

Estimation of Some Cytokines in Serum and Gingival Fluid in Diabetic Patients with Periodontitis

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Abstract Cytokines play a central role in the host's response to the periodontal film. Although the pathways through which diabetes affects periodontal status are well understood, less is known about the impact of periodontal diseases on the diabetes-related inflammatory state. This review attempts to explain the immunobiological connection between periodontal diseases and type 2 diabetes mellitus. **Subjects and methods:** Patients were selected among patients presented to the outpatient clinic of Oral Medicine and Periodontology Department, Faculty of Oral and Dental Medicine, AL-Azhar University. Patients were divided into four groups (Periodontitis group without diabetes, Diabetic group with periodontitis, Diabetic group without periodontitis and control group). Serum and GCF of IL-6 and TNF- α were detected by ELISA. C-peptide was assessed by immune-enzymometric assay and glycated hemoglobin (HbA1c %) was assessed by quantitative colorimetric method. **Results:** Serum levels and GCF levels of TNF- α were significantly higher in diabetic patients with periodontitis group compared to control group and diabetic group and periodontitis group. Also, serum levels and GCF levels of IL-6 were significantly higher in diabetic patients with periodontitis compared to control group and diabetic group and periodontitis group. Highest percent of glycated haemoglobin (HbA1c) was found in diabetic patients with periodontitis then diabetic group.

Conclusion: Diabetes and periodontitis are closely related. Results give evidence that periodontal destruction and diabetes mellitus have a synergistic effect in elevating the proinflammatory cytokines.

Keywords: Gingival Crevicular Fluid (GCF), Tumor Necrosis Factor- α (TNF- α), Interleukin-6 (IL-6), glycated haemoglobin (HbA1c)

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1. Introduction

The most documented condition related to periodontal disease (PD) is diabetes mellitus (DM). PD is considered to be the sixth complication of DM, both having inflammation process and increased oxidative stress as the primary etiologic features (Velea et al., 2013).

The National institute of Dental and Craniofacial Research (2003) demonstrated that the patients with type 1 diabetes showed 9.8% prevalence of periodontal disease compared with 1.7% in non-diabetic subjects. However, patients with type 2 diabetes are three times more likely to develop periodontal disease than non-diabetic people. Periodontitis and type 2 diabetes are co morbid conditions, both characterized by infectious susceptibility (Bassim et al, 2008).

Both of these diseases have a relatively high incidence globally in the general population with a number of common pathways in their pathogenesis. DM and periodontitis are polygenic disorders with some degree of

immuno-regulatory dysfunction. Numerous reports indicate a higher incidence of periodontitis in diabetics compared to healthy controls. This relationship appears bi-directional as the presence of one condition tends to promote the other, and that the meticulous management of either may assist treatment of the other (Malik et al., 2011).

Diabetes and periodontal disease are common chronic diseases observed in the U.S. population. These diseases are thought to be associated biologically, and a number of reviews and studies have proposed mechanisms to explain the relationship, including: 1) microvascular disease, 2) changes in components of gingival crevicular fluid, 3) changes in collagen metabolism, 4) an altered host response, 5) altered subgingival flora, 6) genetic predisposition, and 7) non enzymatic glycation.

In addition, in vitro studies of monocytes from people with diabetes have shown a hyper responsive phenotype with over expression of pro-inflammatory mediators such as interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), and prostaglandin E2. In similar in vivo studies, patients with periodontitis and diabetes were found to have significantly higher levels of local inflammatory mediators

compared to systemically healthy individuals with periodontal disease (Southerland et al., 2005).

Evidence has consistently indicated that diabetes is a risk factor for increased severity of gingivitis and periodontitis. Conversely, periodontitis may be a risk factor for worsening glycemic control among patients with diabetes and may increase the risk of diabetic complications. Periodontitis may initiate or propagate insulin resistance in a manner similar to that of obesity, by enhancing activation of the overall systemic immune response initiated by cytokines (Tunes et al., 2010).

