

Periodontal status and secretory activity of neutrophilic leukocytes among 25-44-year-olds in Kaunas city, Lithuania

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Summary

Objective. The aim of the present study was to describe the prevalence of periodontal diseases and oral hygiene status among 25-44-year-olds in Kaunas city and to study the secretory function of peripheral venous blood neutrophilic leukocytes (NL) exposed to various antigens in patients with gingivitis and periodontitis.

Material and Methods. The prevalence of periodontal diseases (according to CPITN), oral hygiene status (according to OHI-S index) was examined in 420 subjects. Secretory function of peripheral venous blood neutrophilic leukocytes (NL) in 112 subjects was determined. Secretory function of NL affected by antigens, such as opsonized zymosane and anatoxine *S. aureus* was investigated by lysozyme (LZ) activity in patients with gingivitis and periodontitis who were internal diseases free. Lysozyme activity was analyzed by the spectrophotometric method, supplemented with *Micrococcus luteus* 2665.

Results. The prevalence of periodontal diseases was 90.2%. Gingival bleeding and dental calculus were diagnosed in 73.2% and periodontal pockets in 16.6% of subjects. Oral hygiene in 55.7% of participants was satisfactory. NL secreted higher levels of LZ in incubation media of all participants with healthy periodontium and of patients with gingivitis and periodontitis. In patients with gingivitis and periodontitis NL affected by antigens secreted higher levels of LZ in incubation media compared with control media.

Conclusions. Higher prevalence of periodontal diseases and more periodontal lesions were diagnosed in patients with unsatisfactory oral hygiene. Response of NL to bacterial origin stimuli in patients with gingivitis and periodontitis was found specific and dependent on the periodontal lesion severity level. Once NL are exposed to corpuscles, prone to phagocytosis, an increased secretion of LZ can be explained by overall increase in secretion of NL lysosomic enzymes. That explains the mechanism of inflammatory origin of periodontal tissue damage.

Key words: periodontal status, oral hygiene, secretory function of neutrophilic leukocytes, lysozyme.

Introduction

Periodontal diseases are highly prevalent among the population all over the world and prevention as well as treatment of these

diseases are among most topical problems in dentistry [1,2,3,4,5]. Findings of epidemiological studies in Lithuania showed that the main cause of teeth loss in population under 40 years was dental caries. Periodontal dis-

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eases are the second major cause of teeth loss [6]. Healthy mode of life, proper oral hygiene, general health and immunity have an impact on the development of dental diseases [7,8].

To unify the data of the prevalence and severity of periodontal diseases in different countries, the WHO has recommended the CPITN index. Different studies data showed that the percentage of people with periodontal diseases is increasing with age.

Number of recent studies showed a relation between the origin of periodontal diseases and the immune system of the macro-organism. The interaction between the components of immune system and antigens often results in destruction of native tissues [8,9,10].

Many studies verify the role of bad oral hygiene in aetiology and pathogenesis of inflammatory periodontal diseases [6,11,12]. Plaque bacteria is an expositional factor, however, the exact mechanism of periodontal tissue destruction has not been clearly defined [10,13,14]. Bacteria are able to penetrate deep gingival tissues and release toxic substances, which can permeate through and damage biological membranes [11,12]. The most important location of bacterial invasion into deep periodontal tissues is gingival sulcus [14]. The immune response of the macro-organism to periodontal pathogenic bacteria and its toxins is ambiguous [15,16].

Studies on local and systemic parameters of humoral and cell mediated immunity are thought to be very important in defining the origin of inflammatory periodontal diseases and the basics for evidence-based methods in treatment of periodontal diseases by anti-inflammatory medications [15,17].

Neutrophilic leukocytes (NL) are main cells protecting the macro-organism from infection. NL contain numerous biologically active substances and cytoplasmic granules. Phagocytosis of the microorganisms and their destruction inside the phagolysosome

is one of the stages in defense process [17]. Bacteria and cancer cells infected by viruses, might be destroyed by NL by releasing their lysosomal granules content into environment and through the generation of active forms of oxygen [17,18,19].

