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Alkaline Phosphatase Activity Changes of Blood Neutrophil Leukocytes Among Patients Suffering from Diabetes Mellitus Type I and Periodontal Diseases

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SUMMARY

The aim of this work was to investigate secretion function of peripheral venous blood neutrophil leukocytes (NL) in the effect of stimulators of bacterial origin: *E. coli, S. aureus, opsonized zymozan* for patients with insulin dependent diabetes mellitus (1 TDM) and periodontal disease, patients with periodontal disease without systematic pathology and healthy donors. Activity of NL degranulation was analyzed for 32 patients with 1 TDM, 37 patients with periodontal disease and somatically healthy. 35 healthy donors, with healthy periodontal tissues formed the control group.

Neutrophil leukocytes, that are affected by stimulators of bacterial origin in non-cellular environment among patients with 1 TDM and periodontal disease, without generic diseases secrete considerably higher amount (p= 0,001) of alkaline phosphates than neutrophil leukocytes of patients without periodontal disease in analogical medium.

Neutrophil leukocytes affected by microbial origin stimuli in non cellular environment, can release lysosomic ferments which might cause destruction of periodontal tissue.

Keywords: neutrophil leukocytes, alkaline phosphatase, insulin-dependent diabetes mellitus (1 TDM).

INTRODUCTION

World Health Organization characterizes diabetes mellitus (DM) as a metabolic disorder of diverse etiologic factors during which is observed chronic hyperglycaemia with hydrocarbon, albumen and adipose metabolic disorder caused by deficiencies in insulin secretion, insulin action, or both. Long- term damage, dysfunction and failure of various organs appear in the process of diabetes. According to epidemiological research data of National register (2001) 2.31% urban residents and 1.98 % rural residents suffer from DM and glucose tolerance disorder is diagnosed respectively to 5.64 % and 4.66 % (1, 2).

The number of DM patients increases every year. According to death- rate structure DM takes the third place in the world after heart, blood-vessel diseases and cancer (3).

DM is divided into 2 main types: Type 1 diabetes mellitus (1 TDM) was called insulin - dependent diabetes mellitus earlier. This type is caused by destruction of insulin-producing â cells of the pancreas. Type 2 diabetes (2 TDM)– non-insulin dependent diabetes mellitus. This type results from disorder of insulin secretion or insulin resistance. Irrespective of what are etiologic factors of DM, characteristic features of this disease are disorders of hydrocarbon, albumen and adipose always predetermine insulin deficiency or ineffective interaction with cells receptors (2, 3).

1 TDM is clinically revealed only when 50-90 â cells of the pancreas are dead (4, 5). Approximately half of the people with DM are undiagnosed (8). This way a dental odontologic examination can become the first indication of the disease. Mouth cavity symptoms are very important for DM: dryness of mucous membrane (xerostomia), burning mouth or tongue (glossopyrosis), oral candidiasis, impaired wound healing (6).

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DM often includes damage of gums and periodantitis tissues (7, 8, 9, 10, 11). It is proved, that there are at least two factors that influence appearance of periodantitis diseases of connective tissues: microorganisms and the products of their activity and reactivity of the macro-organism (12). In the course of DM disease there is a decreased salivary flow contributes to increase concentration of glucose level and it results to multiply bacterial substrate. It increases susceptibility for beginning of caries and disorders of periodontal tissues (13).

Recently has been established a direct connection between degree of severity of DM and level of periodontal tissues lesion (14, 15, 16). Investigations show that patients with uncontrolled or poorly controlled DM increased tendency of periodontal tissues for oral infection is not directly dependent on quantity of dental fur, increase of glucose concentration has a huge influence in this case (14, 15, 16, 17). It is established that patients with uncontrolled or poorly controlled DM damage of periodontal tissues is more distinct than non-diabetic persons with periodontal pathology (7, 8, 9, 10, 11). Presumption is that normalizing the blood glucose level should stop the progression of periodontal disease. However, it is observed that even if DM is well controlled, patients have more often and more severe periodontal disorders than non-diabetic people (18). This way, it is possibly to think that a clear role for appearing periodontal pathology falls on polymorphonuclear leukocyte (PMN) disorder function.

