Original article

Crevicular C-telopeptide and C-propeptide of type I collagen are markers of parodontal disease evolution in diabetic and non-diabetic patients

Telopeptida C şi propeptida C creviculare – markeri de evoluție în boala parodontală la pacienți diabetici şi non-diabetici

Roxana I. Vasluianu[1*], Didona Ungureanu[2], Daniela Jitaru[2], Andreea D. Ioanid[3], Norina C. Forna[1]

1."Gr.T.Popa"University of Medicine and Pharmacy, Faculty of Dental Medicine, Department of Removable Dentures
2."Gr.T.Popa"University of Medicine and Pharmacy, Faculty of Medicine, Department of Biochemistry
3. Specialist of dental medicine

Abstract

The alveolar bone is the place where some permanent structural reshaping occurs through resorption and apposition processes induced by the modification of the forces direction on the level on odontal support, so that they withstand efficiently the mechanical stress. Collagen is the most abundant protein in the human body and at the same time, the most important one. The assessment of the C-telopeptide of type I collagen (CTx) and of the C-terminal propeptide of type I procollagen (PICP) was carried out on a lot of 174 patients, between 50-75 years old, out of which 130 were women and 44 men. The lot of patients was divided according to sex and pathology. The determination of the markers implied taking samples of crevicular fluid on the level of the gingival sulcus, with filter paper. The bone resorption rate was significantly modified ($p<0.001$) in the case of the patients with diabetes or osteoporosis compared to the witness lot whilst the evolution of the bone apposition was not significantly modified. From the results obtained in our study, we could draw the conclusion that the PICP marker of bone apposition was not correlated with the clinical pathology of the patients, unlike the CTx marker of bone resorption and atrophy of the alveolar bone that was significantly correlated from the statistical point of view with the clinical pathology of the investigated patients.

Key words: *C-telopeptide of the type I collagen - CTx, C-propeptide of the type I procollagen - PICP, crevicular fluid*

[*]**Corresponding author:** Roxana Ionela Vasluianu, "Gr.T.Popa"University of Medicine and Pharmacy, Faculty of Dental Medicine, Department of Removable Dentures, Pasaj Banu, Nr.4, Iasi, 707027 Romania.
Tel. +40232251128, Fax +40232251128, Mobile +40722926248, e-mail: roxana302000@yahoo.com

Rezumat

Osul alveolar este sediul unor permanente remanieri structurale prin procese de rezorbție și apoziție induse de modificarea direcției forțelor de la nivelul suportului odontal, astfel încât acesta să reziste eficient la solicitările de ordin mecanic. Colagenul este proteina cea mai abundentă din organismul uman și cea mai importantă totodată. Evaluarea telopeptidei C terminale a colagenului de tip I (CTx) și a propeptidei C terminale a procolagenului de tip I (PICP), s-a efectuat pe un lot de 174 de pacienți, cu media de vârstă cuprinsă între 50-65 ani, dintre care 130 femei și 44 bărbați. Lotul de pacienți a fost împărțit în funcție de sex și patologie. Determinarea markerilor a presupus prelevarea de lichid crevicular de la nivelul sulcusului gingival, cu hârtie de filtru, la pacienți asanați în prealabil. Rezultatele obținute indică importanța markerului CTx în comparație cu PICP și intervalele biologice pentru care rata de rezorbție osoasă este modificată. Rata rezorbției osoase este semnificativ statistic modificată (p<0,001) la pacienții cu diabet sau osteoporoză în comparație cu lotul martor comparativ cu evoluția apoziției osoase, care este modificată nesemnificativ statistic la loturile cu patologie diabetică sau osteoporoză comparativ cu lotul martor. Din rezultatele obținute în studiul nostru se poate concluziona că markerul PICP de apoziție osoasă nu se corelează cu patologia clinică a pacienților, spre deosebire de markerul CTx de rezorbție osoasă și atrofie a osului alveolar, care se corelează semnificativ statistic cu patologia clinică a pacienților investigați.

