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Serum and Salivary Markers in Aggressive Periodontitis Patients in Mosul

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Abstract

The aims of the study is to assess and compare serum levels of cytokines (IL-8,IL-6,IL-1β,IFN-γ and TNF-α) and salivary markers (sIgA, peroxidase, peroxidase specific activity and total protein) in aggressive periodontitis patients and periodontally healthy individuals in Mosul. This study was carried out on a total number of 49 subjects, 24 Aggressive Periodontitis patients,14 males and 10 females, aging between 20 - ≥40 years, and 25 healthy subjects as control group which consisted of 14 males and 11 females aging between 20-45 years. All patients were systemically healthy. Five ml of venous blood and saliva were withdrawn from each patient and control subject, for assessments of serum cytokines (IL-8,IL-6,IL-1β,IFN-γ and TNF-α) and salivary markers (sIgA, total protein and peroxidase specific activity) using ELISA and biochemical test. The comparison of serum cytokines and salivary markers between study and control groups showed significance increase in serum IL-6 and TNF-α level in Aggressive Periodontitis group. Males showed increase in IL-8, IL-6, IFN-γ, TNF-α level, peroxidase and peroxidase specific activity comparing with females, but there increase was not significant. Within Aggressive Periodontitis group, serum IL-1β and salivary sIgA showed significance differences according to age specially in ≥ 40 year age group. There was unclear correlation between serum cytokines and salivary parameters according to the increase of periodontal pocket depth.

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This study also showed direct correlation between serum IL-1β and Salivary peroxidase, also between (IgA, total protein and peroxidase Specific activity in saliva). Finally we found a direct correlation between peroxidase specific activity and total protein.

Keywords: Aggressive Periodontitis; Serum and Salivary Markers.

1. Introduction

Aggressive Periodontitis (AP), as the name implies, is a destructive type of periodontitis characterized by severe periodontal attachment loss and rapid destruction of alveolar bone around the teeth in conjunction with formation of periodontal pockets. AP affects systemically healthy individuals aged <30years but older patients may be affected, The global prevalence of AP is much lower than chronic periodontitis and seems to range from 1 % to 15% of population [1,2]. Some types of AP seems to be inherited in a Mendelian manner [3,4,5], with racial and sex tendency, thus adolescence black males have higher risk for the disease than adolescence whites females, although there is variation between ethnic groups and populations [6]. AP consists of two forms "localised aggressive periodontitis" (LAP) and "generalized aggressive periodontitis" (GAP) [7], replaces the older term "localized juvenile periodontitis". In general the prevalence of LAP is nearly 1% and that of GAP is 0.13%. However in Asia the prevalence rate of 1.2% for LAP and 0.6% for GAP in Baghdad and Iran population, whereas 0.47% in Japanese population [8]. Besides infection with specific microorganisms [4,9], a host tendency seems to play a key role in the pathogenesis of AP as evidenced by present of phagocyte abnormalities, hyperresponsive of monocyte / macrophage and elevated cytokines level [2,10,11]. Bacteria and their products accumulate in the gingival sulcus and mediate connective tissue destruction whereas stimulating synthesis of proinflammatory cytokines (IL) such as interleukins IL-1b, IL-6, IL-8, and tumor necrosis factors (TNF-a), which are synthesized by many stimulated cells [12,13]. Numerous studies have suggested that the proportion of proinflammatory cytokines could influence the course and development of periodontitis [13,14].

2. Materials and Methods

2.1. Subject Groups

This study was carried out on total number of 49 subjects , 24 AP patients were selected from patients referred to the periodontic clinic in Oral Surgery / College of Dentistry/Mosul University, Iraq and some of private clinics in Mosul. Participated patients were to 14 males and 10 females, aging between 20 - ≥40 years, and 25 healthy subjects as control group consisted of 14 males and 11 females aging between 20-45 years. In this study all patients were systemically healthy and had not undergone periodontal treatment in the previous 6 months. Smokers, alcoholisms, menstruation or pregnancy were excluded from this study. Medical history data were determined by chart review and patient interview. The diagnosis for AP status was established for all patients according to the 1999 International Classification of Periodontal Diseases and Conditions. Patients were based on full mouth periodontal probing and analysis of the alveolar bone level on the perapical and interproximal radiographs [15].

