Effect of Periodontal Therapy on Salivary Total Protein, TNF-alpha and IL-1 beta in Chronic Periodontitis Patients

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Abstract

Objective: Periodontitis is a chronic infectious disease of tissues surrounding and supporting the teeth, it causes by microbial plaque accumulation and its severity depend on interaction between pathogenic bacteria and host immune response. This study aimed to evaluate the effect of scaling and root planing on the mean values of salivary total protein (TP), tumor necrosis factor-alpha (TNF-α) and interleukin 1 beta (IL-1β).

Methods: Comparative study conducted on 40 volunteers: 20 of them with chronic periodontitis and 20 with healthy periodontium. Scaling and root planing was conducted for chronic periodontitis patients and unstimulated saliva was collected from all studied subjects at baseline before and after 4 weeks of periodontal treatment to estimate the mean levels of TP, TNF-α and IL-1β.

Results: High mean values of clinical periodontal parameters (plaque index, gingival index, probing pocket depth and clinical attachment loss), TP, TNF-α and IL-1β were detected in chronic periodontitis patients as compared to controls ($p<0.000$), and significant reduction in their mean values were seen after 4 weeks of periodontal therapy as compared to base line before treatment ($p<0.000$).

Conclusions: Biochemical (TP) and immunological parameters (TNF-α and IL-1β) detected at increased levels in chronic periodontitis patients and reduced after periodontal therapy, so further studies are necessary to evaluate the diagnostic and prognostic values of these markers.

Keywords: *Total protein, TNF-α, IL-1β, Chronic periodontitis.*

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Introduction

Periodontal diseases are chronic inflammatory disease in which gingivitis and periodontitis are the most common that depend on a dynamic interactions between the microbial challenge and host inflammatory response[1]. The diagnose of periodontal disease relies almost and fundamentally on traditional periodontal diagnostic parameters including plaque index (PI), gingival index (GI), probing pocket depth (PPD), bleeding on probing (BOP), clinical attachment loss (CAL) and alveolar bone loss)[2]. However, these traditional diagnostic procedures are limited in that only disease history can be assessed and not the current disease status (these measure are useful in detecting evidence of past disease or verifying periodontal health, but they provide only limited information about patients and sites at risk for future periodontal breakdown[2]. Therefore, advances diagnostic research in oral and periodontal diseases are moving toward developing methods whereby periodontal risk can be justified and quantified by objective measures like biomarkers[3]. Nowadays various researches are conducting to evaluate possible compounds in the oral fluids through which it may be possible to assess the presence and severity of these diseases as well as to identify the patients at risk for these diseases[4]. Therefore, analysis of saliva can contribute to the diagnosis and prognosis of the disease[5]. Total protein is a vital component of saliva and play role in various saliva functions like lubrication, physical protection, cleansing, buffering, maintenance of tooth integrity, taste and antibacterial activity[4-6]. Cytokines are small polypeptides with a wide range of inflammatory, metabolic and immunomodulatory properties, which are produced by a variety of cells including macrophage/monocyte system, dendritic cells, lymphocytes, neutrophils, endothelial cells and fibroblasts[7,8]. All periodontopathogenic bacteria as well-known as extracted lipopolysaccharides have primarily been shown to stimulate monocytes to produce cytokines such as IL-1β and TNF-α[9,10]. Interleukin-1β and TNF-α represent proinflammatory cytokines that stimulate a cascade of reactions which occur during periodontal disease, including induction of adhesion molecules and other mediators that facilitate and amplify the inflammatory response, stimulation of matrix metalloproteinase and bone resorption[11]. Tumor necrosis factor-α can be detected in saliva and gingival crevicular fluid of both healthy and periodontitis patients[12] and the increased concentration in periodontitis correlated closely with the tissue destruction and immune response[13]. Interleukin-1β is a highly proinflammatory cytokine and the margin between clinical benefit and toxicity in humans is exceedingly narrow[14]. The balance between the local levels of cytokines, stimulated in response to periodontopathogenic bacteria and their products is important in determining the outcome of an immune response to a given pathogen[15]. Study reported that IL-1β, IL-6 and TNF-α are present in low quantities in clinically healthy gingival tissue[16], this means that cytokines are prominent factors of normal tissue homeostasis[17]. Evidence suggested that interleukin can induce and activate metalloproteinase and tissue inhibitors of metalloproteinase[18,19], which are believed to be critical in initiating the collagenolytic cascade and regulation of connective tissue degeneration under both physiologic and pathologic conditions. A study demonstrated that the biological activity of a variety of cytokines could be directly related to periodontal destruction such as periodontal attachment loss, destruction of collagen and alveolar bone resorption[20]. Another study reported that IL-1β level in inflamed periodontal tissue was highly correlates with clinical parameters including gingival and plaque indices and degree of inflammation[21]. This study aimed to evaluate the effect of scaling and root planing on clinical periodontal parameters (PI, GI, BOP, PPD and CAL), salivary TP and inflammatory cytokines (IL-1β, and TNF-α) after 4 weeks of periodontal therapy and compare them with base line records before treatment**.**

