

MAŁGORZATA NĘDZI-GÓRA, RENATA GÓRSKA

Elastase Concentration in Saliva in Patients with Chronic Periodontitis

Stężenie elastazy w ślinie pacjentów z przewlekłym zapaleniem przyzębia

Department of Periodontology and Oral Diseases Warsaw Medical University, Poland

Abstract

Background. The traditional diagnosis of periodontal disease is based on clinical and radiological examination. Ideal diagnostics should be based not only on clinical parameters of periodontal tissues, but also on biochemical indices which could indicate a potential progression of the disease. Elastase is one of numerous proteolytic enzymes released by neutrophilic granulocytes in the course of periodontitis.

Objectives. The aim of the study was to determine the concentration of elastase in saliva in patients with chronic periodontitis compared to healthy individuals.

Material and Methods. The enzyme-linked immunosorbent assay method was employed to determine the concentration of elastase in saliva in patients with chronic periodontitis and with pocket depth (PD) \geq 4 mm and PD $<$ 4 mm, as well as in saliva of healthy individuals.

Results. A significantly higher concentration of elastase was observed in patients with periodontitis compared to healthy individuals. Also a significant difference in elastase concentration in saliva was observed between the PD \geq 4 mm and PD $<$ 4 mm groups and between the PD \geq 4 mm and control groups as well as no statistically significant differences were observed between the PD $<$ 4 mm and control groups.

Conclusions. The elastase concentration in saliva can be considered as one of biochemical indicators of severity of periodontitis (*Dent. Med. Probl.* 2011, 48, 4, 474–480).

Key words: chronic periodontitis, elastase, saliva.

Streszczenie

Wprowadzenie. Tradycyjne rozpoznanie choroby przyzębia wykorzystuje badanie kliniczne i radiologiczne. Idealna diagnostyka powinna uwzględniać nie tylko kliniczne wskaźniki stanu tkanek przyzębia, ale również wskaźniki biochemiczne, które mogłyby wskazywać na potencjalną progresję choroby. Elastaza jest jednym z wielu enzymów proteolitycznych uwalnianych przez granulocyty obojętnochłonne w przebiegu zapalenia przyzębia.

Cel pracy. Określenie stężenia elastazy w ślinie pacjentów z przewlekłym zapaleniem przyzębia w porównaniu do osób ze zdrowym przyzęciem.

Materiał i metody. Metodą immunoenzymatyczną oznaczono stężenie elastazy w ślinie pacjentów z przewlekłym zapaleniem przyzębia i kieszonkami przyzębnymi o głębokości (PD) \geq 4 mm oraz PD $<$ 4 mm oraz w ślinie osób zdrowych.

Wyniki. Stwierdzono znamienne wyższe stężenie elastazy w ślinie pacjentów z zapaleniem przyzębia w porównaniu do osób zdrowych. Wykazano również istotność różnic w stężeniu elastazy w ślinie między grupami PD \geq 4 mm i PD $<$ 4 mm oraz PD \geq 4 mm i kontrolną oraz brak różnic statystycznie istotnych między grupami PD $<$ 4 mm i kontrolną.

Wnioski. Stężenie elastazy w ślinie można brać pod uwagę jako jeden ze wskaźników biochemicznych stopnia nasilenia zapalenia przyzębia (*Dent. Med. Probl.* 2011, 48, 4, 474–480).

Słowa kluczowe: przewlekłe zapalenie przyzębia, elastaza, ślina.

The traditional diagnosis of periodontal disease is based on clinical and radiological examination. Ideal diagnostics should be based not only on clinical parameters of periodontal tissues at the

moment of examination, but also on clinical and biochemical indices which could indicate a potential progression of the disease. Unfortunately, until now no ideal indicators have been developed which

could predict progression of the disease, primarily in patients with possible periodontal lesions at early age, or which could detect a risk of such lesions at the subclinical level. We still have at our disposal solely methods of examining the clinical attachment loss (CAL) and radiological methods of alveolar bone loss (BL) assessment. Unfortunately, observation of these indicators in time is possible only after a few millimetres of clinical attachment or bone have been lost. Therefore for many years researchers have been trying to develop diagnostic tests which could help to identify patients (or certain sites in a patient's periodontium) particularly susceptible to occurrence and fast progress of periodontal disease.