Tumor necrosis factor α (TNF- α), Interleukin (IL)-6, and IL-1, all mediators important in periodontal inflammation, have been shown to have important effects on glucose and lipid metabolism, particularly following an acute infectious challenge or trauma. TNF- α has been reported to interfere with lipid metabolism and to be an insulin antagonist. IL-6 and IL-1 have been reported to antagonize insulin action. (Kumari et al., 2011)

The level of inflammatory cytokines in the crevicular fluid is also related to glycemic control. Previous study reported that diabetic patients with periodontitis, whose HbA1c levels were over 8%, had approximately twice the amount of interleukin-1 β (IL-1 β) in their crevicular fluid in comparison to patients with indexes below 8%. The net effect of these changes in the immune response of diabetics is an increase in periodontal inflammation, a loss of epithelial insertion and alveolar bone. (Engbretson et al., 2004)

Studies have provided evidence that control of periodontal infection has an impact on improvement of glycemic control evidenced by a decrease in demand for insulin and decreased hemoglobin A-1c levels (Fiehn et al., 2005).

2. Patients and Methods

2.1. Patients:

In the current study 40 patients were selected from outpatient clinic of Oral Medicine and Periodontology Department, Faculty of Oral and Dental Medicine Al-Azhar University. There were divided into four groups as following:

Group I: It includes 10 patients suffering from periodontitis without diabetic history.

Group II: It includes 10 patients suffering from diabetes with periodontitis.

Group III: It includes 10 diabetic patients without periodontitis.

Group IV: It includes 10 healthy subjects free from any systemic diseases with healthy periodontium.

Samples of 5mls venous blood were withdrawn by venipuncture from all individuals included in this study and divided into two parts:

1- 1ml of whole blood stored in EDTA purple tubes for determination of HbA1c level.

2- Serum after centrifugation stored at -20°C for analysis of TNF- α , IL-6, and C-peptide.

Medical history was taken for all individuals.

Determination of TNF- α had been carried out using commercially available kit AviBion Human TNF- α ELISA kit (Ani Biotech Orgenium Laboratories. Cat. No.

TNF α 021). This kit is an in vitro enzyme-linked immunosorbent assay for quantitative measurement of human TNF- α in cell culture supernatants, serum, plasma, cerebrospinal fluid, urine, synovial fluid and other body fluids. This assay employs an antibody specific for human.

IL-6 level is measured by quantitative sandwich immunoassay (ELISA) using commercially available AviBion Human IL-6 ELISA kit (Ani Biotech Orgenium Laboratories Cat. No. IL06001). The assay employs an antibody specific for human IL-6 coated on a 96-well plate. Determination of Glycohemoglobin (HbA_{1c}) by Stanbio method for quantitative colorimetric determination of glycohemoglobin in whole blood using commercially available kit Stanbio Glycohemoglobin Cat.No.:0350-060. Determination of C-peptide had been carried out by a micro plate immunoenzymometric assay using commercially available kit (Monobind Inc, Acc-Bind ELISA Microwells, Cat. No. 2725-300A).

2.2. Statistical Analysis

Quantitative data were presented as mean, median, standard deviation (SD). Data showed non-parametric distribution so Kruskal-Wallis test was used to compare between the four groups. Mann-Whitney U test was used for pair-wise comparisons between the groups when Kruskal-Wallis test is significant. Spearman's correlation coefficient was used to determine significant correlations between the different variables. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

3. Results

In the present study, the mean values of serum TNF- α were (63.5 \pm 44.6) in group I, (163.2 \pm 34.5) in group II, (133.7 \pm 41.2) in group III and (52.1 \pm 14.5) in group IV.

There was a statistically significant difference between four groups in serum TNF- α , with group II (diabetic patients with periodontitis) showing the highest serum TNF- α level. Group IV (normal healthy subjects) recorded the lowest serum TNF levels.

The mean values of levels of TNF- α in GCF were (98.9 \pm 51.5), (158.8 \pm 26), (44 \pm 12) and (46.2 \pm 12.9) for groups I, II, III, and IV respectively. There was a statistically significant difference between group I and group II, group I and group III, group I and group IV and group II and III. However, there was a non significant difference between group III and IV. Group II showed the highest mean level of TNF- α in GCF followed by group I then group IV and III.

