https://dx.doi.org/10.5272/jimab.1642010_27-30
Journal of IMAB - Annual Proceeding (Scientific Papers) vol. 16, book 4, 2010

GINGIVAL TISSUE IL-1beta AND PGE_2 LEVELS IN PATIENTS WITH CHRONIC PERIODONTITIS AFTER ADDITIONAL THERAPY WITH NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

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ABSTRACT

Background: The understanding of the pathogenesis of periodontitis makes various progresses in the last decades. Today it is well known that the synthesis of high levels of pro-inflammatory mediators from gingival tissues in response to periodontopathogens results in destruction of soft and hard periodontal tissues and clinical expression of periodontal disease. There is enough evidence that PGE_2 and IL-1β are important mediators in the initiation and progression of periodontal disease. Detection of numerous cytokines in high levels in gingival tissues and crevicular fluid may be indicator for activity of periodontitis. The reduction of IL-1β and PGE_2 levels after periodontal therapy may be a potential criterion for successful periodontal therapy. The occurrence of increased IL-1β and PGE_2 levels in GCF or gingival tissue is able to indicate risk from progression of destruction in specific periodontital site. The current conception of the pathogenesis of periodontitis suggests that additional host modulation approach may inhibit the production of pro-inflammatory mediators in periodontal tissues and may enhance the treatment result.

Aim of the study: To evaluate the effectiveness of additional host modulation therapy with NSAID (Aulin®) in non-surgical therapy of chronic periodontitis by measurement of IL-1β and PGE_2 gene expression levels in patient's gingival tissues.

Materials and methods: Evaluation of prostaglandin E_2 (PGE_2) and interleukin-1β (IL-1β) gene expression levels in gingival tissue of chronic periodontitis patients before and after non-surgical periodontal therapy (scaling and root planing) was performed. Prostaglandin E_2 (PGE_2) and interleukin-1β (IL-1β) gene expression levels in gingival tissue of patients with chronic periodontitis receiving conventional mechanical therapy alone or with additional host modulation therapy with NSAID (Aulin®) – 100 mg per day were compared. PCR analysis- TagMan RT-PCR for evaluation of gene expression levels of IL-1β and PGE_2 in gingival tissue of periodontal patients was applied.

Results: Statistically significant differences were found between additional Aulin® therapy group and conventional therapy group. Received correlative coefficient with Spearman analysis was respectively t = -0.72 ($p< 0,05$) for IL-1β and t = 0.81($p<0,05$) for PGE_2. The negative values of ddCt in test group reveal lower level of inhibition of gene expression. The comparative analysis of the collected data demonstrates fewer differences between both groups. The deviations in gene expression levels of IL -1β and PGE_2 are higher in the patients treated with adjunctive medication with Aulin®.

Conclusion: This study confirms the effectiveness of non-surgical therapy in moderate and severe periodontitis. Additional use of non-steroidal anti-inflammatory agent Aulin® results in higher inhibition of the pro-inflammatory cytokines IL-1β and PGE_2. This data may be the base for modifying the conventional non-surgical therapy by including anti-inflammatory agents in the treatment of chronic periodontitis.

Key words: proinflammatory mediators, gene expression, $NSAID_s$, nonsurgical periodontal therapy, host modulation, TagMan RT-PCR.

BACKGROUND

The understanding of the pathogenesis of periodontitis makes various progresses in the last decades. Today it is well known that the synthesis of high levels of pro-inflammatory mediators from gingival tissue in response to periodontopathogens results in destruction of soft and hard periodontal tissues and clinical expression of periodontal disease (9, 10). There is enough evidence that **PGE_2** and **IL-1β** are important mediators in the periodontal inflammation and bone destruction and are involved in tissue response regulation. It is well known that **IL-1β** stimulate bone loss and have inhibitory effect to bone forming (4, 6). Prostaglandin E_2 (**PGE_2**) is one of the key mediators in the periodontal inflammation by stimulating the suppression of lymphocyte production, decreasing the collagen synthesis by fibroblasts and influencing osteoclastic bone resorption (11, 12, 15).

