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NITRITE-ANION AS A MARKER OF INFLAMMATORY-DYSTROPHIC PROCESSES IN PERIODONTAL DISEASES

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Abstract

Background: Diagnosing the persistent but not clinically pronounced inflammation in periodontal tissues often is an important clinical problem. Measurement of Nitrite-anion concentration in saliva is informative in diagnosing the periodontal pathology at different stages of its development as well as in differentiation of superficial inflammation of periodontal tissues in the case of gingivitis and profound destruction of gums and bone in case of periodontitis.

Aim of our investigations was the examinations of Nitric oxide level in saliva of patients with periodontal pathology by measuring accumulation of Nitrite anion.

Materials and methods. Were examined 47 patients (20-40 years old), including 28 persons with generalized inflammatory diseases of the gums: chronic simple (14) and hypertrophic gingivitis (14) and 19 patients with chronic generalized periodontitis. The level of Nitrite anion in salive of patiens was meassured. Saliva was obtained in the quantity of 1 ml from each patient on an empty stomach.

The method of analysis was as follows: 0,2 ml of sodium hydrocarbonate was added to 0,2 ml of saliva and stored in ice for 10 min, than 0,4 ml of distilled water and 1,2 ml of 4% $ZnSO_4$ were introduced into this solution. The mixture was centrifuged for 20 min and 1,2ml of translucent supernatant was separated from it and mixed with 1,2ml of Gries reactive. Results were obtained within 15 min in a spectrophotometry method (λ = 550 nm).

Results. In the patients with simple chronic gingivitis the level of Nitrite anion in saliva differs significantly from the control samples only in the group with exacerbation of simple chronic gingivitis (51,9±2,4 pmol/L). In the patients with chronic hypertrophic gingivitis amounts of Nitrite anion in saliva were 11,6 ±1,6 pmol/L, but in the case of exacerbation they were increased 4-5 times as much as 56,1 ±2,7 pmol/L. Levels of Nitrite anion increased considerably in the exacerbation of both forms of gingivitis and amounted to 51,9 ±2,4 pmol/L and 56,1 ±2,7 pmol/L, respectively. These results indicate that level of NO synthesis in saliva doesn't depend on the type of inflammation in the gums (simple or hypertrophic).

All the patients with chronic generalized periodontitis were tested at the active stage of disease and at the stage of remission. Increased amounts of nitrite anion in saliva of the patients with chronic generalized periodontitis were reported at the active stage of chronic

generalized periodontitis (84,9 ± 3,8 pmol/L), which exceeds the healthy control group more than 10 times.

Remission in the patients with generalized periodontitis was attained after complex treatment, which included surgical pocket elimination and was characterized with absence of pockets, inflammation and gums bleeding on probing. Levels of nitrite anion in saliva were lowered to 8,5 ±0,7 pmol/L, which makes an insignificant difference with healthy persons (4,9 ±0,6 pmol/L).

Conclusion. The level of nitric-anion which depicts the level of Nitric oxide in the saliva probably depicts not only the activity of periodontal inflammatory process, but also the aggressiveness of alveolar bone destruction.

All human studies were conducted in compliance with the rules of the Helsinki Declaration of the World Medical Association "Ethical principles of medical research with human participation as an object of study". Informed consent was obtained from all participants.

Keywords: *gingivitis, periodontitis, Nitric oxide, levels of Nitrite anion in saliva*

Introduction

Periodontal diseases are characterized by inflammatory and dystrophic-inflammatory processes in tooth surrounding tissues and the bone, which can be easily diagnosed in clinical observation. An important clinical problem, however, is in diagnosing the persistent but not clinically pronounced inflammation in periodontal tissues [1, 2, 3]. Thus, measurement of Nitric oxide (NO) derivates in saliva can be greatly informative in diagnosing the periodontal pathology at different stages of its development as well as in differentiation of superficial inflammation of periodontal tissues in the case of gingivitis and profound destruction of gums and bone in case of periodontitis.