Numerous studies were devoted to determining specific bacterial species (strains) present in dental plaque, which are responsible for faster progression of periodontal disease of its occurrence at an early age. Nowadays the so-called red complex bacteria *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola* and *Agregatibacter actinomycetemcomitans* are regarded as indicator species [1].

Many clinical studies tried to establish various substances in gingival crevicular fluid, saliva or gingival tissue which could be indicators of disease progression. These substances include such inflammatory mediators as: PGE₂, interleukins e.g. IL-1- β , enzymes e.g. metalloproteinase and elastase [2, 3].

Elastase is a neutral serine proteinase (endopeptidase) "stored" in azurophilic granules of granulocytes, at the number of 3 pg per cell. The enzyme is capable of degrading a large spectrum of various molecules in human tissues, including periodontal tissues, such as collagen, laminin, fibronectin, proteoglycans and elastin [4]. Elastase seems to play a particular role in the early stages of periodontal disease, as contrary to e.g. collagenases it can destroy intact non-collagenous proteins. In healthy periodontium these proteins surround collagen fibers protecting them against activity of collagenases [5, 6].

Elastase is one of numerous proteolytic enzymes (proteases) released by neutrophilic granulocytes as a result of such phenomena as tissue destruction or bacterial infection. Elastase is present in granules of granulocytes in the form of both a proenzyme and an active enzyme, however their activating mechanisms are not fully known. Outside the cell, elastase activity control is performed mainly by serine proteinase inhibitor – alpha-1-antitrypsin (A1AT), produced and released also by neutrophils [7].

Neutrophilic granulocytes are dominant leukocytes in epithelium of periodontal pockets and

adjacent periodontal tissues. They protect periodontal tissues against bacterial infections and subsequent tissue destruction by means of oxydative and non-oxydative mechanisms. The latter seem to be of highest significance in anaerobic conditions of the periodontal pocket. Most antibacterial substances are located in azurophilic granules of neutrophilic granulocytes. They are capable of destroying phagocytized bacteria, but they can be also released extracellularly during phagocytosis [8].

Numerous *in vitro* studies confirmed that neutrophilic granulocytes activated by bacteria from dental plaque – release lysosomal enzymes, including elastase [9–13]. Elastase reaches one of highest levels among proteinases marked quantitatively in gingival crevicular fluid (GCF) [14].

The gingival (periodontal) crevicular fluid, which is transudate from serum or inflammatory transudate, is a reflection of blood serum ingredients as well as cellular response and processes occurring in marginal periodontium. Examination of gingival crevicular fluid plays an important role in scientific research on pathophysiology of periodontal disease. Cellular (e.g. granulocytes) and humoral (e.g. enzymes) elements of crevicular fluid penetrate into saliva. An analysis of GCF-originated mediators in mixed saliva could be a fast screening method for periodontal disease [15].

The aim of the study was to assess elastase concentration in saliva in patients with chronic periodontitis compared to individuals with healthy periodontium.

Material and Methods

The study included a group of 32 patients (17 females and 15 males) with diagnosed chronic periodontitis [16]. Average age was 41.76 years. Within the group two subgroups were distinguished according to periodontal pocket occurrence: pocket depth (PD) \geq 4 mm (16 patients) and PD < 4 mm (16 patients). None of the patients reported any coexisting systemic disease and was subject to long-term pharmacological treatment. Individuals who during the previous three months were treated with antibiotics or anti-inflammatory medications, especially non steroidal anti-inflammatory drugs and aspirin, were excluded from the study. The control group consisted of 13 individuals (7 females and 6 males) with healthy periodontium – without clinical attachment loss, without periodontal pockets > 3 mm and with bleeding index (BOP) < 10%. Due to the above criteria, average age in the control group was 32.2 years.

5 ml of stimulated mixed saliva was collected from each individual between 9.00 and 13.00 hours (at least 2 hours after a meal). The collected material was frozen at -20 degrees Celsius and stored in that temperature until biochemical tests were performed.

Elastase concentration in saliva was determined with enzyme-linked immunosorbent assay ELISA. The test sensitivity was 1.98 ng/ml.

