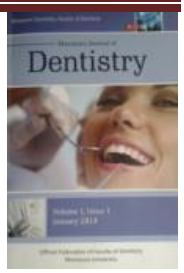
Evaluation of Salivary and Serum Platelet Activating Factor Level in Chronic Periodontitis in Diabetic and non-Diabetic Patient



Ramzi Ibrahim sarab[1], Laila Ibrahim Ragab Sidky[2] , Mohamed Mahmoud Anees[3] and Ziyad Mohamed Essam Ei-Din Tawhed[4]
[1]BDS, Faculty of Dentistry, al-zawaia University, Libya.
[2]Professor of Oral Medicine, Periodontology, X-Ray & Diagnosis, Faculty of Dentistry, Mansoura University, Mansoura, Egypt.
[3]Assistant Professor of , of Oral Medicine and Periodontology, Faculty of Dentistry, Mansoura University, Mansoura, Egypt.
[4]Assistant of Clinical Pathology, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

Abstract:
Objectives: 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 bio films 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 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 redical 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 PAFactivity ,suggesting that PAFin 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 y ±55).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 5females 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 30y ± 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 -70oC 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 EIAab 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 for sites on a pre-coated monoclonal 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

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= .00. 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<.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= 0.00)0. 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>.05.

Comparison of clinical attachment between groups by One Way ANOVA test showed significant differences in clinical attachment between different groups p= .00. Group IV (type II 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= .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 serum. Multiple comparisons by post hoc test between groups using the Bonferroni correction showed that there were 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= .007. 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=0.026*), 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, loose 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 diabeticand 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 potentand 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 glucosated 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 etipathogenesis 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 diabetes [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 byTNF-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 PAFacetylhydrolase(PAF-AH) activity in EPCs. This enzyme either hydrolyses PAF (AH activity) or transfers acetyl groupsfrom PAF to lysophospholipids (transacetylase 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 biosynthesisas it produces lyso-PAF necessary for PAF production. The latter is due to platelet-activating factor acetylhydrolase (PAF-AH) which is a Ca2+-independent phospholipaseA2 that catalyses the conversion of PAF to the inactivemetabolite lyso-PAF [55].

Patients with poor glycaemic control showed a higher PAF-R. Inhibitory effects of PAF-receptor antagonists on insulitis support the evidence that PAF is involved in insulitis [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 acetylhydrolase 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 hepaticglycogenolysis [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 hypertriglyceridemia 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 eleveation 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

1-Pucher J, Stewart J. Periodontal disease and diabetes mellitus. Currdiab Rep. 2004 Feb; 4 (1):46-50.

2-Gibbons RJ, Van Houte J: Bacterial Adherence and the Formation of Dental Plaques. In: Bacterial Adherence.EH Beachey, Eds.; Chapman and Hall London, 1980: 61-1045.

3-Atkinson MA, Maclaren NK: What causes diabetes? Scientific American, 1990; 263(1):62-71.

4-Loe H: Periodontal disease.The sixth complication of diabetes. Diabetes care, 1993; 16 (1):329-334.

5-Dumitrescu AL, Inagaki K: Interrelationship between periodontal disease and mortality in CVD, metabolicsyndrome & diabetes mellitus. In: Etiology and Pathogenesis of periodontal diseases. Dr Alexandriana L, Dumitrescu (Eds.) Ist Edn. Springer -Verlag, Berlin Heidelberg, 2010: 125 - 157. 1.

6-Loe H. Periodontal disease. The sixth complication of diabetes mellitus. Diabetes Care. 1993 Jan; 16 (1):329-34.

7-Prescott, S. M., Zimmerman, G. A., Stafforini, D. M. & McIntyre, T. .: Platelet activating factor and related lipid mediators. Annual Review of Biochemistry 2000; 69: 419-445.

8-Snyder, F.: Platelet-activating factor and related acetylated lipids as potent biologically active cellular mediators. The American Journal of Physiology 1990; 259: C697-C708.