Patients and methods

Setting and time

The study was carried out in periodontics clinic and biochemistry laboratory of basic science department of College of Dentistry/ Hawler Medical University from January, 2018 to March, 2018.

Subjects

Subjects with periodontitis and clinically healthy periodontium were recruited in this study. Chronic periodontitis group had two periodontal pockets depth of ≥ 4 mm in each quadrant and clinical attachment loss of ≥ 5mm in one or more sites in two or more teeth[22] and clinically healthy subjects have no clinical sign of gingivitis (mean of GI =0.04, and BOP =0) or periodontitis (gingival sulcus depth ≤3 , PPD ≥4 =0 , and clinical attachment loss CAL=0). The inclusion criteria

were: presence of at least 20 teeth, systemically healthy, no history of periodontal treatment or drug intake for the last 6 months. The excluding criteria were: smokers, alcoholic, pregnant, lactating and post-menopausal females. Study protocol was reviewed and approved by the institutional ethical committee of College of Dentistry/ Hawler Medical University. Furthermore, all participants were informed about the purpose of this study and signed written consent forms.

Intra oral clinical examination

All participants underwent complete clinical examination after salivary sample collection for control group, and for chronic periodontitis patients group at base line before treatment and after 4 weeks of treatment. Clinically, for measuring plaque score according to PI[23] by using a straight sharp explorer and measure the amount of bacterial plaque for four surfaces of all the examined teeth and given a score from 0-3. Measurement of the extent and severity of gingival inflammation according to GI[24,25] inspection by naked eyes and by gentle probing through using Williams periodontal probe for four gingival surfaces of all examined teeth (facial, lingual, mesial and distal) and given a score from 0-3. Measurement of BOP[26] was performed by passing periodontal probe gently along the inner surface of gingival sulcus or pocket wall, bleeding was noted after 30 seconds and recorded as absent (-) or present (+). The bleeding is expressed in percent (number of sites positive for bleeding divided by the number of measured sites multiplied by 100.

Probing pocket depth was assessed by inserting calibrated Williams periodontal probe from gingival margin to the base of the sulcus or pocket at four surfaces of each tooth, no pressure was used; the probe was allowed to fall by its own weight and the sites for measurement were mesio-buccal (labial), mid- buccal (labial), disto-buccal (labial), mid-lingual (palatal)[27]. And clinical attachment level was assessed by measuring the distance by a calibrated Williams periodontal probe from cemento-enamel junction (CEJ) to the base of the pockets, when CEJ was obliterated by the gingival margin, the CAL was measured indirectly by subtracting the distance in millimeters from the gingival margin to the CEJ from PPD at each site, and in case when there was a gingival recession, loss of attachment was measured by adding the distance from gingival margin to the CEJ to PPD at each sites[27].

Patients with chronic periodontitis were under conventional periodontal treatment consisting of motivation, oral hygiene instructions, scaling (supra and sub gingival), root planing and polishing.