There is great number of evidence that **PGE_2** assist regulation of **IL-1β** production and is involved in the

inflammatory reaction and tissue destruction. It is accepted that **PGE_2** and **IL-1β** are key cytokines of inflammation and clinical attachment loss and bone loss. The rates of pro-inflammatory cytokines **PGE_2** and **IL-1β** in crevicular fluid and gingival tissue in patients with chronic periodontitis are increasing proportionally to severity of periodontal disease (6,8,10,15). The examination of these cytokines may enhance the understanding of pathogenesis of periodontitis and their assessment in the treatment process may result in better control of patient's disease.

The modification of destructive host response against periodontopathogens by inhibition of pro-inflammatory cytokines has a potential therapeutic means in the treatment of periodontitis. Reduction in the levels of pro-inflammatory mediators by using $NSAID_s$ may reduce host modulation bone resorption in chronic periodontitis (12, 13, 14). Numerous studies exhibit better treatment effect with additional use of non-steroidal anti-inflammatory drug (NSAID) in non-surgical periodontal therapy (3, 14, 15).

$NSAID_s$ include a suppressing effect of prostaglandin synthesis via COX- 1 and COX- 2 and may act as inhibitor of gingival inflammation and bone destruction (5, 11, 12, 14). Detection of elevated levels of **PGE_2** and **IL-1β** in gingival tissue may be recognized as indicator for activity of periodontitis and may provide the evaluation of recurrence and progression of disease. Reducing the production of **PGE_2** and **IL-1β** after treatment may be indicator for successful periodontal therapy. Higher levels of this cytokines in crevicular fluid and gingival tissue of periodontal patients in supporting periodontal treatment can be capable predictor of further destruction.

Aim of the study:

To evaluate the effectiveness of additional therapy with NSAID (Aulin®) in non-surgical therapy of chronic periodontitis by measurement of **PGE_2** and **IL-1β** gene expression levels in patients gingival tissue.

MATERIALS AND METODS

It were measured **PGE_2** and **IL-1β** gene expression levels in gingival tissue of chronic periodontitis patients before and after non-surgical periodontal therapy (control group), gene expression levels of **PGE_2** and **IL-1β** in gingival tissue of chronic periodontitis patients before and after conventional mechanical therapy plus additional therapy with COX-2 inhibitor **Aulin®** (test group). The comparative analysis of **PGE_2** and **IL-1β** gene expression levels in gingival tissue of two patient groups: suffering from chronic periodontitis receiving conventional mechanical therapy alone and those receiving mechanical therapy and additional host modulation therapy with NSAID (Aulin®) was made.

1. Patients selection – all of patients having moderate to severe chronic periodontitis were randomly divided in two groups:

A. **Test group** – 20 chronic periodontitis patients receive non-surgical periodontal therapy with adjunctive systemic administration of NSAID (**Aulin®**) – for 14 day, 100 mg per day.

B. **Control group –** 10 chronic periodontitis patients receive non-surgical periodontal therapy alone.

2. Tag Man Real Time – PCR analysis was applied for **PGE_2** and **IL-1β** gene expression levels evaluation in gingival tissue of all patients involved in the study.

Quantitative TaqMan Real Time-PCR (Polimerase Chain reaction in Real time) was used for gene expression analyses of amount of inflammation mediators IL-1β and PGE_2 in gingival tissue of patients with periodontitis.

This method is based on PCR technology. The difference is that here we use TaqManProbe which is fluorescently labeled and allow detection in Real time. The 5'-3' exonuclease activity of the Taq polymerase enzyme is cutting the Reporter Dye and fluorescence rise with every cycle. This allows detection in real time (2, 7).