Alkaline phosphatase - is the hydrolases group ferment which together with water decompose chemical relations including C-O, C –N, and C-S. Considering decomposed relation there are few subclasses of hydrolases: peptidase, esterase and others.According to active substrate and optimal pH phosphatase is alkaline and acid. Alkaline phosphatase is found in all tissues. Especially a lot of it is in bones, lever secrete epithelium. While acting, these ferments, acid molecule chips off complex esters and can make disorder in periodontal tissues (19, 20, 21).

Neutrophil leukocytes stimulated by lipopolysaccharide of micro-organisms performing a protective function begin to spread excess of lizosomic ferments that destroy

structure of surrounding tissues: â glucuronidase, alkaline phosphatase, elastase, etc.

Investigations show that DM and periodontal diseases patients' PNL protective functions become weaker: chemothaxis (22, 23, 24, 25, 26), adherence (8), phagocytosis (23, 27, 28, 29, 30). The accumulative bacteria secret lipopolysaccharide (LPS) and injure mucous membrane, and under-tissues (20, 21). At the same LPS induct immune reaction, stimulate immune component cells including neutrophil leukocytes (20, 21). Stimulated neutrophil granuliocytes function not only as fagocytes but, in addition, secret biologically active materials: alkaline phosphatase, elastase, â-glucuronidase and others (31, 32). When stimulated neutrophil leukocytes lysosomes membrane decompose or its conductibility increase, alkaline phosphatase with other ferments go to inter cell environment, participate in extra cells digestion process and injure structure of macro-organisms (19, 20). Research of subgingivalin microflore, are found the same microorganisms for DM and periodontal diseases patients and for periodontal diseases non-diabetes patients (33). Clinical damage of periodontal tissues is more distinct for patients with DM and periodontal diseases comparing to non-diabetics (34). We can assume that varied PNL response when suffering from periodontal pathology can have its influence: neutrophil leukocytes contacting with microorganisms secrete increased amount of ferments, which have a destroying influence upon periodontal structure.

Numerous studies have identified a clear role for the PMN in maintenance of gingival and periodontal health. Reduced PMN function has been found in patients with diabetes. This impairment of function was noted in assays of PMN chemotaxis, adherence, and phagocytosis. Studies of PMN defects suggest that this function could lead to impaired host resistance to infection (23, 26, 27, 28).

The severity of periodontitis has been correlated with defective chemotaxis; diabetic patients with severe periodontitis had depressed PMN chemotaxis compared to those with mild periodontitis or non-diabetic subjects with severe or mild periodontitis (35, 36).

Further, decreased PMN chemotaxis has been reported in a family with a history of diabetes and periodontitis, suggesting that the PMN defect was of genetic origin (37) A local effect has been suggested since the PMN phagocytic activity of gingival sulcular PMNs was less than of peropherial blood PMNs and, irrespective of the diabetic state, the functional activity of PMNs collectad from diseased sites was less than that at healthy sites (35).

We now that neutrophil leukocytes contacting with microorganisms secrete increased amount of ferments, which have a destroying influence upon periodontal structure. Because, that no many studies about PMN secretion function, and it is not easy to estimate the range of leukocyte function disorders because it is difficult to estimate when not protective but damaging features of mouth tissues start to predominate (20, 21). So, our purpose was to investigate the venous peripheral neutrophil leukocytes (NL) activity of alkaline phosphates among different groups of people:

- Healthy donors (group I) ;
- Patients with periodontal disease without systematic pathology (group II);
- Patients with I TDM and periodontal disease (group III);

affected by stimuli of bacterial origin: opsonized zymozan, non-opsonized Escherichia coli (E. clli ATCC 25922) and non-opsonozated Staphylococcus aureus (S. aureus 256).

MATERIALS AND METHODS

Research groups. There were selected 20 – 50 year-old persons.

Inflammation of periodontal tissue - destructive lesions were rated by Russel periodontal index- PI (38): from 0.0 till 8.0 points.

35 healthy donors (group I) constituted the first (control group) (20 males and 15 females); persons without systematic pathology with healthy periodontal tissues, they were rated PI from 0.0 till 2.0 points.

