http://dx.doi.org/10.5272/jimab.2014203.563

Journal of IMAB
ISSN: 1312-773X
http://www.journal-imab-bg.org

OPEN ACCESS

Journal of IMAB - *Annual Proceeding (Scientific Papers)* 2014, vol. 20, issue 3

CORRELATION OF GENE EXPRESSION OF MAIN INFLAMMATORY CITOKINS AND THE SEVERITY OF CHRONIC PERIODONTITIS

Christina Popova, Velitchka Dosseva-Panova, Antoaneta Mlachkova
Department of Periodontology, Faculty of Dental Medicine, Medical University - Sofia, Bulgaria.

ABSTRACT:

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 periodontal pathogens results in destruction of soft and hard periodontal tissues and clinical expression of periodontal disease. The occurrence of increased IL-6, IL-1β, TNFα and PGE_2 levels in GCF or gingival tissue is capable to indicate risk of progression of destruction in specific periodontal site. Detection of gene expression of numerous major cytokines in high levels in gingival tissues and crevicular fluid may be indicator for activity of periodontitis and provides the rationale for the additional periodontal therapy. 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 thus may enhance the treatment result.

Key words: chronic periodontitis, gene expression, IL-6, IL-1β, TNF-α, PGE_2.

INTRODUCTION:

The current conception of the pathogenesis of periodontal diseases suggests that periodontitis is an inflammatory disease of bacterial origin that results in the progressive destruction of the supportive periodontal tissues. Although bacteria are necessary for the initiation of periodontal disease, the immune-inflammatory response that develops in periodontal tissues in response to the presence of plaque bacteria is of high importance and results in destruction of periodontium and clinical signs of periodontitis [1 - 8]. There are literature data for a higher level of gene expression of the major pro-inflammatory cytokines at higher severity of chronic periodontitis and current progression of destruction [2, 4, 5, 9 - 13]. Therefore, the current literature focuses on producing as a result of the local inflammatory response cytokines such as part of an imbalance between macro-organism and pathogenic bacteria from the dental biofilm [8, 10, 11, 14 - 18]. As a result of bacterial infection different host cells such as keratinocytes, endothelial cells, fibroblasts, macrophages, and other express multiple cytokines such as interleukin 1-alpha, interleukin 1-beta, interleukin-6 and tumor necrosis factor-alpha. During inflammation gingival fibroblasts are stimulated by cytokines released to synthesize cytokines themselves, and also MMPs (matrix metalloproteinases), and PGE_2 (prostaglandin E_2) that additionally cause tissue damage and stimulate bone resorption. [19, 20].

Aim:

Evaluation of the gene expression of IL-6, IL-1β and TNF-α as well as PGE_2 in the gingival tissues in relation with severity of chronic periodontitis.

MATERIALS AND METHODS:

Two groups of patients with chronic periodontitis were enrolled in this study - 20 patients examined for gene expression of IL-1β and PGE_2 and 20 patient tested for gene expression of IL-6 and TNF-α. The patient gingival tissue was investigated using TaqMan Real-Time PCR (Progene lab.). The gingival samples were taken from sites in contact to deep periodontal pockets.

RESULTS:

Significant differences were established according to the gene expression of IL-6 and TNF in relation with deeper periodontal pockets and severe attachment loss ($P < 0.05$) in patients with chronic periodontitis. The similar results are obtained for IL-1β and PGE_2. [Table 1. and Table 2.]

Significant differences were established according to the gene expression (marked as dCt) of IL-6 between samples taken from the gingiva in conjunction of pockets ≤ 4 mm and periodontal pockets with depth > 6 mm), as well as of TNFα in two deeper probing depths (4 - 6 mm / > 6 mm).[Fig. 1. and Fig. 2.] As regards to severity of disease gene expression of TNF (dCt 4-6 mm) shows significant differences. In patients with a higher severity of disease dCt increased (i.e. lower gene expression). [Fig. 3]

Changes in gene expression of IL-1β and PGE_2 demonstrated in Fig. 4 and Fig. 5. show a reduction of the gene expression of the major cytokines associated with periodontitis with healing as a result of elimination of inflammation and a reduction of the pockets.

CONCLUSION:

The results of this study suggest for existing correlation of chronic periodontitis severity and the gene expression of main factors associated with destructive host response

TABLES AND FIGURES

Table 1. Comparative analysis of gene expression in samples taken from different depths, expressed as dCt (Ct (gene – Ct (reference gene - ACTB))) of IL6 and TNF.