9-Behrens, T. W. & Goodwin, J. S.: Control of human T cell proliferation by platelet activating factor. International Journal of lmmuno pharmacology 1990; 12: 175-184

10-Denizot, Y., Dupuis, F., Praloran, V., Ishihara, Y., Zhang, J. B., Quinn, S. M., Schenkein. Effects of platelet-activating factor on human T and B cells - an overview. Research in Immunology 1994; 145: 109-116.

11-Ishii, S. & Shimizu, T.: Platelet-activating factor (P AF) receptor and genetically engineered P AF receptor mutant mice. Progress in Lipid Research 2000; 39, 41-82.

12-Miller Csfioley JD , Bailey, CampellCL, Humphies RL, Jacobson jw, Redding SW, Ebersole jt. Cuitent developments in salivary diagnostics, Bio mark med 2010;4(1):71-89.

13-Cox, C. P., Ward low, M. L., Jorgensen, R. & FaI T, R. S. The presence of platelet activation factor (P AF) in normal human mixed saliva. Journal of Immunology 1981; 127: 46-50.

14-Kazarov EV, Dorn BR, Shelburne CE, Dunn, Jr. WA, Rogulske-Fox A. Human atherscleroticplaque contains viable invasive Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis. Arterioscler Thromb Vasc Biol 2005; 25(3):E17-E18.

