**Evaluation of Salivary and Serum Platelet Activating Factor Level in Chronic Periodontitis in Diabetic and non-Diabetic Patient**

**Abstract:**
The aim to evaluate salivary and serum levels of platelets activating factor in chronic periodontitis patient with and without diabetes.

**Methods:** A total of forth patients with moderate to severe chronic periodontitis were selectively collected for contribution in the present study.

**Results:** showed significant differences in platelet activating factor in saliva and serum between different groups. Group IV (type II diabetes mellitus) showed the highest level of platelet activating factor in saliva and serum followed by group III (type I diabetes mellitus) then group II (chronic periodontitis). Group I (control) showed the lowest level of platelet activating factor in saliva and serum. A positive correlation was observed between PAF levels and periodontal parameters. PAF levels in both serum and saliva in patients had significantly positive correlation with periodontal parameters.

**Conclusions:** PAF may be used for evaluation of the severity of chronic periodontitis in healthy and diabetic patients. It may also be responsible for the development and increased incidence of complications which are commonly found in diabetes.

**Keywords:** Salivary, platelet activating factor, serum platelet activating factor, Diabetic, non-Diabetic, chronic Periodontitis.

**Introduction**

Periodontitis is defined as infections of the tissues surrounding the teeth. This infection is usually caused by anaerobic gram-negative microorganisms. Infection causes destruction of supporting alveolar bone and can lead to tooth loss [1]. The destructive process of periodontitis is thought to begin with the accumulation of biofilms which contain significant bacterial masses on the tooth surface at or below the gingival margin [2].

Diabetes mellitus is a disease of metabolic dis-regulation, which develops either from either a deficiency in insulin production (IDDM) or impaired insulin utilization (NIDDM). Insulin-dependent diabetes mellitus (IDDM) results from the destruction of the insulin producing β-cells of the pancreas which may involve an autoimmune or a virally mediated destructive process. Non-insulin-dependent diabetes mellitus (NIDDM) results either from defects in insulin molecule or from altered cell receptors for insulin and manifests as insulin resistance rather than insulin deficiency [3].

Diabetes is characterized by the classic triad of symptoms like polyuria, polydipsia and polyphagia. Classic complications of diabetes include retinopathy, nephropathy, neuropathy, cardiovascular disease and impaired wound healing. Periodontal disease is considered as the sixth greatest complication of diabetes [4].

There is a strong relationship between periodontal health or disease and systemic health or disease. This means a two-way relationship in an individual may have powerful influence on individual’s systemic health or disease as well as most customary understood role that systemic disease may have an influence on individual’s periodontal health or disease [5].

In order to understand the cellular/molecular mechanism responsible for such a cyclical association, one must identify the common physiological changes associated with diabetes and PD that produce a synergy when condition coexist [6].

Platelet-activating factor (PAF) is one of the most potent and versatile proinflammatory mediators found in mammals [7]. It can be produced and released from a variety of cells, especially activated inflammatory cells like macrophages, and lymphocyte [8]. PAF promotes aggregation, chemotaxis, granule secretion and oxygen radical generation by leucocytes and their adherence to the endothelium [9-11].

Saliva has been discussed lately as important biological material that diagnostic tests which may contribute in the diagnosis and explaining the pathogenesis of many disease [12]. The presence of PAF in human mixed saliva was first reported in 1981, pure parotid saliva apparently has no detectable PAF activity suggesting that PAF in mixed saliva originated from a source other then this salivary gland [13,14].

**Patients and methods**

This study comprised 40 subjects; 30 patients having chronic periodontitis, selected from the outpatient clinic of Oral Medicine and Periodontology Department, Faculty of Dentistry, Mansoura University. In addition to 30 subjects with matched age and sex were selected to act as a control group.
Patient Selection

The first study group (group I) included 10 patients suffering from chronic periodontitis without diabetes, they were 4 males and 6 females patients with age ranged from 30 to 60 years (mean 46 ± 5.5). They were diagnosed by presence of clinical loss of attachment and radiographic bone loss. The second study group (group II) included 20 patients (10 patients were IDDM and the other 10 were INDDM) suffering from chronic periodontitis. They were 5 males and 5 females for each type of diabetes with age ranged from 30 to 60 years (mean 33 y ± 58). They were diagnosed by presence of clinical loss of attachment and radiographic bone loss. Control group III: The control group (group III) included 10 healthy subjects free from any periodontal diseases. They were 8 males and 2 females with age ranged from 30 to 60 years (mean 30 y ± 34).

