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Appraisal Of Interleukin-18 In The Serum Of Bengal's Population Suffering From Periodontal Disease

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Abstract

Background: Every day the human body is subjected to different types of bacterial challenges, which it encounters by producing several cytokines. One such cytokine is interleukin-18 (IL-18). It is a pro-inflammatory and tumour suppressive cytokine.

Aims: IL-18 might have a role to play in the deterioration from oral health to periodontal disease. So this study was carried out to interpret the level and role of IL-18 in the serum of individuals with healthy gingiva, chronic periodontitis and aggressive periodontitis before and after periodontal treatment.

Settings and Design: Ninety individuals chosen for the study were divided equally into healthy control group (1A), chronic periodontitis (2A) and aggressive periodontitis (3A) with thirty individuals each. Criteria for the division were the subject's gingival index, probing pocket depth, clinical attachment loss and radiographic evidence of bone loss.

Methods and Material: The individuals underwent treatment (scaling in case of Group 1A and scaling and root planing (SRP) followed by flap surgery in Groups 2A and 3A) to form post-treatment groups 1B, 2B and 3B respectively. Thus a total of 180 serum samples were collected and tested by ELISA. Statistical analysis was done by posthoc Tukey's test to observe the intergroup variations.

Results: The mean serum IL-18 concentration was greatest in Group 2A (55.12 pg/ml) followed by Group 3A (39.06 pg/ml) and lowest (11.49 pg/ml) in Group 1A. The post-treatment groups showed reduction in the mean serum IL-18 concentration.

Conclusions: As the inflammation increased, there was a concomitant increase in the level of IL-18 and vice versa following periodontal therapy.

Keywords: Aggressive periodontitis; Chronic periodontitis; Cytokines; Interleukin; Serum

Introduction

It is a well-established fact that bacterial attack leads to the commencement of gingival inflammation and then deterioration to periodontal tissue destruction in a susceptible host. The host's body encounters such attacks by eliciting numerous cytokines [1, 2]. This study was designed to appraise the role, if at all any of one such cytokine interleukin-18 (IL-18) in periodontal health and disease in the population of Bengal, a state in India.

Originally named as interferon gamma (IFN- γ) inducing factor, it was re-christened as IL-18 after molecular cloning and later to IL-1F4 due to its resemblance in structure, receptor family and signal transduction pathways with IL-1 [3]. IL-18, a pro-inflammatory cytokine is produced primarily by the

antigen-presenting cells, monocytes/macrophages, Kupffer cells and by non-immune cells such as intestinal and airway epithelial cells [3]. It is formed at the sites of chronic inflammation, autoimmune diseases, in a variety of cancers and in several diseases. IL-18, in the company of IL-12 induce IFN-γ production from natural killer (NK) and T-cells and stimulate a Th1 cell response, while in its absence, IL-18 encourage the production of Th2 cytokines like IL-4, IL-5, IL-10 and IL-13, stimulate allergic inflammation and induce prostaglandin E_2 production [4]. Thus IL-18 has the unique capacity to induce either Th1 or Th2 differentiation, depending upon the immunological context [4].

Methods And Material

A total of ninety subjects were incorporated in this study. They were of the age group 20-50 years, non-smokers, free from any known systemic disease and had not experienced any periodontal therapy or had received any antibiotics and anti-inflammatory drugs in the last six months. Written informed consent from the individuals participating in the study along with ethical clearance from the institution's ethical committee were obtained. This study was conducted in accordance with the Helsinki Declaration of 1975 as revised in 2000. Orthopantomograph supplemented by intraoral periapical radiographs were done to judge the bony architecture. Based on their gingival index (GI), probing pocket depth (PD), clinical attachment loss (CAL) and radiographic evidence of bone loss, these ninety individuals were then divided equally into three groups of thirty members each as follows-

Group 1A (healthy control)- individuals having clinically healthy periodontium, GI score-0, PD of ≤3 mm and CAL -0 with no indication of bone loss on radiographs.

Group 2A (chronic periodontitis)- individuals with signs of clinical inflammation consistent with local etiological factors, GI score > 1, PD ≥ 4 mm, CAL ≥ 3 mm, with radiographic evidence of bone loss [5].

Group 3A (aggressive periodontitis)- individuals with non-contributory medical history, rapid attachment loss and bone destruction, familial aggregation of cases, amount of deposits which are inconsistent with the severity of periodontal tissue destruction and showing generalized interproximal attachment loss affecting at least three permanent teeth other than first molars and incisors [6, 7].

The individuals of group 1 A underwent non-surgical periodontal therapy, which was accomplished within 24 hours to form post-treatment group 1B [8]. Individuals of groups 2A and 3A underwent periodontal flap surgery after scaling and root planing (SRP) to form groups 2B and 3B respectively. Serum samples were collected six to eight weeks after scaling or flap surgery. Thus a total of 180 serum samples were gathered over the study period.