Cuvinte cheie: *telopeptida C a colagenului de tip I - CTx, propeptida C terminală a procolagenului de tip I – PICP, lichid crevicular.*

Introduction

The bone tissue is a much differentiated support element that is characterized by two fundamental features: high mechanical resistance due to minerals and considerable elasticity due to its organic components. The alveolar bone is the place where some permanent structural reshaping occurs through resorption and apposition processes induced by the modification of the forces direction on the level on dental support, so that they withstand efficiently the mechanical stress. Bone reshaping is controlled by local and systemic factors that regulate the structural and metabolic functions (1, 2).

Collagen is the most abundant protein in the human body and at the same time, the most important one. Soft organs contain a relatively small amount of collagen, while the bone contains 23% from the dry mass. The main function of collagen is to provide resistance and to maintain the structural integrity of the tissues (3). Collagen undergoes some important metabolic synthesis and degradation processes. The modifications in the metabolism of collagen explain a series of metabolic mechanisms that occur in diabetes mellitus and osteoporosis (4, 5). Type I collagen comprises 90% of the organ matrix of the bone and it is the most abundant collagen in the bone tissue. The degradation products of collagen are considered to be important markers of the bone turnover in several osteolytic or bone metabolic diseases (6). The relation between diabetes and the level of the CTx and PICP markers, through sampling on the level of the crevicular fluid, is a present day interest subject due to the high percentage of type I collagen on the level of the marginal paradontium.

The aim of the study was to evaluate the bone markers, CTx and PICP, in order to establish a relation between the resorption and the apposition degree of the alveolar bone in patients with diabetes mellitus and osteoporosis.

Material and methods

The evaluation of the C-terminal telopeptide of type I collagen and C-terminal propeptide of type I procollagen was carried out on a group of 174 patients, between 50-75 years old, out of which 130 were women and 44 men. The lot of patients was divided according to sex and pathology. The patients included in the study

did not smoke and did not drink alcoholic beverages. The study was not conducted on children, pregnant women, patients with neoplasm or other chronic diseases except diabetes mellitus or osteoporosis. The purpose of the determinations was to emphasize the bone resorption and/or regeneration degree on the level of the alveolar bone. The patients were distributed into 4 groups, according to the general condition:

- M = control group with a balanced general condition (44 patients: 16 women and 28 men),
- DM2 = group with type 2 diabetes mellitus (72 patients: 56 women and 16 men),
- DM1 = group with type 1 diabetes mellitus (32 patients: 12 women and 20 men),
- Os = group of patients with osteoporosis (26 female patients).

Upon the assessment of the group, we took into account the age, sex, type of diabetes mellitus, osteoporosis and affection degree of the odonto-parodontal support. The variation of the edentation on the level of the maxilla and the mandible was also studied in the research, by evaluating the area of partial extended edentation (PEE), partial reduced edentation (PRE), extended edentation (EE), complete edentation (CE) depending on the studied pathological cases. The lots of patients suffering from diabetes were made up of patients registered at the Diabetology Clinic within „Sf. Spiridon" Hospital from Iaşi; the patients were diagnosed with type I or type II diabetes and they were under the strict monitoring of the diabetologist who kept repeating the biochemical analyses specific to the existent disease. The lot of patients with osteoporosis and the control lot were made up of patients that came to the Clinical Base of Stomatological Education within the Faculty of Dentistry, „Gr. T. Popa" University of Medicine and Pharmacy, Iaşi.

The study was carried out in collaboration with: Immunology and Genetics Laboratory of "St. Spiridon"Hospital, where we performed the determinations of the bone markers; Diabetology Clinic within "St. Spiridon" Hospital, from where the patients for the study lot were selected; Clinical Base of Stomatological Medical Education where clinically healthy patients and patients with osteoporosis came.

The dental-periodontal status was emphasized through the clinical examination that established the gingival retraction degree, the gingival inflammation degree and the examination of the periodontal bags with the help of the periodontal probe. The clinical examination was accompanied by paraclinical exams such as: retro-dental-alveolar radiography, orthopantomography and the determination of the bone resorption and apposition. The patients included in the study presented different degrees of evolution of the periodontal disease.

The determination of the markers implied taking samples of crevicular fluid on the level of the gingival sulcus, with filter paper, from previously decontaminated patients. The sampling site was isolated with cotton rolls and slightly air dried and then 2 strips of filter paper were applied at a single tooth, being inserted on the gingival sulcus till coming across a slight resistance. They were maintained in the sulcus for 25-30 seconds. The samples were then introduced into Eppendorf tubes with PBS solution. Both propeptides were measured through ELISA technique.

