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 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% 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 taste in mouth (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 leucocyte (PMN) disorder function.

DM often includes damage of gums and periodontitis tissues (7, 8, 9, 10, 11). It is proved, that there are at least two factors that influence appearance of periodontitis 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 leucocyte (PMN) disorder function.

Alkaline phosphatase is the hydrolases group ferments 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..
microorganisms secrete increased amount of ferments, which has 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 1 TDM and periodontal disease (group III);
- Affected by stimuli of bacterial origin: opsonized zymozan, non-opsonized Escherichia coli (E. coli 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).

<table>
<thead>
<tr>
<th>Research groups</th>
<th>NLIT+ phosphate buffer</th>
<th>NLIT+neops. E. coli</th>
<th>NLIT+neops. S. aureus</th>
<th>NLIT+opsonized zimozan</th>
<th>P (Reliability)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I n=35</td>
<td>90,0 ± 18,5 (11)</td>
<td>109,8 ± 12,6 (12)</td>
<td>106,0 ± 15,8 (13)</td>
<td>P 110,4 ± 11,3 (14)</td>
<td>P 11-14&lt;= 0,001</td>
</tr>
<tr>
<td>II n=37</td>
<td>123,1 ± 19,2 (21)</td>
<td>199,9 ± 13,0 (22)</td>
<td>184,3 ± 21,6 (23)</td>
<td>182,7 ± 12,0 (24)</td>
<td>P 13-23&lt;= 0,001</td>
</tr>
<tr>
<td>III n=32</td>
<td>150,0 ± 12,0 (31)</td>
<td>310,0 ± 14,0 (32)</td>
<td>245,0 ± 14,0 (33)</td>
<td>234,0 ± 11,0 (34)</td>
<td>P 21-33&lt;= 0,001</td>
</tr>
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Crops grown in laboratory of microbiology of KMUC was used for stimulation of leukocytes: *E. coli* ATCC 25922 and *S. aureus* 256. *Zymozan* was opsonised using R. Zeiger and other methods (39).

**Preparation 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 from biochemical laboratory research in table 1.

Activity of alkaline phosphatase in control peripheral venous blood NLIT of healthy donors and people suffering from periodontitis 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 analogue 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 *opsonized 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 analogue 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 ferments activity of healthy donors in analogue 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 analogue medium and more than three times exceeded NLIT of the first group.

**DISCUSSION**

During recent decades it has been proved that bacteria of tooth fund 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, phagocyte 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). Secretion from NL second-rate granules alkaline phosphatase might cause destruction of connective tissue. Amount of 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, adhesion, phagocytosis (22, 24, 25, 26, 27, 29, 30, 49).

Data of our investigations showed that, NL affected by *non-opsonised E. coli* and *S. aureus*, opsonised *zymozan*, patients with periodontitis without systematic pathology and patients with periodontitis and also 1TDM, 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 analogue media.

Functional efficiency 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 NLIT of patients with periodontal disease, reacting to mouth bacteria and their toxins, secrete bigger amount of lysosomal ferments to non-cellular 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 analogue 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**