The second research group (group II) was constituted of 37 people (20 males and 17 females); people without systematic pathology, but with periodontal disease. After clinical and radiological investigations inflammation process of periodontal tissues in this group**,** was rated according to PI from 4.0 till 8.0 points

The third research group (group III) was constituted of 32 people (20 males and 12 females); people 1 TDM and periodontal disease. PI was rated from 4.0 till 8.0 points. All patients of this group have been ill for 3-8 years, they have been treated for 3-8 years in Endocrinology Clinic of KMUC and got corrective-treatment with insulin medicine. Distinct diabetes complications (insufficiency of organs) were not observed.

Reagents. Buffering solution of Dietanolamin ph 9,8 1,0 mol/l, Magnesia Cloridum 0,5 mmol/l, paranitrophenilphosphate 10 mmol/l, phosphate Dulbeko buffering solution Hanks balanced salt solution (ph 7,3) was obtained from Sigma Chemical Co (USA).

Table 1. Alkaline phosphatase activity (U/l).

Research groups	NLIT+ phosphate buffer	NLIT+*neops. E. coli*	NLIT+*neops. S. aureus*	NLIT+*opsonized zimozan*	P (Reliability)
I n=35	90,0 ± 18,5 (11)	109,8 ± 12,6 (12)	106,0 ± 15,8 (13)	P 110,4 ± 11,3 (14)	P 11-14<= 0,001 P 11-13<= 0,001 P 11-12<= 0,001 P 13-12< 0,2 P 13-14< 0,2
II n=37	123,1 ± 19,2 (21)	199,9 ± 13,0 (22)	184,3 ± 21,6 (23)	182,7 ± 12,0 (24)	P 21-24<= 0,001 21-23<= 0,001 21-22<= 0,001 P 23-24< 0,5 22-23<= 0,001
III n=32	150,0 ± 12,0 (31)	310,0 ± 14,0 (32)	245,0 ± 14,0 (33)	234,0 ± 11,0 (34)	31-34<= 0,001 31-33<= 0,001 31-32<= 0,001 33-32<= 0,001 33-34< 0,2
	11-21<= 0,001 11-31<= 0,001 21-31<= 0,05	12-22<= 0,001 12-32<= 0,001 22-32<= 0,001	13-23<= 0,001 13-33<= 0,001 23-33<= 0,01	14-24<= 0,001 14-34<= 0,001 24-34<= 0,001	

Crops grown in laboratory of microbiology of KMUC was used for stimulation of leukocides: *E.coli ATCC 25922* and *S. aureus 256*. *Zymozan* was *opsinisated* using R. Zeiger and other methods (39).

Preparing of incubation medium of neutrophil leukocytes (NLIT):

The incubation media of leukocytes were prepared by Talstad and another method (43). Activity of alkaline phosphatase (ALP) was estimated by spectrophotometric method using "Instrumentation Laboratory" automatical biochemical analyser 'Monarch".

RESULTS

Data of biochemical laboratory research in table 1.

Activity of alkaline phosphatase in control peripheral venous blood NLIT of healthy donors and people suffering from periodantitis without systematic pathology and people with 1 TDM differed p= 0,05. Activity of alkaline phosphatase of neutrophil leukocytes of patients with periodontal disease and 1 TDM affected by *opsonized zymozan* was high and reached 234,0 ± 11,0 U/l, which was statistically reliably (p= 0,001) higher than activity of alkaline phosphatase of healthy donors in analogical medium which was equal to 110,4=11,3 U/l. Ferments activity NL of patients with periodontal disease and without systematic pathology affected by *opsonised zymozan* was less than of patients with periodontal disease and 1TDM. It was equal to 182,7 ± 12,0 U/l. At the same time definitely exceeded analogical medium of healthy donors.

Activities of alkaline phosphatase NLIT affected by *non-opsonized S. aureus,* statistically data reliability was higher than NLIT of the second and the third groups comparing to ferment activity of healthy donors in analogical media.

It is important to note that the most significant increase of ferment activity was established in the third research group, NL affected by *non-opsonized E. coli*, it was equal to 310,0 ± 14,0 U/l and distinctly exceeded (p = 0,001) of the second group in analogical medium and more than three times exceeded NLIT of the first group.