Statistical Analysis

The concentration of elastase in three groups of patients ($PD \geq 4$ mm, $PD < 4$ mm and control) was compared. In order to determine if the differences between the groups are significant (after checking for normality of the distribution with Kolmogorov-Smirnov test), one-way analysis of variance was used. In case of a rejection of the hypothesis about lack of differences in the level of examined parameters between groups, Tukey's multiple comparison test was used to assess differences between group pairs. Difference was regarded as statistic significant if p-value was below 0.05.

Results

Mean concentration of elastase in saliva of patients with periodontitis was 11204.44 ng/ml, whereas in the control group the result was 2849.38 ng/ml. The difference was statistically significant ($p < 0.01$) (Table 1). After dividing the study group into two subgroups, $PD \geq 4$ mm and $PD < 4$ mm, the distribution of examined parameters did not significantly differ from the normal one. Variance analysis showed significant differences between examined parameters (p for variance analysis < 0.001) (Table 2). In Tukey's multiple comparison test significant differences were observed in elastase concentration in saliva between $PD \geq 4$ mm and $PD < 4$ mm groups as well as between $PD \geq 4$ mm and control groups, however no statistically significant differences were observed between $PD < 4$ mm and control groups (Table 3).

Discussion

Cross-sectional examinations showed an increased level of elastase in diseased sites compared to healthy sites or healthy patients [2, 17–19], as well as increased level of elastase in the course of experimentally induced gingivitis [20]. Furthermore, a reduced level of elastase in gingival cre-

vicular fluid was observed also after surgical and non-surgical treatment [21–23], and in patients treated pharmacologically for rheumatoid arthritis [24].

Studies by Alpagot et al. [25] indicate that level of elastase in gingival crevicular fluid – as well as patient's age and tobacco smoking – is one of the risk factors for periodontitis in patients with diabetes.

Studies by Wells et al. [17] indicated an increased level of elastase in gingival crevicular fluid of patients with periodontitis compared to healthy individuals. Palcanis et al. [2] in their research tried to develop a diagnostic test, based on measurement of elastase concentration level in gingival crevicular fluid, which would allow to determine the risk of activity and progress of the disease in given examined sites. The authors proved that elastase concentrations were significantly higher in sites showing progression of periodontal disease (clinical attachment loss and alveolar bone loss during 6 months). The total elastase level in samples of gingival crevicular fluid was assessed by means of spectrofluorometric method. Similar results were obtained by Armitage et al. [26]. They demonstrated that examined sites where high elastase levels were observed bear a higher risk of progressive alveolar bone loss, determined by means of digital radiography. Determination of sites with a higher risk of progressive alveolar bone loss would allow to develop a standard treatment procedure for such sites, a procedure that would involve further treatment without turning to maintenance phase in such sites, or application of additional, e.g. surgical treatment methods, or application of additional chemical substances, e.g. antibiotics administered topically. Patients with such sites would also require more frequent check-up visits. Also Jin et al. [27, 28] observed significantly higher elastase levels in gingival crevicular fluid in patients with so-called refractory periodontitis compared to patients with similar progression of periodontitis who responded positively to applied treatment.

Subsequent studies on the role of elastase in destruction of periodontal tissues were based on assessing its activity in gingival crevicular fluid [21, 22, 29, 30]. Eley et al. [21, 22, 29] demonstrated that elastase activity in gingival crevicular fluid impacts the severity of periodontal disease and is associated with clinical attachment loss. Research by Jin et al. [27, 28] proved that activity of granulocytic elastase in gingival crevicular fluid is positively correlated with response to applied treatment of periodontitis. An association between histologically confirmed clinical attachment loss and increased elastase activity was dem-

Table 1. Comparison of elastase concentration in saliva between study and control groups**Tabela 1.** Porównanie stężenia elastazy w ślinie między grupą badaną i kontrolną

	Group (Grupa)	N	Mean (Średnia)	SD ¹	P
Elastase (Elastaza)	control	13	2849.38	2418.146	< 0.001
	study	32	11204.44	9365.738	

¹SD – standard deviation.

