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Trace Elements Analysis in Gingiva in Patients with Chronic Periodontitis

Analiza pierwiastków śladowych w tkance dziąsłowej u pacjentów z przewlekłym zapaleniem przyzębia

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Abstract

Background. In the literature, the dependence between organism's disease states and elemental composition of tissues involved in the disease process is being emphasized. Basing on the available literature, one can state almost complete lack of studies connecting ultrastructural changes in inflammatory forms of periodontopathy with elemental analysis of surrounding soft tissues.

Objectives. Establishing contents of specific trace elements in soft tissues of marginal periodontium in patients with chronic periodontitis as compared to patients with healthy periodontium.

Material and Methods. The examination comprised 17 gingival biopsy specimens from patients with chronic periodontitis and 13 from healthy patients by standard methods in terms of morphology. Patients included in the study were all Caucasian, males and females, aged 29–53. Biopsy specimens were examined by standard methods in terms of morphology but elemental composition analysis of gingival tissue was done by a new, not previously used method developed by the author of this study.

Results. Elemental content of a gingival tissue during the course of periodontitis is different than that of a healthy tissue, especially in respect to zinc and magnesium.

Conclusions. Elemental content of gingival tissue in a course of deep periodontitis is different than that of a healthy tissue, especially in relation to zinc and magnesium, which can be an evidence of their role in healing process of inflammatorily changed soft tissues. There is a tendency to increase the amount of calcium and decrease of phosphorus in the gingival tissue of the study group. The obtained results can be used in future to determine the rules of supplementing specific microelements deficiencies in the course of periodontal diseases, which will complement basic treatment (**Dent. Med. Probl. 2005, 42, 2, 227–231**).

Key words: periodontitis, trace elements, gingival tissue.

Streszczenie

Wprowadzenie. W piśmiennictwie podkreśla się zależność między stanami patologicznymi organizmu a składem pierwiastków w tkankach objętych procesem chorobowym. Opierając się na dostępnej literaturze, można stwierdzić niedosyt badań wiążących zmiany mikrostrukturalne tkanki dziąsłowej w zapalnych postaciach periodontopatii z jej analizą pierwiastkową.

Cel pracy. Oznaczenie zawartości poszczególnych pierwiastków śladowych w dziąśle u pacjentów z przewlekłym zapaleniem przyzębia w porównaniu z osobami ze zdrowym przyzębiem.

Materiał i metody. Grupę badaną stanowiło 17 wycinków tkanki dziąsłowej pobranych od pacjentów z rozpoznaniem przewlekłego zapalenia przyzębia, grupę kontrolną natomiast 13 wycinków od osób ze zdrowym przyzębiem. Pacjenci zakwalifikowani do badań byli rasy białej, kobiety i mężczyźni w wieku 29–53 lat. Analizy morfologicznej wycinków dokonano według metod standardowych, a do określenia składu pierwiastków wykorzystano nową, określoną przez autorów metodykę badań.

Wyniki. Stwierdzono, że zawartość pierwiastków w tkance dziąsłowej w przebiegu zapalenia przyzębia jest inna niż w tkance zdrowej, zwłaszcza w odniesieniu do cynku i magnezu.

Wnioski. Zawartość pierwiastków w tkance dziąsłowej w przebiegu zapalenia przyzębia jest odmienna niż w tkance zdrowej; dotyczy to przede wszystkim cynku i magnezu, co może świadczyć o ich roli w procesie gojenia zapalnie zmienionych tkanek miękkich. Wyniki uzyskane na podstawie przeprowadzonego testu wskazują, iż istnieje

je tendencja do wzrostu zawartości wapnia a spadku fosforu w tkance dziąsłowej grupy badanej. Uzyskane wyniki mogą być użyte w przyszłości do określenia zasad suplementacji niedoborów poszczególnych mikroelementów w przebiegu zapalenia przyzębia, co uzupełni leczenie podstawowe (**Dent. Med. Probl.** 2005, 42, 2, 227–231).

Słowa kluczowe: zapalenie przyzębia, pierwiastki śladowe, tkanka dziąsłowa.

The most common disease of periodontium is chronic periodontitis. Bacteria present in a dental plaque are considered as the main etiologic factor in marginal periodontitis. There are also many systemic and local factors, which are modifying the genesis and course of the disease. Among general factors, one distinguishes endocrine glands disturbances, blood disorders, genetic syndroms, age, drugs taken and dietary habits. Diet has double influence on periodontium: exogenous (food consistency) and endogenous (deficiencies of specific nutrients like vitamins, protein, trace elements). In the literature, the dependence between disease states of an organism and elemental composition of tissues involved in a disease process, is being emphasized [1, 2]. For years, attempts have been made for using micro- and macroelements in treatment of many systemic and local illnesses, the etiology of which is related to trace elements deficiency.

