ABSTRACT

Background: The effectiveness and limitations of causal therapy of chronic periodontitis have been thoroughly documented in the literature, and the intensity of the individual destructive host response is taken into consideration, as well. These facts are the basis in research of additional therapeutic approaches to modify tissue response by regulating the amount and activity of pro-inflammatory mediators and those of destruction. Various drugs are being studied to modulate the host’s response in chronic periodontitis. The use of non-steroidal anti-inflammatory drugs that inhibit the expression and activity of important mediators has led to positive results.

Aim: To assess changes in PGE2 gene expression levels as a result of non-surgical therapy and additional application of a non-steroidal anti-inflammatory agent in chronic periodontitis.

Material and methods: Thirty patients with moderate to severe periodontitis without systemic diseases were involved in the study. Clinical and laboratory methods were used to evaluate non-surgical periodontal treatment.

Results: The data obtained from the clinical measurements - PD, CAL, BL and BOP shows that no statistically reliable relationship between gene expression of PGE2 and most clinical parameters was found in the control and test groups. Reduction in the values following the therapy was recorded in a lot of patients, but only in the test group (taking NSAIDs) with a pocket depth (PD) ≥5mm a statistically significant, inverse correlation between the PGE2 gene expression levels and changes in the pocket depth was established.

Conclusion: Additional therapy with NSAIDs in chronic periodontitis has higher efficacy, manifested by correlation of deep pockets reduction and changes in PGE2 expression.

Keywords: chronic periodontitis, host response, non-surgical therapy (SRP), NSAIDs, pro-inflammatory mediators.

INTRODUCTION:

In current periodontal literature chronic periodontitis is well defined as a periodontal attachment and bone destruction due to the expansion of plaque-associated inflammation in response to a persistent dental biofilm. Studies are being carried out to investigate the aetiology and pathogenesis of this widespread disease. Most of the studies are focused on microbiological and immunological aspects in relation to the variations in chronic periodontitis clinical manifestation. Recent knowledge of the disease nature highlights the fundamental role of controlling the bacterial biofilm by mechanical therapy, which in most cases of chronic periodontitis is effective in stabilizing the periodontium and providing long-term attachment maintenance [1, 2]. Studies have shown that in cases of severe chronic periodontitis, the advanced bone loss is associated with a destructive host response to periodontal pathogens. Therefore, controlling the severe cases of chronic periodontitis, it is of critical importance to include the bacterial finding in the diagnosis as well as detecting the patient’s susceptibility due to some genetic and / or other factors [3]. Establishing diagnostic markers, revealing the characteristics of the host response would lead to a better assessment of the disease and patient, adequate treatment planning with higher effectiveness and correct predictability of the results of its long-term control. Confirming this, Ebersole et al. and other authors in recent years have shown that the expression of certain immune response factors are associated with disease progression and may be criteria for the host susceptibility and severity of chronic periodontitis [4, 5, 6, 7]. The degree of expression of such factors, accepted as markers, may have an important role in the disease prognosis evaluation and therapeutic approaches [8, 9, 10, 11, 12].

For many years tissue response to bacterial load has been evaluated by the production of various cytokines due to their important role in almost all inflammatory processes in human body, including those developing in periodontal structures [13]. There is a significant number of publications proving that the mediators resulting from the metabolism of arachidonic acid by the enzyme cyclooxygenase (COX-1 and 2) - prostaglandins (PGE2), prostacyclin (PGL2), tumor- necrosis factor-α (TNF-α), interleukin (IL-6), etc. have a major role in the processes of inflammation and bone destruction [14, 15, 16, 17, 18, 19].
High levels of COX-2 expression in periodontitis result in elevated levels of prostaglandin E₂ (PGE₂) and (PGE₂₀) - basic mediators of periodontal tissue destruction [8, 15, 18, 19]. Prostaglandin E₂ is known to act as a pro-inflammatory mediator, primarily associated with bone loss in periodontitis, as well as with the stimulation of other inflammatory agents’ production, especially IL-1β [18, 20, 21].