Saliva sample collection

Seven ml of un-stimulated saliva samples were collected from all 40 patients at 9-10 a.m. The patients were in a seated position with the head inclined forwards and spitting method was used for collection of un-stimulated saliva from the patients with chronic periodontitis at base line before treatment and after 4 weeks of periodontal treatment. Furthermore, un-stimulated saliva samples were collected from all controlled healthy patients. Saliva was collected in sterilized plastic test tubes during 5 minutes period and immediately was centrifuged at 3000 rpm for 20 minutes to obtain clear supernatant, then divided into 3 parts and placed in Eppendorf tubes, finally stored at -20ºc for later estimation of TNF-α, IL-1 beta and TP.

Immunological investigation

The measurement of salivary TNF-α and IL-1β by indirect enzyme linked immunosorbent assay (ELISA technique) supplied by specific kits (IL-1β kit/KOMA BIOTECH INC, USA), (TNF-α kit/ KOMA BIOTECH INC, USA), which used for estimation of body fluid, and the procedure was performed according to the manufacturer's instructions.

Biochemical investigation

The estimation of salivary TP (g/ml) based on the Biuret method[28]. Protein forms a colored complex with cupric ions in alkaline medium. Based on this principle, salivary protein estimation was done by mixing undiluted saliva with the reagent (45 g of Rochelle salt and 15g of copper sulfate in 400 mL of 0.2 N sodium hydroxide. Five grams of potassium iodide was added to make up to 1L with 0.2N sodium hydroxide) and measuring the colored product using a photoelectric colorimeter at a wavelength of 546nm (colorimeter visible light of spectrophotometer). Standard solution of 6 g of bovine albumin dissolved in 100 mL of normal saline containing 0.1 g/dL sodium azide was used[28].

Statistical analysis

The SPSS (version 22) was used, paired T-test was used to compare before and after treatment and independent T-test were used for comparison between the two study groups, P value <0.05 was considered as statistically significant.

Results

A total of 40 subjects comprised of 15 females and 25 males with an age of 25-45 years were enrolled in this study and the mean age for female and male were 38.47±5.84, 37.20 ± 5.98 years, respectively.

Clinical results

Table 1, showed high significant reduction in the mean values of PI, GI, BOP, PPD and CAL after 4 weeks of treatment for chronic periodontitis patients (0.80±0.33), (0.911±0.217), (38.89±3.00%), (3.31±0.29mm) and (3.52±0.34mm) as compared to their mean values at base line before treatment (1.93±0.34), (1.80±0.37) (78.68±5.03%), (4.60±0.30mm) and (5.65±0.23mm), respectively, (P≤0.000). Table 2, showed the comparison between control and chronic periodontitis patient groups in case of base line before treatment and after 4 weeks of periodontal therapy in relation to the mean values of PI, GI, PPD, CAL and BOP and the results showed high significant differences in the mean values of PI, GI, BOP, PPD and CAL scores in control group (0.12±0.08) (0.04 ±0.02), (0), (1.79±0.29mm) and (0) as compared to periodontitis patients at base line before treatment (1.93±0.34) (1.80±0.37) (78.68±5.03%) (4.60±0.30mm) (5.65±0.23mm) (P≤0.000), respectively. Furthermore, high significant differences were found in relation to the mean values of PI, GI, BOP, PPD and CAL between the control (0.12±0.08), (0.04±0.02), (0) and (1.79±0.29mm) (0) and chronic periodontitis groups after 4 weeks of treatment (1.93±0.34), (1.80±0.37), (78.68±5.03%), (4.60±0.3mm) and (5.65±0.23mm), respectively, (P≤0.000).