¹SD – odchylenie standardowe.

onstrated by Renvert et al. [31] in a study on beagle dogs. Chen et al. [30] tried to develop diagnostic tests for assessment of progression and progression risk of periodontal disease based on determining the activity of metalloproteinase-8 and elastase in

gingival crevicular fluid in patients with chronic periodontitis. Elastase activity was established by means of fluorogenic substrate. The studies showed that both clinical parameters of periodontal tissues and total levels of examined enzymes [ng/sample] in gingival crevicular fluid are lowered as a result of non-surgical periodontal treatment. Furthermore, collagenase activity was correlated with gingival index and bleeding index. Amounts of the two examined enzymes were also correlated with each other. Figueredo et al. [32] demonstrated an increased level of active elastase in gingival crevicular fluid collected from sites indicating significant destruction of periodontal tissues. Also Rescala et al. [33] revealed higher levels of elastase activity in deep sites compared to shallow sites in patients with chronic periodontitis. And Rosalem et al. [34] observed significant reductions in elastase activity in deep sites in patients with generalized

Table 2. Comparison of elastase concentration in saliva between PD ≥ 4 mm, PD < 4 mm and control groups**Tabela 2.** Porównanie stężenia elastazy w ślinie między grupami PD ≥ 4 mm, PD < 4 mm i grupą kontrolną

Elastase (Elastaza)	N	Mean (Średnia)	SD ¹	Min	Max	PANOVA
PD ≥ 4 mm	16	17870.1	8804.2	5500	37000	< 0.001*
PD < 4 mm	16	4538.8	2996.9	320	9700	
Control (Grupa kontrolna)	13	2849.4	2418.1	330	6500	

* In Tukey's multiple comparison test significant differences were observed between PD ≥ 4 mm and PD < 4 mm groups as well as between PD ≥ 4 mm and control groups, no statistically significant differences were observed between PD < 4 mm and control groups.

¹SD – standard deviation.

* Wg testu wielokrotnych porównań Tukeya różnice istotne statystycznie zauważono między grupami PD ≥ 4 mm i PD < 4 mm oraz między grupą PD ≥ 4 mm a grupą kontrolną. Nie było różnic istotnych statystycznie między grupą PD < 4 mm a grupą kontrolną.

¹SD – odchylenie standardowe.

Table 3. Tukey's test results. Multiple comparisons of elastase concentration in saliva between PD ≥ 4 mm, PD < 4 mm and control groups**Tabela 3.** Wyniki testu Tukeya. Porównania wielokrotne stężeń elastazy w ślinie między grupami PD ≥ 4 mm, PD < 4 mm i grupą kontrolną

Dependent variable (Zmienna zależna)	(I) Group 1 ((I) Grupa 1)	(J) Group 1 ((J) Grupa 1)	Mean difference (I–J) (Średnia, różnice)	Standard error (Błąd standardowy)	Significance (Istotność statystyczna)	95% Confidence Interval (95% przedział ufności)	
						lower limit	upper limit
Elastase (Elastaza)	PD ≥ 4 mm	PD < 4 mm	13331.375(*)	2017.478	0.000	8429.92	18232.83
		Control	15020.740(*)	2130.694	0.000	9844.23	20197.25
	PD < 4 mm	PD ≥ 4 mm	-13331.375(*)	2017.478	0.000	-18232.83	-8429.92
		Control	1689.365	2130.694	0.709	-3487.14	6865.87
	control	PD ≥ 4 mm	-15020.740(*)	2130.694	0.000	-20197.25	-9844.23
		PD < 4 mm	-1689.365	2130.694	0.709	-6865.87	3487.14

* Mean difference is significant at 0.05 level.

* Średnia różnic jest istotna statystycznie na poziomie 0,05.

chronic periodontitis after non-surgical periodontal treatment. Jin et al. [35, 36] studied a relationship between elastase activity together with PGE2 level in gingival crevicular fluid and the presence of periopathogenic bacteria (*Actinobacillus actinomycetemcomitans*, *Bacteroides forsythus*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola*) in patients with untreated periodontitis. The authors demonstrated that topical immunological response to periopathogens is varied depending on intensity of immunological response measured by the level of elastase and PGE2 in gingival crevicular fluid.