2.2. Serum collecting

Five ml of venous blood was withdrawn from each patient and control subjects . Blood samples were left to clot in a plain tube at 37˚C for 30 minutes, centrifuged at 3000 rpm for 5 minutes, and serum was separated for laboratory assessments. A 0.5 ml Eeppendorf tubes were used for preserving serum samples at -20˚C for subsequent specific laboratory investigations of serum using of IL-8,IL-6,IL-1β,IFN-γ and TNF-α [16].

serum cytokines levels were detected with ELISA technique:

1. Human Interleukin-1β ELISA kit, ABO Switzerland Co., Ltd.
2. Human Interleukin-6 ELISA kit, ABO Switzerland Co., Ltd.
3. Human Interleukin-8 ELISA kit, Immune Leader.
4. Human interferon-γ ELISA kit, ABO Switzerland Co., Ltd.
5. Human Tumor Necrosis Factor-α ELISA kit, ABO Switzerland Co., Ltd.

2.3. Saliva Collecting

Non-stimulated saliva was collected from AP and control groups . Subjects were instructed not to brush their teeth, or eat or drink 2 hour before the time of saliva collection . Unstimulated whole saliva was collected between 10:00 a.m. and 11:00 a.m. Subjects rinsed with water, and saliva samples were collected via passive drooling into a sterilized disposable collector cup, Saliva was separated at 3000 rpm for 10 min. The clear supernatant fraction was dispensed in a 0.5 ml Eppendorff tubes and stored at -20˚C until biochemical analysis [17].

Salivary markers used:

1. Salivary sIgA ELISA kit, Dia Metra.

2. Saliva total protein biochemical test kit, BIOLABO S.A.

3. Salivary peroxidase test.

Protocols for preparing the kits and placing serum and saliva samples were implemented according to the instructions of the manufacturer.

2.4. Statistical analysis

T-Test, Pearson Chi-Square Test, Post Hoc Test, ANOVA Test, Duncan Test, Correlations, Means, Means plots, was used to analyze data.

3. Results

The present study was carried out on 49 subject, 24 AP patients 14(58.3%) males, 10(41.7 %) females and 25 control subjects (14 males, 11females). (Figure 1)

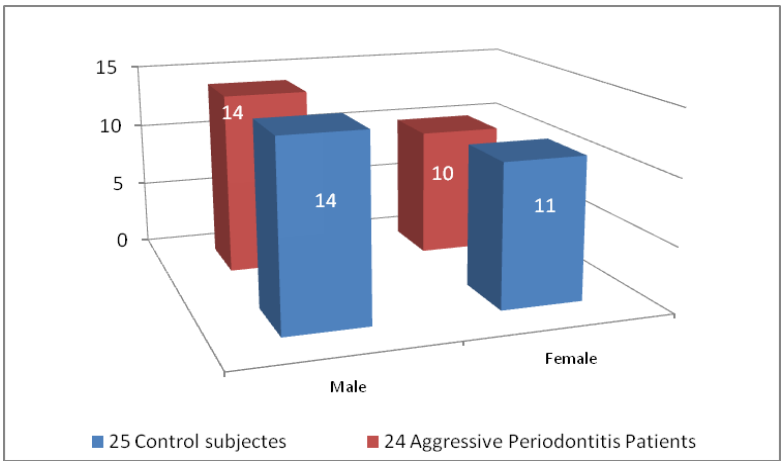

Figure (1): study groups distributed according to gender

In this study the mean value of serum cytokines levels in AP group were IL-8 (33.846 pg/ml), IL-6(17.433 ng/L), IL-1β(36.571 pg/ml), IFN-γ (305.23 ng/L), TNF-α(310.124 ng/L), whereas in control group were IL-8 (31.026 pg/ml), IL-6 (13.471 ng/L), IL-1β (28.689 pg/ml), IFN-γ (287.30 ng/L), TNF-α (279.94 ng/L). Furthermore salivary markers levels in AP group showed sIgA(76.95 µg/ml), Peroxidase (0.174 U/ml), total protein(4.074 mg/ml), peroxidase specific activity(0.0674 U/mg). whereas control group showed sIgA(90.71µg/ml), peroxidase (0.0038 U/ml), total protein(2.701 mg/ml), peroxidase specific activity(0.001 U/mg) levels. In the study group the distribution of patients according to age showed presence of 12(50%), 6(25%), 6(25%) of patients in 20-29, 30-39, ≥40 years, age group respectively (Figure 2).

