of bone tissue that shows changes in the bone structure [20] and bone density [32].

Hongbing [33] shows changes in the microarchitecture of the bone tissue, causing increased fragility of bones and decreased remodeling ability, which helps us to understand increased risk of bone damage, typical for this type of patients with unchanged bone quantity and bone density.

Metabolic aspects of diabetes as systemic disorder, directly affect the healing of bone fractures and defects in the jaw bone occurred by osteotomy or through a pathological process [34-36]. Bone healing takes place in three phases: the inflammatory phase, the phase of repair and the bone remodeling phase. After the remodeling phase the bone gets its previous building, structure and mechanical durability.

The time required for healing of alveolar bone defects (occurring after operative extraction of impacted teeth, apicotomy or cystectomy) is different and depends on numerous local and general factors including diabetes [37-38].

In our research, the average percentages of bone density in the defect versus density of the surrounding healthy bone on immediate radiography in the experimental group is 29.7 ± 5.1%, on the radiographic control image after 6 months bone density increased to 54.4 ± 7.1% and on the control after 12 months reaches a value of 77.2 ± 8.7% [Table 6].

The average percentages of bone density in the defect versus surrounding healthy bone on immediate radiography in the control group is 23.8 ± 5.2%, on the radiographic control image after 6 months bone density increased to 52.5 ± 7.3% and on the control after 12 months reaches a value of 84.5 ± 9.0% [Table 6].

Table 6: Average radiographic analysis (%) of bone healing compared to the surrounding healthy bone in both groups

	group	average	number	St dev	min	max
RTG immediate	**DG**	29.7	60	5.1	18.4	44.9
	KG	23.8	40	5.2	17.4	36.0
RTG afer 6 months	**DG**	54.4	60	7.1	38.0	68.5
	KG	52.5	40	7.3	35.2	69.1
RTG afer 12 months	**DG**	77.2	60	8.7	62.0	96.0
	KG	84.5	40	9.0	65.8	97.8

Table 7: Mann-Whitney U test

	Rank Sum	Rank Sum	U	Z	p-level
Immediate	3732.500	1317.500	497.500	4.942773	0.000001
After 6 months	3267.500	1782.500	962.500	1.67104	0.0947
After 12 months	2500.500	2549.500	670.500	-3.72555	**0.000195**

The different bone healing between the groups after 12 months surrounding healthy bone in the test group (77.2 ± 8.7 versus 84.5 ± 9.0%) is statistically significant for $p < 0.05$ [Table 7].

Through the above mentioned biochemical analysis, we focus on three main steps of osteogenesis:

- synthesis of extracellular organic matrix (osteoid)
- matrix mineralization
- remodeling (resorption and formation)

Reduced values of bone resorption indicated by significantly lower values of bone resorption marker in subjects with diabetes, suggest reduced bone turnover in this group of respondents. These findings correlate with the findings of Sun [17], Hwang [39], Lappin [40], Bao [41] and Linde [20].

Opposite to our results are the histomorfometric studies of Brandi [5] which did not show changes in bone resorption.

We confirmed our results with another statistical method - MRA (multiple regression analysis). Multiple regression analysis in patients with diabetes determined connection between the values of bone healing (%) compared to the surrounding healthy bone (dependent variable) and the system of independent variables (type of diabetes, duration of diabetes, the degree of regulation-HbA1C, CTX).

The analysis of independent variables showed a significant impact of duration of diabetes on the percentage of bone healing.

For the independent variable-duration of diabetes, the coefficient of partial regression analysis was 0.323 and tested with t -test shows that the impact on bone healing (%) compared to the surrounding healthy bone is statistically significant for $p = 0,013$ [Table 8].

ISSN: 0975-8585

Table 8: Multiple regression analysis (%) of bone healing compared to the surrounding healthy bone in patients with diabetes

INDEPENDENT VARIABLES	R = 0,749 R^2 = 0,561 F = 4.539 p= 0,000063		
	Beta	t – test	p - level
gender	0.127266	1.12802	0.265162
age	0.047782	0.41075	0.683161
BMI	-0.154686	-1.29397	0.202136
Type of diabetes	-0.223339	-1.76261	0.084609
Duration of diabetes	-0.323594	-2.57356	**0.013354***
HbA1C	-0.170630	-1.37227	0.176633
CTX	-0.224291	-1.83379	0.073159

The results of MRA did not show significant impact of CTX on percentage of bone healing in both groups of respondents.

CONCLUSION

Serum values of bone resorption marker CTX in diabetic patients suggested reduced activity of bone metabolism with no influence on bone mineralization process followed by bone density increase in oral surgical defects.

Type of diabetes, duration of diabetes and the degree of regulation (HbA1C) may have an impact on CTX values. The results showed a statistically significant negative correlation between CTX and duration of diabetes. Different types of diabetes do not affect the CTX levels.

The awareness of the possible impact of diabetes and its complications on the mineralization and remodeling of oral-surgical defects followed by simple biochemical analysis will contribute to adequate treatment of these patients in terms of prevention of postoperative complications

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