The results of quoted studies prove the significance of elastase in pathomechanism of periodontitis. In own study the level of elastase was assessed in saliva, not *in situ* in gingival crevicular fluid. It was assumed that mean concentration of elastase in saliva can be a resultant of concentrations in individual periodontal pockets. A significantly higher concentration of elastase in saliva in patients with chronic periodontitis compared to healthy individuals ($p < 0.01$) was observed, which proves the argument that determining the enzyme in saliva can be a fast and simple screening method for periodontal disease.

In this study the concentration of elastase in saliva in patients with chronic periodontitis and pocket depths $PD \geq 4$ mm was compared to con-

centration of elastase in saliva in patients with chronic periodontitis and pocket depths $PD < 4$ mm. A statistically significant difference in elastase concentrations in saliva which was observed between the group with $PD \geq 4$ mm and the group with $PD < 4$ mm, and also between the group with $PD \geq 4$ mm and the control group, can indicate that periodontal pockets $PD \geq 4$ mm are the main source of increased concentration of the enzyme in saliva. In turn, no statistically significant difference between patients with $PD < 4$ mm and the control group can indicate that periodontitis diagnosis which is based solely on clinical attachment loss may be insufficient, and that the progression of the disease and intensity of inflammation is determined by pocket depth (PD). These results are consistent with Offenbacher's et al. [37] proposed new approach to the issue of division and diagnostic criteria for periodontal disease, according to which patients with $PD < 4$ mm are classified as healthy (BOP $< 10\%$) or as patients with gingivitis (BOP $> 10\%$).

Conclusions

Elastase concentration in saliva can be considered as one of biochemical indicators of severity of periodontitis.

References

- [1] NIEMINEN A., ASIKAINEN S., TORKKO H., KARI K., UITTO V.J., SAXÉN L.: Value of some laboratory and clinical measurements in the treatment plan for advanced periodontitis. *J. Clin. Periodontol.* 1996, 23, 572–581.
- [2] PALCANIS K.G., LARJAVA I.K., WELLS B.R., SUGGS K.A., LANDIS J.R., CHADWICK D.E., JEFFCOAT M.K.J.: Elastase as an indicator of periodontal disease progression. *Periodontology* 1992, 63, 237–242.
- [3] WILLIAMS R.C., BECK J.D., OFFENBACHER S.N.: The impact of new technologies to diagnose and treat periodontal disease. A look to the future. *J. Clin. Periodontol.* 1996, 23, 299–305.
- [4] JANOFF A.: Elastase in tissue injury. *Annu. Rev. Med.* 1985, 36, 207–216.
- [5] UJIE Y., OIDA S., GOMI K., ARAI T., FUKAE M.: Neutrophil elastase is involved in the initial destruction of human periodontal ligament. *J. Periodontal. Res.* 2007, 42, 325–330.
- [6] UJIE Y., SHIMADA A., KOMATSU K., GOMI K., OIDA S., ARAI T., FUKAE M.: Degradation of noncollagenous components by neutrophil elastase reduces the mechanical strength of rat periodontal ligament. *J. Periodontal. Res.* 2008, 43, 22–31.
- [7] FIGUERO C.M., GUSTAFSSON A., ASMAN B., BERGSTRÖM K.: Increased release of elastase from *in vitro* activated peripheral neutrophils in patients with adult periodontitis. *J. Clin. Periodontol.* 1999, 26, 206–211.
- [8] DENNISON D.K., VAN DYKE T.E.: The acute inflammatory response and the role of phagocytic cells in periodontal health and disease. *Periodontology* 2000. 1997, 14, 54–78.
- [9] TAICHMAN N.S., TSAI C.C., BAEHNI P.C., STOLLER N., MCARTHUR W.P.: Interaction of inflammatory cells and oral microorganisms. IV. *In vitro* release of lysosomal constituents from polymorphonuclear leukocytes exposed to supragingival and subgingival bacterial plaque. *Infect. Immun.* 1977, 16, 1013–1023.
- [10] BAEHNI P., TSAI C.C., MCARTHUR W.P., HAMMOND B.F., TAICHMAN N.S.: Interaction of inflammatory cells and oral microorganisms. VIII. Detection of leukotoxic activity of a plaque-derived gram-negative microorganism. *Infect. Immun.* 1979, 24, 233–243.
- [11] DING Y., UITTO V.J., HAAPASALO M., LOUNATMAA K., KONTTINEN Y.T., SALO T., GRENIER D., SORSA T.: Membrane components of *Treponema denticola* trigger proteinase release from human polymorphonuclear leukocytes. *J. Dent. Res.* 1996, 75, 1986–1993.
- [12] DING Y., HAAPASALO M., KEROSUO E., LOUNATMAA K., KOTIRANTA A., SORSA T.: Release and activation of human neutrophil matrix metallo- and serine proteinases during phagocytosis of *Fusobacterium nucleatum*, *Porphyromonas gingivalis* and *Treponema denticola*. *J. Clin. Periodontol.* 1997, 24, 237–248.