Collection Of Serum

The skin over the antecubital fossa was disinfected and two ml of blood was collected by venipuncture using 20-gauge needle and two ml syringe. The blood samples were permitted to clot at room temperature and one hour later, they were centrifuged at 3000 rpm for six minutes to separate the serum component. The samples were stored at -70^0C till the time of the assay. The collected serum samples were analyzed using ELISA technique. The technician who executed the ELISA was unaware of the study groups.

Statistical Analysis

The data were analyzed using statistical software SPSS. £ The data were checked for normal distribution using the Kolmogorov Smirnov test. Mean and standard deviation of the continuous variables were calculated. Correlational analysis was conducted within the groups by using measures from both the pre-treatment groups and the post-treatment groups. Intergroup comparison of the mean levels of IL-18 in the serum samples were performed using a posthoc Tukey's test. The difference in mean was considered statistically significant if $p < 0.05$. Pearson's correlation test was used to evaluate correlation among serum IL-18 concentration and the clinical parameters. p values < 0.05 were considered as statistically significant.

Results

All the individuals maintained their appointments regularly. None of the individuals or the sampling sites was dropped in the course of the study. The descriptive data of the pre-treatment and post-treatment groups are presented in Tables 1 and 2 respectively. Intergroup comparison of the mean

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levels of IL-18 using a posthoc Tukey's test are presented in Tables 3 and 4.

The results suggest that serum IL-18 levels were lowest in health and highest in chronic periodontitis. Post treatment there was a reduction in the level of IL-18 in each group. A statistically significant difference in the mean concentration of IL-18 levels was observed between Groups 1A and 2A and between 1A and 3A. Post treatment, a statistically significant difference in the mean concentration of IL-18 levels was observed between Groups 1B and 2B. IL-18 level in the subjects of Group 2A is positively correlated with GI, PD and CAL (Table 5). There is no significant correlation between the levels of serum IL-18 and clinical parameters such as PI, GI, PD and CAL in healthy and aggressive periodontitis subjects.

Table 1- Descriptive data of the pre-treatment groups

Variables Mean ± sd	Group 1A Healthy control	Group 2A Chronic periodontitis	Group 3A Aggressive periodontitis
Age (years)	26.25±4.03	41.22±3.36	36.95±5.41
PI	0.22±0.02	2.06±0.16	1.89±0.49
GI	0.01±0.00	2.21±0.20	2.10±0.29
PD	1.50±0.08	5.80±0.43	5.25±0.18
CAL	0.00	4.10±0.52	3.99±0.72
Serum IL-18 pg/ml	11.49±3.96	55.12±19.77	39.06±15.37

sd- Standard Deviation, PI- Plaque Index, GI- Gingival Index, PD- Probing Pocket Depth

CAL- Clinical Attachment Loss

Table 2- Descriptive data of the post-treatment groups

Variables Mean ± sd	Group 1B Healthy Control	Group 2B Chronic Periodontitis	Group 3B Aggressive Periodontitis
PI	0.21±0.05	1.19±0.15	1.12±0.19
GI	0.01±0.00	0.94±0.25	1.05±0.09
PD	1.48±0.12	3.02±0.53	2.56±0.91
CAL	0.00	2.31±0.39	1.78±0.56
Serum IL-18 pg/ml	10.56±3.31	28.50±5.66	21.16±4.22

Table 3- Comparison of IL-18 levels in the pre-treatment groups

Groups	Mean difference among groups serum pg/ml	p value

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Healthy		
Chronic Periodontitis	-43.63	0.02*
Aggressive Periodontitis	-27.57	0.03*
Chronic periodontitis		
Aggressive Periodontitis	16.06	0.49

*P<0.05- statistically significant,

Table 4-Comparison of IL-18 levels in the post-treatment groups

Groups	Mean difference among groups serum pg/ml	p value
Healthy		
Chronic Periodontitis	-17.94	0.02*
Aggressive Periodontitis	-10.60	0.34
Chronic periodontitis		
Aggressive Periodontitis	07.34	0.33

*P<0.05- statistically significant,

Table 5- Pearson correlation coefficient test

Groups	IL-18 and GI		IL-18 and PD		IL-18 and CAL	
	r	p	r	p	r	p
Group 2A	0.788	0.034 *	0.892	0.022*	0.652	0.037*

*statistically significant < 0.05

Discussion

There are several studies that have reported enhanced GCF concentration of IL-1 in periodontal disease sites as compared to the healthy sites [9]. However studies relating to the role of IL-18, a member of the IL-1 family in periodontal health and disease were found to be scarce. Our study was unique and stood apart from other studies because we assessed IL-18 levels both prior and post treatment in three separate groups- healthy control, chronic periodontitis and aggressive periodontitis.