Regarding serum levels of IL-6, there was a statistically significant difference between the four studied groups. Group II showed the highest mean level (254.5 \pm 88.6), and group IV recorded the lowest mean serum IL-6 level (38.1 \pm 18.6).

Regarding the level of IL-6 in GCF, group II showed the statistically highest mean level (251.6 \pm 60). The level of IL-6 in GCF was lower in group I (226.9 \pm 62.4). When comparing the mean value of level of IL-6 in GCF in group III (104 \pm 46.2) and group IV (113 \pm 27), there was no statistically significant difference.

The statistical analysis of the data revealed a significant difference in C-peptide level. The highest level was

(1.01±0.61) recorded in group II, followed by group III (0.54±0.22) and group I (0.37±0.51). The lowest mean level was reported in group IV (0.005±0.007)

The diabetic groups with periodontitis (group II) showed the highest percent of HbA1c which was (9.9±1.9). There

was no statistically significant difference between the mean percent of HbA1c in group's I & II and III. The non-diabetic groups (group I and IV) showed lower percent of HbA1c which was (6.4±0.9) and (6.2±0.3) respectively. Data are summarized in Table 1 and Table 2 as following:

Table 1. Mean ± standard deviation (SD) values of TNF-α and IL-6 levels in serum and GCF, C-peptide level and HbA1c % in the four groups

Variable	Group I	Group II	Group III	Group IV	p-value
TNF-α Serum (M ± SD)	63.5±44.6	163.2±34.5	133.7±41.2	52.1±14.5	<0.001
GCF(M ± SD)	98.9±51.5	158.8±26	44±12	46.2±12.9	<0.001
IL-6 Serum(M ± SD)	143.3±81.5	254.5±88.6	173.6±54.2	38.1±18.6	0.001
GCF(M ± SD)	226.9±62.4	251.6±60	104±46.2	113±27	0.001
C-peptide Serum(M ± SD)	0.37±0.51	1.01±0.61	0.54±0.22	0.005±0.007	<0.001
HbA1c Serum(M ± SD)	6.4±0.9	9.9±1.9	6.8±0.9	6.2±0.3	<0.001

Table 2. Correlation coefficient for the correlation between different variables

Variables	Correlation coefficient	P-Value
TNF-α (Serum) and IL-6	0.546	<0.001
TNF-α (Serum) and HbA1c	0.668	<0.001*
TNF-α(GCF) and HbA1c	0.572	<0.001*
Il-6(serum) and HbA1c	0.490	0.001*
Il-6(GCF) and HbA1c	0.414	0.008*

*: Significant at P ≤ 0.05

Levels of TNF-α in serum were positively correlated to levels of IL-6 in serum in the four studied groups (Figure 1).

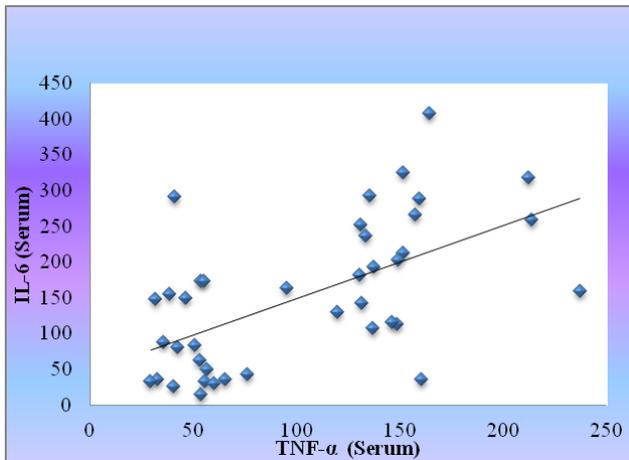


Figure 1. Correlation between TNF-α (Serum) and IL-6 (Serum) in the four studied groups