- [13] AIRILA-MÅNSSON S., SÖDER B., KARI K., MEURMAN J.H.: Influence of combinations of bacteria on the levels of prostaglandin E2, interleukin-1beta, and granulocyte elastase in gingival crevicular fluid and on the severity of periodontal disease. *J. Periodontol.* 2006, 77, 1025–1031.
- [14] COX S.W., ELEY B.M.: Detection of cathepsin B- and L-, elastase-, trypsin-, and dipeptidyl peptidase IV-like activities in crevicular fluid from gingivitis and periodontitis patients with peptidyl derivatives of 7-amino-4-trifluoromethyl coumarin. *J. Periodontol. Res.* 1989, 24, 353–361.
- [15] LAMSTER I.B., AHLO J.K.: Analysis of gingival crevicular fluid as applied to the diagnosis of oral and systemic diseases. *Ann. N. Y. Acad. Sci.* 2007, 1098, 216–229.
- [16] ARMITAGE G.C.: Development of a classification system for periodontal diseases and conditions. *Ann. Periodontol.* 1999, 4, 1–6.
- [17] WELLS B., GILL E., LARJAVA I., SUGGS K., PALCANIS K., JEFFCOAT M.: Crevicular fluid elastase in healthy and periodontitis patients. *J. Dent. Res.* 1990, 69 (Spec Issue), 201 (Abstr 744).
- [18] ZAFIROPOULOS G.G., FLORES-DE-JACOBY L., TODT G., KOLB G., HAVEMANN K., TATAKIS D.N.: Gingival crevicular fluid elastase-inhibitor complex: correlation with clinical indices and subgingival flora. *J. Periodontol. Res.* 1991, 26, 24–32.
- [19] GUSTAFSON A., ASMAN B., BERGSTROM K., SÖDER P.-O.: Granulocyte elastase in gingival crevicular fluid. A possible discriminator between gingivitis and periodontitis. *J. Clin. Periodontol.* 1992, 19, 535–540.
- [20] GIANNOPOULOU C., ANDERSEN E., DEMEURISSE C., CIMASONI G.: Neutrophil elastase and its inhibitors in human gingival crevicular fluid during experimental gingivitis. *J. Dent. Res.* 1992, 71, 359–363.
- [21] ELEY B.M., COX S.W.: Cathepsin B/L-, elastase-, trypsin- and dipeptidyl peptidase IV-like activities in gingival crevicular fluid: a comparison of levels before and after periodontal surgery in chronic periodontitis patients. *J. Periodontol.* 1992, 63, 412–417.
- [22] ELEY B.M., COX S.W.: Cathepsin B/L-, elastase-, trypsin- and dipeptidyl peptidase IV-like activities in gingival crevicular fluid: correlation with clinical parameters in untreated chronic periodontitis patients. *J. Periodontol. Res.* 1992, 27, 62–69.
- [23] JIN L.J., LEUNG W.K., CORBET E.F., SÖDER B.: Relationship of changes in interleukin-8 levels and granulocyte elastase activity in gingival crevicular fluid to subgingival periodontopathogens following non-surgical periodontal therapy in subjects with chronic periodontitis. *J. Clin. Periodontol.* 2002, 29, 604–614.
- [24] MIRANDA L.A., ISLABÃO A.G., FISCHER R.G., FIGUEREDO C.M., OPPERMAN R.V., GUSTAFSSON A.: Decreased interleukin-1beta and elastase in the gingival crevicular fluid of individuals undergoing anti-inflammatory treatment for rheumatoid arthritis. *J. Periodontol.* 2007, 78, 1612–1619.
- [25] ALPAGOT T., SILVERMAN S., LUNDERGAN W., BELL C., CHAMBERS D.W.: Crevicular fluid elastase levels in relation to periodontitis and metabolic control of diabetes. *J. Periodontol. Res.* 2001, 36, 169–174.