15-Shrihari TG. Potential correlation between periodontitis and coronary heart disease – An overview. General Dentistry 2012; 60(1):20-24.
16-Iacopino AM, Cutler CW. Pathophysiological relationships between periodontitis and systemic diseases: recent concepts involving serum lipids. J Periodontol 2000 Aug; 71(8):1375-1384.
17-Abhijit .,Gurav and Varsha, Jadhav.,Reviw Article Periodontitis and risk of diabetes mellitus Journal of Diabetes 3 (2011) 21–28.
18-Mealey B. Diabetes and periodontal diseases. J Periodontol. 1999; 70: 935–49.
19-McManus LM,Marze BT, Schiess AV (1990) Deficiency of salivary PAF in edentulous individuals. J Periodontal Res 25:347–351.
20-Prescott SM, Zimmerman GA, Stafforini DM, McIntyre TM (2000) Platelet-activating factor and related lipid mediators. Annu Rev Biochem 69:419–445.
21-Stafforini DM, McIntyre TM, Zimmerman GA, Prescott SM (2003) Platelet-activating factor, a pleiotrophic mediator of physiological and pathological processes. Crit Rev Clin Lab Sci 40:643–672.
22-Behrens TW, Goodwin JS (1990) Control of human T cell proliferation by platelet-activating factor. Int Immunopharmacol 12:175–184.
23-Denizot Y, Dupuis F, Praloran V (1994) Effects of plateletactivating factor on human T and B cells—an overview. Res Immunol 145:109–116.
24-Cox CP, Wardlow ML, Jorgensen R, Farr RS (1981) The presence of platelet-activating factor (PAF) in normal human mixed saliva. J Immunol 127:46–50.
25-Montrucchio G, Alloatti G, Camussi G (2000) Role of platelet activating factor in cardiovascular pathophysiology. Physiol Rev80:1669–1699.
26-Keles GC, Cetinkaya BO, Isildak I, Koprulu H, Acikgoz G (2006) Levels of platelet activating factor in gingival crevice fluid following periodontal surgical therapy. J Periodontal Res 41:513–518.
27-Rasch MS, Mealey BL, Prihoda TJ, Woodard DS, McManus LM (1995) The effect of initial periodontal therapy on salivary platelet-activating factor levels in chronic adult periodontitis. J Periodontol 66:613–623.
28-Garito, M. L,, Prihoda, T. J. & McManus. L. M.(1995). Salivary PAF levels correlate with the severity of periodontal inflammation. Journal of Dental Research 74, 1048-1056.
29-Baltas G, Kotsifaki H, Antonopoulou S, Kipioti A,DemopoulosCA.Implication of PAF and acetylhydrolase (PAF-AH) activity inperiodontal disease. Adv Exp Med Biol 1996; 416: 135 _/141.
30-Ribaldi E, Guerra M, Mezzasoma AM, Staffolani N, Goracci G, Gresele P. PAF levels in saliva are regulated by inflammatory cells. J Periodontal Res 1998; 33: 237 _/241.
31-Buduneli E, Vardar S, Buduneli N, Berdeli AH, Turkoglu O, Baskesen A, Atilla G (2004) Effects of combined systemic administration of low-dose doxycycline and alendronate on endotoxin-induced perio-dontitis in rats. J Periodontol 75:1516-1523
32-Vardar S, Buduneli E, Baylas H, Berdeli AH, Buduneli N, Atilla G (2005) Individual and combined effects of selective cyclo- oxygenase-2 inhibitor and omega-3 fatty acid on endotoxin- induced periodontitis in rats. J Periodontol 76:99-106.
33-Vardar S, Buduneli E, Turkoglu O, Berdeli AH, Baylas H, Baskesen A, Atilla G (2004) Therapeutic versus prophylactic plus therapeutic administration of omega-3 fatty acid on endotoxin- induced periodontitis in rats. J Periodontol 75:1640-1646.
34-Noguchi K, Morita I, Murota S (1989) The detection of platelet- activating factor in,inflamed human gingival tissue. Arch Oral Biol 34:37-41.
35-ChenH & Zheng p & Zhu H & Zhu J & Zhao L & El Mokhtari & Eberhard J& Lins M& Jepsen S:Platelet-activating factor levels of serum and gingival crevicular fluid in nonsmoking patients with periodontitis and/or coronary heart disease Clin Oral Invest (2010) 14:629–636.
36-Emingil G, Coker I, Atilla G, Huseyinov A (2000) Levels of leukotriene B4 and platelet activating factor in gingival crevicular fluid in renal transplant patients receiving cyelosporine A. J Periodontol 71:50-57.
37-Rasch, M. S., Mealey, B. L., Prihoda, T. J., Woodard, D. S. & McManus, L. M. (1995) The effect of initial periodontal therapy on salivary platelet-activating factor levels in chronic adult periodontitis. 66, 613-623.
38-Zheng P, Chen H, Shi S, Jepsen S, Eberhard J (2006) Periodontal parameters and platelet-activating factor levels in serum and gingival crevicular fluid in a Chinese population. J Clin Periodontol 33:797–802.
39-Shindou H, Ishii S, Yamamoto M, Takeda K, Akira S, Shimizu T**.** 2005. Priming effect of lipopolysaccharide on acetyl-coenzyme A:lyso-platelet-activating factor acetyltransferase is MyD88 and TRIF independent. J. Immunol. 175(2):1177-1183.
40-Jansson, L., Lavstedt, S., Frithiof, L. & Theobald, H. (2001) Relationship between oral health and mortality in cardiovascular diseases. Journal of Clinical Periodontology 28. 762-768.
41-Haynes, W. G. & Stanford, C. (2003) Periodontal disease and atherosclerosis: from dental to arterial plaque. Arteriosclerosis, Thrombosis and Vascular Biology 23, 1309-1311.
42-Geerts, S. O., Legrand, V., Charpentier, J., Albert, A. & Rompen, E. H. (2004) Further ' evidence of the association between periodontal conditions and coronary artery disease. Journal of Periodontology 75, 1274-1280.
43-Jones, k. P., Reynolds , S. P., Gray, M,, Hughes, K. T,, Rolf, S. & Davies, B. H. (1994) Salivary PAF in acute myocardial infarction and angina: changes during hospital treatment and relationship to cardiac enzymes. Thrombosis Research 75, 503-511.
44-Narahara, H., Toyoshima, K. & Johnston, J. M.(1996) Role of platelet-activating factor in parturition. Advances in Experimental Medicine and Biology 416, 269-275.
45-Cavallo-Perin P, Lupia E, Gruden G, Olivetti C, De Martino A, Cassader M, et al. Increased blood levels of platelet-activanting factor in insulin-dependent diabetic patients with microalbuminuria. Nephrol Dial Transplant. 2000; 15:994-9.
46-Nathan N, Denizot Y, Huc MC, Claverie C, Laubie B, Benveniste J, et al. Elevated levels of paf-acether in blood