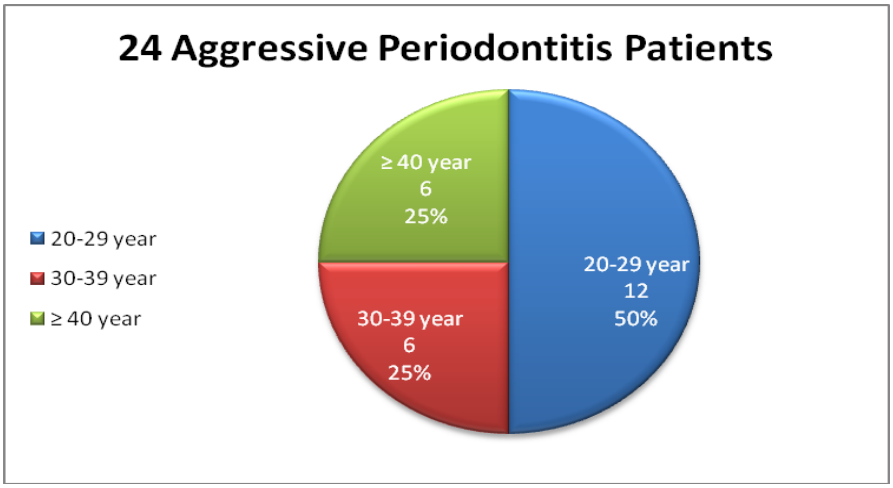

Figure (2): Shown distribution patients g according to age groups

The comparison of serum cytokines (IL-8,IL-6,IL-1β,IFN-γ,TNF-α) and salivary markers (sIgA, peroxidase, total protein, peroxidase specific activity) levels between AP and control group showed significance increase in serum IL-6 and TNF-α level in AP comparing with control group (Figure 3).

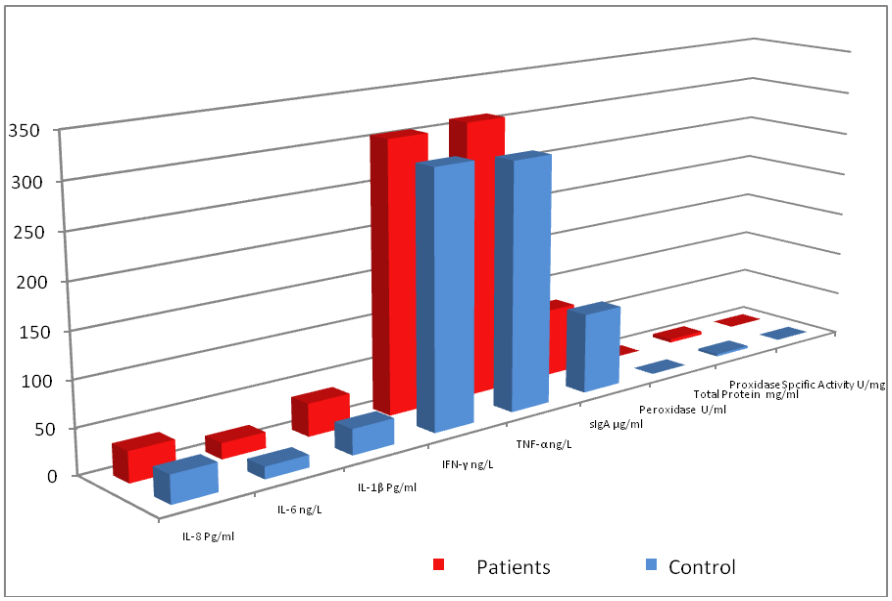

Figure (3): the comparison of serum and salivary markers levels, between patients and control group

Male patients showed increase in IL-8, IL-6, IFN-γ, TNF-α levels and peroxidase, peroxidase specific activity comparing with females, but its increase was not significant (Figure 4).