Proteolytic enzymes in NL lysosomal granules (elastase) and acidic hydrolases (β -glucuronidase, cathepsin D) may erode periodontal tissues. NL proteolytic enzymes released from lysosomal granules degrade collagen, while acidic hydrolases destroy non-collagen components of extracellular matrix in periodontal tissues [11]. Once exposed to stimulating factors, NL show high functional activity: adhesion, chemotaxis, „respiratory burst“, phagocytosis, degranulation, extracellular secretion, antimicrobial and cytotoxic effect [10,14,15,16,18,19]. Therefore, the studies on functional activity of NL might provide important information in etiopathogenesis of inflammatory periodontal diseases.

The aim of the present study was to describe the prevalence of periodontal diseases and oral hygiene status among 25-44-year-olds in Kaunas city and to study the secretory function of peripheral venous blood neutrophilic leukocytes (NL) exposed to various antigens in patients with gingivitis and periodontitis.

Material and methods

A total number of 420 inhabitants of Kaunas city aged 25-44 years were examined. Subjects were divided into two age groups: 25-34 and 35-44 years.

One hundred and twelve voluntaries participated in the experiment: 40 with gingivitis, 37 with periodontitis and 35 donors with healthy periodontium. Patients with gingivitis or periodontitis were systemic and internal diseases free. The control group comprised subjects with no periodontal lesions.

The periodontal status was evaluated by CPITN (Community Periodontal Index of Treatment Needs) [20]. A special periodontal probe was used to determine the periodontal status. Oral hygiene status was evaluated by OHI-S (Green-Vermillion simplified) [21]. The evaluation of the oral hygiene according to OHI-S index comprised: 0 - perfect oral hygiene, 0.1-1.0 - good, 1.1-2.0 - satisfactory, 2.1-6.0 - bad .

Secretory function of peripheral venous blood neutrophilic leucocytes. Peripheral venous blood (5 ml) was taken with heparine (20 IU/ml) vacuum test-tube from all participants. The test-tubes were placed into thermostat at 45° angle for one hour at 37°C temperature. Plasma was aspirated and the leukocyte count was standardized to 1x10⁹ cells/l with Hanks' Balanced Salt Solution. Every test-tube was filled with 1.5 ml of plasma and with 0.1 ml of bacterial origin stimuli. Prepared media were placed into a thermostat at 37°C temperature for one hour. Phagocytes were activated by opsonized zymosane and anatoxine *S. aureus*. Zymosane was opsonized accordingly to R. Zeiger et al [22].

Enzyme analysis. Lysozyme (LZ) was analyzed by spectrophotometric method supplemented with *Micrococcus luteus* 2665 [23].

Statistical analysis of the data was performed with SPSS MS for Windows. The groups were described by the characteristics of general statistics, dispersion and symmetrical association. Verification of the

hypotheses of the relation between qualitative variables was by χ^2 criterion. The comparison of quantitative values was performed by Student's or Fisher's F criterion. Continuous variables were described as Mean \pm SEM. P value <0.05 was as a level of statistical significance.

Results

The prevalence of periodontal diseases was 90.2%. Among the investigated subjects only 9.8% had healthy periodontal tissues (Table 1). The majority (73.2%) in the group of subjects with periodontal diseases was with gingival bleeding and supragingival or subgingival calculus. They comprise a group of patients with gingivitis. Periodontal pockets 4-5 mm and 6 mm or deeper were found in 13.2 % and 3.4% of subjects respectively. They comprise a group of patients with periodontitis.

The largest number of patients with periodontitis was found among 35-44 year-olds. In this group, 4-6 mm pockets were found in 22.3% of patients and differ statistically significant from the 25-34 year-olds group ($p < 0.05$). Mean 1.1 sextant of 4-5mm depth and mean 0.18 sextant of 6 mm or deeper periodontal pockets were diagnosed in 35-44 year-olds (Table 1).