There is evidence in vivo and in vitro that PGE₂ contributes to the formation of a type of osteoclasts associated with bone loss in periodontitis by balancing the receptor-activator of the nuclear factor-kB ligand (RANKL) and osteoprotegenin (OPG) in osteoblasts. It is believed that PGE₂ regulates osteoclastogenesis mainly by the action of one of the fixed 4 subtypes PGE₂ receptors - EP4 (PGE₂-specific-G protein receptors) [22].

Current treatment of periodontal diseases is based mainly on the infection control with the mechanical regular removal of dental biofilm. In some cases, the response to periodontal infection is influenced by systemic factors, and resolution of inflammation may not be successful applying conventional therapy approaches alone (SRP) [1, 3, 6, 23, 24].

As a result of better understanding of the significance of host factors in the onset and progression of periodontal diseases, the focus of clinicians is drawn to the application of additional approaches to modulate host response to a more successful therapy [3, 7, 25, 26, 27]. Over the years some clinical trials have demonstrated the effectiveness of NSAIDs in the treatment of periodontal disease. In patients with a further application of NSAIDs, reductions in gingival inflammation and periodontal pockets depth, control of attachment loss, effective inhibition of bone loss and reduction of recurrences have been reported [8, 24, 26, 27, 28].

It is known that NSAIDs efficacy in controlling the progression of periodontal disease is due to the ability of these agents to affect prostaglandin synthesis, mainly by suppressing the COX-1 and COX-2 arachidonic acid isoenzymes. Many modern selective COX-2 inhibitors are also known to provide additional inhibition of collagenase-2 (MMP-8), which can reduce the risk of periodontitis progression by inhibiting connective tissue destruction [6, 28]. The obtained data regarding prostaglandins (PG) have indicated a clear correlation between disease severity and their increased levels in gingival fluid and gingival tissue [9, 15, 21]. Long-term studies have reported less disease progression by inflammatory factors inhibition with NSAIDs as adjunctive therapy in periodontal diseases [32]. Additional studies are needed to assess the effectiveness of periodontal therapy by changes in the expression of pro-inflammatory factors.

AIM OF THE STUDY:

To assess the changes in gene expression levels of prostaglandin E₂ (PGE₂) as a result of non-surgical therapy and an additional application of a non-steroidal anti-inflammatory agent in chronic periodontitis.

MATERIAL:

1. PATIENT SELECTION: Two groups are formed basing on clinical and radiographic diagnostic criteria for patients with chronic periodontitis:
   - A test group of patients (20) who underwent mechanical periodontal therapy with administration of a non-steroidal anti-inflammatory therapy (Aulin® 100 mg twice daily for 14 days’ period).
   - Control group of patients (10) who underwent only mechanical periodontal therapy.

Criteria for patients’ inclusion in the study:
- With moderate to severe periodontitis - attachment loss (CAL) - 4-6mm, pocket depth (PD) - 4-6mm, loss of alveolar bone (BL) - 4-6mm measured by conventional orthopantomography without conducted periodontal therapy for the last 6 months, without systemic disease and systemic medication for the last 6 months, with a minimum of 20 available teeth.

Criteria for excluding patients from the study:
- Systemic diseases - diabetes, hepatitis, immunodeficiency, viral diseases, immunosuppressive therapy or anti-inflammatory agents, pregnancy and breastfeeding.

2. METHODS OF STUDY:
   - Clinical - periodontal status of patients was recorded using the following clinical parameters:
     - bleeding on probing (BOP);
     - pocket depth in mm (PD);
     - attachment loss in mm (CAL).
     The same clinical parameters were used in re-evaluation after the completion of initial treatment (6th week after the end of the initial therapy).
   - Laboratory methods - measured levels of gene expression for PGE₂ in gingival tissues before and after non-surgical periodontal treatment by TaqMan RT-Real Time-PCR (reverse transcript polymerase chain reaction in real time) [29, 30, 31]. A fluorescent specific TaqMan probe was used. The sample used for analysis is gingival tissue adjacent to pockets with depth ≥5 mm and volume of not less than 3 mm³.
     - Statistical methods - the clinical parameters are evaluated with the IBM SPSS Statistics 19.0 and Sigma Stat software. A p<0.05 was considered statistically significant.

