EFFECTIVENESS OF NON-SURGICAL PERIODONTAL THERAPY BY GINGIVAL EXPRESSION OF IL-1β AND IL-6

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SUMMARY:

Chronic periodontitis is a multifactorial disease that is characterized by attachment loss and loss of alveolar bone. Periodontal pathogens from the subgingival microbiota are suggested as a major etiological factor for the periodontitis. The host inflammatory response against bacteria and their virulence factors underlies the current understanding of the pathogenesis of chronic periodontitis. The destructive immune response was shown to be associated with an extremely increased production of inflammatory cytokines in periodontal tissues. The expression of several cytokines likeIL-1β and IL-6 is responsible for alveolar bone resorption, and loss of connective tissue attachment in chronic periodontitis.

Authors reported a reduction in the levels of inflammatory cytokines after successful periodontal therapy. Measuring the levels of inflammatory factors may be diagnostic approach to assess the effectiveness of the therapy of periodontitis.

Keywords: chronic periodontitis, cytokines, IL-1β, IL-6, periodontal therapy, periodontal diagnosis.

INTRODUCTION:

Inflammatory periodontal diseases are related with a response to complex dental biofilm that result in destruction of connective tissue attachment and alveolar bone [1, 2, 3, 4]. Different periodontal diseases showed some variations in the histological and biochemical characteristics, but the molecular mediators and destructive mechanisms of pathological processes are similar. In the gingival tissue and the gingival crevicularfluid of patients with chronic periodontitis were detected high levels of pro-inflammatory factors: RANKL - (Receptor activator of NF-KB ligand), pro-inflammatory cytokines such as: IL-1β and IL-6 and TNF-α [1, 5, 6].

Studies on the expression levels of IL-1β and IL-6 in relation to the periodontal treatment will lead to: completing the understanding of the pathogenesis of periodontal destruction, assessing the severity of periodontitis, assessing the effectiveness of therapy or refractory, prediction of a recurrence and determination of progression of periodontitis.

There is evidence that non-surgical therapy of chronic periodontitis is effective leading to reduction of bacteria load and pathogenic species levels in periodontal pockets, elimination of inflammation and significant reduction of periodontal pockets depth. This also results in a shift of the local destructive response including expression levels of some pro - inflammatory factors IL- 1β and IL-6. Their evaluation may serve to evaluate the effectiveness of non-surgical therapy [1, 2, 6, 7].

AIM:

➤ Assessment of the effectiveness of non-surgical periodontal therapy in chronic periodontitis IL-1β and IL-6 gingival gene expression.

MATERIALS AND METHODS:

In this study were used the following assessment methods:

1. Clinical methods: Selection of patients - 20 patients with diagnosis chronic moderate to severe periodontitis.

Including criteria:
• presence of a minimum of 20 teeth,
• presence of periodontal pockets with PPD ≤7 mm to ≥7 mm,
• patients without periodontal therapy in the past 6 months,
• patients without systemic antimicrobial treatment in last 6 months.

Survey excluded: pregnant women, patients with systemic diseases and systemic medication.

Clinical parameters:

Hygiene Index (HI); Bleeding on probing (BOP); Periodontal pocket depth (PPD); Clinical attachment loss (CAL); Gingival recession (R); Furcation degree - Hamp 1975 classification (F).

2. Molecular methods:

➤ Gene expressionof IL-6 and IL-1β in the gingival tissue was determined by comparative quantitative analysis - TAQ MAN REAL-TIME PCR - (REVERSE TRANSCRIPT PCR) method.

• Gingival biopsies were taken for assessment of gene expression of IL-6 and IL-1β (in relation to the pockets with depth ≥7 mm- before and after the non-surgical periodontal treatment).

3. Statistical methods:
• Descriptive analysis
• Test c2 and exact Fisher test
• nonparametric Shapiro-Wilk test

RESULTS:
In this study was assessed the effectiveness of mechanical treatment of chronic periodontitis. The average age of the participants in the study was 47.9 years with standard deviation 10.5 SD (12 women and 8 men); the severity of periodontitis was assessed including the prevalence of periodontal sites with different pocket depth (3-5 mm, 5-7mm and over 7 mm).