Oral hygiene data obviously showed inadequate toothbrushing in examined population. Good oral hygiene (OHI-S <1) was found only in 30.7% of the examined sub-

Table 1. Oral hygiene status (according to Green-Vermillion index) in 25-44 year-olds in Kaunas city

Age groups	n	Oral hygiene index(OHI-S)									
		<1.0		1.01-2.0		2.01-3.0		3.01-6.0		Mean OHI-S	
		n	%	n	%	n	%	n	%	Mean	SE
25-34 years	196	69	35.2	110	56.1	17	8.6*	-	-	1.28*	0.05
35-44 years	224	60	26.7	124	55.4	35	15.6	5	2.2	1.66	0.06
Total	420	129	30.7	234	55.7	52	12.4	5	1.2	1.47	0.06

* $p < 0.05$ between age groups

Table 2. Periodontal status (according to CPITN Index) among 25-44 year-olds in Kaunas city

Age groups	Number of examined subjects	Percent of examined people with CPITN codes «0-4»					Mean number of sextants involved per person with CPITN codes «0-4»					Excluded Sextants «X»						
		Periodontal pockets					Periodontal pockets					Excluded Sextants «X»						
		«0»	«1»	«2»	4-5 mm «3»	?6 mm «4»	«0»	«1»	«2»	4-5 mm «3»	?6 mm «4»	Mean	SE	Mean	SE			
25-34	196	11.7	14.3	63.3	10.71	-	1.11	0.05	1.26	0.08	3.05	0.15	0.58	0.02	-	-	-	-
35-44	224	8.0	11.6	57.1	15.6?	6.7	0.88	0.05	0.96	0.05	2.86	0.12	1.11	0.06	0.18	0.01	0.01	0.001
Total	420	9.8	13.0	60.2	13.2	3.35	0.99	0.03	1.11	0.04	2.96	0.08	0.84	0.02	0.09	0.01	0.005	0.001

*p< 0.05 between age groups

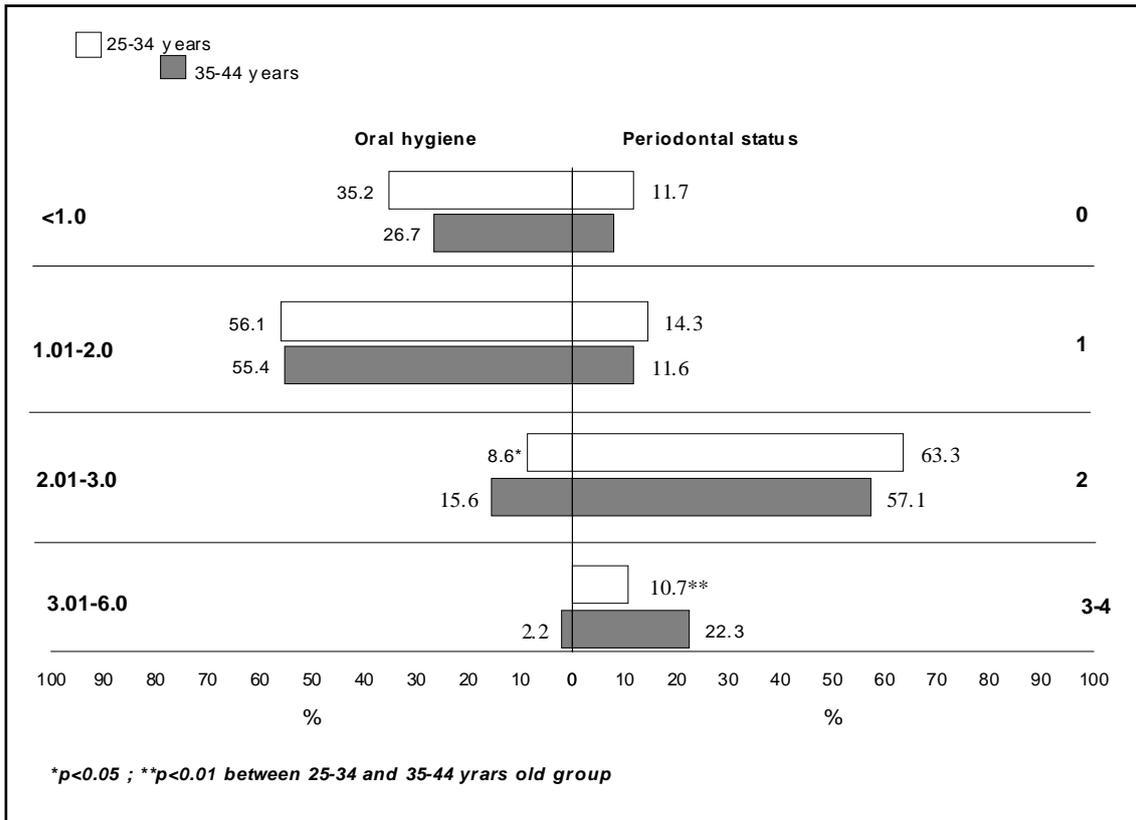


Figure 1. Relationship between oral hygiene and periodontal status among 25-44 year-olds in Kaunas city (Lithuania)