The participating patients were free from any systemic disease and they were nonalcoholic and nonsmoker. They had neither history of using systemic antibiotics in the last three months nor periodontal treatment for at least three months before starting the study. Glycosylated hemoglobin was used to assess the degree of control of diabetes as HbA1c reflects average plasma glucose over the previous eight to 12 week and its use can avoid the problem of day-to-day variability of glucose values [15].

Patients having levels of 7-9 % HbA1c were included, where the normal level is less than 7 %, level of controlled diabetic patients is 7-9 %, level of poorly controlled patients is above 9 % and level of very poorly controlled patients is above 12 %. Periodontal indices as probing pocket depth, bleeding index 29, Plaque index (PI) 30, gingival index (GI) 31 and clinical attachment level (CAL) was registered for each participant. The mean value for each patient was obtained by dividing the sum of teeth scores by the number of teeth examined.

Sampling:

Patients and controls in this study were subjected to assay of platelet-activating factor in serum and saliva by using ELISA technique. Three ml of venous blood was collected from all patients and control by clean venipuncture using plastic disposable syringes. The whole blood samples were delivered into pyrogen/endotoxin free plastic collecting tube. Blood samples were allowed to clot before centrifugation for 10 minutes at approximately 3000 rpm and serum samples were taken and stored frozen at -70°C until assay of platelet-activating factor by using ELISA technique.

Refraining from intake of any food or beverage (water excepted) one hour before the test session was done. Smoking, chewing gum and intake of coffee also was prohibited during this hour. Rinsing the mouth was done several times with deionized (distilled). Three milliliter of unstimulated, whole mixed saliva was collected between 8:00 AM and 1:00 PM from all patients. Saliva was expectorated directly into disposable glass tubes (16 x 100 mm) 19.

Assay of platelet-activating factor in serum and saliva

The determination of platelet-activating factor was done by using immunoassay kit allows for in vitro quantitative in serum and (cat. No.E0526Ge provided by EIAb Science Co., China. The ELISA is based on the competitive binding enzyme immunoassay technique. The microtiter plate provided in this kit was pre-coated with an antibody specific to platelet-activating factor. During the reaction, the sample or standard competed with a fixed amount of biotin-labeled antibody specific to platelet-activating factor. Excess conjugate and unbound sample or standard were washed from the plate. Next, Avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. Then a TMB substrate solution was added to each well. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of the samples was then determined by comparing the O.D. of the samples to the standard curve.

A linear standard curve was generated by plotting the average OD absorbance of standards on the vertical axis versus the corresponding platelet-activating factor standards concentrations on the horizontal axis. The concentrations of platelet-activating factor in each sample in serum and saliva was determined by extrapolating the OD values of samples by using the standard curve.

Statistical analysis

The SPSS statistical package for social science version 22 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Descriptive statistics were performed in terms of (mean, median, standard deviation, range, minimum, maximum) for numerical data and in terms of frequency distribution and percentages of categorical data (gender). One way ANOVA was used to detect possible differences between groups for numerical data followed by multiple comparisons (post hoc test) between groups using the Bonferroni correction. For categorical data Fischer exact test was used to compare between groups. Comparison of HbA1c between the 2 diabetic groups was performed using the independent samples t-test. P is significant if < 0.05 at confidence interval 95%.

Results

Concerning the comparison of gender (males and females) between groups, there was no significant differences in gender between different groups (Fisher exact test, p= .26).

Regarding the comparison of plaque scores between groups performed by One Way ANOVA test, there were highly significant differences between different groups at p= 0.00. Group II (chronic periodontitis) showed the highest plaque scores, followed by group IV (type II diabetes mellitus) and then group III (type I diabetes mellitus). Group I (control) showed the lowest plaque scores. Multiple comparisons by post hoc test between groups using the Bonferroni correction showed there were significant differences between each 2 groups at p<0.05 except between group III and group IV, there was no significant difference p>0.05.

Comparison of gingival scores between different groups by One Way ANOVA test revealed highly significant differences in gingival scores between different groups at p= 0.00. Group IV (type II diabetes mellitus) showed the highest gingival scores, followed by Group II (chronic periodontitis) and then group III (type I diabetes mellitus). Group I (control) showed the lowest gingival

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scores. Regarding the multiple comparisons by post hoc test between groups using the Bonferroni corrections, group I differed significantly from group II, III and IV at p<0.05, while there was no significant difference between group II, III and IV at p>0.05.

