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IMPACT OF SMOKING ON THE IL-1B, IL-8, IL-10, IL-17 AND TNF-α PRODUCTION IN CHRONIC PERIODONTITIS PATIENTS

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ABSTRACT

Background: *Cigarette smoking is a significant risk factor in the pathogenesis and progression of periodontal disease, leading to an increase in periodontal tissue destruction as a consequence of altered production of cytokines, inflammatory markers and host-cell function.* ***Objectives:*** *The purpose of the present investigation is to study the serum cytokine profile in chronic periodontitis subjects and the influence of cigarette smoking on the serum levels of cytokines.* ***Subjects and Methods:*** *Sixty subjects with chronic periodontitis (30 smokers and 30 non-smokers) and 30 healthy control individuals participated in this study. Serum samples separated from the whole blood of patients and healthy individuals. The levels of cytokines were determined by an enzyme-linked immunosorbent assay.* ***Results:*** *The study showed statistically significant elevation in levels of IL-1β, IL-8 and IL-17 in chronic periodontitis patients compared to healthy control ($p<0.001$). Moreover, the IL-1β, IL-8 and IL-17 levels were also significantly higher in smokers than non-smokers patients ($p<0.05$). On the other hand, there are no significant differences in levels of IL-10 and TNF-α between patients and healthy controls and nor between smokers and non-smokers ($p>0.05$).* ***Concussion:*** *The present findings suggest that serum levels of IL-1β, IL-8 and IL-17 reflect the activity of periodontal destruction, as well as the enhanced production of these inflammatory cytokines (IL-1β and IL-17) and chemokin (IL-8) in the presence of smoking may have clinical consequences. Thus these results could be one of several explanations why smoking aggravates periodontitis.*

Keywords: Periodontitis, Smoking, Cytokines

INTRODUCTION

Periodontitis is defined as inflammatory disease of supportive tissue of teeth caused by specific microorganism which lead to progressive destruction of periodontal membrane and alveolar bone, with formation of periodontal pockets and gingival recession (Savage *et al.*, 2009).The incidence and progression rate of periodontal disease depends on complex interaction between

periodonotopathic bacteria, cells of the host immune system and environmental factors (Page *et al.*, 1997). These interactions are mediated by cytokines produced by heavy lymphocytic infiltration into periodontal tissues, and these cytokines represent an important component of the immune response to bacterial lipopolysaccharides (LPS), (Deo and Bhongade, 2010; Buduneli and Kinane, 2011). Cytokines are the mean of communication between immune and non-immune cells (Kinane and Lindhe, 1997). It has been postulated that cytokines are central to the pathogenesis of number of diseases, including periodontal disease (Gemmell, 2004). However, evaluation of serum cytokines is crucial in determining the systemic inflammatory response in periodontitis patients. It is worthy to mention that for many years, the pathogenesis of periodontitis was involved an immunological Th1/Th2 paradigm. According to this view, the tissue destructive Th1 cells and cytokines would arise in the early period of the disease, while the tissue protective Th2 cells and cytokines would arise in the late phase. However, in several clinical contexts, the Th1/Th2 balance/imbalance is not sufficient to explain the progression and/or remission of periodontitis observed in patients (Gaffen and Hajishengallis, 2008; Garlet, 2010; Arun *et al.*, 2011).Th17 subset of CD4+ T cells was identified in 2003, and added greater complexity to Th function. Th17 cells are generally considered to be proinflammatory, through the production of IL-17. These cells and their cytokines have been associated with the pathogenesis of numerous autoimmune and inflammatory diseases, including rheumatoid arthritis, inflammatory bowel diseases, psoriasis (Shahrara *et al.*, 2008; Abraham and Cho, 2009; López, 2012), and periodontitis (Vernal *et al.*, 2005). The majority of studies have reported increased IL-17 levels associated with the development of chronic periodontitis (Laine *et al.*, 2012).