Nitric oxide (NO) is a free radical involved in the regulation of many physiological processes. Inflammation is characterized by the raise of nitric anion concentration in biological fluids, in particular by the direct activation of inducible nitric oxide synthase (iNOS) by cytokines (especially IL-1 and TNF), biological active amines, bacterial endotoxins, NO production can be more easily assessed indirectly by measuring accumulation of its stable metabolites - nitrate and nitrite [4, 5]. In recent years it has become apparent that NO also has important effects on bone cell function; specifically, high levels of NO inhibit bone resorption and formation and may act to suppress bone turnover in severe inflammation [6, 7, 8].

The aim of our investigations was the examinations of NO levels in saliva of patients with periodontal pathology by measuring accumulation of its stable metabolite Nitrite anion.

Materials and methods

Were tested 47 patients (20-40 years old), including 28 persons with generalized inflammatory diseases of the gums: chronic simple (14) and hypertrophic gingivitis (14) and 19 patients with chronic generalized periodontitis, (inflammatory-dystrophic disease of the periodontium) (Fig. 1).

In each patient with generalized periodontitis, minimum 20 teeth were preserved and none the less than 10-12 true periodontal pockets and radiographic symptoms of bone destruction were revealed. All the patients were not reported to have serious general pathology. 10 healthy persons, 20-25 years old with intact periodontium formed the control group.

The level of inflammation in periodontal tissues was estimated with bleeding index [9, 10, 11]. Gingival bleeding varies in severity, duration and the ease with which it is provoked. The severity of the bleeding depends on the intensity of the inflammation. NO production was assessed indirectly by measuring accumulation of its stable metabolite - Nitrite anion in saliva using Gries reaction [12].

In our research, Nitric oxide production was studied in saliva, by measuring accumulation of its stable metabolite Nitrite anion. Saliva was obtained in the quantity of 1 ml from each patient on an empty stomach. The method of analysis was as follows: 0,2 ml of sodium hydrocarbonate was added to 0,2 ml of saliva and stored in ice for 10 min, than 0,4 ml of distilled water and 1,2 ml of 4% $ZnSO_4$ were introduced into this solution. The mixture was centrifuged for 20 min and 1,2ml of translucent supernatant was separated from it and mixed with 1,2 ml of Gries reactive. Within 15 min results were obtained in a spectrophotometry method (λ = 550 nm).

Results

Were examined patients with three types of diseases of the periodontium: simple chronic gingivitis, hypertrophic gingivitis and chronic generalized periodontitis. Patients' in-depth information is shown in the table 1.

In simple chronic gingivitis the level of nitrite anion in saliva differs significantly from the control samples only in the group with exacerbation of simple chronic gingivitis (51,9±2,4 pmol/L). These two groups cannot always be distinguished clinically by gingival bleeding on probing (table 1).

Similar differences were observed in hypertrophic gingivitis. Clinical signs included gingival overgrowth in the form of diffuse swelling of interdental papillae. Gums were bluish red and in case of exacerbation, they were friable with intensive bleeding on probing. In chronic hypertrophic gingivitis amounts of Nitrite anion in saliva are 11,6 ±1,6 pmol/L, but in the case of exacerbation they were increased 4-5 times as much as 56,1 ±2,7 pmol/L.

However, no significant differences were observed in the level of Nitrite anion in the saliva of patients with simple chronic and hypertrophic gingivitis. (Fig. 2).

Levels of Nitrite anion increased considerably in the exacerbation of both forms of gingivitis and amounted to 51,9 ±2,4 pmol/L and 56,1 ±2,7 pmol/L, respectively. These results indicate that level of NO synthesis in saliva doesn't depend on the type of inflammation in the gums (simple or hypertrophic). (Fig.3).

All the patients with chronic generalized periodontitis were studied at the active stage of disease and at the stage of remission. Increased amounts of nitrite anion in saliva were reported at the active stage of chronic generalized periodontitis (84,9 ± 3,8 pmol/L), which exceeds the healthy control group more than 10 times (Fig. 4-6).

