Effect of Periodontal Therapy on Serum and Salivary Interleukin-2 Levels in Chronic Periodontitis

Haween T. Nanakaly, B.Sc., M.Sc., Ph.D. [(1)]

ABSTRACT

Background: Interleukine-2 is a multifunctional cytokine, considered a central regulator of host resistance against a variety of pathogens and has been recently demonstrated to exert an active role in the pathogenesis of periodontal diseases. The purpose of this study was to evaluate the effect of scaling and root planning on level of IL-2 in serum and saliva of patients with chronic generalized periodontitis, in relation to clinical parameters.

Materials and Methods: A total of 50 subjects were enrolled, of which 25 had chronic generalized periodontitis and 25 periodontally healthy subjects as control. The clinical parameters included: gingival index, pocket probing depth, clinical attachment level and bleeding on probing. The level of IL-2 in serum and saliva was estimated by using enzyme linked immunosorbent assay kit at baseline and after 4 weeks of the treatment (scaling and root planning).

Results: Mean IL-2 level in serum and saliva of patients with chronic periodontitis at baseline(382.15± 96.02 and 501.82± 88.08 ng/L) were significantly higher than in controls (10.98± 3.04 and 20.89 ± 7.16 ng/L; p=0.001) respectively. Post-periodontal therapy, IL-2 levels in serum and saliva increased significantly (534.18 ± 127.70 and 710.87 ± 198.15; P= 0.001.) compared to basal levels, as well as to control group (p< 0.001). Statistically significant positive association was found between salivary IL-2 level and the pocket depth in the chronic periodontitis post therapy (r = 0.45, p= 0.02).

Conclusion: Short-term nonsurgical periodontal therapy leads to in a significant improvement in clinical periodontal parameters and a marked increase in IL-2 levels

Keywords: Non-surgical periodontal therapy, chronic periodontitis, serum, saliva, Interleukine-2. (J Bagh Coll Dentistry 2016; 28(2):73-78).

INTRODUCTION

Periodontal disease (PD) is an inflammatory disease, is also considered as an immunological disease as localized chronic inflammation associated with gingiva exhibits distinctive immunologic features which include elevated cellular and humoral immune responses [(1)]. It results from an interaction of the host defence mechanism with microorganisms in biofilm accumulating on the teeth surface, consequences in progressive destruction of periodontal tissues and alveolar bone, with formation of periodontal pockets and gingival recession, leading to tooth loss [(2,3).]

Cytokines are water-soluble glycoproteins that are secreted by various cells in the body, usually in response to an activating stimulus and induced responses through binding to specific receptors. Their primary role is intercellular signaling that act as intracellular regulatory factors at both local and systemic level. They are central to the pathogenesis of an ever-increasing number of diseases, including periodontal disease [(4)] Cytokines have a key role in the function and differentiation of T cells, and they designate the T-helper subsets to amplify or control the amplitude of the inflammation and the destruction related to inflammation [(5)]. The macrophages and T cells are dominated in the early/stable lesions of chronic periodontitis (CP), leading to the respect that this response is developed by the Th1 cytokines. While, in the advanced/ progressive lesions of CP, B cells and plasma cells are dominate and Th2 cytokines [(6,7)] . In diseased states, cytokines may be secreted not only by resident cells but also by locally infiltrated immune-competent cells [(8)]. The balance between Th1 and Th2 cells phenotypes determines the successful resolution of inflammation. The Th1 response is maintained by Interleukin-2 (IL-2) and interferon-gamma (IFN-γ), which inhibits Th2 response [(9)].

Contributing inflammatory mediators and tissue destructive molecules have been detected in the gingival tissues, gingival crevicular fluid (GCF), saliva and serum of patients affected by periodontitis. Qualitative and quantitative changes in the composition of these biomarkers could have diagnostic and therapeutic significance. Furthermore, periodontal therapy reinforces the immune response and leads to the recovery of inflammation by reducing the level of inflammatory mediators in serum, saliva and GCF with significance difference [(10)].