Index	**Depth**									**P - coefficient**		
	≤ 4 mm			**4-6 mm**			**> 6 mm**			**Paired t-test**		
	n	X	**SD**	**n**	X	**SD**	**n**	**X**	**SD**	**≤ 4 mm / 4 - 6 mm**	**≤ 4 mm/ > 6 mm**	**4-6 mm/ > 6 mm**
dCT (IL6)	**77**	14.5	2.5	**10**	15.9	4.2	**77**	16.8	3.4	0.45	**0.048***	0.48
dCT (TNF)	**16**	7.3	3.6	**16**	7.3	2.6	**76**	8.6	1.8	0.94	0.11	**0.064***

n – Number of patients studied, **X** - arithmetic average value, **SD** – standard deviation
* Statistically significant differences are underlined (P coefficient < 0.05)

Table 2. Comparison of gene expressions relative to the disease severity.

Index	**P - coefficient (t-test / Mann-Whitney U test)**	(severity 1)	(severity 2)
dCtIL6**(=4 mm)**	0.094	15.35	12.98
dCt TNF (= **4 mm)**	0.103	8.4	5.6
dCt IL6 **(4 - 6 mm)**	0.603	16.7	15.6
dCt TNF (4-6 mm)	**0.056***	8.6	6.2
dCt IL6 **(> 6 mm)**	0.637	16.1	17.1
dCt TNF (> **6 mm)**	0.247	8.9	7.9
ddCTIL6**(dCt(4-6 mm) -dCt(=4 mm))**	0.488	1.1	3.3
Degree of change rL6 **(dCt (4 - 6 mm) - dCt (= 4 mm))**	1.0	0.2	0.1
ddCTIL6 **(dCt(>6 mm) -dCt(=4 mm))**	0.188	2.4	4.2
Degree of change IL6 **(dCt (> 6 mm) - dCt (= 4 mm)**	0.413	0.12	0.05
ddCTIL6**(dCt(>6 mm) -dCt(4-6 mm)**	0.161	-0.89	3.6
Degree of change IL6 **(dCt (>6 mm) - dCt (4 - 6 mm)**	0.160	1.9	0.1
ddCTTNF**(dCt(4-6 mm) -dCt(=4 mm))**	0.580	-0.02	0.7
Degree of change TNF **(dCt (4 - 6 mm) - dCt (= 4 mm))**	0.955	0.4	0.7
ddCTTNF**(dCt(>6 mm) -dCt(=4 mm))**	0.596	1.2	2.1
Degree of change TNF **(dCt (> 6 mm) - dCt (= 4 mm)**	0.244	0.7	0.3
ddCTTNF**(dCt(>6 mm) -dCt(4-6 mm)**	0.291	0.9	1.9
Degree of change TNF **(dCt (>6 mm) - dCt (4 - 6 mm)**	0.205	1.4	0.3

Fig. 1 and **Fig. 2**. dCt of gens (a) IL-6 and (b) TNFα in (1) ≤ 4 mm, (2) 4 – 6 mm, (3) > 6 mm

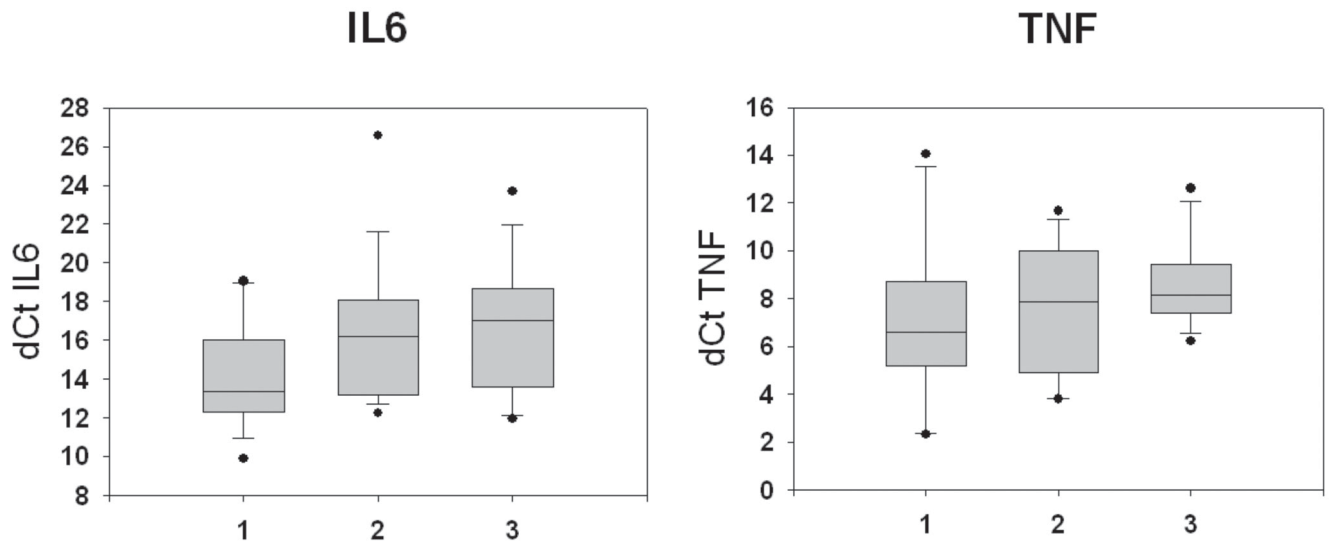


Fig. 3. dCt of gene TNF in patients with different severity- (1) moderate and (2) severe.