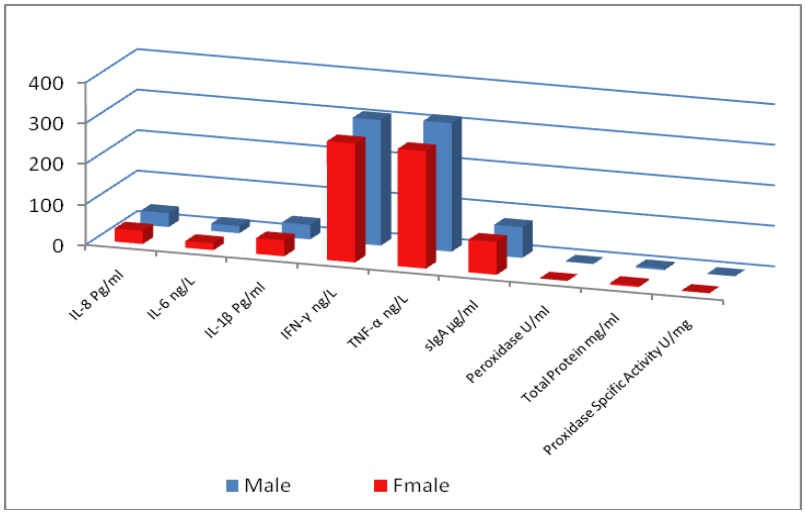

Figure (4): the comparison of serum and salivary markers levels, within aggressive periodontitis patients, according to gender

According to three age groups, AP group showed significance differences in serum IL-1β and salivary sIgA, particularly at ≥ 40 year age group (Figure 5).

In this study the increase in some serum cytokines and salivary markers were seem as increase of periodontal pocket depth specially at 4,5,6 mm pocket depth. On the other hand, serum IL-8, TNF-α and salivary total protein

showed decrease in their levels at 7mm pocket depth. However all these differences were not significant. (Figure 6).

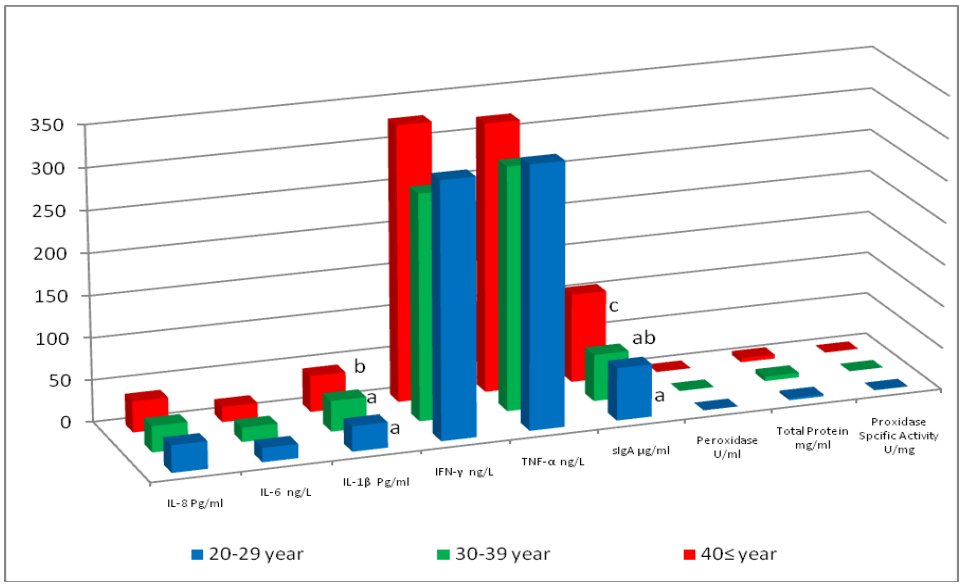

Figure (5): the comparison of serum and salivary markers levels within aggressive periodontitis patients according to three age groups