Interleukin-2 (IL-2), is a pro-inflammatory cytokine derived from T-helper 1 cells in response to antigenic stimuli. IL-2 regulates a series of processes in different cells of the immune system including; acts as a T cell growth factor, B- cell activation and the stimulation of immunoglobulin (Ig) secretion by B-lymphocytes, stimulates monocytes/macrophages, promotes proliferation and differentiation of natural killer cells (NK) cells to increase their cytolytic functions, is also essential for the development of Th1, Th2, Treg, and Th17 differentiation and osteoclast activity

(1)Lecturer. Department of Basic Sciences, College of Dentistry, Hawler Medical University. Ph.D. (Clinical Immunology)

[11]. IL-2 has been also implicated in the stimulation of osteoclast activity in bone resorption, and it has been suggested that IL-2 plays an active role in the pathogenesis of periodontal diseases [12]. It is recognized that the metabolic products of periodontopathic bacteria decrease cytokine production including IL-2 [13]. Furthermore, serum levels of IL-2 in patients with chronic periodontitis were higher than in healthy controls [14] suggesting that an elevated serum IL-2 level in untreated chronic periodontitis has the potential to be a biomarker for periodontal tissue destruction. Recent evidence has suggested that IL-2 responses to periodontal pathogens decreased from the mononuclear cells in periodontitis patients [15].It is preferred to determine whether non-surgical periodontal therapy can change the levels of these markers, so that a novel link between periodontal disease and other systemic inflammatory diseases can be explored. Thus, the aim of this study was to compare serum and salivary level of IL-2 between healthy subjects and patients with chronic periodontitis, to determine whether the levels of IL-2 changed after one month of non-surgical periodontal therapy (scaling and root planning, SRP), and to analyse the correlation between serum and salivary IL-2 levels with clinical periodontal parameters.

MATERIALS AND METHODS

Study groups

The present prospective cohort trail was conducted at the Clinic of Periodontology and Microbiology Laboratory of the College of Dentistry, Hawler Medical University from mid November 2014 to May 2015. The study protocol was approved by the Ethical Committee of College of dentistry/ Hawler Medical University. Written informed consent was obtained from all patients for their participation in the study. This study included 50 participants: 25 patients suffering from a severe generalized chronic periodontitis (30 to 45 years; 13 females and 12 males) and 25 periodontally healthy controls (30-42 years; 14 females and 11 males).

Clinical history was recorded for all thestudy group (personal data and medical history). Each subjects had at least 20 standing teeth, periodontitis patients with at least five teeth with sites probing 5mm or deeper and radiographic of alveolar bone loss for screening purposes or presented a sever (loss of supporting bone ≥ 1/3 of root length), generalized (≥ 30% affected sites) periodontal disease. The control group enclosed individuals without any history of periodontal disease and attachment loss, as well as with probing pocket depth (PD) ≤ 3 mm and with bleeding index (PBI) simplified < 20% to exclude the presence of gingivitis. Subjects had received no previous periodontal treatment, antimicrobial therapy or periodontal surgery in the preceding six months, subjects with systemic diseases, tumors, pregnancy, lactating mothers, alcoholism and smokers were excluded. Generalized chronic periodontitis was diagnosed according to the criteria accepted by the American Academy of Periodontology [16].

Sample collection and analysis

All the samples, prior to and after periodontal therapy were collected between 8:00-11:00 A.M. Participants were requested to refrain from eating, drinking (except water), chewing gum, brushing their teeth and using mouth rinsing within at least 2 hours prior to saliva collection to exclude any possible influences. Subjects were asked to rinse their mouth with distilled water thoroughly to remove any food debris, following which they expectorated at least 3 mL of un-stimulated whole saliva into a 5mL sterile tubes before periodontal examination according to the method described by Navazesh [17]. Collected samples were placed on ice pack immediately, then transported to the laboratory andcentrifuged at 3500 rpm for 10 minutes. The supernatant was kept frozen at -40 º C as aliquots until assayed.