DISCUSSION

During recent decades it has been proved that bacteria of tooth fur and products of their vital activity cause inflammation processes of periodontal tissues (12, 41, 42). However, bacteria aren't the only factors, which cause destruction of periodontal tissue. Another important factor of this disease pathogens is a macro-organism, the immune system response to influence of microorganisms (43). Active pathogenic infection in the mouth deranges interaction among immune-competitive cells: lymphocytes, lymphokines, macrophages or antibodies, complement system, polymorphonuclear leukocytes.

Neutrophil leukocytes as immune-competitive cells carry out a protective function of an organism. There are the first cells that migrate into tissues, phagocytate microbes and their complexes, devastate injured tissue (44). Penetrating into inflammated place NL, their functioned activity is changes, displaying certain biological effect, increased degranulation and generation of active oxygen forms.

Increase of NL functional activity is important to unspecific immune reaction of organism, stopping penetration of microbes, the factor which might injure macro-organism's tissue (21, 45). Not only local, but also systematic factors have a huge role to development of pathology of periodontal tissue (46). Periodontal diseases of tissues are frequently diagnosed to DM patients. There is relation between injured periodontal tissue and DM development level (47). Secreting from NL second-rate granules alkaline phosphatase might cause destruction of connective tissue. Amount oh this ferment is increasing in fluid of gums furrows during non-treated periodontitis (48). Increase of ALP activity correlates with level of activity of inflammation process of periodontal tissue (43). Studies of others authors of PMN defects shows reduced PMN chemotaxis, adherence, phagocytosis (22, 24, 25, 26, 27, 29, 30, 49).

Data of our investigations showed that, NL effected by *non-opsonised E. coli* and *S. aureus, opsonised zymozan,* patients with periodontitis without systematic pathology and patients with periodontitis and also 1 TDM, APL activity considerably increases (p= 0,001), comparing to control media of the second and third groups. The highest APL activity is estimated to people of the third group. NL affected by *non-opsonised E. coli*, which is statistically reliable, (p = 0,001) was bigger not only than control medium of the same group, but bigger than the first and second research groups of analogical media.

Functionald afficiency of neutrophil leukocytes of patients with 1 TDM and periodontitis depends on used different bacterial origin stimulators. Data of investigation shows that latter make more significant influence upon NL degranulation.

Based on performed research we can state that blood neutrophil leukocytes of patients with periodontal disease without systematic pathology and patients with 1 TDM and periodontal disease, reacting to mouth bacteria and their toxins, secrete bigger amount of lysosomal ferments to noncellular environment and have possibility to cause destruction of periodontal tissue.

CONCLUSIONS

1. Peripheral venous blood neutrophil leukocytes, of patients with 1 TDM and periodontal disease and also patients with periodontal disease without somatic diseases when affected by stimulus of bacterial origin in non-cellular environment, secrete abundant amount of alkaline phosphates, which statistically reliably exceeds the amount of this ferment of healthy donors in analogical medium.

2. Neutrophil leukocytes of patients with 1 TDM and periodontal disease and also patients with periodontal disease without somatic diseases secrete the biggest amount of alkaline phosphates comparing to control media of research groups, after affecting them by bacterial origin stimulator: *E. coli.*

REFERENCES

1. Lietuvos medikų diabeto sąjungos leidinys. *Diabetologija* 2001; 9:49-53.
2. Danilevičius J. Nauja cukrinio diabeto klasifikacija, nauji kriterijai ir naujos problemos. *Lietuvos endokrinologija* 2000; 8 (3,4).
3. Danilevičius J. Nauja cukrinio diabeto klasifikacija, nauji kriterijai ir naujos problemos. *Lietuvos endokrinologija* 2000; 8(3,4).
4. Palmer JP, Mc Culloch DK. Prediction and prevention of IDDM. *Diabetes* 1991; 40: 943-947.
5. Poussier Ph, Schiffrin A, Ciampi A, Tam E, Lalla D, Belmote MM, Berger R. The risk for developing disease for sibblings with insulin dependent diabetes mellitus. *Clin. Invest. Med* 1991;14:1-8.
6. Benson L, Braem M, Van Acker K, Willemsa G. Orthodontic treatment considerations in patients with diabetes mellitus. *Am J Orthod Dentofacial Orthop* 2003; 123(1): 74-8.
7. Akintewe TA, Kulasekara B, Adetuyibi A. Periodontitis diabetica. A case report from Nigeria. *Trop Geogr Med* 1984; 36:85-86.
8. Arch CB, Rosenberg WMC, Scott GW, Mac Donald DM. Progresive bacterial synergistic gangrene in patient with diabetes mellitus. *J R Soc Med* 1984; 77 (Suppl 4):1-3.
9. Bartolluci EG, Parkes RB. Accelerated periodontal break down in uncontrolled diabetes. Pathogenesis and treatment. *Oral Surg Oral Med Oral Pathol* 1981; 52:387-390.