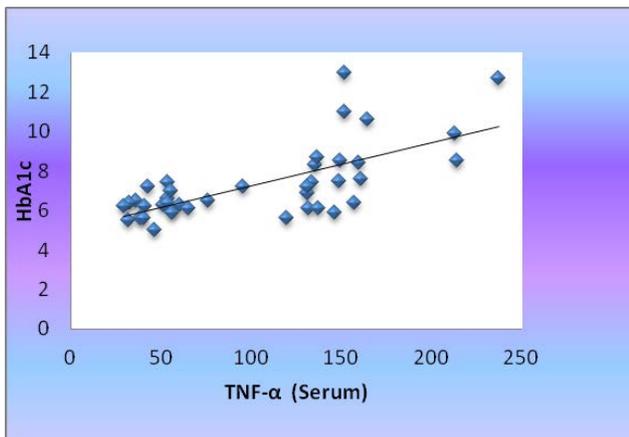


Figure 2. Correlation between TNF-α (Serum) and HbA1c in the four studied groups

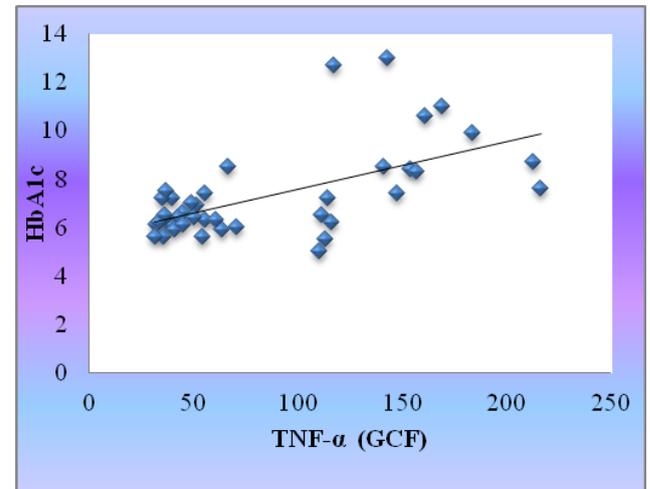


Figure 3. Correlation between TNF-α (GCF) and HbA1c in the four studied groups

The four groups demonstrated a positive correlation between levels of TNF-α in serum & GCF and percent of HbA1c. (Figure 2 & Figure 3)

Levels of IL-6 in serum were positively correlated to percent of HbA1c in the four groups.

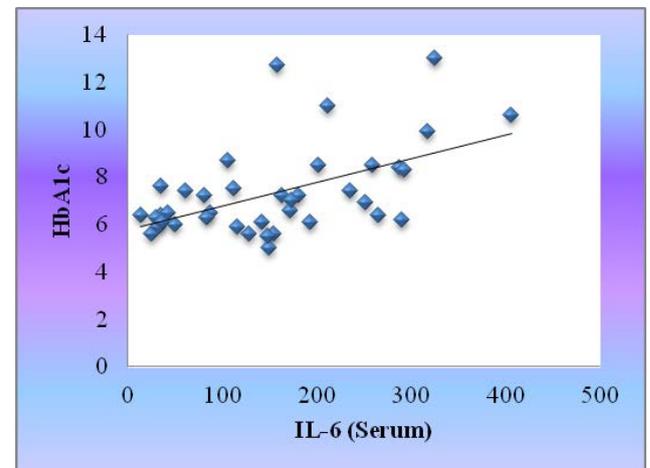


Figure 4. Correlation between IL-6 (Serum) and HbA1c in the four studied groups

There was a positive correlation between levels of IL-6 in GCF in the four studied groups and percent of HbA1c.

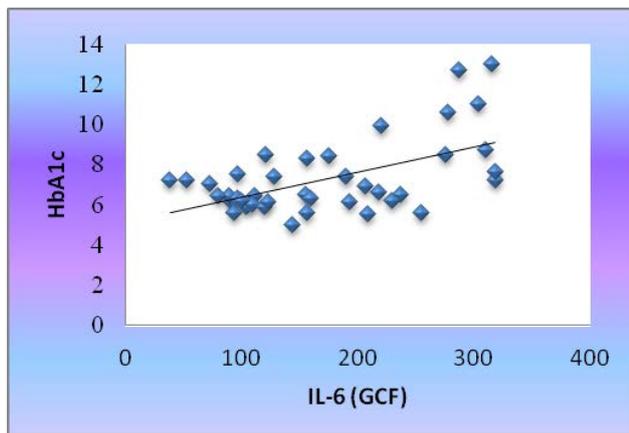


Figure 5. Correlation between IL-6 (GCF) and HbA1c in the four studied groups

4. Discussion

An elevated chronic systemic inflammatory state induced by periodontal disease may contribute to insulin resistance through “feed-forward” mechanism, worsening glycemic control. Also, periodontitis may contribute to the elevation of serum inflammatory mediators through enhanced in-vitro production of TNF- α , IL-6 and prostaglandin(PGE₂) by monocytes as has been shown in patients with diabetes and periodontitis (Malik et al., 2011).