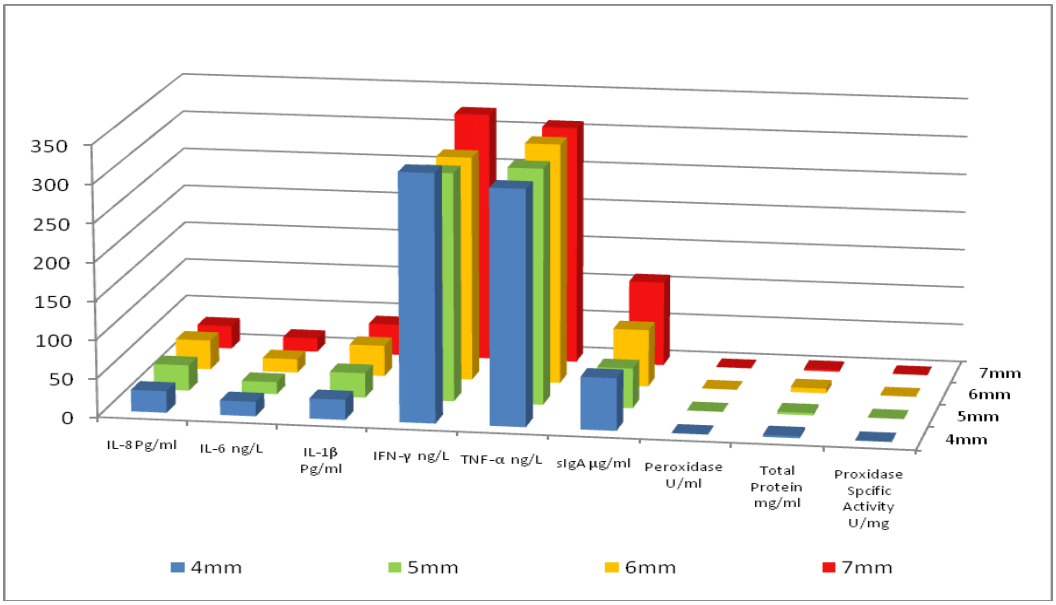

Figure (6): the comparison of serum and salivary parameter levels within aggressive periodontitis patients group according to periodontal pocket depth

Results in Table (1) showed presence of direct correlation between serum IL-1β and salivary peroxidase. Also presence of direct correlation between salivary IgA with saliva total protein and saliva peroxidase specific activity. Finally presence of direct correlation between Salivary peroxidase specific activity and saliva total protein.

Table 1: Co-relations between serum and Salivary markers within aggressive periodontitis patients group

		IL-8	IL- 6	IL- 1β	IFN-γ	TNF-α	Saliva IgA	Saliva total protein	Saliva peroxides	Saliva peroxides Specific activity
IL- 8	**Pearson Correlation**	1	- 0.005	0.598	0.255	0.440	0.022	- 0.353	0.700	- 0.406
IL- 6			1	0.381	0.169	0.297	0.434	0.385	0.432	0.182
IL- 1β				1	0.102	0.244	0.469	0.218	**0.710***	0.018
IFN-γ					1	0.677	0.700	0.289	- 0.172	0.414
TNF-α						1	0.545	0.452	0.100	0.508
Saliva IgA							1	**0.774***	- 0.019	**0.714***
Saliva total protein								1	- 0.173	**0.942****
Saliva peroxides									1	0.294
Saliva peroxides Specific activity										1