The results of the study are shown on histograms, presenting the periodontal measurements in the initial diagnosis and after periodontal treatment as well as biochemical measurements. The data from the histograms are displayed as rectangles. The horizontal axis (the axis X) presents the research categories of data (e.g. quantitative or biochemical parameter). The vertical axis (the axis Y) describes the distribution of the presented parameter (patient's contribution in the study). The height of the rectangle represents the frequency or density of the cases. The highest point of the curve shows the average frequency of the distribution of the monitored parameter.

Histograms in the Figure 1 and Figure 2 represent hygiene status and the associated with dental plaque gingival inflammation, expressed by bleeding on probing. It is seen that in the majority of patients with severe chronic periodontitis the percentage of free plaque surfaces is less than 10% (HI <10%). After treatment the percentage of the free surfaces of the plaque increased to more than 70% (Figure 1). Figure 2 shows the reduction (percentage of bleeding sites).

Fig. 1. The percentage (%) of hygiene index increases up to 70% after non-surgical therapy.
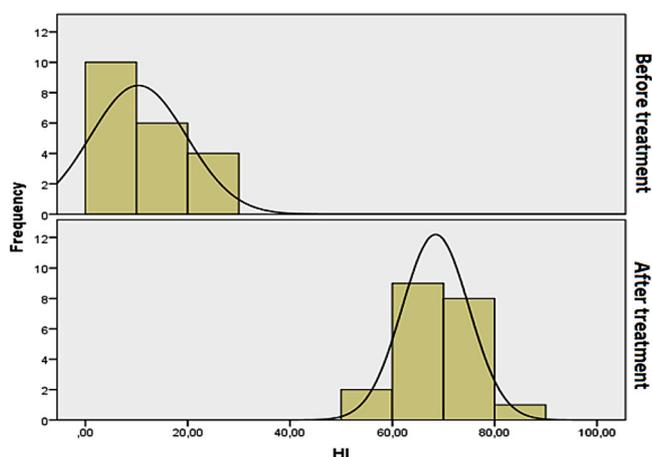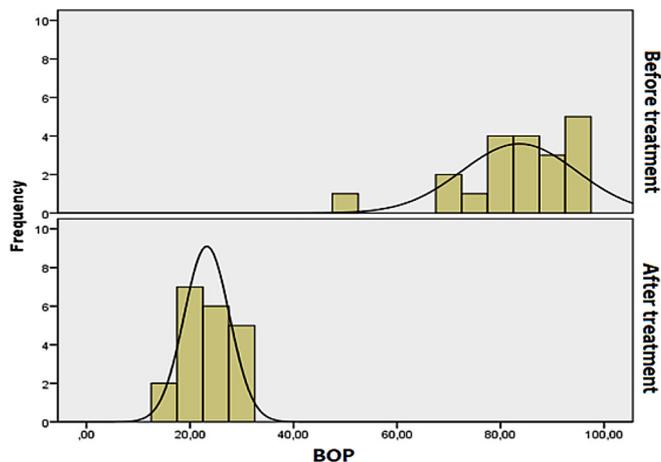

Fig. 2. Reduction of bleeding on probing from 85% to 25%

Histograms in Figures 3, 4 and 5 show the changes in the periodontal probing depth after non-surgical periodontal treatment in the target groups of periodontal sites (3-5 mm, 5-7mm and >7 mm).
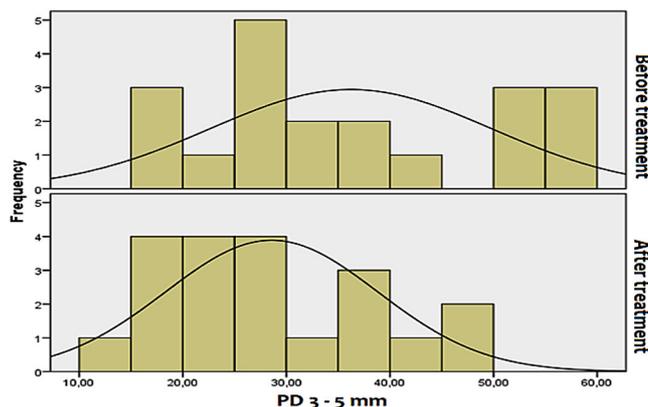
Fig. 3. Reduction of sites with PD <5mm from 40% to 27%