Cigarette smoking is considered to be one of the most important environmental risk factors, which is closely related not only with the risk but also the prognosis of periodontitis (Miki and Hanioka, 2010). It is well accepted that smoking alters the host response, including vascular function, neutrophil/monocyte activities, adhesion molecule expression, antibody production, as well as cytokine and inflammatory mediator release (Ryder, 2007; Gaudy *et al.*, 2009). However; cigarette smoking has been implicated in the activation of a complex inflammatory cascade resulting in the production of a variety of potent cytokines and chemokines, which in turn contribute to development of periodontal disease. Hence; the present study was undertaken to study the serum cytokine profile (IL-1β, IL-8, IL-10, IL-17 and TNF-α) in chronic periodontitis subjects and the influence of cigarette smoking on the serum levels of these cytokines in patients with chronic periodontitis.

Subjects and Methods

The test group consisted of 60 Iraqi patients (mean age: 42.72± 7.84 years; range: 30–55 years) with chronic periodontitis. Patients who smoked a minimum of 10 cigarettes per day for 2 years were included in the smoker periodontitis group (30), the remainder of the patients who never smoked were assigned to the non smoker periodontitis group (30). They were selected among people referring to periodontics departments in College of Dentistry, Baghdad University for diagnosis and treatment of periodontitis from November 2012 till January 2013, who were

volunteers to participate in this study. Diagnosis was made by specialized dentists (single examiner conducted the periodontal assessment in order to minimize the variation in the data), all the cases had received no treatment with no complain of other chronic or systemic diseases.The control group included 30 healthy unrelated patients, age and sex matched (mean age: 39.48±4.89 years; range: 25–55 years). All of them didn't have medical history or clinic evidence of any chronic or acute diseases. They were from the staff and graduate students of College of Dentistry.Plaque index (PI), gingival index (GI), probing pocket depth (PPD), clinical attachment level (CAL) and bleeding on probing (BOP) were employed as clinical periodontal parameters in our study. Whole blood from patients and healthy individuals was collected. Serum samples were separated from the whole blood, aliquated and stored at -20ºC until used. The level of IL-1β, IL-8, IL-10, IL-17 and TNF-α were determined by using commercially available enzyme-linked immunosorbent assay (ELISA) kits and performed as recommended in leaflet with kits (IL-1β, IL-8, IL-10 and TNF-α- BioSource Europe S.A. Company/ Belgium ; IL-17- Ray Bio/ USA).

Statistical Analysis: Statistical analyses were done using SPSS v13. The age, number of teeth and clinical periodontal parameters were expressed as mean ± standard deviation, the significance of differences in mean was assessed using the student's t-test and ANOVA test.The outcome quantitative variable (IL-1β, IL-8, IL-10, IL-17 and TNF-α) were non-normally distributed. Such variable is described by median. The difference in median of quantitative non-normally distributed variable groups was calculated by Kruskal-Wallis-test and Mann-Whitney-test. Analyses where the *P*-value was <0.05 were considered to be statistically significant.

RESULTS

This study was performed on 60 patients with periodontitis and 30 healthy individuals without any periodontal disease. There were 38 males and 22 females in the periodontitis patients, and there were 19 males and 11 females in the healthy individuals group.

Table 1 showed that the mean age of periodontitis patients was 42.72±7.84 years, whereas for healthy subjects was 39.48±4.89 years with no significant differences ($p>0.05$). Moreover, the smoker group had a mean number of 24.37±2.58 teeth present in the mouth at the time of examination. The non smokers and healthy group had slightly higher numbers of teeth present, with a mean number of 27.07±1.14 teeth and 27.84±0.94 teeth, respectively. All clinical periodontal parameters were significantly higher in the patients group as compared to the healthy control. On the other hand, the smoker group had a significantly higher mean levels of PI, PPD and CAL compared to non-smokers ($p<0.001$), while there was no significant differences in each of GI and BOP between two groups of patients ($p>0.05$), as clearly show in Table 2.

The study showed statistically significant elevation in median levels of IL-1β, IL-8 and IL-17 in chronic periodontitis patients (82.10; 17.10; 465.0 pg\ml respectively) compared to healthy control (65.38; 9.40; 202.50 pg\ml respectively), ($p<0.05$; $p<0.001$). Moreover, the IL-1β, IL-8 and IL-17 levels were significantly higher in smokers (91.60; 19.90; 617.50 pg\ml respectively), than non

smokers patients (70.30; 11.10; 321.50 pg\ml respectively), ($p<0.05$; $p<0.001$). On the other hand, there are no significant differences in levels of IL-10 and TNF-α between patients and healthy controls and nor between smokers and non-smokers ($p>0.05$), as observe in Table 3.