**. Correlation is significant at the 0.01 level. *. Correlation is significant at the 0.05 level.

4. Discussion

In the study group, high percentage of patients with AP 12(50%) were in age between 20-29 years, (absence of adolescent patients in this study may be related to clinic system). Whereas another 12(50%) of patients were separated to 6(25%) patients to each group 30-39 and ≥40 years. This results is compatible with others [1,18], who showed that AP affected at age of < 30 years, but older and younger patients also may be affected [3,4]. According to gender this study showed that AP males were more than females but there were no significant differences. The males to females percentage concerning AP was 58.3/41.7. Although menstruation and pregnancy females were excluded from this study, these criteria may shift female patient's total percentage. This finding is acceptable since many studies showed that the prevalence of AP was nearly equally distributed in males and in females [8,18], although females attend dental clinic more than males, asking seek beauty care [19]. The comparison between serum cytokines (IL-8,IL-6,IL-1β,IFN-γ,TNF-α) and salivary (sIgA, Peroxidase, total protein, peroxidase specific activity) levels in AP and control groups, showed the mean value of IL-6 in AP group was (17.43 ng/L) , which was significantly higher than the control group (13.47 ng/L), ($P<0.05$). while the mean value of TNF-α in the AP group was (310.124 ng/L) which was higher than in the control group (279.94ng/L) with significant difference ($p<0.05$), although other parameters showed increase in their levels, but there was no significant differences. In the AP group, the mean value of serum TNF-α was significantly increased compared with healthy individuals, furthermore, serum TNF-α level was significantly decreased in its

concentrations at 6 months post-therapy, but its level remained higher than healthy subjects [18,20,21]. Recent study mentioned a positive correlation between the periodontal disease and high serum levels of TNF-α and suggesting the use of TNF-α level as a marker for AP [22]. In contrast treatment with TNF-α inhibitors therapy has improved periodontal inflammatory condition [20,23,24]. IL-6 and TNF-α are a pro-inflammatory cytokines (inflammation key) that have important regulators of the immune response associated with periodontal disease. In the AP group, the mean values of IL-6 was significantly increased compared with control group [25,26,27]. IL-6 is an important parameter in periodontal research because it plays a crucial role in proliferation and differentiation of B cells into plasma cells. Furthermore IL-6 contributes in bone resorption [28]. IL-6 is present at higher level in inflamed tissue, GCF and plasma. Furthermore treatment with IL-6 receptor inhibition therapy has improved periodontal inflammatory condition [20,23,25]. Noh and his colleagues suggested that tissue IL-6 and TNF-α may be related with pathophysiology of periodontitis, and the estimate of these cytokine levels may be advantageous to detect of patients with periodontitis [29]. The comparison of serum cytokines (IL-1β,IL-8, IFN-γ) and salivary (sIgA, peroxidase, total protein, peroxidase specific activity) levels between AP and control groups showed no significant increase in AP comparing with control group. Interleukins 8 is a CXC chemokine and the most potent chemo attractant of neutrophils (first line of host defense) migrates into the inflamed region through the bloodstream [30,31]. In the current study, serum interleukins-8 in patients with AP was not significantly different from that of the healthy subjects. This result is compatible with others [32]. Interleukin-1, is a pro-inflammatory cytokine has several functions associated with microbial immune response and active stimulators of osteoclastic activity in periodontom. Higher production of IL-1 has been associated with enhanced localized response to infection [33]. In the current study serum IL-1β level was not significantly elevation in AP compared in healthy subjects, while is compatible with Stefanovska and his colleagues result who found low levels of IL-1 in AP patient's serum [34]. Furthermore Armitage found an elevation in level of IL-1β in AP patient's tissue which is due to macrophages hyper responsive [2]. In contrasts de Lima Oliveira and his colleagues found significant reductions in GCF IL-1β after therapy, and suggested that periodontal therapy improved GCF cytokine profiles by lowering IL-1β [35]. Gurban & Drugarin showed elevated level of IFN-γ in AP at different stages of disease [36].Whereas Mattuella, *et, al.,* suggested, no significant difference in the concentration of plasma cytokine IFN-*γ* between AP and healthy group [37]. These results are strongly compatible with our findings. In the present study, the mean values of salivary peroxidase and peroxidase specific activity in AP group were (0.0675) U/mg ,(0.1746) U/ml, which was higher than in healthy group (0.0153) U/mg, (0.0383) U/ml with no significant difference (P>0.05). Study finding was achieved by Taha & Al-Taei [18] and Mirza & Al-Azzawi [38] showed that peroxidase activity had a wide distribution in AP inflammation gingiva tissue, specifically in the subepithelial connective tissue, while epithelial tissue showed low activity. Moreover Gomes, *et, al.,* found significant increase in myloperoxidase (MPO) level, suggesting use it as an inflammatory marker in aggressive periodontitis [39]. In contrast Saxén, *et, al.,* noted decrease in salivary peroxidase and MPO in juvenile periodontitis group suggesting that this suppression could be characteristics of aggressive periodontitis [40]. In this study AP group showed decrease in salivary IgA levels (76.958) µg/ml, compared with healthy subjects (90.701) µg/ml, with no significant differences. Daniel, noted that low salivary IgA levels found in students repeatedly colonized by Actinobacillus actinomycetemcomitans which is the most prevalent bacteria in aggressive periodontitis [41]. In contrast Al-Rassam & Taha showed a significant increase in salivary sIgA level within chronic periodontitis comparing with healthy individuals [42]. The results of the