jects. Satisfactory oral hygiene was found in 55.7%, and $OHI-S >3$ in 1.2% of subjects. The mean $OHI-S$ index among 25-34 year-olds was 1.28 ± 0.05 , and 1.66 ± 0.06 among 35-44 year-olds (Table 2).

Better oral hygiene was related with less severity of periodontal diseases. Gingivitis was mostly diagnosed, and in subjects with $OHI-S \geq 3$ mostly periodontitis was diagnosed (Figure 1).

With an aim to evaluate the possible risk factors of inflammatory periodontal diseases, the 77 patients (40 with gingivitis and 37 with periodontitis) were selected for the analysis of secretory function of NL in peripheral venous blood.

LZ activity in incubation media (IM) of NL significantly increased after affection with opsonized zymosane in patients with gingivitis and periodontitis, as well as in donors ($p \pm 0.001$), in comparison with control media (phosphate buffer) (Figure 2). LZ

activity did not differ significantly in IMNL of patients with gingivitis (30.3 ± 4.2 mg/l) and periodontitis (32.7 ± 4.1 mg/l) affected by opsonized zymosane in comparison with the enzyme activity in analogues IMNL of donors. LZ activity significantly increased (Figure 2) and was determined in IMNL with anatoxine *S. aureus* of patients with gingivitis and periodontitis ($p \leq 0.001$), compared with control media (phosphate buffer) and with the enzyme activity in analogues IMNL of donors. LZ activity did not differ significantly in IMNL of donors with intact periodontium affected by anatoxine *S. aureus* in comparison with control media (phosphate buffer).

Discussion

Examination of periodontal status among 25-44 year-olds of Kaunas city showed high prevalence of periodontal dis-