For determining the bone markers, we used: CROSSLAPS FOR CULTURE ELISA kit, made by the company Imunodiagnostic systems, UK, for *C-telopeptide of type I collagen.* The determination was done through ELISA technique (enzyme-linked immunosorbent assay) and the detection limit was 0.44 nM CrossLaps. The standard curve was generated using 8 standard concentrations: 0 nM; 1.32 nM; 2.65 nM; 5.29 nM; 10.59 nM; 21.18 nM; 42.35 nM; 84.70 nM. METRA CICP kit made by the company Quidel Corporation-USA was used for *C-terminal propeptide of type I procolagen*, quantification by ELISA technique. Six standard concentrations were provided: 0 ng/mL; 1 ng/mL; 2 ng/mL; 5 ng/mL; 20ng/mL; 80 ng/mL and the detection limit was 0.2 ng/mL.

The t-Student test and the Pearson correlation coefficient were used for statistical pro-

Table 1. Statistical evaluation of the CTx marker for the studied lots

Gender		DM2	DM1	M	Os
F	M ± DS	19.357 ± 11.99	10.65 ± 5.660	26.57 ± 18.85	15.57 ± 9.968
	p	p < 0.001	p < 0.01	p = 0.01	p < 0.001
M	M ± DS	5.75 ± 1.625	7.50 ± 3.098	35.62 ± 31.48	-
	p	p<0.001	p<0.01	p=0.001	-

Tabel 2. Statistical evaluation of the PICP marker for the studied lots

Gender		DM2	DM1	M	Os
F	M ± DS	8.597 ± 6.992	8.48 ± 4.765	7.54 ± 7.58	8.49 ± 6.641
	p	p < 0.001	p < 0.001	p < 0.05	p = 0.001
M	M ± DS	12.24 ± 2.261	10.64 ± 5.451	9.90 ± 10.38	-
	p	p < 0.001	p < 0.01	p < 0.01	-

cessing, using the SPSS 13.0 statistic and Microsoft Excel 1997 softwares. The statistical interpretation of data took into account the corresponding differences for a significance threshold p<0.05.

Results

As any other component of the human body, the parodontal structures also undergo the ageing phenomenon, especially in the case of the patients with metabolic diseases.

The obtained results aimed at the correlation between the obtained values for women and for men, depending on pathology, establishing a relation regarding the destructive or regenerative potential of the paradontium in correlation with the two investigated fractions of collagen I, as well as establishing the importance of the type I collagen determinations through taking samples on the level of the crevicular fluid.

The average age of the patients from the investigated lots ranged between 50-65 years. The lot of patients with type 2 DM represented 41.4% of the total number of investigated patients (21.54% women, 18.9% men) against the witness lot that represented 28.93% (10.74% women, 18.19% men). The lot with type 1 DM represented 21.34% of the total number of investigated patients, and the lot of female patients with osteoporosis represented 10%.

The investigated female patients from the lot with type 1 diabetes mellitus (lot DM1/F) registered an average value of the bone resorption marker (CTx- C-telopeptide of type I collagen) ranging in the interval 1-15 nM emphasizing a high degree of resorption and atrophy of the alveolar bone, as compared to the female patients from the witness lot (lot M/F) for which the average value of the CTx marker is over 20 nM indicating a small degree of bone resorption. Thus, the average value of CTx is 2.5 and respectively 1.3 times higher for the female patients from the witness lot in comparison with the obtained average for the lot of female patients with DM1 and respectively DM2.

At the same time, the differences obtained for the marker CTx were significant from the statistical point of view (p<0.001) between the lots DM2, DM1 in comparison with the lot M both for the female as well as for the male patients included in our study. The average value of CTx for the witness lot was 1.7 times higher than in the case of the female patients with osteoporosis (lot Os). Those results showed that the

bone resorption at the level of the oral cavity had an average rate for the patients with type 2 diabetes or with osteoporosis as compared to the witness lot, while for the male patients both with type 1 diabetes and type 2 diabetes, the bone resorption with the alveolar bone atrophy had a much higher degree (*Table 1*).