A total of 3 mL of blood was drawn from antecubital fossa by vein puncture of all participants at baseline and 1 month after periodontal treatment using a 5cc syringe. Blood was transferred to an appropriately labelled sterile plain tube. Samples were allowed to clot for 1 hour at room temperature, and sera were separated from blood cells by centrifuging at 3000 RPM for 5 minutes. The extracted serum was immediately transferred to a plastic vial and stored at -40 º C until the time of assay[18].Serum and salivary IL-2 levels were measured with an ELISA kit using Human Interleukine-2(IL-2) provided by My Biosource International Inc., USA (Catalog # MBS164623)] according to manufacturer's instructions. The standard range was 5- 2000 ng/L with a sensitivity of 2.51ng/L.

Clinical Parameters

Clinical measurements and radiographic examinations of all participants were performed by a single experienced examiner after collection of serum and saliva samples. Periodontal evaluation included Gingival index (GI), Probing pocket depth (PPD), Clinical attachment level (CAL) and Bleeding on probing (BOP) using

UNC 15 probe were measured at six sites per tooth.

Treatment

Following sampling, the patients received non-surgical periodontal treatment which comprised instruction about oral hygiene and full-mouth scaling and root planning. The clinical parameters were re-evaluated one month after completion of non-surgical treatment and second serum and saliva samples were re-sampled. The study group before phase I therapy was called the BT group, and the same group after completion of 1 month of therapy was called the AT group. Subjects in the control group received no periodontal treatment during the study

Statistical analysis

The data were analysed using a SPSS 21 (Statistical package for the social sciences). Mean and standard deviation (Mean ± SD) are calculated for all the parameters (IL-2, GI, PPD, CAL, BOP) of both subgroups of the test group (at baseline and after 4 weeks of therapy) and control group. Tukey's multiple comparison test was performed to determine the difference of serum and salivary IL-2 levels between the groups. Correlations between IL-2 levels and clinical parameters were assessed by Spearman correlation coefficient analysis. A P-value of $p<0.05$ was considered statistically significant.

RESULTS

Subject variables are listed in Table 1; it showed mean value, standard deviation of Age, GI, PPD, CAL, BOP, and IL-2 in serum and saliva of study and control groups.

Demonstrable levels of IL-2 were found in all serum and saliva samples of study and control groups. The mean values of serum IL-2 levels in patients with chronic periodontitis (BT and AT) and in controls were (382.15± 96.02, 534.18± 127.70, and 10.98± 3.04 ng/L) respectively. For mean values of salivary IL-2 in patients CP (BT and AT) and in controls were (501.82± 88.08, 710.87± 198.15, and20.89± 7.16 ng/L) respectively. IL-2 levels in both serum and saliva in patients with CP increased significantly (p= 0.01, p=0.001) after periodontal therapy, and significantly higher at baseline values than controls ($p < 0.01$, $p < 0.001$) (Table 2).

By Spearman correlation coefficient analysis, showed that there was a significant positive correlation between the increase of salivary IL-2 level with the reduction of PPD after non-surgical periodontal therapy in treatment group (r=0.448, p= 0.025). However, no significant relation of serum and salivary IL-2 levels with other clinical parameters including GI, CAL as shown in (Table 3).

Table 1: The descriptive statistics (mean± SD) for age, GI, BOP, PPD, CAL, serum and salivary IL-2 Levels in control and study group before and after non-surgical periodontal therapy

Parameters	Chronic Periodontitis (N=25)		Control (N=25)
	Before therapy (Mean ± SD)	After therapy (Mean ± SD)	(Mean ± SD)
Age (Years)	38.44 ± 3.59	38.44 ± 3.59	35.72 ± 3.48
GI	2.56± 0.51	1.40± 0.50	1.24± 0.44
PPD	4.73± 0.62	3.12± 0.70	2.40± 0.65
CAL	4.30± 0.56	1.93± 0.44	0.00± 0.00
BOP	48.63± 17.22	11.63± 10.58	0.00± 0.00
Serum IL-2(ng/L)	382.15± 96.02	534.18± 127.70	10.98± 3.04
Saliva IL-2(ng/L)	501.82± 88.08	710.87± 198.15	20.89± 7.16

Data are presented as mean ± standard deviation; GI= gingival index; BOP=bleeding on probing; CAL= Clinical attachment level; PPD=probing pocket depth; IL-2= Interleukin-2.