Table-1. Age and Number of Teeth Present among Studied Groups

	Study groups		Patients groups	
	Healthy control (n=30)	Chronic periodontitis (n=60)	Smokers (n=30)	Non-smokers (n=30)
Age (years) Mean±S.D	39.48±4.89	42.72±7.84^{aNS}	42.60±8.31	42.83±7.48^{bNS}
number of teeth Mean±S.D	27.84±0.94	25.72±2.40^{a**}	24.37±2.58	27.07±1.14 ^{b**}

a: comparison between chronic periodontitis and healthy control group; b: comparison between smokers and non-smokers chronic periodontitis groups; NS: not significant; ** :highly significant.

Table-2. Clinical Periodontal Parameters among Studied Groups

	Studygroups		Patients groups	
	Healthy control	Chronic periodontitis	Smokers	Non-smokers
Plaque index	0.66±0.20	1.47±0.57a**	1.67±0.51	1.27±0.57b**
Gingival index	0.54±0.14	1.29±0.43a**	1.31±0.35	1.19±0.45b^{NS}
Clinical attachment loss	0.0	1.9±0.6a**	2.97±0.72	1.73±1.03b**
Pocket depth	1.01±0.59	2.35±0.56a**	2.54±0.54	2.16±0.51b**
Bleeding on prob	6.00±11.75	23.76±30.61a**	14.03±22.54	20.49±34.67b^{NS}

Table-3. Descriptive Statistics of Serum IL-1β, IL-8, IL-10, IL-17 and TNF-α Levels among Studied Groups

	Study groups		Patients groups	
	Healthy control	Chronic periodontitis	Smokers	Non-smokers
IL-1β	65.38 89.09±56.30	82.10a* 118.06±105.77	91.60 126.54±110.89	70.30^{b*} 111.58±102.18
IL-8	9.40 13.93±19.25	17.10^{a*} 23.22±23.01	19.90 28.37±24.51	11.10^{b*} 22.08±21.78
IL-10	9.00 8.45±8.13	3.40^{aNS} 8.59±9.88	4.00 8.28±9.85	3.40^{bNS} 8.91±10.08
IL-17	202.50 226.54±76.78	465.00^{a**} 508.17±316.35	617.50 582.77±310.62	321.50^{b**} 433.57±309.20
TNF-α	5.30 17.82±23.10	6.32^{aNS} 26.20±28.84	7.60 29.93±31.06	5.30^{bNS} 22.46±26.42

*: significant

DISCUSSION

Periodontitis is an infectious disease caused by anaerobic Gram-negative bacteria (Nishida *et al.*, 2006). Several studies in recent years were conducted to confirm the role of cytokines in the

pathogenesis of chronic periodontitis. However; it appears that the secretion of cytokines may be influenced by certain environmental factors, cigarette smoking is one of those of the greatest significance (Al-Ghamdi and Anil, 2007).

This study is the first in Iraq to investigate the influence of smoking on cytokines production in chronic periodontitis. The current results denoted that the median levels of IL-1β, IL-8 and IL-17 are significantly higher in the systemic circulation of periodontitis patients than those in healthy control. Our results are consistent with other studies (Vernal *et al.*, 2005;Zahraa and Batool, 2012; Cetinkaya *et al.*, 2013). In 2005 Keles et al. found that the concentrations of IL-1β were significantly higher in serum and gingival tissue biopsies samples in patients as compared to healthy control (Keles *et al.*, 2005). Furthermore; Gorska and colleagues suggested that IL-1β level is a sensitive and reliable marker of chronic inflammatory disease activity and IL- 1β elevation may demonstrate tissue destruction (Gorska *et al.*, 2003). On the other hand, this is contrary to the findings of Elkhouli who mentioned that there were no differences in the levels of IL-1β between patients and healthy control (Elkhouli, 2011). Our study also showed greater levels of IL-8 in patients, the present result is in agreement with (Giannopoulou *et al.*, 2003).