RESULTS AND DISCUSSION:

Demographic characteristics and comparison of patients of both groups

When comparing the selected parameters, the patient groups are required to be statistically unified according to known confounding factors gender and age. Preliminary analysis in the follow-up patients showed that the two groups did not differ significantly from these factors – Table 1. This means that the most common factors influencing the outcome of the therapeutic approach are correctly considered.
Table 1: Demographic characteristics of the studied patients

<table>
<thead>
<tr>
<th>CRITERION</th>
<th>CONTROL GROUP (N=10)</th>
<th>TEST GROUP (N=20)</th>
<th>TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVERAGE AGE ± SD (YEARS)</td>
<td>38,70±6,52</td>
<td>36,00±6,48</td>
<td>0,292 T-TEST OF STUDENT</td>
</tr>
<tr>
<td>GENDER - NUMBER (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MALES</td>
<td>4 (40,0)</td>
<td>14 (70,0)</td>
<td>0,139 FISHER’S EXACT TEST</td>
</tr>
<tr>
<td>FEMALES</td>
<td>6 (60,0)</td>
<td>6 (30,0)</td>
<td></td>
</tr>
</tbody>
</table>

The age of the patients enrolled in the study ranged from 26 to 54 years. The average age of patients in both groups showed no statistical differences, which means that patients were standardized by this factor. No statistically significant differences in the distribution of males and females in the test and control groups were recorded, as well. In the test group patients, 25-34 year-old and 35-44 years old are 45%, followed by the 45-54 year-old (10%), while in the control group- 25-34 year-old patients occupied the first position (40%), followed by the 35-44 year-old and 45-54 year-old respectively (30%).

Another necessary criterion for correct comparison of the therapeutic results is the similar severity of periodontal disease in both groups of selected patients. Clinical attachment loss (CAL) and bone loss are of significant importance to determine the severity of periodontitis, followed by pocket depth (PD) and bleeding on probing (BOP). The data in Table 2 shows that measured loss of attachment (CAL 3-4 mm) in the control group was in 38.0% of the sites tested and in the test group the percentage of sites with the same loss of attachment was 42.8% - this means there are no statistically significant differences. In the control group 62.0% of the studied sites had attachment loss > 5 mm, and in the test group, they were 57.0%. The presence of bleeding on probing in both groups was 100%, and in terms of measured pocket depth PD ≥5mm, the result was: 6.5% in the control group and 6.7% in the test group. Such data suggests standardization of both groups’ patients according to the severity of periodontitis.

The measured bone loss values in both groups are presented in Table 2. The percentage of sites of 1-2mm bone loss was 14.3% for the control group and 16.3% in the test group; the percentage of bone loss of 3-5 mm was 72.6% for the control group and 70.9% for the test group; the percentage of bone loss ≥6 mm was 13.2% for the control group and 12.6% for the test group. It was not established the statistically significant difference in bone loss, that suggest standardization in both groups (p>0.05) according to this criterion.

Table 2: Comparative analysis of the studied patients according to with clinical measurement