Remission in the patients with generalized periodontitis was attained after complex treatment, which included surgical pocket elimination and was characterized with absence of pockets, inflammation and gums bleeding on probing. Levels of nitrite anion in saliva were lowered to 8,5 ±0,7 pmol/L, which makes an insignificant difference with healthy persons (4,9 ±0,6 pmol/L). (Fig.7-9).

Discussion

The role of the increased level of nitric oxide in saliva in patients with periodontal diseases (inflammatory and dystrophic-inflammatory) is not quite understandable. In one aspect, more intensive inflammation in the periodontium which is manifested, for instance, by the higher bleeding index [13] is characterized by the increased level of nitrite-anion in saliva of patients with gingivitis [14]. In patients with the active stage of generalized periodontitis, however, the level of nitrite-anion in saliva was significantly higher (when compared with gingivitis patients) but often manifested in clinical examination with a lower gingival bleeding index. This fact probably depicts the effect of pro-inflammatory cytokines (IL-1, TNF), inducing active destruction of alveolar bone in patients with generalized periodontitis. It can also be due to the more profound and intensive penetration of periodontal pathogens, which produce greater amounts of bacterial endotoxins (when compared with gingivitis patients), inducing higher levels of NO. NO levels are elevated in individuals with Generalized Periodontitis and are correlated with a periodontal clinical parameter. The form of periodontal disease and its severity are related to salivary nitrite concentration, indicating that NO may serve as a potential biological marker for detection and/or monitoring of Generalized Periodontitis [15].

It is quite possible that there exists other genesis of increased level of nitrite-anion in saliva of patients with other oral ifections and generalized periodontitis, accompanied by general pathology.

The innate defense factors identified in saliva can manage cariogenic bacteria and may prevent the subsequent development of dental caries [16]. Although other components of saliva have crucial roles in the management of caries, the beneficial antimicrobial role of NO in the oral cavity has been a remarkable issue in recent years [17].

A recent study reported that excretion of NO in exhaled breath was markedly changed in *H. pylori*-infected nonulcerative dyspepsia and peptic ulcer patients. In the same study, molecular NO in exhaled breath was suggested to be used as a potential biomarker for noninvasive diagnosis and selective differentiation of nonulcerative dyspepsia and peptic ulcer diseases. Also, active uptake of nitrate from the bloodstream occurs in the salivary glands [5, 18, 20].

NO in the saliva could potentially be used as a biomarker in dyspepsia, but not in the identification of gastric *H.pylori* infection [21].

In our investigations we try to examine patients with prevalence of periodontal pathology, though the present data clearly indicate the informative value of the levels of nitrite-anion in saliva that depicts not only the activity of periodontal inflammatory process, but also the aggressiveness of bone destruction. This information could be useful particularly in patients with generalized periodontitis when they are examined during the stages of maintenance care to confirm the disease remission or determine its proceeding course.

Conclusions

Our investigations prove that concentration of Nitrite-anion in saliva can appear as a marker of

inflammatory-dystrophic processes in periodontal tissues.

Nitric-anion concentration in the saliva of patients, with chronic simple and hypertrophic gingivitis differs significantly from the control samples only in case of exacerbation. Increased amounts of nitrite-anion in saliva, that exceeds the health group indices more than 10 times, were reported at the active stage of chronic generalized periodontitis. The level of nitric-anion which depicts the level of Nitric oxide in the saliva probably depicts not only the activity of periodontal inflammatory process, but also the aggressiveness of alveolar bone destruction.

Acknowledgments

The authors declsre that there are no conflicts of interest.