Basing on the available literature one can state almost complete lack of studies connecting ultrastructural changes in periodontitis with elemental analysis of surrounding soft tissues. So far, data about quantitative and qualitative microelement composition of a tooth and surrounding tissues during the course of periodontitis, has been rather fragmentary and results have obtained in many cases are divergent. The goal of the study was to determine contents of specific trace elements in gingival papilla in patients suffering from chronic periodontitis as compared to patients with healthy periodontium.

Material and Methods

In the study, the authors used 30 biopsy specimens of gingival tissue, obtained from white male and female patients at the age between 29 and 53. Patients included in the study were treated at the Department of Conservative Dentistry with Endodontics, Medical School of Silesia in Katowice (Poland), between 1998 and 1999. The study group consisted of 17 biopsy specimens of gingival tissue obtained from patients with a diagnosis of chronic periodontitis (group CP) and control group consisted of 13 patients with healthy periodontium (group Z). Patients exposed to heavy metals (f.e. because of occupational exposure), suffering from systemic illnesses, smokers, and taking medicines affecting elemental composition

of body tissues (f.e. steroids) were excluded from the study.

The gingival tissue for the study was obtained within free gingiva (with dimensions not exceeding 1 mm). It was collected during routine surgical interventions within a range of periodontium (f.i. gingivectomy, curettage) and during routine dental extractions (in cases when performing gingivoplasty became necessary) for study and control group. From a single patient, two biopsy specimens of gingival tissue, with above mentioned dimensions, were collected. Tissues removed in such a manner in a course of daily surgical interventions, become post operative waste and as such undergo utilization. On account of this, the study did not require a consent from Bioethical Commission. Patients were informed about the study being conducted and consented to usage of their tissue for the purpose of this study.

Immediately after collection, the material was fixed in glutaraldehyde within two hours and then immersed in a phosphoric buffer for a period of 12 hours.

Morphological Analysis of Gingival Tissue

First, biopsy specimen after fixing (glutaraldehyde and osmium tetroxide) and buffering (phosphoric buffer) was embedded in an epoxy resin and then they were cut into slices 2–3 micrometres thick. Specimens, routinely dyed with methylene blue were assessed using a light microscope in order to make the first selection. In order to observe the specimens in a transmissional electron microscope, ultra thin cuts (70 nm thick) were obtained by using a diamond knife and then routinely contrasted. Cuts were analysed in a transmissional electron microscope type JAMM 100 CX made by Jeol, with accelerant voltage of 80 kV. This assessment was done in order to make more specific selection of studied material, aimed at detecting the presence or absence of changes in cytological structure of studied tissue.

Elemental Composition Analysis of Gingival Tissue

The second biopsy specimen, intended for observation in a X-ray micro-analyser, after fixing in 4% glutaraldehyde on phosphoric buffer, was

Table 1. Content of specific elements in gingival tissue of study and control group and statistical tests results related to elemental content of gingival tissue in both groups

Tabela 1. Zawartość poszczególnych pierwiastków w tkance dziąsłowej grupy badanej i kontrolnej oraz wyniki testów statystycznych dotyczących zawartości pierwiastków w tkance dziąsłowej obu grup

	Median \pm SD (Mediana \pm SD)		Mann-Whitney Test (Test Manna-Whitneya)	Mean \pm SD (Średnia \pm SD)	
	Study group (Grupa badana)	Control group (Grupa kontrolna)		Study group (Grupa badana)	Control group (Grupa kontrolna)
Ca	44.63 \pm 24.36	0 \pm 29.11	0.0957	49.40 \pm 34.42	22.30 \pm 30.21
Mg	0 \pm 0.31	–	0.3660	0.42 \pm 0.76	–
Na	9.34 \pm 17.22	0 \pm 2.13	0.2343	15.99 \pm 18.25	3.25 \pm 6.15
P	11.39 \pm 12.69	29.67 \pm 10.75	0.0734	13.05 \pm 14.49	26.71 \pm 9.72
S	11.04 \pm 12.79	15.82 \pm 31.88	0.5338	15.36 \pm 17.92	29.85 \pm 30.06
K	0 \pm 3.51	2.29 \pm 8.44	0.4846	6.41 \pm 12.58	13.64 \pm 22.28
Zn	0 \pm 1.14	–	0.3660	1.30 \pm 2.25	–

dehydrated in alcoholic series and propylene oxide. The tissue prepared in such a manner was embedded in epoxy-resins mixture in form of blocks, which were subsequently trimmed and sliced until studied material was exposed. Before microanalysis, carbon coat was sprayed on the exposed surface of tissue. Chemical composition analysis was done using a scanning electron microscope with field emission Hitachi S-4200, linked to a roentgen spectrometer Voyager 3500 by Norar Instruments.