Fig. 4. Reduction of sites with PD up to 7mm from 15% to 10%
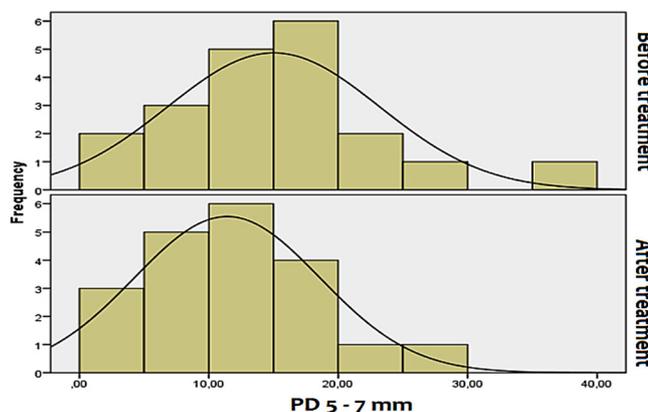
Fig. 5. Reduction of sites with PD>7mm from 2.50% to 1.50%

Fig. 6. Reduction of the gene expression of IL-1β
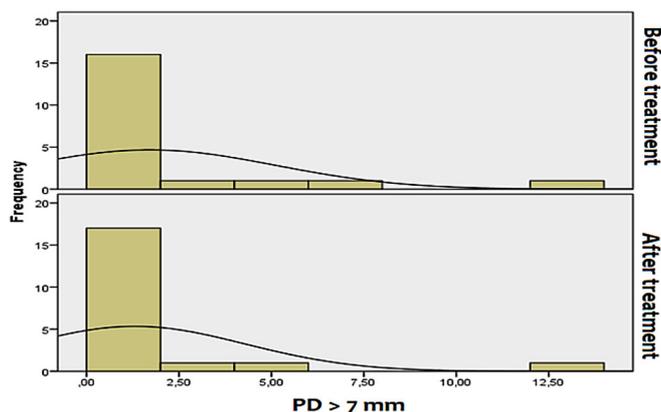
Presented on the charts data clearly show that the therapy (non-surgical) results in a reduction in the number (expressed as a percentage) of periodontal sites with pocket depth 5-7mm and sites with pocket depth more than 7 mm (Figure 4 and Figure 5). Figure 3 presents reduction of sites with periodontal probing depth 3-5 mm.

In Figures 6 and 7 are shown the changes in gene expression of the two studied cytokines - IL-1β and IL-6 after the non-surgical periodontal therapy compared to initial levels. The reduction of gingival expression of investigated markers is associated with improving the gingival status and confirms the effectiveness of the non-surgical periodontal treatment in patients with chronic periodontitis.

Fig. 7. Reduction of the gene expression of IL-6

STATISTICAL CERTIFICATE OF CLINICAL PARAMETER'S REDUCTION

Table 1 presents the statistically significant differences in examined parameters before and after non-surgical periodontal therapy in the current study. From our results it becomes clear that statistically significant differences in clinical parameters were found: HI (hygiene index), BOP (bleeding on probing), PD - 5mm, PD 5 - 7mm and PD> 7mm.

Table 1. Statistically significant differences in studied clinical parameters before and after non-surgical periodontal treatment