When comparing bleeding scores between groups by One Way ANOVA test, there were significant differences in bleeding scores between different groups at p<0.05. Group IV (type II diabetes mellitus) showed the highest bleeding scores, followed by Group II (chronic periodontitis) and then group III (type I diabetes mellitus). Group I (control) showed the lowest bleeding scores. Multiple comparisons by post hoc test between groups using the Bonferroni correction revealed significant differences between each 2 groups (Bonferroni, p<0.05) except between group III and group IV, there was no significant difference at p>0.05.

Comparison of pocket depths between groups by One Way ANOVA test showed that there were significant differences between different groups at p=0.001. Group II (chronic periodontitis) showed the highest pocket depths, followed by Group IV (type II diabetes mellitus) and group III (type I diabetes mellitus). Group I (control) showed the lowest pocket depths. Multiple comparisons by post hoc test between groups using the Bonferroni correction showed that group I differ significantly from group II, III and IV at p<0.05, while there was no significant difference between group II, III and IV p>0.05.

Comparison of clinical attachment between groups by One Way ANOVA test showed significant differences in clinical attachment between different groups at p=0.001. Group II (chronic periodontitis) and then group III (type I diabetes mellitus) showed the highest clinical attachment, followed by Group II (chronic periodontitis) and then group III (type I diabetes mellitus) group I (control) showed the lowest clinical attachment. Multiple comparisons by post hoc test between groups using the Bonferroni correction revealed that there were significant differences between each 2 groups at p<0.05 except between group II and group III and between group III and group IV where there were no significant differences between these groups at p>0.05.

Comparison of platelet activating factor in serum between groups by One Way ANOVA test showed highly significant differences in platelet activating factor in serum between different groups at p=0.001. Group IV (type II diabetes mellitus) showed the highest platelet activating factor in serum, followed by Group III (type I diabetes mellitus and then group II (chronic periodontitis). Group I (control) showed the lowest platelet activating factor in saliva. Multiple comparisons by post hoc test between groups using the Bonferroni correction showed significant differences between each 2 groups at p<0.05 except between group I and group II and between group II and group III where there were no significant differences between these groups at p>0.05.

Comparison of platelet activating factor in saliva between groups by One Way ANOVA test showed significant differences in platelet activating factor in serum between different groups at p=0.001. Group IV (type II diabetes mellitus) showed the highest platelet activating factor in saliva, followed by Group III (type I diabetes mellitus) and then group II (chronic periodontitis). Group I (control) showed the lowest platelet activating factor in saliva. Multiple comparisons by post hoc test between groups using the Bonferroni correction showed significant differences between each 2 groups at p<0.05 except between group I and group II and between group III and group IV where there were no significant differences between these groups at p>0.05.

Comparison of glucosated haemoglobin (HbA1c) between the 2 diabetic groups by One Way ANOVA test showed no significant difference. Spearman correlation coefficient demonstrated significant correlation between platelet activating factors in serum and plaque index (p=0.010, r=0.403) gingival index (p=0.034, r=0.336), bleeding index (p=0.004, r=0.441), pocket depth (p=0.001, r=0.507) and clinical attachment (p=0.002, r=0.474).

Spearman correlation coefficient demonstrated significant correlation between platelet activating factors in saliva and plaque index (p=0.047, r=0.275) gingival index (p=0.049, r=0.272), bleeding index (p=0.000, r=0.718), pocket depth (p=0.048, r=0.275) and clinical attachment (p=0.000, r=0.655).

**Discussion**

Periodontitis is a chronic oral infection that results in loss of attachment, bone destruction and eventually the loss of teeth. The signs and symptoms of periodontitis include swollen gums, discolored gums, bleeding on brushing, increased spacing between the teeth, pus between the teeth and gums, a bad taste, and halitosis. The major etiology of periodontitis is bacterial plaque [16].

Diabetic complications result from micro- and macrovascular disturbances. With respect to the periodontal microflora, no appreciable differences in the sites of periodontal disease have been found between diabetic and non-diabetic subjects [16]. A great deal of attention has been directed to potential differences in the immunomodulatory responses to bacteria between diabetic and non-diabetic subjects. Neutrophil chemotaxis and phagocytic activities are compromised in diabetic patients, which can lead to reduced bacterial killing and enhanced periodontal destruction [16].

Inflammation is exaggerated in the presence of diabetes, insulin resistance, and hyperglycemia. [53] Various studies have revealed elevated production of inflammatory products in diabetic patients.[17] Chronic hyperglycemia in addition to the inflammatory response will eventually lead to complications in diabetes mellitus [18].