The statistical analysis of the CTx parameter for the female lots showed significant statistical differences between the control lot and the lot with DM1 ($p<0.001$), the values of two series being negatively correlated ($r= -0.515$, $p= 0.001$). More precisely, an increase of the value of CTx for the female control lot associated a decrease of its value for the DM1 lot. Significant statistical differences were also established between the female control lot and the female lot with DM2 ($p<0.001$), but now the established correlations were positive ($r= 0.505$, $p= 0.001$). The increase or decrease of the value of CTx for the witness lot would trigger the same type of modifications for the female lot with DM2. For the female lot with osteoporosis, as compared to the female lot from the witness lot, the statistical differences were also significant from the statistical point of view, the established correlations being negative ($r= -0.526$, $p<0.001$), the same as for the female lot with DM1.

The male patients, both those from the lot of patients with type 1 diabetes (lot DM1/M) as well as those from the lot of patients with type 2 diabetes mellitus (lot DM2/M), registered an average value of the CTx marker also ranging in the unfavorable interval of bone resorptions. More precisely, the CTx for the lot M (35.62 nM) was 6 times, respectively 4.7 times higher than the value obtained for the lot DM2 (5.75 nM) and respectively DM1 (7.50 nM), thus emphasizing a significant decrease from the statistical point of view ($p<0.001$) of CTx for the lots of patients with diabetes as compared to the witness lot.

In our study, for the bone apposition marker PICP (C-terminal propeptide of type I procollagen) the obtained differences for the studied lots were not significant from the statistical point of view ($p>0.05$). The average value obtained by that marker for the witness lot both for women and for men was in the same interval (0-10 ng/mL) with the average PICP obtained for the lot DM1, DM2 and the lot Os. For the lot of male patients with type 1 and 2 diabetes, the average value of PICP ranged in the interval with an average rate of bone apposition (10-15 ng/mL) (*Table 2*).

The statistical analysis of the PICP parameter for the lots of women showed insignificant statistical differences between the witness lot and the lot with DM1 ($p>0.05$), the values of the two series being negatively correlated ($r= -0.844$, $p<0.001$). The decrease of the PICP value for the female witness lot associated an increase of its value for the DM1 lot. Insignificant statistical differences were also established between the female witness lot and the female lot with DM2 ($p>0.05$), the established correlations being now positive ($r= 0.421$, $p= 0.007$).

In this study, we have emphasized the importance of the CTx marker in comparison with PICP and the biological intervals for which the bone resorption rate is modified. The bone resorption rate is significantly modified from the statistical point of view ($p<0.001$) for the patients with diabetes or osteoporosis in comparison with the evolution of the bone apposition that is not significantly modified from the statistical point of view ($p>0.05$) for the lots with diabetic or osteoporosis pathology comparatively with the witness lot.

All the results obtained following the determination of the bone markers on the level of the crevicular fluid have been correlated with the odonto-parodontal status assessed through clinical and radiological examination. There is a real correlation between the affection degree of the marginal parodontium and the value of the C telopeptide of type I collagen.

Variation of the edentation for the studied lots depended on the studied pathology**.** The edentation on the level of the MX and the MD

was evaluated by studying the areas of PEE, PRE, EE, CE depending on the studied pathological cases. Taking into account the edentation MX and MD of the studied patients, both for the diabetic or osteoporosis lots, as well as for the witness lot, the patients with PEE and PRE prevailed. Taking into account MX, no CE was registered for the witness lot, and the percentage for patients with PEE was equal to that for EE. Complete edentation was registered only for the patients with type 1 diabetes. For the diabetic lots, PRE was more frequent than ES and for the lot of female patients with osteoporosis ES was more frequent than PRE.

For the patients with DM1, we noticed important modifications at the level of the marginal paradontium through the appearance of the vertical osseous lysis, dental mobility and the presence of gingival pockets. The early appearance of edentation was correlated with the general pathology.

Discussions

Gingival of crevicular fluid is a pathophysiological fluid that flows into the oral cavity. The epidemiological studies have shown that the parodontal disease is much more common in the case of the patients with diabetes as compared to the patients that do not suffer from this affection. Insulin has an anabolic effect on the bone and there is the hypothesis that an insufficient level of insulin contributes to the unbalance of the bone metabolism. The inflammatory mediators produced as a response to an infection play an important role in the increase of the insulin resistance and an altered protein gradient may contribute to periodontal disease in patients with type 2 DM (6,7).