TaqMan Real Time-PCR can be used for qualitative, quantitative and comparative studies.

In this study the examined material was gingival tissue, harvested from interproximal area in distal dentition.

3. Analysis of **PGE_2** and **IL-1β** gene expression levels in gingival tissue was performed by:

Gene Expression Analyses of IL-1β and PGE_2 in gingival tissues

· Isolation of the total RNA from gingival tissue was made using RiboPure isolation kit (Roboscreen Germany)

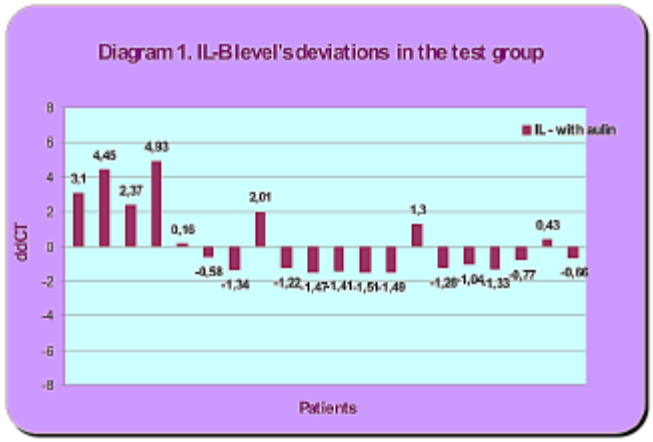
· Followed by reverse transcription of the total RNA

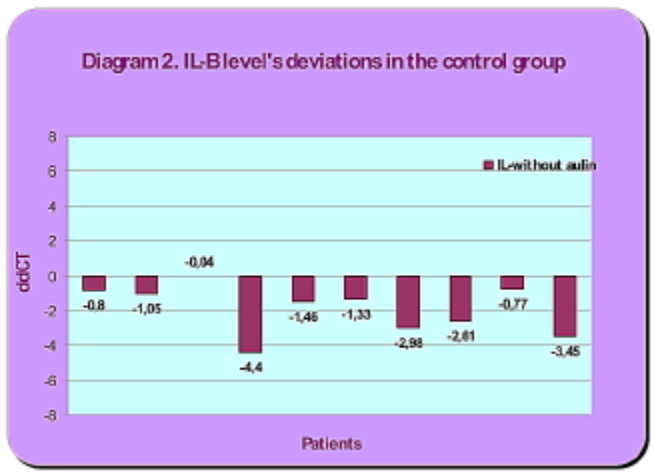
· Amplification with specific TaqMan Gene Expression Assays for IL-1β and PGE_2 (Applied Biosystems) on ABI 7500 and RQ –software (1, 2, 7).

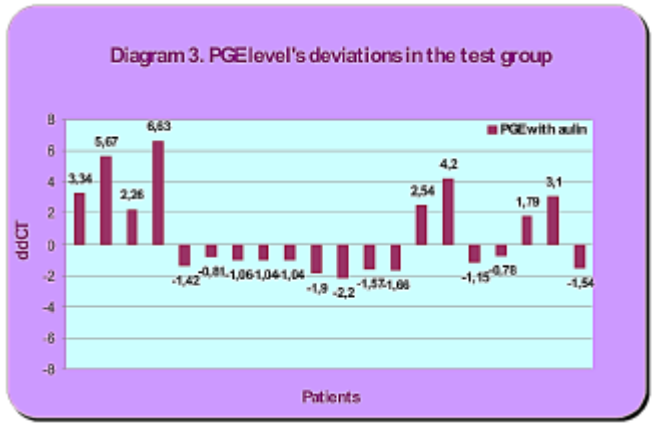
The statistic method used for data procession was **Spearman analysis**.