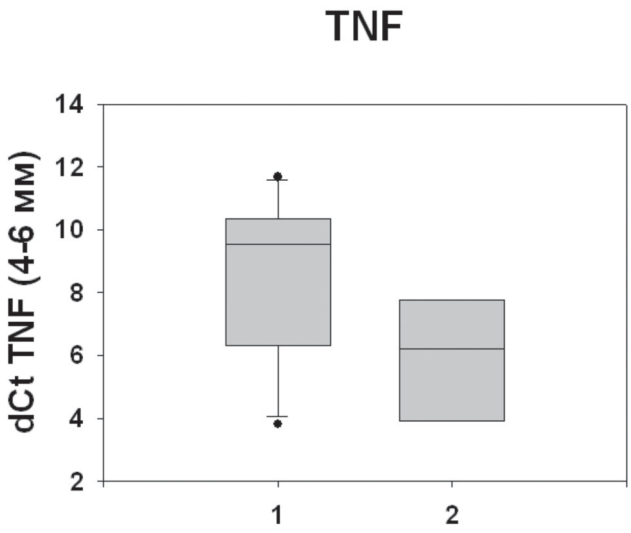
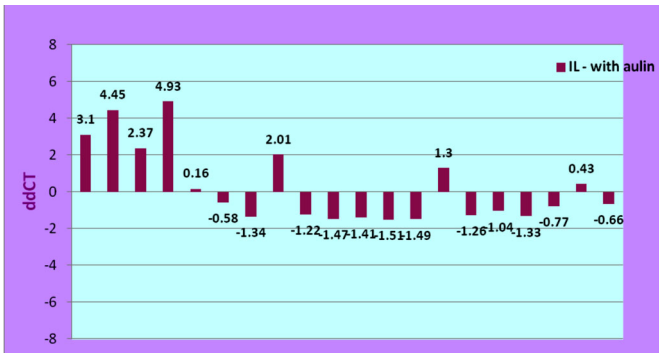
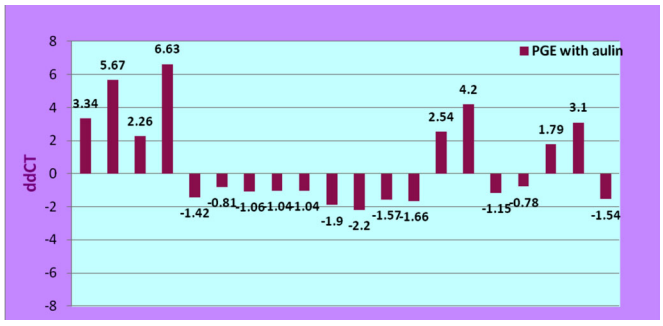


Fig. 4 and **Fig. 5.** Changes in IL-1β and PGE_2 levels in patients with Aulin® therapy added to periodontal treatment.





Acknowledgement:

These studies were supported by Grant projects, Scientific Researches, Medical University – Sofia (Agreement No 14/2010 and No 1/2012)

REFERENCES:

1. Bartold P, Van Dike T. Periodontitis: a host-mediated disruption of microbial homeostasis. Unlearning learned concepts. *Periodontol 2000.* 2013; 62(1):203-217. [PubMed] [CrossRef]

2. Ebersole J, Dawson D, Morford L, Peyyala R, Miller C, Gonzales O. Periodontal disease immunology: 'double indemnity' in protecting the host. *Periodontol 2000.* 2013 Jun;62(1):163-202 [PubMed] [CrossRef]

3. Kinane D, Attstrom R. Advances in the pathogenesis of periodontitis: group B consensus report of the fifth European Workshop in Periodontology. *J Clin Periodontol* 2005; 32(Suppl 6): 130-131. [PubMed]

4. Kornman K. Host modulation as a therapeutic strategy in the treatment of periodontal disease. *Clin Infect Dis.* 1999 Mar;28(3):520-526. [PubMed] [CrossRef]

5. Masada M, Persson R, Kenney J, Lee S, Page R, Allison A. Measurement of interleukin -1alfa and interleukin -1beta in gingival crevicular fluid: implications for the pathogenesis of periodontal disease. *J Periodontal Res.* 1990 May;25(3):156-163. [PubMed]