Depending on the value of CTx and PICP, it could be established whether for the studied patients the evolution of the periodontal disease was accentuated and whether there was or not a bone appoition process, thus being the only paraclinical examination that could emphasize that aspect.

The best therapeutic variant, at the level of the odonto-periodontal support depended on the values of CTx and PICP. For the patients where the value of CTx indicated an accentuated bone lysis process, not accompanied by the bone regeneration process, we could not apply the same treatments (prosthetic, odontal, periodontal) as for the patients that had marginal periodontitis with slow evolution due to the reduced strenght of the periodontal support. For the patients diagnosed with diabetes and osteoporosis, a higher degree of periodontal affection could be noticed, also showed by the values of CTx and PICP.

The multifactorial analyses related to the resorption of the residual crests are still rare. The processes that take place at the level of the alveolar bone, as well as the metabolic processes on this level can be different than other bone structures (8,9). The study of the hard support tissues is rendered difficult by the fact that the bone is one of the most unstable tissues in the organism (4).

Osteoclasts are are responsible for resorption of bone, and increased activity of these cells is associated with several common bone diseases, including post-menopausal osteoporosis (10). The results of the researches have shown that the alveolar bone presented rapid rates of bone loss for the subjects with systemic osteoporosis, with low mineral bone density, with low bone turnover, even if the exercised pressures on the alveolar bone are normal, low or absent (11). In the applications of the recent studies, the female sex and the systemic factors seem to be more important than those related to the wearing of a prosthetic device, especially on the level of the mandibular bone (12,13).

Various studies have shown a correlation between the level of the PICP and the bone resorption rate, while the clinical relevance of PICP regarding the evaluation of the metabolic diseases is regarded with skepticism. Propeptides share these properties with most of the parameters of the metabolism of collagen (14,15).

The negative correlation occured due to the fact that the bone regeneration process was

reduced after the age of 50 years or even absent in the case of the people that suffered from diseases such as diabetes or osteoporosis, a reason for the early appearance of the periodontal disease. The bone regeneration process had a less obvious character (attenuated) than the bone lysis process that was much more relevant for the periodontal disease and significant, positive correlations could be noticed between the general affection of the patient and the periodontal affection degree (16). The minimal surgical intervention may need to be revised to include alternative strategies for the elimination or management of these pathologies (17,18).

The alveolar bone essentially behaves as a bone of the organism that undergoes a great load, such as the macroscopic modeling and internal structural remodeling. The mechanical stress absorbed by the parodontium, as a result of the functional, parafunctional and therapeutic load, greatly influences the shape and the turnover with a high remodeling degree. The bone turnover can be assessed/ quantified through C terminal telopeptide of type I collagen, becoming a sensitive marker of the bone modifications (3).

From a practical point of view, the thermal stability of propeptides represents an advantage because the extension of transport and storage time is well tolerated without significant signal loss (7).

Conclusions

The degradation products of collagen are considered important markers of the bone turnover in several osteolytic and bone metabolic diseases.

The predilection of the patients with DM for the parodontal disease is much higher and the determination of the biochemical markers, CTx and PICP, in the crevicular fluid, represents one of the present day methods for paraclinical emphasizing of the bone resorption and atrophy of the maxilla bones, as well as of the bone regeneration level.

Thus, we can say that type 1 diabetes is a pathology that leads at the level of the mouth cavity to a smaller degree of bone resorption both for the female, as well as for the male patients.

From the results obtained in our study, we can draw the conclusion that the PICP marker (C terminal propeptide of type I procollagen) of bone apposition is not statistically correlated with the clinical pathology of the investigated patients unlike the CTx marker of bone resorption and atrophy of the alveolar bone that is intensely correlated with the clinical parameters of the studied patients.

Abbreviations

CE – complete edentation
CTx - C- telopeptide of type I collagen
DM1 - type 1 diabetes mellitus
DM2 - type 2 diabetes mellitus
EE – extended edentation
F- female
M - witness lot with a balanced general condition
M-male
MD– mandibular
Mx - maxillar
Os – osteoporosis
PEE – partial extended edentation
PICP - C-terminal propeptide of type I procollagen
PRE – partial reduced edentation

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