current study showed higher levels of salivary total protein in AP patients (4.0748) mg/ml compared with healthy individuals (2.701) mg/ml which was not statistically significant. This result is in agreement with other studies [43,44]. Recent study showed statistically significant an increase in concentration of salivary total protein saliva in chronic periodontitis patients compared to healthy individuals [17]. The increased protein levels in AP could be due to the inflammatory process that activates the sympathetic system to enhance the synthesis and secretion of some proteins (as evidenced by increased amylase levels , cathepsin C activity) in the saliva of AP patients, thereby increasing the protective potential of saliva against the diseases [43,45]. In contrast Rocha, et, al., showed reduced expression of mucin glycoprotein-2 (MG2) and lactoferrin in AP [46]. Furthermore other study suggested the increased levels of total protein could partly be also due to an increased leakage of plasma proteins into saliva due to inflammation [47]. Thus total protein is a vital component of saliva and is responsible for most of its functions like lubrication, physical protection, cleansing, buffering, maintenance of tooth integrity, taste and digestion and antibacterial activity [48]. In the present study, male patients showed increase in IL-8, IL-6, IFN-γ, TNF-α level and peroxidase, peroxidase specific activity comparing with female group, but these increases still not significance. In contrast Rashmi, et, al., found higher level of TNF-α in periodontitis females GCF compared to males but also the mean difference was not statistically significant [20]. Furthermore Al-Rassam & Taha, showed no significant differences in serum and salivary parameter in chronic periodontitis patients according to gender [42]. According to age groups, AP group showed significance differences in serum IL-1β and salivary sIgA, particularly in ≥ 40 year age group. This result may be associated with progressive and severity of AP with age. In this study the increase in some serum cytokines and salivary parameters were related to the increase of periodontal pocket depth especially at 4,5,6 mm. From the other hand serum IL-8, TNF-α and salivary total protein decreased at 7mm pocket depth, however all these differences are not significant. Yue, *et, al.*, found presence of significance correlation between cytokine levels in GCF and saliva with AP clinical parameters, furthermore assess of cytokines in saliva may be benefit as a quick method for estimation of periodontal disease activity [49]. The present study showed significant correlation between serum IL-1β and saliva peroxides, both parameter are associated with infection status. IL-1β is pro-inflammatory cytokines that responsible for immune responses regulation. Furthermore salivary peroxidase is a bacterial inhibitor and removes accumulation of H_2O_2 that created by oral bacteria. Moreover presence of significance correlation between salivary IgA with saliva total protein and salivary peroxidase specific activity. Finally presence of significant correlation between saliva peroxides specific activity and saliva total protein.

5. Conclusion

Presence of significant increase in serum IL-6 and TNF-α levels in Aggressive Periodontitis patients comparing with control group. Aggressive Periodontitis showed significance differences in serum IL-1β and salivary sIgA according to age with no clear difference in parameters level according to periodontal pocket depth and gender. Presence of direct correlation between serum IL-1β and salivary peroxidase. More studies are required to identify the specific role of each cytokine in the initiation and progression of aggressive periodontitis.

This work is dedicated to the memory of Dr.Al-Rassam's wife and his daughter.

6. Recommendation

More studies are required to identify the specific role of each cytokine in the initiation and progression of aggressive periodontitis

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