Since their discovery in 2003, there have been numerous studies on Th17 cells, and they have emerged as key players in the pathogenesis of some inflammatory diseases, but their role in pathogenesis of periodontal disease is not fully understood. IL-17 is a pro-inflammatory cytokine and the prototype cytokine for a subset of Th17 cells (Gaffen and Hajishengallis, 2008). Several reports mentioned that periodontitis patients have higher IL-17 levels when compared to the healthy population (Takahashi *et al.*, 2005; Lester *et al.*, 2007). An increase in IL-17 was also observed in our study with a chronic periodontitis subject. In contrast to this finding other study conducted by Avani et al. in Indian population, who observed that the concentration of IL-17 in chronic periodontitis patients was close to 0 pg/µl (Avani *et al.*, 2009). It is noteworthy as well that Th17 has been shown to be a key Th cell subset associated with bone resorption and osteoclast generation (Sato *et al.*, 2006). Our findings indicated that in addition to Th1 and Th2 responses in the periodontitis pathogenesis, Th17 immune track also causes the host immunological response to the periodontal pathogens.

It is possible that high levels of serum IL-1β, IL-8 and IL-17, is derived initially from gingival tissues as reponse to bacterial stimulation, es-pecially by LPS, may mediate inflammatory responses in tissues distant from the oral cavity in those patients. However; these results are consistent with the hypothesis that locally produced cytokines finds its way into the systemic circulation in chronic periodontitis.

An anticipated, the current results are in line with other studies reported that there was no significant difference in median levels ofIL-10 and TNF-α between patients and healthy control (Bodet *et al.*, 2006; Ladez *et al.*, 2012).While other study in Iraq (Zahraa and Batool, 2012),

reported that there was siginficant elevation in TNF-α levels and reduction in the levels of IL-10 among chronic periodontitis paeitnts when compared to healthy control.

The effect of smoking on cytokines production in periodontal patients has been extensively investigated and conflicting results have been reported. In one hand Bostrom and associate (Bostrom *et al.*, 2000) observed no influence of smoking on the levels of IL-1β, on the other hand (Rawlinson *et al.*, 2003) found that the level of IL-1β was lower in smokers vs. non smokers, suggested that production of pro-inflammatory biomarkers is depressed in smokers, but these mediators are still present at concentrations capable of pathogenesis. However, similarly to our findings, a higher amount of IL-1β was observed in smokers when compared to non smokers were reported by Kamma and colleagues (Kamma *et al.*, 2004). Also in this study, IL-8 had a significant elevation in smoker patients than non smokers, however; results of other reports are different. Some, like our study reported increase secretion of IL-8 in smoker periodontitis pateints (Moi *et al.*, 1997), but other found that smoking was reduce the sensitivity of peripheral neutrophils to stimulation of IL-8 in smokers (Fredriksson *et al.*, 2002). It may be suggested that tobacco smoke activates more cells of the periodontium to express IL-8, thereby resulting in a local accumulation of PMN's (Yoshimura *et al.*, 1997). When the influence of smoking is examined in regards to IL-17 levels, this study showed increase in IL-17 among smokers. This is in agreement with the observations of Buduneli and colleagues (Buduneli *et al.*, 2009).

Finally in contrast to the findings by (Cesar-Neto *et al.*, 2007), who stated that IL-10 and TNF- α were depressed in smokers with periodontitis as compared to non smokers with periodontitis, current finding failed to show any significant differences between two groups of patients. Elevation of serum IL-1β, IL-8 and IL-17 levels detected in current study among smokers could be due to response to LPS of gram negative bacteria were increased in smokers (Bostrm *et al.*, 1999). Another explaination for this elevation may be due to nicotine in cigarette smoke that affects the host inflammatory response to oral pathogens by upregulating release of prostaglandin and IL-2 leading to accelerated periodontal tissue destruction (Axelsson, 2005). These new findings deserve further investigation to confirm the differences observed in this study. Within the limitations of this study, we conclude that these results suggest that serum levels of IL-1β, IL-8 and IL-17 reflect the activity of periodontal destruction, as well as the enhanced production of these inflammatory (IL-1β and IL-17) cytokines and chemokin (IL-8) in the presence of smoking may have clinical consequences. Thus these results lead to uncovering the basis for the etiological role of cigarette smoking in peridontitis.

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