6. Taylor JJ. Cytokine regulation of immune responses to Porphyromonas gingivalis. *Periodontol 2000.* 2010 Oct; 54(1):160-194. [PubMed] [CrossRef]

7. Tipton DA, Flynn JC, Stein SH, Dabbous MKh. Cyclooxygenase-2 inhibitors decrease interleukin-1beta-stimulatet prostaglandin E_2 and IL-6 production by human gingival fibroblasts. *J Periodontol.* 2003 Dec; 74 (12):1754-1763. [PubMed] [CrossRef]

8. Vardar S, Baylas H, Huseyinov A. Effect of selective cyclo- oxygenase-2 inhibition on ginival tissue levels of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ and clinical parameters of chronic periodontitis. *J Periodontol.* 2003 Jan;74(1):57-63. [PubMed] [CrossRef]

9. Popova Chr, Mlachkova A, Kicheva M. Tag Man RT-PCR method for evaluation of IL-1β and PGE_2 levels in the gingival tissue in patients with chronic periodontitis. *Problems of dental medicine.* 2009; XXXV(2):8-15. [in Bulgarian]

10. Delima AJ, Oates T, Assuma R, Schwartz Z, Cochran D, Amar S, et al. Soluble antagonists to interleukin-1(IL-1) and tumor necrosis factor (TNF) inhibits loss of tissue attachment in experimental periodontitis. *J Clin Periodontol.* 2001 Mar;28(3):233-240. [PubMed]

11. Ejeil AL, Gaultier F, Igondjo-Tchen S, Senni K, Pellat B, Godeau G, et al. Are cytokines linked to collagen breakdoun during periodontal disease progression? *J Periodontol.* 2003 Feb; 74(2):196-201. [PubMed] [CrossRef]

12. Geivelis M, Turner DW, Pederson ED, Lamberts BL. Measurements of interleukin-6 in gingival crevicular fluid from adults with destructive periodontal disease. *J Periodontol.* 1993 Oct;64(10);980-983. [PubMed] [CrossRef]

13. Raunio T, Nixdorf M, Knuuttila M, Karttunen R, Vainio O, Tervonen T. The extent of periodontal disease and the IL-6_174 genotype as determinants of serum IL-6 level. *J Clin Periodontol.* 2007 Dec;34(12):1025-1030. [PubMed] [CrossRef]

14. Dinarello CA. The many worlds of reducing interleukin-1. *Arthritis Reum.* 2005 Jul;52(7):1960-1967. [PubMed] [CrossRef]

15. Giulietti A, Overbergh L, Valckx D, Bouillon R, Decallonne B, Mathieu Ch. An overview of real- time quantitative PCR: Applications to quantify cytokine gene expression. *Methods.* 2001 Dec;25(4):386-401. [PubMed] [CrossRef]

16. Roberts FA, McCaffery KA, Michalek SM. Profile of cytokine mRNA expression in chronic adult periodontitis. *J Dent Res.* 1997 Dec;76 (12):1833-9. [PubMed] [CrossRef]

17. Salvi GE, Lang NP. Host response modulation in the management of periodontal diseases. J Clin *Periodontol.* 2005 Oct;32(Suppl. 6): 108-129. [PubMed] [CrossRef]

18. Van Dyke T. Control of inflammation and periodontitis. *Periodontol 2000.* 2007 Oct;45(1):158-166. [PubMed] [CrossRef]

19. Taylor J. Cytokine regulation of immune responses to Porphyromonas gingivalis. *Periodontol 2000.* 2010 Oct; 54(1):160–194. [PubMed] [CrossRef]

20. Kirkwood KL, Taba M Jr, Rossa C Jr, Preshaw PM, Giannobile WV. Molecular biology of the host-microbe interaction in periodontal diseases: selected topics: molecular signaling aspects of pathogen-mediated bone destruction in periodontal diseases. In Carranza's Clinical Periodontology / [edited by] Newman MG, Takei HH, Klokkevold PR, Carranza FF. 10th ed., *W.B Saunders Company, Philadelphia,* 2006; Chapter 15.

Please cite this article as: Popova Chr, Dosseva-Panova V, Mlachkova A. CORRELATION OF GENE EXPRESSION OF MAIN INFLAMMATORY CITOKINS AND THE SEVERITY OF CHRONIC PERIODONTITIS. *J of IMAB.* 2014 Jul-Sep;20(3):563-566. doi: http://dx.doi.org/10.5272/jimab.2014203.563

Received: 17/06/2014; Published online: 19/08/2014;

Address for correspondence:
Professor Christina Popova, PhD, DM;
Department of Periodontology, Faculty of Dental Medicine,
1, St. Georgi Sofiiski str., 1431 Sofia, Bulgaria
E-mail: hrpopova@yahoo.com,