INTRODUCTION

Etiopathogenesis of chronic periodontitis continues to be unclear. A significant involvement of oral cavity anaerobic bacteria in aetiology of periodontitis is recognised, in particular involvement of *Porphyromonas gingivalis*, *Tannerella forsythiensis* and *Treponema denticola* (1). However, the periodontopathogens may also represent a physiological component of oral microflora in healthy individuals (2). In turn, the data are presented indicating that proinflammatory cytokine response of Th17 cells may play a significant role in pathogenesis of periodontitis, expressed by high levels of IL-17 and TNF-α in gingival tissue from periodontitis patients (3-5). Nevertheless, till today it has not been established whether augmented secretion of IL-17 and TNF-α is linked to a specific clinical form of chronic periodontitis. In addition, recently it has been demonstrated that *Lactobacillus* spp. capable of H₂O₂ production, may be of importance in prevention against progression of CP, most probably reducing secretory activity of Th17 cells and restricting growth of periodontopathogens.

MATERIALS AND METHODS

The studies were conducted on 28 patients (28-42 years of age, mean 35.7±3.8 years), among whom two groups were distinguished. Group 1 included 14 patients (28-42 years of age, mean 35.2±3.6 years; 7 women and 7 men; DMFT - decayed, missing and filled teeth 13.62±2.66) with moderate chronic periodontitis, in whom the average duration of the disease amounted to 20.1±2.1 months (range: 18-25 months). Group 2 included 14 patients (32-42 years of age, mean 36.1±3.7 years; 8 women and 6 men; DMFT 13.53±3.18) with severe chronic periodontitis, in whom the average duration of the disease was 28.1±4.1 months (range: 22-35 months). Group 3 (control) consisted of 15 patients (27-39 years of age, mean 33.9±4.1 years; 8 women and 6 men; DMFT 13.53±3.18) with severe chronic periodontitis, in whom the average duration of the disease was 28.1±4.1 months (range: 22-35 months). Group 3 (control) consisted of 15 patients (27-39 years of age, mean 33.9±4.1 years; 8 women and 6 men; DMFT 13.53±3.18) with severe chronic periodontitis, in whom the average duration of the disease was 28.1±4.1 months (range: 22-35 months). Group 3 (control) consisted of 15 patients (27-39 years of age, mean 33.9±4.1 years; 8 women and 6 men; DMFT 13.53±3.18) with severe chronic periodontitis, in whom the average duration of the disease was 28.1±4.1 months (range: 22-35 months). Group 3 (control) consisted of 15 patients (27-39 years of age, mean 33.9±4.1 years; 8 women and 6 men; DMFT 13.53±3.18) with severe chronic periodontitis, in whom the average duration of the disease was 28.1±4.1 months (range: 22-35 months).

The study aimed at evaluation of IL-17 and TNF-α levels and at analysis of oral lactobacilli in patients with chronic periodontitis (CP) in the context of their protective effect on a course of the disease. The study was conducted on 14 patients with moderate CP (group 1) and 14 patients with severe CP (group 2). Control group (group 3) included 15 individuals with gingivitis. Levels of IL-17 and TNF-α were estimated using an ELISA. Strains of *Lactobacillus* were isolated in Rogosa agar, H₂O₂-production was determined in TMB-Plus agar. In group 1, the mean content of IL-17 was 19.66±6.1 pg/ml, and that of TNF-α was 4.95±0.91 pg/ml, in group 2 IL-17 content was 34.7±6.65 pg/ml, and that of TNF-α was 6.94±0.78 pg/ml, in group 3 content of IL-17 was 0.65±0.58 pg/ml, content of TNF-α was 0.17±0.14 pg/ml. Analysis of lactobacilli manifestation in the control group and in the group with moderate CP in most of the persons demonstrated presence of H₂O₂-producing *Lactobacillus*, while in the group with severe CP presence of *Lactobacillus* was demonstrated in only 5 patients. Conclusions: development of CP is linked to persistent excessive cytokine response of Th17 cells, the intensity of which may affect clinical course of the disease; in parallel, H₂O₂-producing oral lactobacilli may prevent against progression of CP, most probably reducing secretory activity of Th17 cells and restricting growth of periodontopathogens.

Key words: periodontitis, gingivitis, *Lactobacillus*, hydrogen peroxide, tumour necrosis factor-α, interleukin-17
parallel to long dental axis, shifting peak of the probe in permanent contact with the tooth, down to the periodontal pocket bottom, recording depth of the periodontal pocket at every surface of the examined tooth. The measurements provided grounds for calculation of the mean periodontal pocket depth in individual patients.