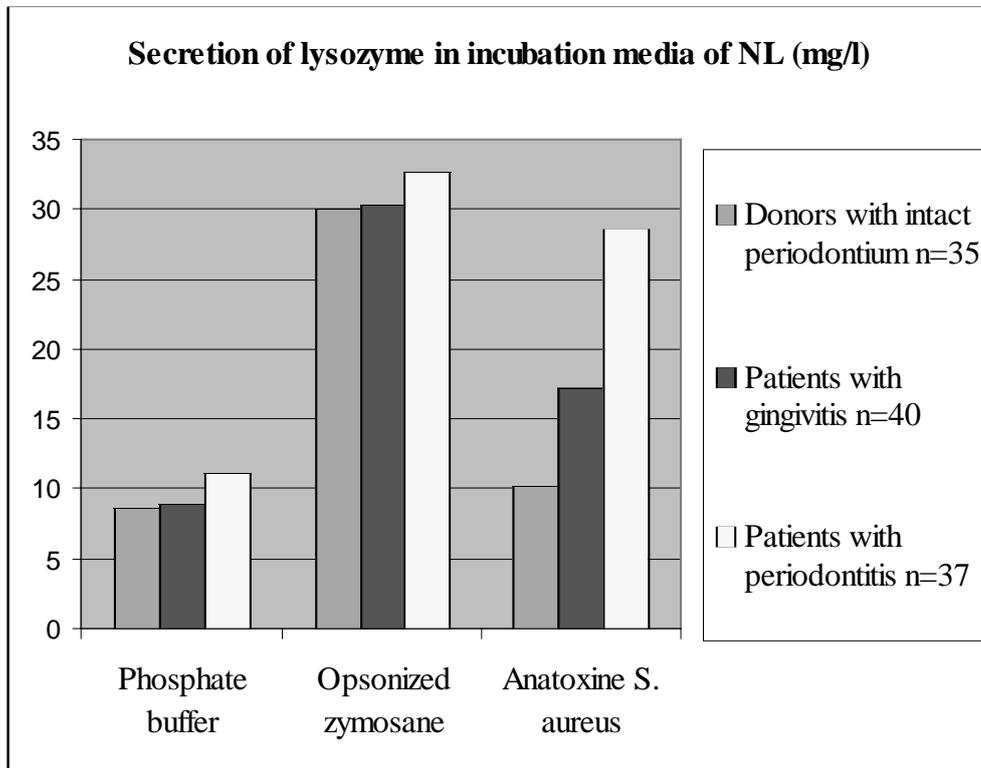


Figure 2

eases. Healthy periodontium was found only in 9.8% of subjects. The data showed the tendency of periodontal diseases to increase with and increasing necessity of treatment by planning effective oral health care [20,24,25]. Our study showed the necessity to study periodontal diseases in different age groups. It will help to monitor periodontal diseases more objectively and periodontal treatment more effectively.

Comparison of prevalence of periodontal disease by CPITN index and oral hygiene status show more severe periodontal lesions in 35-44 year-olds with unsatisfactory oral hygiene. Thus, it leads to assumption that dental plaque plays an important role in the development of periodontal diseases [8,9, 24]. Oral hygiene improvement is very important in reducing the prevalence of periodontal diseases among Lithuanian population calling for implementation of periodontal diseases prevention program among

adults.

Recent studies show that dental plaque is a risk factor in developing inflammatory periodontal diseases [11]. The other important factor in aetiopathogenesis of this condition is the immune response to microorganisms [9]. Released granule components from infiltrating leukocytes, such as lysosomal enzymes and reactive oxygen species, which are normally intended to degrade ingested microbes, can also lead to tissue destruction and amplification of the inflammatory response [26,27,28]. In localized aggressive periodontitis, in particular, uncontrolled neutrophil recruitment and activation has been demonstrated to lead to the aberrant release of an array of noxious agents intended to fight the bacteria, with the potential to cause further tissue damage [26,28]. The increased activity of NL secretion in non-cellular environment in patients with periodontitis was determined by expos-

ing the incubation media to corpuscles prone to phagocytosis and bacterial toxins. Bacterial toxins are capable to penetrate intact sulcular epithelium; therefore, they can activate NL by increasing their secretory activity [14,17].

Differences were found between the control group and groups of subjects with gingivitis and periodontitis in NL degranulation according to LZ activity induced by opsonized zymosane and anatoxine *S. aureus*. Anatoxine *S. aureus* and particularly opsonized zymosane significantly induce LZ activity that is dependent upon periodontal status. The highest activity was determined in patients with periodontitis, particularly when induced by opsonized zymosane.

Our data showed that once NL is affected by anatoxine *S. aureus*, LZ level increases in subjects with gingivitis and periodontitis in comparison with donors ($p \leq 0.001$).

Having determined the increase of LZ activity in NL exposed to various antigens, a discussion can be started on the activation of secretion of the entire system of lysosomic enzymes in these cells. Subsequently, proteolytic enzymes can be incorporated into the explanation of mechanism of development of periodontal diseases and give basics for oral hygiene importance in prevention of inflammatory periodontal diseases.

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Conclusions

The prevalence of periodontal diseases among 25-44 year-olds was 90.2%. Mild periodontal lesions and periodontal pockets were diagnosed in 73.2% and 16.6% of subjects, respectively.

More than half of the examined subjects (55.7%) evidenced satisfactory oral hygiene (OHI-S 1.1-2.1) whereas only one third demonstrated good oral hygiene (OHI-S 0-1.0). More severe periodontal lesions were diagnosed in patients with poor oral hygiene.

Secretory activity of lysozyme of neutrophilic leukocytes in incubation media affected with anatoxine *S. aureus* showed significantly higher increase in subjects with gingivitis and periodontitis than in the healthy.

Under the action of various antigens the secretion of leukocytic enzymes to non-cellular environment by neutrophilic leukocytes is increased in patients with gingivitis and periodontitis. Higher secretion levels were found in periodontitis than in the gingivitis group.

Increase in secretion of in incubation media of neutrophilic leukocytes affected by various antigens in subjects with gingivitis and periodontitis provides scientific basics for oral hygiene importance in prevention of inflammatory periodontal disease.

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