As GCF transverse the inflamed tissue, it carries molecules involved in the destructive process (Loos and Tjoa, 2005).

The added advantage in GCF collection is that, it is a non-invasive procedure as compared to gingival tissue biopsy. In the present study, levels of the proinflammatory cytokines were assessed in the serum and GCF as the composition of GCF reflects the changes occurring in the local periodontal environment (Champagne et al., 2003).

On the other hand, in DM, adipokines like leptin and resistin highly activate cells releasing interleukin-6. This, in turn, stimulates greater hepatic CRP synthesis which may also increase insulin resistance. Inflammatory and infectious stimuli such as lipopolysaccharides and cytokines increase leptin levels in acute phase of periodontitis (Buduneli and Gümiş, 2013).

In the present study design, the influence of age and sex on biomarkers concentrations was minimized by including an equal number of males and females in the study and selecting the patients within the specified age group. The variability in concentrations in each group could be due to different stages of the disease process at the time of collection of GCF and serum samples.

Hemoglobin A1c (HbA1c) test is used to monitor the patients overall glycemic control and provides an estimate of the average blood glucose level over the preceding 90-120 days period (Daily et al, 2006).

Evaluation of patient’s HbA1c was carried in this study. The binding of glucose to hemoglobin is highly stable, therefore, hemoglobin remains glycosylated for the life span of the erythrocyte (~123±23 day).The HbA1c test is used to measure glycohemoglobin levels and provides an estimate of the average blood glucose level over the preceding 30-to 90 day period. HbA1c levels correlate well with the development of diabetic complications and

may become established as a test for the diagnosis of diabetes at some time in future (Gurralla et al., 2013).

Levels of biomarkers have been evaluated in the present study by the use of ELISA.

ELISA tests are generally highly sensitive, specific, easy and reliable techniques that are commonly used nowadays in most laboratory assays (Frodge et al., 2008).

Results of the present study demonstrated significantly different levels of TNF- α in GCF and serum in the four groups.

In serum, TNF- α showed the greatest mean level in group II diabetic patients with periodontitis where it was 163.2±34.5, followed by group III (diabetic patients without periodontitis) where it was 133.7±41.2 and group I (non diabetic patients with periodontitis) it was 63.5±44.6 and finally in group IV (normal healthy controls) where it was 52.1±14.5.

Regarding TNF- α level in GCF, TNF- α in group II showed the highest mean level (158±26) where DM is associated with periodontitis.

It was noticed that group III appears to show higher level of mean serum TNF- α than group I as sample obtained was serum.

On the other hand, group I appears to show higher TNF- α level in GCF than group III.

Furthermore, group III& group IV showed the lowest mean of TNF- α (44±12) & (46.2±12.9) where diabetes mellitus was present without periodontitis or the subjects were normal healthy individuals, respectively.

On comparing the IL-6 levels in non diabetic patients with periodontitis in the four studied groups there were a statistically significant difference between them in serum and GCF. Group II showed the highest mean (254±88.6) & (251.6±60) and group IV showed a lower mean (38.1±18.6) & (113±27) in serum and GCF respectively.

Furthermore, no statistically significant difference between diabetic patients with healthy periodontium and the normal healthy periodontium.

This would highlight the role of periodontal disease in pathogenesis of diabetes mellitus.

These results confirmed with Adriankaja et al., 2009 who evaluated the levels of IL-6 in 725 subjects, NDM and DM type 2 with periodontal healthy or gingivitis and concluded that high levels of serum IL-6 were detected in DM group when biofilm was presented.