Clinical attachment level (CAL) was measured at 6 surfaces of every tooth using the WHO 621 Hu-Friedy periodontal probe (scale of up to 11.5 mm). The CAL value was calculated on the basis of the measurement establishing the distance between enamel/cementum junction and depth of periodontal pocket. In cases when gingival margin overlapped the enamel/cementum junction two measurements were conducted: the depth of periodontal pocket and the distance between gingival margin and the line of enamel/cementum junction. Deduction of the value representing distance between gingival margin and the line of enamel/cementum junction from the value expressing the depth of periodontal pocket yielded the value orienting us in the site of connective tissue attachment. Data obtained in this way provided basis for calculation of mean CAL value in individual patients (8, 9).

Moderate chronic periodontitis was diagnosed when GI=0; CAL: 3-4 mm and two teeth were present with gingival pockets of >4mm in depth. In turn, patients with severe chronic periodontitis manifested the following variables: GI=0, CAL>5mm and 4 teeth with gingival pockets of >4mm in depth. On the other hand, moderate gingivitis was diagnosed when GI=1.1-2.0; CAL<1 (10, 11).

The investigated material involved gingival crevicular fluid, sampled using a 1 ml syringe with a thin, blunt, endodontic needle from three deepest periodontal pockets. The studies included patients in whom sampling of GCF was not followed by antibacterial rinses. The obtained gingival fluid was tested for presence of IL-17, TNF-α and for manifestation of Lactobacillus genus, producing or not producing H2O2. Estimations of gingival fluid (at the 1:10 dilution in PBS) IL-17 and TNF-α levels were conducted by ELISA, using Quantikine Human IL-17 and Quantikine HS Human TNF-α immunoassay kits (R&D Systems). Assays were carried out according to the manufacturer’s recommendations using human recombinant standards. The minimum detectable concentrations (sensitivity) in TNF-α assays ranged from 0.038 to 0.191 pg/ml. The minimum detectable concentration of IL-17 was <15 pg/ml. The optical density was measured at 450 or 490 nm, according to respective recommendation of manufacturer. At every experiment a standard curve was drawn basing on the obtained values of optical density (OD) for individual concentrations of the standards (0-32 pg/ml for TNF-α and 0-500 pg/ml for IL-17).

The final result of the studied cytokine concentration involved a product of the readout on the standard curve (0-32 pg/ml for TNF-α and 0-500 pg/ml for IL-17) and the applied dilution (x10).

In turn, Lactobacillus spp was cultured on Rogosa agar and the cultured isolates obtained in anaerobic conditions were identified using API 50 CHL (bioMerieux). The capacity of hydrogen peroxide production among Lactobacillus strains was defined in culture of the obtained isolates in presence of 5% CO2 at the temperature of 37°C for 48 hours in a differentiating medium, TMB-Plus agar, prepared according to Rabe and Hillier (12). Development of an altered colour of the growing colonies (appearance of a blue colour) indicated production of hydrogen peroxide.

Results obtained in the studies were analyzed using the computer software STATISTICA 8 for Windows. The statistical analysis included non-parametric tests, permitting calculation of a broad range of various position measurements (mean) and dispersion (standard deviation). In the comparative analysis related to GI, PPD, CAL and PL.I and cytokine levels in studied groups the non-parametric Kruskal-Wallis test and Dunn’s test were applied. Analysis of the correlation between IL-17 levels and manifestation of oral lactobacilli in CP was conducted using a non-parametric method based on Spearman’s rank correlation coefficients. Significance in frequency of Lactobacillus spp. presence is the studied groups was analysed using the exact Fisher’s test. In every test, hypotheses were verified at the significance level of p=0.05.

**RESULTS**

In the study, the stage of periodontitis advancement was defined using WHO criteria. The obtained values of GI, PPD, CAL indices permitted to distinguish three research groups: group 1 including 14 patients with moderate chronic periodontitis and group 2 including 14 patients with severe chronic periodontitis. Group 3 (control group) included 15 persons with gingivitis.

Statistical characteristics of the obtained mean values of GI, PPD, CAL, PL.I in the three examined patient groups is shown in Table 1.

Table 1. Results of examined indices in patients with moderate chronic periodontitis (group 1), severe chronic periodontitis (group 2) and in control group with gingivitis (group 3).