- [26] ARMITAGE G.C., JEFFCOAT M.K., CHADWICK D.E., TAGGART E.J. JR, NUMABE Y., LANDIS J.R., WEAVER S.L., SHARP T.J.: Longitudinal evaluation of elastase as a marker for the progression of periodontitis. *J. Periodontol.* 1994, 65, 120–128.
- [27] JIN L.J., SÖDER P.O., ASMAN B., SÖDER B., PURIENE A., BERGSTRÖM K.: Variations in crevicular fluid elastase levels in periodontitis patients on long-term maintenance. *Eur. J. Oral Sci.* 1995, 103, 84–89.
- [28] JIN L.J., SÖDER P.O., ASMAN B., BERGSTRÖM K.: Granulocyte elastase in gingival crevicular fluid: improved monitoring of the site-specific response to treatment in patients with destructive periodontitis. *J. Clin. Periodontol.* 1995, 22, 240–246.
- [29] ELEY B.M., COX S.W.: A 2-year longitudinal study of elastase in human gingival crevicular fluid and periodontal attachment loss. *J. Clin. Periodontol.* 1996, 23, 681–692.
- [30] CHEN H.Y., COX SW, ELEY B.M., MÄNTYLÄ P., RÖNKÄ H., SORSA T.: Matrix metalloproteinase-8 levels and elastase activities in gingival crevicular fluid from chronic adult periodontitis patients. *J. Clin. Periodontol.* 2000, 27, 366–369.
- [31] RENVERT S., WIKSTRÖM M., MUGRABI M., KELLY A., CLAFFEY N.: Association of crevicular fluid elastase-like activity with histologically-confirmed attachment loss in ligature-induced periodontitis in beagle dogs. *J. Clin. Periodontol.* 1998, 25, 368–374.
- [32] FIGUEREDO C.M., GUSTAFSSON A.: Activity and inhibition of elastase in GCF. *J. Clin. Periodontol.* 1998, 25, 531–535.
- [33] RESCALA B., ROSALEM W. JR, TELES R.P., FISCHER R.G., HAFFAJEE A.D., SOCRANSKY S.S., GUSTAFSSON A., FIGUEREDO C.M.: Immunologic and microbiologic profiles of chronic and aggressive periodontitis subjects. *J. Periodontol.* 2010, 81, 1308–1316.
- [34] ROSALEM W., RESCALA B., TELES R.P., FISHER R.G., GUSTAFSSON A., FIGUEREDO C.M.: Effect on non-surgical treatment on chronic and aggressive periodontitis: clinical, immunologic and microbiologic findings. *J. Periodontol.* 2011, 82, 979–989.
- [35] JIN L.J., SÖDER P.O., LEUNG W.K., CORBET E.F., SAMARANAYAKE L.P., SÖDER B., DAVIES W.I.: Granulocyte elastase activity and PGE2 levels in gingival crevicular fluid in relation to the presence of subgingival periodontopathogens in subjects with untreated adult periodontitis. *J. Clin. Periodontol.* 1999, 26, 531–540.
- [36] JIN L., SÖDER B., CORBET E.F.: Interleukin-8 and granulocyte elastase in gingival crevicular fluid in relation to periodontopathogens in untreated adult periodontitis. *J. Periodontol.* 2000, 71, 929–939.
- [37] OFFENBACHER S., BARROS S.P., BECK J.D.: Rethinking periodontal inflammation. *J. Periodontol.* 2008, 79, 1577–1584.

Address for correspondence:

Małgorzata Nędzi-Góra
Department of Periodontology and Oral Diseases
Warsaw Medical University
ul. Miodowa 18
00-246 Warszawa
Poland
tel./fax: +48 22 502 20 36
e-mail: mnedzi-gora@wp.pl

Received: 25.07.2011

Revised: 10.10.2011

Accepted: 26.10.2011

Praca wpłynęła do Redakcji: 25.07.2011 r.

Po recenzji: 10.10.2011 r.

Zaakceptowano do druku: 26.10.2011 r.