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>CONTROL GROUP (N=10)</th>
<th>TEST GROUP (N=20)</th>
<th>t/p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BEFORE THERAPY</td>
<td>AFTER THERAPY</td>
<td>t/p</td>
</tr>
<tr>
<td>BOP %</td>
<td>100,0%</td>
<td>31,8%±4,90%</td>
<td>t=17,51 p&lt;0,001</td>
</tr>
<tr>
<td>CAL 1-2mm</td>
<td>0,0%</td>
<td>0,0%</td>
<td></td>
</tr>
<tr>
<td>CAL 3-4 mm</td>
<td>38,0%±7,10%</td>
<td>61,9%±11,00%</td>
<td>t=5,73 p&lt;0,001</td>
</tr>
<tr>
<td>CAL ≥ 5 mm</td>
<td>62,0%±6,70%</td>
<td>38,1%±11,00%</td>
<td>t=5,99 p&lt;0,001</td>
</tr>
<tr>
<td>PD &gt; 5mm</td>
<td>6,5%±5,50%</td>
<td>1,2%±2,90%</td>
<td>t=2,69 p&lt;0,05</td>
</tr>
<tr>
<td>Bone Level 1-2mm</td>
<td>14,3%±4,60%</td>
<td>16,3%±7,80%</td>
<td>t=0,60 p&gt;0,05</td>
</tr>
<tr>
<td>Bone Level 3-5mm</td>
<td>72,6%±4,10%</td>
<td>70,90%±5,50%</td>
<td>t=0,64 p&gt;0,05</td>
</tr>
<tr>
<td>Bone level ≥ 6mm</td>
<td>13,2%±4,00%</td>
<td>12,60%±3,5%</td>
<td>t=0,36 p&gt;0,05</td>
</tr>
</tbody>
</table>

The analysis (Table 2), shows statistically significant changes in most periodontal parameters as a result of the treatment – reduction in bleeding on probing was observed in both groups - from 100% to 29.1% in the test group (p<0.001) and from 100% to 31.8% (p<0.001) in the control group. Pockets ≥5 mm in the control group of patients from initial 6.5% reached 1.2% (p<0.05), in the test group PD ≥5mm changed from 6.9% to 0.8% (p<0.02). Attachment loss data shows that the sites with a loss of attachment > 5 mm were reduced from 57.0% the sites with
attachment loss > 5 mm decreased to 25.9% (p<0.001) in the test group. For the control group, attachment loss > 5mm changed from 62.0% to 38.1% (p<0.001). The proportion of sites with a loss of attachment 3-4 mm increased from 42.8% to 73.2% (p<0.001) in the test group; in the control group from 38.0% before therapy the percentage increase to 61.9% (p<0.001). No statistically significant differences in 1-2 mm attachment loss in both groups.

While examining PGE2 gene expression in the gingival tissue of studied patients, the obtained negative ΔΔCt values in both the test group and the control one reflect a lower degree of inhibition of gene expression of the examined cytokine, which is important to register for the correct interpretation of the results.

Diagram 1 show the changes in gene expression levels of PGE2 in patients in the test group, treated additionally with NSAID Aulin®, and the changes in gene expression of PGE2 in control group with conventional therapy only. In 8 of the patients from the experimental group (40%) the values of the change in gene expression were positive varying from 1.79 to 6.63, which is considered as a high level of inhibition of gene expression; in 12 of the patients (60%) the changes in gene expression were negative within the range of -0.78 to -2.2, which is considered to be a lower degree of inhibition of gene expression. In the control group, a positive change occurred only in one of the patients - gene expression 0.18. In the rest of the patients of the group, the change in PGE2 gene expression levels was negative varying from -0.66 to -6.45. Such values were assessed as a lower degree of inhibition of PGE2 gene expression.

Diagram 1: Changes in PGE2 levels in group (with Aulin®) and group (without Aulin®)

Table 3 presents a comparative analysis (ΔΔCt) in PGE2 expression of two groups. In patients who additionally received NSAID Aulin®, the inhibition of PGE2 expression was more marked. However, in patients who did not take Aulin®, the expression of PGE2 was suppressed to a much lower extent.

Table 3: Change analysis in PGE2 gene expression

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>CONTROL GROUP WITHOUT AULIN®</th>
<th>TEST GROUP WITH AULIN®</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>XSD</td>
<td>n</td>
<td>XSD</td>
</tr>
<tr>
<td>ΔΔCt (PGE2)</td>
<td>10 -3.014 2,242</td>
<td>20 0.563 2,729</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Statistically significant differences were found between Aulin® treated group and the group treated without Aulin® (p<0.05). The change in PGE2 gene expression is greater in patients treated with conventional therapy supplemented with Aulin®.
ANALYSIS OF THE CORRELATION BETWEEN CHANGES IN PGE2 GENE EXPRESSION AND STUDIED PARAMETERS

Table 4 shows that:

- In the control group patients without Aulin®, there is no correlation between changes in PGE2 gene expression levels and periodontal measurements;
- In the group of patients receiving Aulin®, a statistically significant reversed correlation was found between changes in PGE2 gene expression and pocket depth changes - only for the pockets > 5 mm.