References

1. Monastyrsky V.A., Hrynovets V.S. Discovery that resulted in significiant changes in views of the pathology of periodontal tissues. Danylo Halytsky Lviv National Medical University, 2017; 124.
2. Michałowjć Z., Bachanek T., Ogonovsky R., Marzec Z., Kopciał E., Pitura K., Jarosz Z., Samborski D., Wolańska E., Hendzel B., Hrynovets V., Szybiński V., Hrynioh V., Szabelska A., Dzida K. Wpływ wybranych czynników środowiska na zawartość mikroelementów w warzywach z okolic jeziora Pisoczno (UA). Aspekty Środowiskowo-rekreacyjne i prawne zdrowia człowieka. Monografia pod redakcją Artura Wdowiaka, Andrzeja Tuckiego.– Włodawa, 2015; 175-184.
3. Ripecka Olga, Deneha Igor, Hrynovets Volodymyr, Hysyk Marta. Estimation of Professional Hygiene Efficiency in Patients with Generalized Periodontitis. Polish Journal of Environmental Studies. 2012; 21 (6A): 76-79.
4. Bachanek T., Ogonovski R., Hendzel B., Szybiński W., Jarmolińska K., Hrynovets W., Brzozowska A., Denega I., Wolańska E. Stan uzębienia młodzieży w wieku 18 lat w województwach lwowskim i lubelskim. Dent. Med. probl. 2013; 50 (3) 308-314.
5. Surdilovic D, Stojanovic I, Apostolovic M, Igic M, Kostadinovic L. The role of nitric oxide in saliva in reduction of caries. Acta Fac Med Naissensis. 2008; 25: 93-95.
6. Chia-Chen Chang, Yi-Shyan Liao, Yi-Ling Lin, Ruei-Ming Chen. Nitric oxide protects osteoblasts from oxidative stress-induced apoptotic insults via a mitochondria-dependent mechanism. J. Orthop. Res., 2006; 24 (10): 1917-25. doi: 10.1002/jor.20244
7. Stepanov J. M., Kononov I. N., Zhurbina A. I., Filippova A. I.: Arginine in medical practice. J. Acad. Med. Sci. Uk. 2004; 10 (2): 39.
8. Van't Hof R. J. & Ralston S. H.: Nitric oxide and bone. Immunology 2001; 203-255. doi: 10.1046/j.1365-2567.2001.01261.x
9. Deneha I. S., Kukhta S. Y., Hrynovets V. S.: Informativeness of the index of bleeding in periodontitis. Act. Probl. Stomat. 1996; 13.
10. Deneha I., Ripetska O., Hrynovets V. New Approach to the Estimation of the Nature of Generalized Periodontitis. Pol.J.Environ.Stud. 2013; 22, (5A) 21-25.
11. Hrynovets I., Mahlovanyy A., Deneha I., Ripetska O., Hrynovets V., Buchkovska A Application of different medicinal films in dental practice. Gorlice, 2016; 105.
12. Feelisch M., Stamler J. S.: Methods in Nitric Oxide Research. J. Wiley & Sons, Chichester 1996.
13. Ripetska O., Deneha I., Hrynovets V., Hysyk M. Diseasess of the Periodontium. Etiology.Pathogenesis. Diagnosis. Treatment. Lviv: Liga-Press, 2004; 174.
14. Ripecka Olga, Deneha Igor Concentration of nitrit-anion in saliva of patients at different stages of periodontal diseases. Annales Universitatis Mariae Curie-Sklodowska.- Lublin-Polonia, 2008; (55): 295-298.
15. Reher VG, Zenóbio EG, Costa FO, Reher P, Soares RV. Nitric oxide levels in saliva increase with severity of chronic periodontitis. J Oral Sci. 2007 Dec; 49 (4): 271-276. doi: 10.2334/josnusd.49.271
16. Lenander-Lumikari M, Loimaranta V. Saliva and dental caries. Adv Dent Res. 2000; 14: 40-47. doi: 10.1177/08959374000140010601. doi: 10.1177/08959374000140010601
17. Hegde Mithra N, Hegde Nidarsh D, Ashok A, Shetty S. Salivary Nitric Oxide (NO_2+NO_3) as biomarker of dental caries in adults: An invivo study. IRJP. 2012; 3 (11) :100-102.
18. Loscalzo J. The identification of nitric oxide as