The results of the present study specify that the concentration of IL-18 in the serum was lowest in periodontal health (group 1A), even higher in aggressive periodontitis (group 3A) and maximum in case of chronic periodontitis (group 2A) subjects. This upsurge in the IL-18 concentration were in harmony with the reports of previous studies [10-11].

Besides the concentration of IL-18 in the serum was detected to be decreased six-eight weeks after periodontal therapy. This decline in the concentration in each group could be credited to the post-treatment reduction in the inflammation of the gingiva which in turn would lead to a diminished release of cytokines into the serum [12].

Another outcome noted was that notwithstanding the reduction in the mean concentration of IL-18 post treatment, the level did not touch the baseline level of the healthy control group. This could be due to the non-resolving inflammation of the gingiva influenced by some other cytokine like IL-6 and IL-12 [13]. In 2005, Johnson and Serio opined that periodontal inflammation might not successfully resolve because of a decreased concentration of anti-inflammatory cytokines like IL-12 within the inflamed gingival tissue and accumulation of other cytokines like IL-6

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and IL-18 [13]. IL-18 was also reported to play a vital role in maintaining the chronicity of inflammation in rheumatoid arthritis, inflammatory bowel disease and some allergies [11]. Offenbacher et al. (2010) analyzed 33 GCF biomarkers in experimental gingivitis in humans and noted that the pattern of biomarker expression during the generation and resolution of inflammation differed considerably among subjects with similar clinical responses. Thus the effect of one cytokine depends upon the other cytokines and mediators in the local environment. This may explain the wide variability seen between the individuals in the same group [14].

Ishida et al. (2004) stated that IL-18 amplified the chemotaxis of natural killer (NK) cells and encouraged the production of activated matrix metalloproteinases (MMP)-2, pro-MMP-2 and MT1-MMP from these cells [15]. Juvenile idiopathic arthritis individuals with incipient connective tissue attachment loss were reported to have higher serum IL-18 levels signifying a role of IL-18 in periodontitis [16]. In severe inflammatory and septic conditions, high plasma IL-18 concentrations have displayed poor clinical outcome. So IL-18 was proposed as a marker in monitoring severe inflammatory conditions particularly in Gram-positive sepsis suspected cases [17]. Kim et al (2009) explored the effect of IL-18 on expression of type I and collagen genes in dermal fibroblasts. Their results suggested that IL-18 down-regulated collagen production in human dermal fibroblasts via extracellular signal-regulated kinase (ERK) pathway [18]. IL-18 is associated with obesity, atherosclerosis, insulin resistance/glucose intolerance, cardiovascular disease and multiorgan dysfunction [19-22]. IL-18 assessment might add prognostic information to lipid and inflammatory markers in patients with or without clinically established atherosclerotic disease. [23]

Literature reports have showed that IL-18 could induce the release of MMP-9 and IL-1β, both of which were reported to have pro-inflammatory and tissue degradation effects [5]. IL-18 was found to activate macrophages and other immune cells to secrete pro-inflammatory cytokines and chemokines. IL-18 might promote a priming effect on neutrophils which could upregulate the production of IL-1□ a pro-inflammatory cytokine [24]. Due to its chemotactic, pro-inflammatory and angiogenic properties, IL-18 might be a factor in the progression of inflammation. [13] Thus the accumulation of IL-18 in the periodontal tissues might be associated with persistence and maintenance of gingival inflammation. A concomitant rise in the level of IL-18 was observed in this study as the PD increased. This observation was consistent with the findings of Johnson and Serio (2005). They had reported higher concentrations of IL-18 adjacent to the sites with PD >6 mm than that of the healthy sites. [13] Thus IL-18 could play an important role in gingival inflammation as relatively higher levels of IL-18 were found in gingival samples with increasing sulcular depth.

The study design had initially three pre-treatment groups with three post-treatment groups added six-eight weeks after the completion of periodontal therapy. Such a designing of the study enabled better evaluation of the role of IL-18 in the different stages of periodontal health and disease and to gauge the role of periodontal therapy on the concentration of IL-18 in the serum. Though most of the healing was supposed to be completed by six weeks, the repair process could continue for a longer period. Perhaps a delay in the post-treatment sample collection by a few weeks might have produced further attenuation in the inflammation of the periodontal tissues and hence further decrease in the IL-18 levels. However further studies involving larger sample sizes are necessary to properly appraise the role of IL-18 in periodontal health and disease.

Conclusion

Increase in the serum levels of IL-18 is directly proportional to the increased level of inflammation of the periodontal tissues while a decrease in inflammation following periodontal therapy lead to a reduction in the levels of the interleukin. Further longitudinal prospective studies involving larger sample sizes are merited to affirm the role of IL-18 in periodontal health and disease.

Footnotes:

□□ RayBio Human IL-18 ELISA kit (Assaypro, USA)

£ (Chicago, IL, USA) version 11.0

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