<table>
<thead>
<tr>
<th>Periodontal status</th>
<th>Studied groups</th>
<th>p-value for difference</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n=14</td>
<td>n=14</td>
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<tr>
<td>GI</td>
<td>1.73±0.29</td>
<td>2.35±0.27</td>
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<tr>
<td></td>
<td>group 1 vs. group 3 p&gt;0.05</td>
<td>group 2 vs. group 3 p&lt;0.001</td>
</tr>
<tr>
<td>PPD</td>
<td>4.36±0.22</td>
<td>7.03±1.39</td>
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<tr>
<td></td>
<td>group 1 vs. group 3 p&lt;0.001</td>
<td>group 2 vs. group 3 p&lt;0.001</td>
</tr>
<tr>
<td>CAL</td>
<td>3.47±0.41</td>
<td>6.94±1.23</td>
</tr>
<tr>
<td></td>
<td>group 1 vs. group 3 p&lt;0.001</td>
<td>group 2 vs. group 3 p&lt;0.001</td>
</tr>
<tr>
<td>PL.I</td>
<td>2.22±0.17</td>
<td>2.16±0.22</td>
</tr>
<tr>
<td></td>
<td>group 1 vs. group 3 p&lt;0.001</td>
<td>group 2 vs. group 3 p&lt;0.01</td>
</tr>
</tbody>
</table>
In both groups of patients periodontal pockets were found to contain high concentrations of examined cytokines. In the group of patients with moderate chronic periodontitis (group 1) mean values of IL-17 and TNF-α amounted to, respectively, 19.66±6.1 pg/ml and 4.95±0.91 pg/ml. In turn, in the group of patients with severe chronic periodontitis (group 2) mean values of IL-17 and TNF-α amounted to, respectively, 34.7±6.65 pg/ml and 6.94±0.78 pg/ml and were significantly higher than cytokine levels obtained in group 1 (p<0.0001 and p=0.0003, respectively). In the control group of individuals with gingivitis (group 3) mean values of IL-17 and TNF-α amounted to, respectively, 0.65±0.58 pg/ml and 0.17±0.14 pg/ml and were significantly lower than cytokine levels obtained in group 1 and in group 2 (for all the analyses p<0.0001). The mean variations of concentrations of cytokine IL-17 and TNF-α and statistical comparisons in the periodontitis group and in the control group are shown in Table 2. In analysis of lactobacilli manifestation, presence of H2O2-producing Lactobacillus spp. was demonstrated in 10 patients of group 1 (71.4%) while in group 2 two patients (14.3%) of patients with severe chronic periodontitis, a significant decrease was detected in frequency (Spearman r=-0.4211) was disclosed between IL-17 levels and Lactobacillus spp. presence. In patients of group 2, with severe chronic periodontitis group and in the control group are shown in Table 2. In analysis of lactobacilli manifestation, presence of H2O2-producing Lactobacillus spp. was demonstrated in 10 patients of group 1 (71.4%) while in group 2 two patients (14.3%) of patients with severe chronic periodontitis, a significant decrease was detected in frequency of Lactobacillus spp. presence, as compared to the control group (p=0.0078). Manifestation of Lactobacillus spp. and their ability to produce H2O2 are shown in Table 3. In the studies a significant (p=0.0256) negative correlation (Spearman r=-0.4211) was disclosed between IL-17 levels and frequency of oral lactobacilli manifestation in CP.

**DISCUSSION**

Population of Th17 cells is characterized by secretion of mainly IL-17, and also of other proinflammatory cytokines, including TNF-α, IL-22 and IL-26 (13). IL-17 induces recruitment of neutrophils together with inflammatory reaction, which in addition is synergically amplified by TNF-α, IL-22 and IL-26. Moreover, IL-17 triggers neutrophilia while IL-22 induces anti-microbial peptides, providing a nonspecific immune response against bacterial and fungal pathogens (14-17). Thus, the cytokine response of Th17 cells plays an important role in host defense, and may represent also a principal mediator in pathogenesis of several inflammatory diseases, as shown by results of recent years (3, 12, 17-19). In our studies, presented in this paper we have demonstrated a marked increase in levels of IL-17 and TNF-α in gingival crevicular fluid in patients with either moderate or severe chronic periodontitis, even if in the latter the estimated cytokines level was significantly higher. Such an elevation of IL-17, as well as of TNF-α levels in chronic periodontitis was earlier described also by other authors (3-5). Nevertheless, in this study for the first time overproduction of IL-17 and TNF-α have been shown to be typical for both the defined clinical forms of moderate and severe chronic periodontitis. In parallel, the obtained results have permitted to distinguish patients with a high cytokine response of Th17 cells (representing the „high responders”) and those with moderate response of Th17 cells (representing „moderate responders”). In „moderate responders” the increase in maximum cytokine levels has not exceeded the 20-fold maximum values in the control group. In patients representing the „high responders” the increase in maximum cytokine levels corresponded to the same or higher level than that representing 20-fold maximum level in the control group.