of patients with type 1 diabetes mellitus. Diabete Metab. 1992; 18: 59-62.
47-De Castro SH, Faria Neto HC, Gomes MB.Arq Bras Cardiol Platelet-activating factor acetylhydrolase (PAF-AH) activity in patients with type 1 diabetes mellitus.2007 Feb; 88(2):179-84.
48-Akiba S, Naka M, Hashizume T, Sugatani J, Fujii T, Sato T: Stimulation of plateletactivating factor synthesis in polimorphonuclear leukocytes from streptozocin induced diabetic rats. *J Biochem (Tokyo)*117:425–433, 1995.
49-Memon RA, Saeed SA, Jabbar A, Jafri A, Gilani AH, Saleem S, et al. Altered platelet activating factor metabolism in insulin dependent diabetes mellitus. J Pak Med Assoc. 1995; 45: 122-5.
50-Shukla, S.D., Paul, A. and Klachko, D. M. Hypersensitivity of diabetic human platelets to platelet activating factor. Thromb. Res., 1992; 66:239-46.
51-Zhang Y, Herbert BS, Rajashekhar G et al (2009) Prematuresenescence of highly proliferative endothelial progenitor cells isinduced by tumor necrosis factor-alpha via the p38 mitogenactivated protein kinase pathway. FASEB J 23:1358–1365.
52-Balestrieri ML, Castaldo D, Balestrieri C, Quagliuolo L, Giovane A,Servillo L (2003) Modulation by flavonoids of PAF and related phospholipids in endothelial cells during oxidative stress. J Lipid Res44:380–387.
53-Balestrieri ML, Giovane A, Milone L, Servillo L (2010)Endothelial progenitor cells express PAF receptor and respondto PAF via Ca(2+)-dependent signaling. Biochim Biophys Acta1801:1123–1132
54-Servillo L, Balestrieri C, Giovane A et al (2006) Lysophospholipid transacetylase in the regulation of PAF levels in human monocytes and macrophages. FASEB J 20:1015–1017.
55-Karasawa K, Harada A, Satoh N, Inoue K, Setaka M. Plasma platelet activating factor-acetylhydrolase (PAF-AH). Prog Lipid Res 2003;42:93–114.
56-Balestrieri.M.L & Servillo.L & Esposito.A: Poor glycaemic control in type 2 diabetes patients reducesendothelial progenitor cell number by influencing SIRT1signalling via platelet-activating factor receptor activation Diabetologia (2013) 56:162–172.
57-Beck JC, Goodner CJ, Wilson C, Wilson D, Glidden D, Baskin DG, et al. Effects of ginkgolide B, a plateletactivating factor inhibitor, on insulitis in the spontaneously diabetic BB rats. Autoimmunity 1992;9:225–35.
58-Jobe LW, Ubungen R, Goodner CJ, Baskin DG, Braquet P, Lernmark A ˚ . Protection from BB rat diabetes bymthe platelet-activating factor inhibitor BN50730. Autoimmunity 1991;16:259–66.
59-Lee ES, Jiang J, Sund GC, Simonson WT, Graham J, Dietsch G, et alRecombinant human platelet-activatifactoracetylhydrolase reduces the frequency of diabetes in the diabetes-prone BB rats. Diabete 1999;48:43–9.
60-Evans, R. D., lie, V. and Williamson, D. H. Metabolic effects of platelet-activating factor in rats in vivo: Stimulation ofhepatic glycogenolysis and lipogenesis. Biochem. J., 1990;269:269-72.
61-Memon, R. A., Feingold, K. R. and Grunfeld, C. The effects of cytokines on intermediary metabolism. Endocrinologie, 1994;4:56- 63.
62-Winocour, P. D. Platelet abnormalities in diabetes mellitus. Diabetes. 1992;41:26-31.