Both diabetes and periodontitis are chronic diseases. Diabetes has many adverse effects on the periodontium, including decreased collagen turnover, impaired neutrophil function, and increased periodontal destruction [19].

Salivary PAF most likely originates from the crevicular space and derives from inflammatory cells within the gingival and/or periodontal tissues [20]. Platelet-activating factor (PAF) is one of the most potent and versatile proinflammatory mediators found in mammals [21]. It can be produced and released from a variety of cells, especially activated inflammatory cells such as macrophages, thrombocytes, and lymphocytes [22]. PAF promotes the aggregation, chemotaxis, granule secretion,
and oxygen radical generation from leukocytes and the adherence of leukocytes to endothelium [21,22]. PAF increases the permeability of endothelial cell monolayers and stimulates the contraction of smooth muscle [23].

Therefore, the aim of this study was to evaluate salivary and saliva levels of platelets activating factor in chronic periodontitis patient with and without diabetes.

Since the presence of PAF in human mixed saliva was first reported, only few studies have tried to reveal the relationship between PAF and periodontal inflammation [24-25]. In the periodontal literature, PAF has been analyzed in saliva [26-30]. It has been demonstrated that PAF released by inflamed periodontal tissues could be measured within the gingival sulcus fluid [12].

Our results showed significant differences in platelet activating factor in saliva between different groups. Group IV (type II diabetes mellitus) showed the highest level of platelet activating factor in saliva followed by group III (type I diabetes mellitus) then group II (chronic periodontitis). Group I (control) showed the lowest level of platelet activating factor in saliva.

Previous studies reported that PAF levels in saliva and gingival fluid of patients having gingival and periodontal diseases are elevated compared with healthy controls [32]. Analyzing the role of PAF in the pathogenesis of periodontal disease, there are few experimental studies related to the gingival tissue PAF levels in periodontal therapy [33].

In addition, data indicating significantly higher levels of PAF in inflamed gingiva from periodontitis patients than in healthy gingiva have suggested that the higher levels of PAF in the inflamed gingival tissue might have been due to an increase in PAF production [34].

The elevated levels of PAF in GCF in patients having periodontitis and gingivitis suggested an important role of this lipid mediator in the pathogenesis of gingivitis, periodontitis and a potential involvement of this mediator in the development of systemic diseases [35]. PAF is considered to be an important mediator of alveolar bone and connective tissue destruction in periodontitis [36].

The importance of this mediator in the course of periodontitis was underlined by the reduction of PAF levels in saliva after initial periodontal therapy [37].

Comparison of the studied periodontal parameters (CAL, PPD, BOP, GI) between groups showed significant differences in periodontal parameters between different groups. Group IV (type II diabetes mellitus) showed the highest periodontal parameters followed by Group II (chronic periodontitis)and then group III (type diabetes mellitus). Group I (control) showed the lowest values. Comparison of the studied PI between groups. There were significant differences in periodontal parameters between different groups. Group II (chronic periodontitis) showed the highest periodontal parameters followed Group IV (type II diabetes mellitus) by and then group III (type diabetes mellitus). Group I (control) showed the lowest values. We also found significant correlation between platelet activating factors in saliva and plaque index, gingival index, bleeding index, pocket depth and clinical attachment.

A positive correlation has been observed between PAF levels and severity of periodontitis, suggesting that PAF may have a causative role in the disease process [38]. Indeed, periodontal therapies not only reduced the indices of disease but also resulted in reductions in salivary PAF [20]. The specific role of PAF in the pathogenesis of PD is not known [28].

Our results showed significant differences in platelet activating factor in serum between different groups. Group IV (type II diabetes mellitus) showed the highest level of platelet activating factor in saliva followed by group III (type I diabetes mellitus) then group II (chronic periodontitis). Group I (control) showed the lowest level of platelet activating factor in serum. Comparison of glycosated haemoglobin (HbA1c) between the 2 diabetic groups by One Way ANOVA test showed no significant difference excluding the role of glucose level in the pathogenesis. We also found significant correlation between platelet activating factors in serum and plaque index, gingival index, bleeding index, pocket depth and clinical attachment.

A significant positive correlation was observed between periodontal parameters and the levels of PAF in serum from patients suffering from periodontitis [39].

Based on these observations, it is conceivable that the proinflammatory mediator PAF synthesized within the oral cavity among others may play a significant role in the initiation or progression of non-oral inflammatory processes to promote the manifestation of systemic diseases. These orally derived mediators may increase the inflammatory reaction in atherosclerotic lesions and potentially the risk for cardiac events, as several studies have shown that periodontitis is a significant risk factor for coronary heart diseases [40,41]. Interestingly, salivary PAF levels are transiently increased in subjects presenting with myocardial infarction [42]. In addition, PAF appears to have a role in reproductive pathophysiology including labour in normal and pre-term pregnancy [43].