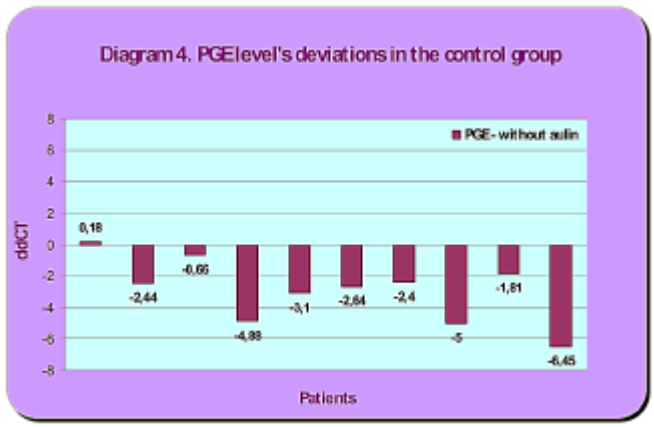
RESULTS AND DISCUSSION

The following two diagrams (diagram 1 and diagram 2) represent the results of gene expression levels of Il-1â in gingival tissue of chronic periodontitis patients from two groups.









The following two diagrams (diagram 3 and diagram 4) represent the results of gene expression levels of PGE_2 in gingival tissue of chronic periodontitis patients from two groups.

The PGE_2 and IL-1β (ddCt) gene expression level deviations between two groups of patients were compared. In 42% of the patients treated with Aulin® an inhibition of the IL-1β was registered and in 37% of these patients an inhibition of the PGE_2 was registered. It can be noticed that the inhibition of PGE_2 gene expression is higher then the suppression of IL-1β gene expression. This occurrence is logical concerning the fact that PGE_2 influence the IL-1β expression in a previous stage of the inflammatory process (10).

In patients receiving conventional periodontal treatment without adjunctive Aulin® the PGE_2 and IL-1β gene expression depict lower level of inhibition (table 1).

Negative values of ddCt in both groups reflect lower level of inhibition of gene expression (diagrams 1-4).

Statistically significant differences were found between test and control group in the inhibition of PGE_2 and IL-1β gene expression (table 1).

Table1. Comparative analysis in gene expression level's deviations of IL1β and PGE_2

Criterion	Control group (without Aulin)			Experimental group (with Aulin)			ð
	n	X	SD	n	X	SD	
ddCt (IL1β)	10	-1,862	1,461	20	0,191	2,033	0,016
ddCt (PGE_2)	10	-3,014	2,242	20	0,563	2,729	0,002

The received correlative coefficient with Spearman analysis is respectively t = - 0,72 (p<0,05) for IL-1β, and t = 0,82 (p< 0,05) for PGE_2. The comparative analysis of average values demonstrates slight difference between both of groups. However the alterations in PGE_2 and IL-1β gene expression levels are higher in patients additionally treated with Aulin®.

The descriptive statistic by Sigma Stat software shows statistically significant differences in PGE_2 and IL-1β gene expression levels with higher inhibition in the experimental group supporting the hypothesis of this study.

CONCLUSION

Results of this study confirm the effectiveness of non-surgical therapy in moderate and severe periodontitis.

The significant difference in PGE_2 and IL-1β gene expression levels betweenboth groups confirm the advantage of the experimental therapy with additional administration of non-steroidal anti- inflammatory agent (Aulin®). Additional use of non-steroidal anti-inflammatory agent results in higher effectiveness of initial treatment of chronic perodontitis.

The laboratory examination demonstrated inhibition of gene expression levels of important pro-inflammatory cytokines like PGE_2 and IL-1β, and confirms the approach to modification of the host response in the treatment of chronic periodontitis.

The recorded variations in PGE_2 and IL-1β gene expression levels in this study suggested the rationale for additional investigations upon various factors, influencing the production of different cytokines in gingival tissues.

The performed experimental investigation is supported by GRANT project No. 8, contract No.8/2008, financed by Medical University Sofia, Medical sciences counsel. With **acknowledgements** to PROGEN laboratory Sofia.

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