Observation of morphological and cytological structure of tissue biopsy specimens in light and electron microscopes was confirmed, that the selected specimens corresponded with the study and control group in a way they were qualified during clinical examination of patients.

Statistical Analysis

The obtained results did not fulfil the criteria related to parametric tests, so appropriate statistical analyses were done using non-parametric tests. Thus, two of the observations were compared using the Mann-Whitney test and more with ANOVA test by Friedman. Comparisons of linear regression coefficients of the study group to the control group, were done using analysis of covariance (ANCOVA), which is designed to verify the hypothesis that regression coefficients are equal. The authors have decided to show a median and quartile deviation, because most of the data had asymmetric distribution. All tests were done at the significance level of $\alpha = 0.05$.

Results

Table 1 shows the arithmetic mean and standard deviation, median and quartile deviation of the results obtained from microanalysis of gingival

Table 2. Content of elements in gingival tissue of the study group related to arithmetic mean of the control group

Tabela 2. Zawartość pierwiastków w tkance dziąsłowej grupy badanej względem średniej arytmetycznej grupy kontrolnej

	Median \pm SD (Mediana \pm SD)	Mean \pm SD (Średnia \pm SD)
Ca	190.17 \pm 109.26	218.20 \pm 156.87
Na	287.64 \pm 530.18	492.31 \pm 562.08
P	42.65 \pm 47.52	48.85 \pm 54.23
S	37.00 \pm 42.85	51.47 \pm 60.05
K	0 \pm 25.72	47.00 \pm 92.25

ANOVA test by Friedman $p = 0.0616$.

Test ANOVA Friedmana $p = 0,0616$.

tissue biopsy specimens and results of Mann-Whitney U test. Table 2 shows percentage content of elements in a gingival tissue of the study group relatively to arithmetic mean of the control group.

The obtained results based on a performed test indicate that there is a tendency towards rise in calcium content and decline in phosphorus content in a gingival tissue of the study group. In none of the analysed gingival tissue biopsy specimens of the control group, there was zinc or magnesium present. The authors also corrected the results of the study group, using the arithmetic mean of the control group (Table 2). Having done the ANOVA Friedman test, the authors found a tendency towards differences in content between trace elements ($p = 0.0616$).

Epithelial cells of gingival tissue obtained from patients suffering from chronic periodontitis do not show such dense structure. The endothelium of blood vessels is not intact, which is why there is extravasation of red blood cells.

There is a local atrophy of a basement membrane and inflammatory infiltration cells, situated under the epithelium, containing granules of dif-

ferentiated electron density. Within an intercellular space, there are granules noted, which have a lysosomal granules structure. The amount of amorphous intercellular substance to collagen fibers relatively is significantly higher (Fig. 2).

A characteristic feature of gingival tissue biopsy specimens from patients with healthy periodontium, is a dense structure of epithelial cells, between which there is an intercellular space tightly filled with intercellular substance and collagen fibers (Fig. 1). The cells contain numerous, small vacuoles. Blood vessels show normal, intact structure. Remaining types of connective tissue cells are present in a regular content and have a normal structure.

Discussion

Zinc is a necessary component of many enzymes. It participates in an early phase of collagen metabolism, as well as diminishes the production of acids in a dental plaque in a way of enzymatic inhibition, causing oxidation of thiolic groups in main enzymatic systems of carbohydrate metabolism [3, 4]. Chemical composition analysis of canine gingival tissue with the use of atomic absorption spectrophotometry was conducted. In gingival tissue with inflammatory changes a significant increase of zinc level as compared to healthy tissue was noted. Increased amount of this microelement in a pathologically changed tissue is related to the fact, that zinc causes faster wound healing [5]. Significant decrease of zinc level in blood serum of patients with a diagnosis of profound periodontitis was noticed [4]. Bacterial plaque, through lipopolysaccharides, stimulates macrophages and neutrophils to release IL-1. This interleukine influencing an effect on the liver causes the decrease in zinc level and the increase in copper, ceruloplasmin, glycoprotein and lipoprotein levels in peripheral blood. According to author, shifts in ionic and protein composition affect also a gingival tissue, causing increased permeability of gingival and connecting epithelium for bacterial products. Those, in turn, stimulate macrophages to release IL-1 and a "vicious circle" closes. Besides zinc and copper, shifts in calcium and phosphorus content in blood serum were also observed [6]. Next experiments indicate that deficiency of zinc in a diet increases the uptake of C-phenytoin (PHT) and C-albumin (BSA), which, in author's opinion, can be of significance when susceptibility towards periodontal illnesses is being considered [7]. Basing on the results from presented study, it is not surprising that in recent years, more and more attention has been paid to prophylactic