10. Branda L. Facial erythema and edema in a diabetic man with mucormyosis. *Arch Dermatol* 1986; 122:331-334.
11. Urcles SD. Case report: a patient with severe periodontitis in conjunction with adult-onset diabetes. *Compendium Contin Educ Dent* 1983; 4:522-528.
12. Genco RJ. Current view of risk factors for periodontal diseases. *J Periodontol* 1996; 67(10):1041-1049.
13. Fajans S. Scope and heterogenous nature of MODY (review). *Diabetes Care* 1990; 13:49-64.
14. Bacic M, Planuok D, Granic M. CPITN assessment of periodontal status in diabetic patients *J Periodontol* 1988; 59:816-822.
15. Gusberti FA, Syed SA, Bacon G, Grossman N, Loesche WJ. Puberty gingivitis in insulin-dependent diabetic children. I. Cross-sectional observations. *J Periodontol* 1983; 54:714-720.
16. Novaes AB, Pereira A, de Moraes N. Manifestations of insulin-dependent diabetes mellitus is periodontium of young Brazilan patients. *J Periodontol* 1991; 62: 116-122.
17. Sorsa T, Ingiman T, Suomalainen K, et al. Cellular source and tetracycline inhibition of gingival cervicular fluid collagenase of patients with labile diabetes mellitus. *J Clin Periodontol* 1992; 19:146-149.
18. Sastrowijoto SH, van der Valden U, van Steenbergen TJM, Hillemans P, Hart AAM, de Graaff J, et al. Improved metabolic control, clinical periodontal status and subgingival microbiology in insulin-dependent diabetes mellitus. *J Clin Periodontol* 1991;17:233-42.
19. Kondrotas A, Jankauskienė K, Meškelevičius V, et al. Burnos patologinė fiziologija 2001; 16-29, 114-118.
20. Labro MT. Host defense and infection. New York etc.: Hoecht. M. Dekker. 1994; p. 68.
21. Leone CW, Clark WB, Mc Arthur WP. Host responses in the etiology and pathogenesis of periodontal disease. *Curr Opin Dent* 1991; 1(1):29-36.
22. Hill HR, Souls H, Deffloff JL, Quie PG. Impaired leucotactic responsiveness in patients with juvenile diabetes mellitus. *Clin Immunol Immunopathol* 1974; 2:395-403.
23. Kjersmen H, Histed J, Madsbad S, Wandall JH, Johansen KS, Borrgaard N. Polymorphonuclear leucocyte dysfunction during shorterm metabolic changes from normo- to hyperglycemia in type I (insulin dependent) diabetic patients. *Infection* 1988; 16:215-221.
24. Leeper SH, Kalkwart KL, Strom EA. Oral status of „controlled" adolescent type I diabetics. *J Oral Med* 1985; 40:127-133.
25. Molenaar DM, Pagumbo PJ, Wilson WR, Ritts RE. Leucocyte chemotaxis in diabetic patients and their nondiabetic first-degree relatives. *Diabetes* 1976; 25(Suppl 2): 880-883.
26. Mowat HR, Bradon J. Chemotaxis of polymorphonuclear leucocytes from patients with diabetes mellitus. *N Engl J Med* 1971; 284:621-627.
27. Bagdade JD, Nielson KL, Bulger RJ. Reversible abnormalities in phagocytic function in poorly controlled diabetic patients. *Am J Med Sci* 1972; 263:451-456.
28. Marhofer W, Stein M, Moeser E, Federlin K. Impairment of polymorphonuclear leucocyte function and metabolic control of diabetes. *Diabetes Care* 1992; 15:256-260.
29. Repine JE, Clawson CC, Goetz FC. Bacterial function of neutrophils from patients with acute bacterial infections and from diabetics. *J Infect Dis* 1980; 142:869-875.
30. Wilson RM, Rieves WG. Neutrophil phagocytosis and killing in insulin-dependent diabetes. *Clin Exp Immunol* 1986; 63:478-484.