Table 2: Multiple comparison of serum and salivary IL-2 levels between the groups by Tukey's test

Dependent variable	Groups	Mean difference (Mean ±SD)	t-test	p-value
IL-2 (ng/L) Serum	BT v C	371.17± 97.01	19.13	0.00**
	AT v C	523.20± 128.67	20.33	0.00**
	BT v AT	-152.04± 133.04	-5.71	0.00**
IL-2 (ng/L) Saliva	BT v C	480.93± 89.97	26.73	0.00**
	AT v C	689.99± 198.77	17.36	0.00**
	BT v AT	-209.05± 215.90	-4.84	0.00**

BT; Before therapy; AT= After therapy, C=Control; ** Paired sample t- test: $p < 0.001$, Highly significant.

Table 3: Correlation Coefficient between clinical parameters and serum IL-2 levels in both serum and saliva (Spearman correlation coefficient)

Group	Clinical Parameter	Interleukin-2 (Serum)		Interleukin-2 (Saliva)	
		r	P-value	r	P-value
Before Therapy N=25	GI	0.02	0.94	-0.13	0.52
	PPD	0.03	0.87	-0.03	0.87
	CAL	0.38	0.06	0.25	0.24
	BOP	0.29	0.16	0.24	0.25
After Therapy N=25	GI	0.06	0.78	-0.02	0.94
	PPD	-0.16	0.45	0.45	0.02**
	CAL	0.23	0.26	0.15	0.49
	BOP	-0.32	0.12	-0.03	0.89
Control N=25	GI	0.04	0.85	0.17	0.42
	PPD	-0.27	0.20	0.06	0.79
	CAL	----	----	----	----
	BOP	---	----	----	----

r=Spearman rank correlation coefficient

DISCUSSION

The data from present study revealed that a higher level of IL-2 was expressed in serum and saliva in CP subjects at baseline than in healthy individuals ($p< 0.01$), as was a significant increase observed in CP subjects post treatment (AT) in comparison with the control expression ($p< 0.01$). The concentration of IL-2 in the saliva was found to be greater than serum in all groups. This may be due to localized secretion of the IL-2 from the cells of periodontal tissue in response to inflammation. The present study also demonstrated that the increase of salivary IL-2 level correlated significantly with the decrease of PPD, a clinical parameter indicating local inflammation.

Mcfarlance and Meikle [19] reported that the concentration of IL-2 and soluble IL-2 receptor in serum were significantly higher in patients with severe generalized periodontitis than that in control group, they suggested that IL-2 could be related to the pathogenesis of inflammatory periodontal disease. Also Yetkínet al. [20] demonstrated that the serum level of IL-2 was significantly higher in aggressive periodontitis in comparison to control group ($P< 0.05$). However, in study done by Al-Ghurabeiet al. [21] reported that there were no statistically significant differences in the level of IL-2 in sera of periodontitis patients when compared to the periodontally healthy population ($p > 0.05$). They reported that this lack of statistically significance may reflect the various contributions made by periodontal disease to the total burden of inflammation in different patients and relatively small number of patients .Though, our findings was in contrast with the observations of Gemmell and Seymour (1994) [22], who found low levels of IL-2 and IFN-γ in periodontal lesions, and suggested decreased Th1 responses. Gorskaet al. [23] who founded that higher production of (IL-1β, TNF-α, IL-2, IFN-γ, IL-4, IL-10) in patients with severe CP subjects may be a marker of continuous Th1 response against bacterial pathogens colonized in gingival tissue, with simultaneous suppression of Th2 cell activity. significantly higher concentrations of (IL-1β, TNF-a, IFN-γ and IL-2) found in inflamed tissue in comparison with healthy tissue and serum samples support the local production of these cytokines and thus, may indirectly indicate strong activation of the monocyte/ macrophage system and Th1 cells in inflamed gingival sites, they play an important role in the initiation and progression of periodontitis. Moreover, they founded positive associations between severe CAL and high levels of TNF-α, IFN-γ and IL-2 in periodontitis patients who had pocket depths exceeding 5mm. The effect of initial periodontal therapy on GCF cytokine levels in subjects with generalized sever CP were assessed by Thunell et al. [24] using a multiplex immunoassay, found a significant reductions in total amount of a group of cytokines particularly IL-2 at disease sites as a result of initial therapy. It is well established that with increasing inflammation, there are increasing amount of GCF [25]. This could relatively be the reason for higher concentration of IL-2 in healthy sites compared to disease sites in the previous studies [26]. Although the expression pattern is variable, these findings, together with results of present study, indicating that there is an association between IL-2 and periodontal disease.