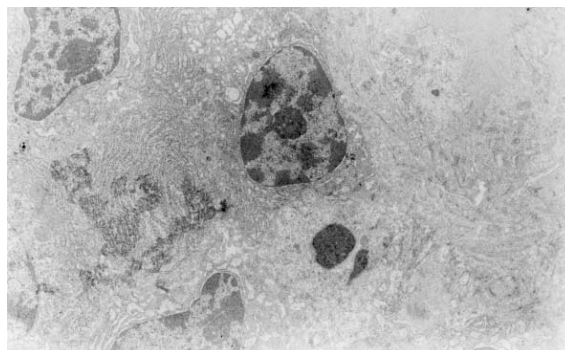


Fig. 1. Gingival tissue biopsy specimen from a patient with healthy periodontium – dense structure of cells, intercellular space tightly filled with intercellular substance and collagen fibers; blood vessels show normal, intact structure

Ryc. 1. Wycinek tkanki dziąsłowej pacjenta ze zdrowym przyzębiem – zwarty układ komórek, cienka przestrzeń międzykomórkowa wypełniona substancją międzykomórkową i włóknami kolagenowymi, naczynia krwionośne – zachowana ciągłość śródbłonnka

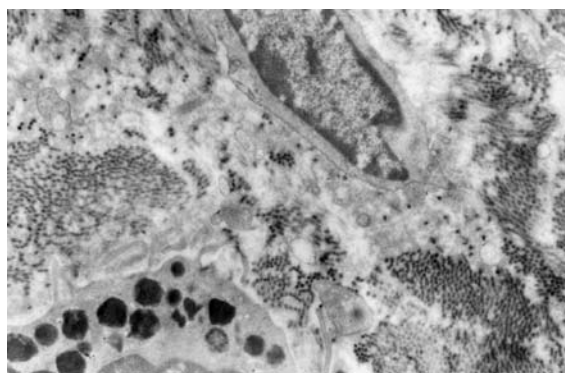


Fig. 2. Gingival tissue biopsy specimen from a patient with chronic periodontitis – lack of dense structure; the amount of amorphous intercellular substance related to collagen fibers is significantly higher

Ryc. 2. Wycinek tkanki dziąsłowej pacjenta z przewlekłym zapaleniem przyzębia – brak zwartej struktury, ilość substancji międzykomórkowej nieupostaciowionej większa od liczby włókien kolagenowych

properties of zinc compounds. They are widely used in mouth washes and toothpastes. In presented study, there was no magnesium present in a gingival tissue of the control group as well. Most researchers dealing with the issue of magnesium content changes in a course of periodontopathy, focused on testing saliva in their studies. It has an important biological function as a prosthetic enolase group participating in a lactic acid fermentation. It is also a necessary activator for alkaline phosphatase and a component of carboxylase. Level of magnesium was compared in saliva during periodontal disease with saliva of healthy people [8]. In the study group, magnesium content

was increased. Mg content was observed in a bone of an alveolar process in deep periodontitis [9]. As compared to the control group, the values in periodontal disease were slightly elevated but were still within the range of accepted limits. According to the authors, the elevated level of magnesium in the bone was increasing the activity of the proteolytic enzymes, which sustained osteolysis.

The authors found a tendency towards calcium content increase and phosphorus content decrease in a gingival tissue of people with periodontal disease. Most likely, it is the effect of increased permeability of connecting gingival epithelium and penetration of mineral components from saliva and gingival pocket's liquid into soft tissues. In a course of CP, an elevation of calcium level occurs,

as compared to the saliva of healthy persons [8]. The authors did not find any difference in content of Na, S and K between study and control groups.

Comparison of the results obtained from micro-analysis of gingival tissue with those showed by other authors should be done with caution because of the lack of uniform study methods. An important factor which can lead to discrepancies in elemental composition of studied material by different authors is the selection of a homogeneous group of people participating in those studies in relation to their age, living area, dietary habits and eliminating the influence of other factors such as cigarette smoking, general illnesses, medications used or work related to heavy metal exposure [10].

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