On the other hand, Longo et al., 2014 found that there were no significant differences among similar groups for IL-6, IL-8 and MCP-1 serum levels.

In the present study, there was a significant positive correlation between TNF- α level in serum and GCF and between TNF- α levels and glycemic control. The highest value was reported in diabetic patients with periodontitis. This was in accordance with Jaganath et al., 2011 who reported poor glycemic control was associated with elevated TNF- α in the GCF.

On the other hand, these results differ from those of Engebreston et al., 2007 who found that plasma level of TNF- α and HbA1c% show no correlation but a dose-response relationship was observed between periodontitis severity and TNF-alpha (p=0.012).

This observation may offer a plausible explanation for increased incidence and severity of periodontitis in patients with diabetes. It can be inferred that glycemic control plays an important role in determining the amount

of TNF- α release. Poor control of diabetes predispose an individual to higher TNF- α production and there by more severe periodontal destruction. Thus, it is expected that TNF- α is a key player in determining the severity of periodontal destruction in type 2 diabetes mellitus. The current finding of elevated TNF- α in diabetic subjects with poor glycemic control raises the possibility that local gingival inflammation may adversely influence the glycemic control in diabetes. TNF- α -from the periodontal environment can enter the systemic circulation and adversely affect insulin sensitivity. The resultant insulin resistance can lead to worsening in the glycemic control which can lead to further periodontal breakdown (*Jaganath and Vijayendra, 2011*).

The present study demonstrated a positive correlation between IL-6 levels in GCF, serum and glycemic blood glucose. This might be explained by the role of IL-6 as a pleiotropic cytokine with a key impact on both immunoregulation and non-immune events in most cell types and tissues outside the immune system (*Choudhary and Ravinder, 2008*).

Mengel et al., 2002 and *Buhlin et al., 2003* reported that periodontitis has been associated with increased circulating levels of IL-6. This increase appears to be correlated with disease severity.

Furthermore, *D'Aiuto et al., 2005* reveal that periodontal treatment can decrease inflammatory mediators due to inflammation control.

Serum levels of high-sensitivity CRP, TNF- α , IL-6, fasting plasma glucose, HbA1c, fasting insulin decreased and adiponectin increased 3 months after periodontal treatment in type 2 DM patients and periodontal treatment may improve glycemic control, lipid profile, reduce serum inflammatory cytokine levels, and increase serum adiponectin levels in poorly controlled type 2 DM patients. (*Sun WL et al., 2011*)

Levels of high sensitivity CRP and stem cell factor in serum and GCF were reported to be increased in patients with periodontitis and DM (*Kalra N et al., 2013*).