PAF has also been implicated in inflammatory bone disorders [44]. It had been previously reported that elevated PAF concentrations in IDDM patients compared to healthy volunteers [45]. PAF levels were increased in children with type I diabetes. This increment was higher in children with new-onset diabetes and may be important in the etiopathogenesis of type I diabetes. Further research to support the use of PAF antagonists in patients with new-onset diabetes [46].

Another experimental study reported that the PAF synthesis capacity of polymorphonuclear leukocytes was increased in diabetic rats [47]. There is also a clinical research that reported an increased serum level of PAF in type I diabetics [48].

PAF has been implicated in the development of type I diabetes. In patients with type I diabetes, a 50-fold increase in blood levels of PAF compared with those of healthy volunteers was noted, whereas the same level of PAF-precursors and PAF-AH activity was maintained [49].

The increase in PAF levels could be due to increased biosynthesis or decreased degradation of PAF. A study demonstrated that there was a decrease in the activity of PAF acetylhydrolase in patients with IDDM [50]. A decrease in the PAF acetylhydrolase activity would indicate that the degradation of PAF would be slowed leading to an...
increase in the serum levels of PAF resulting in an enhancement of its effects. This is supported by the reports that diabetic human platelets showed hypersensitivity to PAF in both aggregations as well as in phosphatidic acid production [51].

The paracrine release of proinflammatory mediators and the expression of proinflammatory adhesion molecules are augmented by TNF-α [52] which, along with high glucose concentration, stimulates the biosynthesis of PAF through the activation of acetyl coenzyme A:lysoPAF acetyltransferase, a membraneboundenzyme of the PAF biosynthetic remodelling route [53]. High glucose concentration also inhibits the PAFacetylhrolase(PAF-AH) activity in EPCs. This enzyme either hydrolyses PAF (AH activity) or transfers acetyl groups from PAF to lysophospholipids (transacylase activity) [54]. Furthermore, besides the downregulation of PAF catabolicenzyme genes (PAF-AH and PAFAH1B1) there is also an upregulation of genes involved in the production of phospholipaseA2, which is a key enzyme involved in PAF biosynthesis as it produces lyso-PAF necessary for PAF production. The latter is due to platelet-activating factor acetylhrolase (PAF-AH) which is a Ca2+-independent phospholipaseA2 that catalyses the conversion of PAF to the inactive metabolite lyso-PAF [55].

Patients with poor glycaemic control showed a higher PAF-R. Inhibitory effects of PAF-receptor antagonists on insulin support the evidence that PAF is involved in insulinitis [56]. Daily intra peritoneal 6.0-mg/kg body weight recombinant plasma PAF-AH injections reduced the frequency of diabetes from 90 to 57% in the diabetes-prone BB rats. Diabetes-prone BB rats protected by recombinant plasma PAF-AH also had a higher percentage of insulin-positive cells in pancreas sections. Recombinant plasma PAF-AH may affect diabetes by reducing the level of PAF in the islets of Langerhans and thereby preserving β-cells by inhibiting the inflammatory process [57].

A decrease in PAF acetylhrolase activity in IDDM patients will eventually result in enhanced effects of PAF. This in turn can influence the actions of PAF in diabetes. PAF has been shown to induce hepatoglycogenolysis [58]. An increase in PAF levels in diabetics can aggravate hyperglycemia. PAF also increases hepatic fatty acid and triglyceride synthesis and thus it may be partly responsible for the hyperglycemia seen in diabetic patients [59]. Finally, the increased responsiveness of platelets of diabetic patients to various aggregating agents including PAF may be responsible for the increased incidence of vascular complications which are commonly found in diabetes [60-62].

**Conclusion**

- This study showed elevation of paf levels in serum and saliva in patient type II diabetes mellitus compared with patient typeI diabetes mellitus.
- Chronic periodontitis have higher level of PAF in comparison with control group.
- The PAF levels in both serum and saliva in patients had significantly positive correlation with periodontal parameters and they increased with the severity of the periodontal inflammation and destruction.
- The correlation analysis between saliva and serum levels verified that PAF is released from periodontitis affected tissues into saliva and contributes to increased serum levels of the mediator measured in periodontitis patients.
- Aggregation agents including PAF may be responsible for the increased incidence of vascular complication which are commonly found in diabetes.

**References**

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