endothelium-derived relaxing factor. Circ Res. 2013; 113: 100-103. doi: 10.1161/CIRCRESAHA.113.301577
19. Som S, Dutta Banik G, Maity A, Chaudhuri S, Pradhan M. Exhaled nitric oxide as a potential marker for detecting non-ulcer dyspepsia and peptic ulcer disease. J Breath Res. 2018 Jan 9;12(2):026005. doi: 10.1088/1752-7163/aa8efb
20. Wang YF, Guo CL, Zhao LZ, Yang GA, Chen P, Wang HK. Effect of helicobacter pylori infection on gastric mucosal pathologic change and level of nitric oxide and nitric oxide synthase. World J Gastroenterol. 2005; 11: 5029-31. doi: 10.3748/wjg.v11.i32.5029
21. Damla Aksit-Bicak, Ebru Emekli-Alturfan, Unsal Veli Ustundag and Serap Akyuz. Assessment of dental caries and salivary nitric oxide levels in children with dyspepsia. BMC Oral Health 2019; 19:11. doi: : 10.1186/s12903-018-0707-z

Figure. 1. Chronic simple gingivitis

Table 1. Nitrite anion level (pmol/L) in the saliva of patients with different types of periodontal pathology

	Type of periodontal disease						
	Simple gingivitis chronic type	Simple gingivitis, exacerbation	Hypertro-phic gingivitis, chronic type	Hypertro-phic gingivitis, exacerba-tion	Generalized periodon-titis, active stage of disease	Genera-lized periodon-titis, remission	Healthy controls
Number of patients	9	5	6	8	12	7	10
Range of blee-ding index	1-2	2-3	1-2	2-4	2-4	0-1	0
level of nitrite-anion	13,5±1,9	50,9±2,4 **	10,6±1,6	54,1±2,7 **	86,9±3,8 *	8,5±0,7	4,9±0,6

*p<0,05, compared with control group
** p<0,05, compared with group of patients with active stage of generalized periodontitis

Figure 2. Hyperplastic gingivitis, oedematous form, II stage of heaviness in the patient M.,26-year-old female

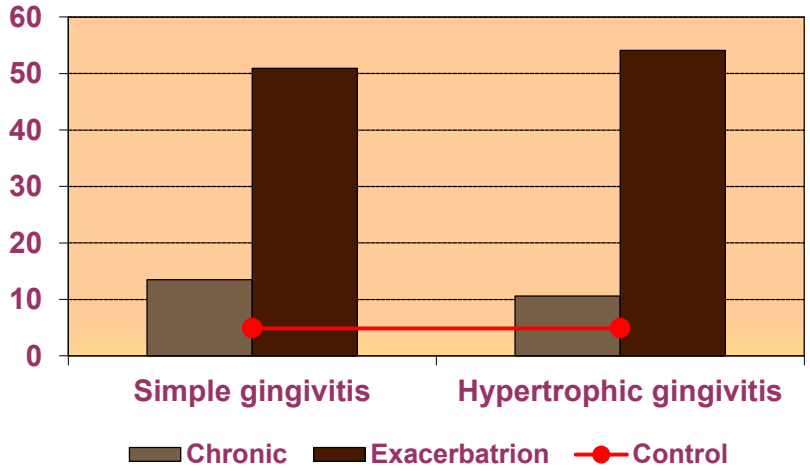

Figure 3. Nitric anion level (pmol/L) in the saliva of patients with different types of gingivitis

Figure 4. Exacerbation of chronic generalized periodontitis, II stage of heaviness, bleeding on probing of the 3rd degree

Figure 5. Slowly progressing not active periodontitis. II-III stages of heaviness in patient L.,42 years old,the depth of periodontal pockets in the region of 42,43 teeth 8mm.