**Table 4:** Correlation coefficients between changes in PGE2 and studied clinical parameters

<table>
<thead>
<tr>
<th>CLINICAL PARAMETERS</th>
<th>PGE2 CHANGES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL GROUP (n=10)</td>
</tr>
<tr>
<td>Bone Level 1-2 mm</td>
<td>0.328</td>
</tr>
<tr>
<td>Bone Level 3-5 mm</td>
<td>0.103</td>
</tr>
<tr>
<td>Bone Level ≥ 6 mm</td>
<td>-0.483</td>
</tr>
<tr>
<td>CAL 1-2 mm</td>
<td>**</td>
</tr>
<tr>
<td>CAL 3-4 mm - reduction</td>
<td>0.577</td>
</tr>
<tr>
<td>CAL ≥ 5mm - reduction</td>
<td>-0.365</td>
</tr>
<tr>
<td>PD &gt; 5mm - reduction</td>
<td>0.128</td>
</tr>
<tr>
<td>BOP - reduction</td>
<td>0.201</td>
</tr>
</tbody>
</table>

* - p<0.05; ** - no changes in the studied indicator

No statistically significant correlation was found between periodontal parameters changes (PD, CAL, BL and BOP) and PGE2 gene expression changes in both groups (Table 4): bone loss of 1-2mm and 3-5 mm; attachment loss of 3-4 mm; pocket depth ≥5 mm and bleeding on probing (BOP) in the test group. In the experimental group, such lack of correlation was found in: bone loss -1-2 mm, attachment loss -3-4 mm and bleeding on probing.

For the pocket depth ≥5mm, a statistically significant (p <0.05), inverse correlation between in PGE2 expression changes and pocket depth changes in the test group was found.

The obtained results in this study suggest the effectiveness of additional administration of NSAIDs on prostaglandin E2 (PGE2) concentration in periodontal tissues that may lead to periodontal therapy with higher efficacy.

Comparative study the efficacy of mechanical periodontal therapy (SRP) alone and SRP plus additionally NSAIDs (Aulin®) shows that both types of therapy lead to statistically reliable healing in correlation with changes in gene expression of PGE2. The obtained in this study data support publications in literature (Heasman et al., 1998, Offenbacher et al., 1986, 1992, 1993; Preshaw et al. 2002) in which the authors report that the PGE2 levels in crevicular fluid in patients with periodontitis are significantly higher than in healthy ones and such levels of PGE2 concentration may be a predictor of periodontitis progression associated with loss of attachment and bone loss [18, 19, 32, 5, 12] Animal experiments and human studies with COX-1 and 2 blockades by NSAIDs have confirmed not only the relationship between periodontitis activity and tissue levels of PGE2 but have shown that elimination or reduction of this mediator of the host response leads to control of the disease progression.

The obtained statistically significant differences in clinical periodontal pockets PD over 5 mm associated with PGE2 gene expression reduction in the test group patients in this study suggest a higher effectiveness of non-surgical periodontal therapy accomplished with NSAID (Aulin®) compared to conventional mechanical therapy. Gene expression of PGE2 can is regarded as a criterion in periodontal therapy effectiveness assessment.

**CONCLUSION:**

No significant correlation between clinical improvements and PGE2 gene expression changes was established as a result of non-surgical periodontal treatment. Only for pocket depth ≥5 mm in the periodontal therapy with additionally NSAID (Aulin®) a statistically significant (p<0.05) inverse correlation was found between PGE2 changes and deep periodontal pockets prevalence changes.
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