Teles et al. [27] conducted a pilot study on 118 subjects; 74 chronic periodontitis and 44 periodontally healthy individuals, and assessed

the salivary levels of different cytokines, including IL-2 by ELISA method. They found that the level of IL-2 was higher in patients with CP than that in control group but statistically not significant ($p > 0.05$) and did not correlate with the clinical parameters. With regard to periodontal diseases, previous data obtained using a protein microarray showed that a higher level of IL-2 was expressed in saliva in periodontitis patients than in healthy controls although differences did not reach statistical significance($p > 0.05$) [28]. In a study by Takeuchi et al. [29]. It was suggested that the expression rate of the interleukin-2 receptor on T-helper cells and B cells was highest in gingival crevicular flow, lower in gingival tissue and lowest in peripheral blood. Moreover, interleukin-2 up-regulates the expression of intercellular adhesion molecule-1 on the surface of human the adhesion of neutrophils is consistently increased [30]. In animal in vitro experiments it was shown that the release of interleukin-2 by mononuclear cells could be activated by a fimbrial protein of Porphyromonas gingivalis [31]. Furthermore, interleukin-2 induced an elevation in the production of osteoclastic acid, corresponding to an increased resorption of bone [32].

There has been absolutely no documented evidence in human regarding the detection of IL-2 in serum and saliva of subjects with CP before and after periodontal therapy, also correlating the local and systemic levels of IL-2 with periodontal clinical parameters which makes this study the first to report such correlation. This study revealed that there was a positive correlation between the increase of salivary IL-2 level with decrease of PPD in CP post therapy ($r= 0.448$, $p= 0.025$). These data suggest that a significant relationship exists between the amount of a proinflammatory cytokine (IL-2) and the destruction of periodontal tissue. Thus, the presence of elevated levels of IL-2 in the saliva of patients with chronic periodontitis, along with the significant correlation with clinical assessments of periodontal tissue destruction, strongly suggests an important role for this mediator in the pathogenesis of periodontal disease.Consequently, we could not find any association between the expression of IL-2 in serum samples from both groups and clinical parameters, indicative of periodontal inflammation, in the entire group as supported in systemic review by Paraskevaset al.[33] who affirmed that chronic periodontitis induces a state of systemic inflammation. The expression pattern is variable, these results, together with our results, indicate that there is an association between IL-2 and periodontal disease, although our study was longitudinal study not cross sectional study or may be due to our follow up, the time interval (one month) between the clinical examinations, before and after treatment may be too short to observe more obvious clinical change in some patients, if we estimated IL-2 in gingival tissue of specific affected site (an inflamed gingival site) may be there is a clear correlation,

In conclusion, based on the finding of the present study, elevated serum and salivary IL-2 levels post-therapy in patients with chronic periodontitis compared to healthy controls may suggest a close association between serum and salivary IL-2 and periodontal status, Therefore, we suggest that serum and salivary IL-2 might be potentially useful in distinguishing health from disease and monitoring periodontal disease activity Further longitudinal studies with a larger population and longer duration should be performed to confirm serum and salivary IL-2 as a marker for periodontal disease.

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