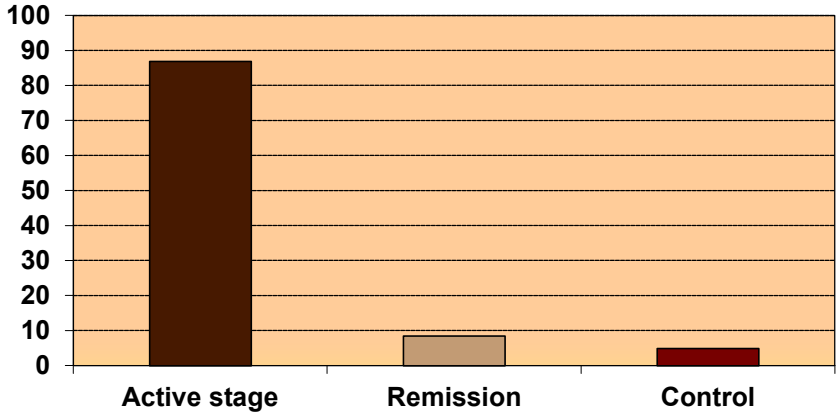

Figure 6. Nitric anion level (pmol/L) in the saliva of patients with Generalized Periodontitis

Figure 7. Rapidly progressing aggressive periodontitis, chronic form, III stage of heaviness in the patient H, 35-year-old female

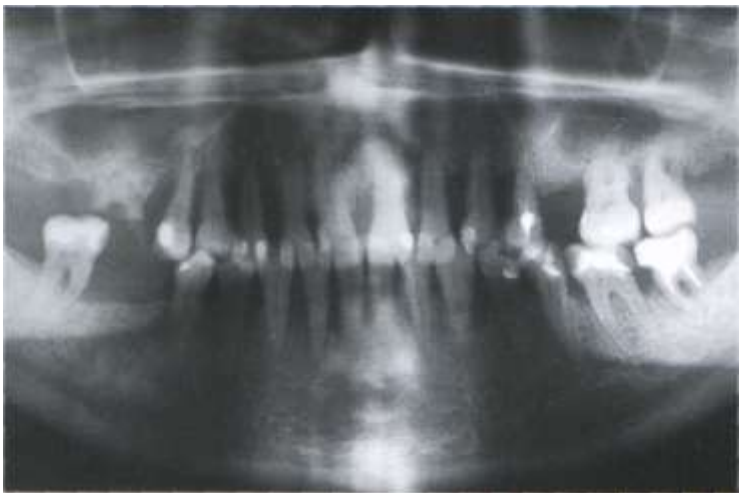

Figure 8. X-Ray of the patient with rapidly progressing aggressive periodontitis; vertical bone loss predominates, broadening of periodontal spaces

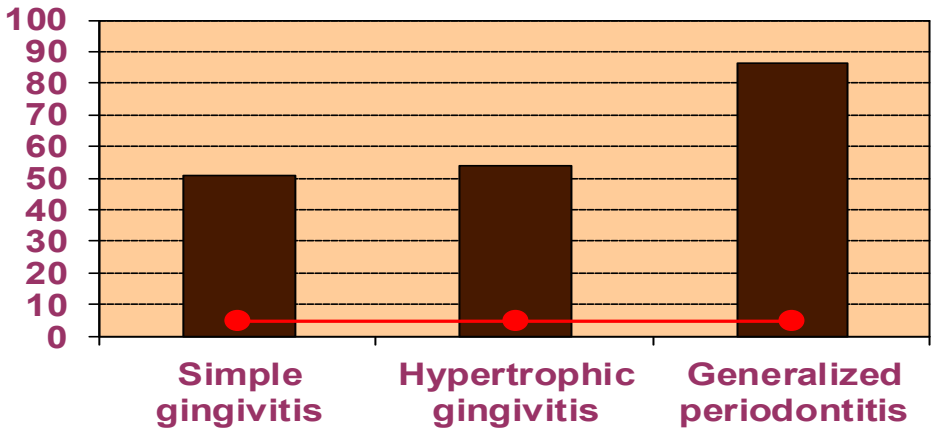

Figure 9. Nitric anion level (pmol/L) in the saliva of patients with active stage of periodontal pathology