![Table 2. Cytokine levels (IL-17 and TNF-α) in gingival crevicular fluid (GCF) of patients with moderate chronic periodontitis or severe chronic periodontitis were highly significantly different (p<0.0001 or p=0.0003) between each other and as compared to the control group with gingivitis (p<0.0001).](image-url)

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>Mean values in pg/ml ± SD and range in parentheses [median values]</th>
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</thead>
<tbody>
<tr>
<td>Patients with moderate chronic periodontitis (group 1, n=14)</td>
<td>IL-17: 19.66±6.1 (11.2–27.4) [20.00]</td>
</tr>
<tr>
<td>Patients with severe chronic periodontitis (group 2, n=14)</td>
<td>IL-17: 34.7±6.65 (25.15–43.58) [33.88]</td>
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<tr>
<td>Control group with gingivitis (group 3, n=15)</td>
<td>IL-17: 0.65±0.58 (0–1.6) [0.47]</td>
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</table>

![Table 3. Capacity to form H2O2 in the obtained Lactobacillus spp. isolates and statistical significance (p) of differences in frequency of Lactobacillus spp. presence.](image-url)

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>Lactobacillus spp.</th>
<th>Production of H2O2 (median values)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n=14)</td>
<td>n=10 (71.43%)</td>
<td>H2O2 (+)</td>
<td>0</td>
</tr>
<tr>
<td>Group 2 (n=14)</td>
<td>n=5 (35.7%)</td>
<td>H2O2 (+)</td>
<td>3</td>
</tr>
<tr>
<td>Group 3 (n=15)</td>
<td>n=13 (86.67%)</td>
<td>H2O2 (+)</td>
<td>0</td>
</tr>
</tbody>
</table>
vitro the periodontopathogens were demonstrated to induce cytokine response of Th17 (21). Therefore, it is possible that Th17 cells play an important physiological indirect role, controlling periodontal colonization by microbes and periodontopathogens in particular. Probably, the effect of the function includes low gingival levels of IL-17, detected also in this study in patients with gingivitis. A marked increase in cytokine response of Th17 cells seems to result from disturbances in its intracellular control or strong expression of target genes due to their transcriptional activation by periodontopathogens. Until now, the intracellular signalling pathway that regulates IL-17 production remains unknown. Nevertheless, the nuclear factor kappa B (NF-κB) was shown to be involved in overproduction of IL-17 (22). Therefore, the final result of activated secretory function of Th17 cells may be dependent on expression of IL-17 gene. Polymorphism of IL-17 gene might determine overproduction of IL-17, as well as the NF-κB-dependent secretion of several proinflammatory, which cooperate with IL-17.

In present studies, manifestation of hydrogen peroxide-producing Lactobacillus spp has been demonstrated in most patients of the control group as well as in patients with moderate chronic periodontitis in gingival crevicular fluid. On the other hand, in gingival fluid of patients with severe chronic periodontitis Lactobacillus spp. has been detected in only 5 persons and in only 2 of them this pertained H2O2-producing strains. However, high gingival concentrations of IL-17 and TNF-α have been disclosed in all patients with severe chronic periodontitis. Moreover, the study has demonstrated negative correlation between levels of IL-17 in GCF on one hand and manifestation of oral lactobacilli on the other. Therefore, it can be concluded that a very pronounced cytokine response of Th17 cells, linked to severe chronic periodontitis, exerting proinflammatory and antibacterial effect reduces in a secondary way manifestation and metabolic activity of oral lactobacilli. In turn, the moderate IL-17 cytokine level may be insufficient for induction of a nonspecific immune response, leading to elimination of H2O2-producing oral lactobacilli, the presence of which has seemed to reduce secretory activity of Th17 cells as well to restrict growth of periodontopathogens and, therefore, to prevent against progression of chronic periodontitis. The suggestion seems to be verified by experimental studies, which show that Lactobacillus strains may exert immunomodulatory effect, acting in a suppressive way on expression of genes coding for proinflammatory cytokines or in a regulatory manner on human dendritic cells (23-25). Moreover, activity of anti-inflammatory probiotic strains of Lactobacillus was noted on urogenital tract mucosa and activity of E. coli Nissle on gastric mucosa were detected (26,27). Recently, strongly antagonistic interaction of oral lactobacilli to Gram-negative periodontal pathogens, Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans (28) was documented.

Results presented in this study indicate that development of chronic periodontitis are linked to persisting cytokine response of Th17 cells, the intensity of which may shape clinical course of the disease. In parallel, H2O2-producing oral lactobacilli may prevent against progress of chronic periodontitis, most probably restricting secretory activity of Th17 cells and growth of periodontopathogens. The data provide rationale for further studies aimed at implementing a new strategy in therapy of chronic periodontitis, considering the role of lactic acid bacteria in severe forms of the disease.

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Conflict of interest: None declared.

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