Immunological and biochemical results

Table 3, showed high significant reduction in the mean values of TNF-α, IL-1β and TP in saliva from (12.1±0.69 pg/ml), (14.32±0.82 pg/ml) and (2.74±0.74 g/ml) at base line before treatment into (7.97±0.20 pg/ml), (8.67± 2.1 pg/ml) and (1.34±0.05 g/ml), respectively, after 4 weeks of treatment (P≤0.000). Table 4, showed the comparison between control and chronic periodontitis groups (at base line before treatment and after 4 weeks of periodontal therapy) using independent t-test in relation to the mean values of TNF-α, IL-1β and TP. The results showed high significant differences in the mean values of TNF-α, IL-1β and TP between control (2.28±0.06 pg/ml) (7.93±0.13 pg/ml) (1.66 ±0.09g/ml) and chronic periodontitis group at base line before treatment (12.1±0.69 pg/ml) (14.32±0.82 pg/ml) (2.74±0.74 pg/ml), respectively (P≤0.000). Moreover, high significant differences in relation to the mean values of TNF-α and TP between control (2.28±0.06 pg/ml) (1.66±0.09g/ml) and chronic periodontitis group after treatment (7.97±0.20 pg/ml) (1.34±0.050 g/ml), respectively (P≤0.000), and non-significant differences was found between control group (7.93±0.13 pg/ml) and periodontitis group (8.67±2.10 pg/ml) in relation to IL-1β after 4 weeks of treatment (P=0.124).

Table 1: Comparison between the mean values of PI, GI, PD, CAL and BOP in patients with chronic periodontitis at base line before treatment and after 4 weeks of treatment (N= 20).

Variable	Time	Mean± Std. Deviation	Paired t-test	df	P-Value
PI	Before	1.93 ± 0.34	11.600	19	0.000$^{(HS)*}$
	After	0.80 ± 0.33			
GI	Before	1.80 ± 0.37	10.293	19	0.000$^{(HS)}$
	After	0.91 ± 0.21			
BOP %	Before	78.68 ± 5.03	29.895	19	0.000$^{(HS)}$
	After	38.89 ± 3.00			
PD(mm)	Before	4.60 ± 0.30	17.112	19	0.000$^{(HS)}$
	After	3.31 ± 0.29			
CAL(mm)	Before	5.65 ± 0.23	20.984	19	0.000$^{(HS)}$
	After	3.52±0.34			

* HS= highly significant.

Table 2: Comparison between control and chronic periodontitis patient groups (at base line, 4 weeks after treatment) in relation to PI, GI, BOP, PD and CAL.

	Groups		Mean±Std. Deviation	Independent t-test	df	P-Value
	Control N (20)	Chronic periodontitis N (20)				
PI	0.12±0.08	Base line	1.93±0.34	23.17	38	0.000(HS)
		After	0.80±0.33	8.75	38	0.000(HS)
GI	0.04±0.02	Base line	1.80±0.37	21.02	38	0.000(HS)
		After	0.91±0.21	17.66	38	0.000(HS)
BOP %	0	Base line	78.68±5.03	69.85	38	0.000(HS)
		After	38.89±3.00	57.84	38	0.000(HS)
PD(mm)	1.79±0.29	Base line	4.60±0.30	29.56	38	0.000(HS)
		After	3.31±0.29	16.47	38	0.000(HS)
CAL(mm)	0	Base line	5.65±0.23	108.86	38	0.000(HS)
		After	3.52±0.34	45.96	38	0.000(HS)

Table 3: Comparison between mean values of TNF-α, IL-1β and TP in patients with chronic periodontitis at base line and after 4 weeks of treatment (N= 20).

	Time	Mean± Std. Deviation	Paired t-test	df	P-Value
TNF-α (pg/ml)	Before	12.18 ± 0.69	27.17	19	0.000(HS)
	After	7.97 ± 0.20			
IL-1β (pg/ml)	Before	14.32 ± 0.82	10.59	19	0.000(HS)
	After	8.67 ± 2.10			
TP (g/ml)	Before	2.74 ± 0.74	8.33	19	0.000(HS)
	After	1.34 ± 0.05			

Table 4: Comparison between control and chronic periodontitis patient groups (at base line and 4 weeks after treatment) in relation to the mean values of TNF-α, IL-1β and TP.