References

- [1] Andriankaja OM, Barros SP, Moss K, Panagakos FS, DeVizio W, Beck J, (2009) Levels of serum interleukin(IL)-6 and gingival crevicular fluid of IL-1beta and prostaglandin E(2) among non-smoking subjects with gingivitis and type 2 diabetes. *J Periodontol*; 80: 307-16.
- [2] Bassim CW, Redman RS, De Nucci DJ, Becker KL, Nysten ES, (2008) Salivary procalcitonin and periodontitis in diabetes. *J Dent Res.*; 7: 630-4.
- [3] Buduneli N and Gümüş P, (2013) Diabetes mellitus and periodontitis: signs of a bidirectional relationship, *European medical Journal*. 1: 30-36.
- [4] Buhlin K, Gusafsson A, Pockley AG, Frostegard J, Klinge B (2003) Risk factors for cardiovascular disease in patients with periodontitis. *Eur Heart J*; 24: 2099-107.
- [5] Champagne CM, Buchanan W, Reddy MS, Preisser JS, Beck JD, Offenbacher S. (2003) Potential for gingival crevice fluid measures as predictors of risk for periodontal diseases. *Periodontol* 2000; 31: 167-80.
- [6] Choudhary N and Ahlawat RS (2008) Interleukin-6 and C reactive protein in pathogenesis of diabetic nephropathy. *Iranian Journal of kidney diseases*; Vol. 1; No.1.
- [7] D'Aiuto F, Nibali L, Parkar M, Suvan J, Tonetti MS (2005) Short-term effects of intensive periodontal therapy on serum inflammatory markers and cholesterol. *J Dent Res*; 84: 269-73.
- [8] Daily GE. Contributions of fasting and post prandial plasma levels to glycosylated hemoglobin and diabetes mellitus related complications: Treating hyperglycemia with insulin. *Insulin*. 2006; 1: 148-157.
- [9] Engebretson S, Chertog R, Nichols A, Hey-Hadavi J, Celenti R, Grbic J, (2007) Plasma levels of tumor necrosis factor-alpha in patients with chronic periodontitis and type 2 diabetes. *J Clin Periodontol*. 34 (1): 18-24.
- [10] Engebretson SP, Hey-Hadavi J, Ehrhardt FJ, (2004) gingival crevicular fluid levels of interleukin-1 β and glycemic control in patients with chronic periodontitis and type 2 diabetes. *J Periodontol*; 75: 1203-1208.
- [11] Fiehn NE, Larsen T, Christiansen N, Holmstrup P, Schroeder TV, (2005). Identification of periodontal pathogens in atherosclerotic vessels. *J Periodontol*. 76: 731-6.
- [12] Frodge I, Brandon D, Jeffrey L, Ebersole and Craig S. M (2008) bone remodeling biomarkers of periodontal diseases in saliva. *J periodontol*; 79: 1913-1919.
- [13] Gurrula S, Muttineni R, Chintala S, Pabolu C, (2013) Reduction of HbA1c levels following nonsurgical treatment of periodontal disease in Type 2 diabetics NTR Univ Health Sci; 2: 109-14.
- [14] Jaganath S, Vijayendra R, (2011) Estimation of tumor necrosis factor-alpha in the gingival crevicular fluid of poorly, moderately and well controlled type 2 diabetes mellitus patients with periodontal disease-A Clinical and Biochemical study, *Indian J Stomatol*; 2 (3): 159-64.
- [15] Kalra N, Pradeep AR, Priyanka N, Kumari M, (2013) Association of stem cell factor and high-sensitivity C reactive protein concentrations in crevicular fluid and serum in patients with chronic periodontitis with and without type 2 diabetes. *J Oral Sci*. 2013; 55: 57-62.
- [16] Kumari M, Naik SB, Namita Shanbhag, (2011). Diabetes and periodontitis, A casual link or a two way street?. *AOSR*; 1 (4): 221-227.
- [17] Longo PL, Artese HP, Rabelo MS, Kawamoto D, Foz AM, (2014) Serum levels of inflammatory markers in type 2 diabetes patients with chronic periodontitis, *J Appl Oral Sci*. 2014 Mar-Apr; 22 (2): 103-108.
- [18] Loos B.G and Tjoa S, (2005) Host-derived diagnostic markers for periodontitis: do they exist in gingival crevice fluid. *Periodontol* 2000; 39: 53-72.
- [19] Malik G, Lehl G, Talwar M, (2011) Association of periodontitis with diabetes mellitus. *Journal of medical college Chandigarh*, vol. 1, No.1.
- [20] Mengel R, Bacher M, Flores-de-jacoby L (2002) Interactions between stress, interleukin-1b, interleukin-6 and cortisol in periodontally diseased patients. *J Clin Periodontol*; 29: 1012-22.
- [21] Southerland JH, Taylor GW, Offenbacher S, (2005) Diabetes and Periodontal Infection: Making the Connection *Clinical Diabetes*, vol. 23 no. 4 171-178.
- [22] Sun WL, Chen LL, Zhang SZ, (2011) Inflammatory cytokines, adiponectin, insulin resistance and metabolic control after periodontal intervention in patients with type 2 Diabetes and chronic periodontitis. *Intern Med*. 2011; 50: 569-74.
- [23] Tunes SR, Foss-Freitas CM, Filho N, (2010) Impact of Periodontitis on the Diabetes-Related Inflammatory Status. *J Can Dent Assoc*; 76:a35.
- [24] Velea AO, Caroline K, Onisel D, (2013) Diabetes mellitus and periodontal disease – A Two –way Road: Current concepts and future considerations (*European scientific journal* Vol. 9, No. 9 ISSN: 1857-7881).