PARAMETER	PAIRT-TEST /P COEFFICIENT /
HI	< 0,01
BOP-distribution	< 0,01
PD-5 mm	< 0,01
PD5-7 mm	< 0,01
PD >7 mm	0,03
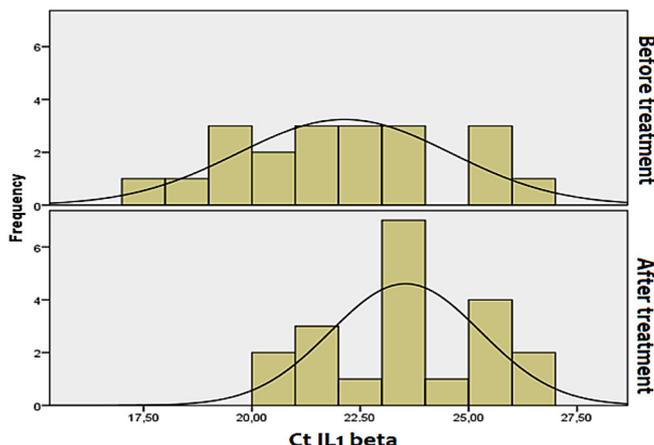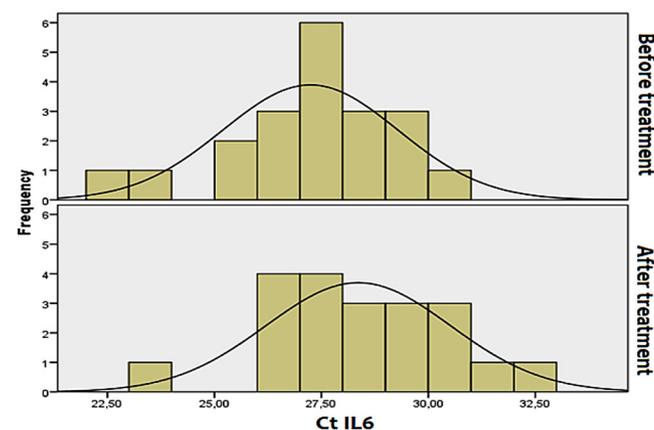
CORRELATIONS BETWEEN THE GENE EXPRESSION OF IL-1β AND IL-6 AND THE CLINICAL PARAMETERS

Table 2 shows statistically significant correlations between investigated parameters in this study in patients with chronic periodontitis. There was established a significant correlation between gene expression of interleukin-6 (expressed as Ct-value) and parameters: bleeding on probing (BOP) and periodontal pockets (PD) 5-7 mm. Furthermore, the change in gene expression of interleukin-6 (expressed as dCt- IL6) was found to correlate with bleeding on probing, and also the change in gene expression of interleukin-6 (expressed as dCt -ILβ) correlates with the parameter - periodontal pockets with probing depth (PD) 5-7 mm (p <0.05).

Table 2. Statistically significant correlations between evaluated in this study clinical and biochemical parameters

BIOCHEMICAL PARAMETER	CLINICAL PARAMETER	CORRELATION COEFFICIENT (R) P<0,05
Ct IL-6	BOP	0,43
Ct IL-6	PD 5 -7mm	0,49
d Ct- IL6	BOP	0,56
d Ct –IL β	PD 5-7mm	0,41

DISCUSSION:

Cytokines are important mediators in the regulation of inflammatory responses in the body. Destruction of tissue in chronic periodontitis may be associated with local levels of destructive cytokines such as IL-1β, TNF-alpha and IL-6 [3, 4, 5, 7, 8]. Teles et al. (2010) investigated the relationship between the biomarkers of gingival fluid (IL-1β, IL-8, MMP-8), and clinical parameters of periodontal disease. They found a positive correlation between these three key biomarkers and clinical criteria [9]. Also, there is evidence from the literature including studies of: Engebretson et al. (2002), and they concluded that patients with severe periodontitis have elevated levels of IL-1β, compared to patients with mild and moderate periodontitis [6]; and Rescala et all. (2010), which also established elevated levels of IL-1β deep periodontal pockets compared to the shallow pockets [7].

Obtained in the present study results support the literature data and suggest the importance of the investigated biomarker (gene expression of two studied cytokine - IL-1β and IL-6 before and after the non-surgical periodontal therapy) in inflammatory immune response of the organism.

CONCLUSION:

• In the present study we have established statistically significant differences in all studied clinical parameters (P <0.01), confirming effectiveness of non surgical therapy in chronic periodontitis;

• The gene expression of both investigated cytokines (IL-1β and IL-6) correlates with the distribution of periodontal inflammation, as well as with the periodontal pockets depth (P <0.05);

• Non surgical therapy in chronic periodontitis results in reduction of main inflammatory factors in gingival tissue - IL-1β and IL- 6 (P <0.1).

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