	Groups		Mean±Std. Deviation	Independent t-test	df	P-Value
	Control N (20)	Chronic periodontitis N (20)				
TNF-α (pg/ml)	2.28±0.06	Base line	12.18±0.69	63.53	38	0.000(HS)
		After	7.97±0.20	119.74	38	0.000(HS)
IL-1β (pg/ml)	7.93±0.13	Base line	14.32±0.823	34.281	38	0.000(HS)
		After	8.67±2.101	1.575	38	0.124(NS)*
TP (g/ml)	1.66 ±0.09	Base line	2.74±0.744	6.450	38	0.000(HS)
		After	1.34±0.050	-13.582	38	0.000(HS)

*= Not significant

Discussion

Clinical results

The results showed that scaling and root planing had a significant positive effect in reduction of gingival inflammation and healing of periodontal tissue throughout its great effect in reduction of the mean values of clinical periodontal parameters (PI, GI, BOP, PPD and CAL) after 4 weeks of periodontal therapy, this may be due to that periodontal therapy disrupts the subgingival plaque biofilm, allowing shift in the microbial population to those more commonly associated with health[29-31]. The results of this study are in agreement with other studies that scaling and root planing by using manual or sonic driven scalers in subgingival pockets resulted in reduction of BOP[29-31], PPD and CAL[29-34]. Other studies evaluate the effect of scaling and root planing on periodontal disease and concluded that the treatment is effective and reliable throughout removing of the elements that initiate and provoke gingival inflammation (i.e. plaque, calculus and endotoxin) from the tooth surfaces[27,35,36,37]. Studies ranging from 4 weeks to 2 years demonstrated up to 80% reduction in BOP and mean PPD reduction of 2 to 3mm[27,35].

Biochemical result

The results showed high mean values of TP in saliva of chronic periodontits patients, and scaling and root planing had a profound significant effect in reduction of its mean values after 4 weeks of periodontal therapy. The high protein levels in periodontitis patients could be due to the inflammatory process that activates the sympathetic system to enhance the synthesis and secretion of TP thereby increasing the protective potential effect of saliva against the diseases, our result is consistent with other studies[38,39], also the increased levels could be due to an increased leakage of plasma proteins into saliva due to inflammation as suggested by a study done by Henskens et al, (1996)[39]. In the present study, the decrease in TP level after treatment perhaps due to reduction in the leakage of protein into saliva due to the inhibition of inflammatory reaction and healing of periodontal tissue. Our result is consistent with other studies which showed similar results[39,40].

Immunological results

The present study showed increase in the mean levels of TNF-α, and IL-1β in saliva of chronic periodontitis patients, and scaling and root planing had a significant effect in reduction of their mean levels after 4 weeks of periodontal therapy. The increase level is closely correlated to tissue destruction and increased host immune response due to inflammation[41-43]. Our results are consistent with other studies that showed similar results[11,13]. Since IL-1beta and TNF-α cytokines are biologically active glycoproteins that secreted by host immuno-inflammatory cells in responses to inflammation and have a role in the inflammatory process, in which the activity of both cytokines coincides with the critical events that occur during periodontal disease, namely loss of attachment and bone resorption. Thus, the majority of tissue destruction that occurs during periodontitis is attributed to IL-1 and TNF activity, so tissue destruction may be very well represented as an over activity of host response to periodontal pathogens that stimulate excessive production of IL-1 and TNF[11,13]. Furthermore, reduction of their levels after treatment could be due to the decrease of their secretion by host immuno-inflammatory cells into saliva due to reduction of inflammatory reaction (reduction of bacterial plaque and their products by scaling and root planing) and healing of periodontal tissue, our results are in agreement with Frodge et al, (2008)[41] and Gamonal et al, (2000)[44]. More studies are required to identify the specific role of scaling and root planing on the mean values of TP, IL-1β and TNF –α in patients with periodontal diseases.

Conclusions

There was association between severity of periodontal inflammation and salivary levels of TP, IL-1β and TNF-α levels in the saliva perhaps due to over production of these mediators due to increased level of periodontal inflammation. Furthermore, their reduced level after treatment might be due to significant reduction of periodontal inflammation, which is represented by reduction in PI, GI, BOP, PPD and CAL. Thus, these markers may be considered as an important biochemical and immunological biomarkers of periodontal inflammation.

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