31. Genco RJ. Host responses in periodontal diseases: current concepts. *J Periodontol*. 1992; 63(Suppl 4): 338-355.
32. Van Dyke TE, Hoop G. *Crit Rev Oral Biol Med* 1990; 2:117.
33. Monrtfeld K, Toolefen T. Serum IgG Lit. antibodies reactive with lipoteichoc acid in adult patients with periodontitis. *J Clin. Periodont* 1989;16:519.
34. Saodaun A. Diabetes and periodontal disease. (A review and update). *J West Soc Periodontol Periodontal Abstr* 1980; 28:116-39.
35. Bissada NF, Manouchehr-Pour M, Haddow M, Spanguolo PJ. Neutrophil functional activity in juvenile and adult onset diabetic patients with mild and severe periodontitis. *J Periodont Res* 1982;17:500-502.
36. Manouchehr-Pour M, Spanguolo PJ, Bissada NF. Impaired neutrophil chemotaxis in diabetic patients with severe periodontitis. *J Dent Res* 1981;60:729-730.
37. McMullen JA, Van Dyke TE, Horoszewicz HU, Genco RJ. Neutrophil chemotaxis in individuals with advanced periodontal disease and a genetic predisposition to diabetes mellitus. *J Periodontol* 1981;52:167-173
38. Russel AL. Epidemiology of periodontal disease. *Int Dent J* 1967;17(2):282-296.
39. Zeiger RS, Twarog FJ, Colten HR. Histaminase release from human granulocytes. *Exp. Med*1976;144(4):1049-1061.
40. Talstad I, Dalen H, Leimann V. Degranulation and enzyme release during phagocytosis of inert particles and of bacteria by polymorphonuclear neutrophil granulocytes. *Acta Pathol Microbiol Immunol Scand [C]* 1983;91: 403-411.
41. Williams RC. Periodontal diseases: the emergence of a new paradigm: A supplement to compendium of continuing education in dentistry. *Periodontal Aspects of Syst Health* – 1998; 19(1):4-10.
42. Williams RC. Periodontal disease. *N Engl J Med* 1990;322:373-382.
43. Nakashima K, Giannopoulou C, Andersen E, et al. A longitudinal study of various crevicular fluid components as markers of periodontal disease activity. *J Clin Periodontol* 1996;23:832-838.
44. Biasi D, Carletto A, Dell Agnola C, et al. Neutrophil migration, oxidative metabolism and adhesion in elderly and young subjects. *Inflammation* 1999;20:673.
45. Conrads G, Herrler A, Moonen I, et al. Flow cytometry to monitor phagocytosis and oxidative burst of anaerobic periodontopathogenic bacteria by human polymorphonuclear leukocytes. *J Periodont Res* 1999;34(3):136-144.
46. Lamster JB, Holmes LG, Gross KB, et al. The relationship of ß-glucuronidase activity in crevicular fluid to probing attachment loss in patients with adult periodontitis. Findings from a mullticenter study. *J Clin Periodontol* 1995;22(1):36-44.
47. Ainamo J, Lathinen A, Uiito V. Rapidly periodontal destruction in adult humans with poorly controled diabetes. A report of two cases. *J Clin Periodontol* 1990;17:122.
48. Chaole JLC, Garner I, Saxby MS, et al. Prediction and diagnosis of attachment loss by enhanced chemiluminescent assay of crevicular fluid alkaline phosphatase levels. *J Clin Periodontol* 1999;26:190-198.
49. Bagdade JD, Stewart M, Walters E. Impaired granulocyte adherence. A reversible defect poorly controlled diabetes. *Diabetes* 1987; 27:677-681.

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