Original Research Paper

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Salivary Interleukin - 6: Assessment in Chronic Generalized Periodontitis Patient with and Without Type 2 Diabetes Mellitus.

Abstract:

Background: Periodontitis is affected by various systemic diseases, among them Diabetes Mellitus is a major systemic factor to influence the severity of chronic periodontitis. Various inflammatory markers are produced in the course of disease that can be reflected in saliva. This study evaluates the salivary concentration of interleukin-6 (IL - 6) in periodontitis patients with type 2 diabetes.

Materials and Methods: Whole saliva samples were collected from sixty four patients who were further divided into four groups; Healthy group (control group; n = 16), Chronic Generalized Periodontitis (PD; n = 16), Diabetes Mellitus (DM; n = 16), and Chronic Generalized Periodontitis along with Diabetes Mellitus (PD + DM; n = 16 patients). Salivary IL-6 concentrations were determined by standard enzyme-linked immunosorbent assay and Clinical parameters were recorded like Gingival Index (GI), Oral Hygiene Index (OHI-S), Probing Pocket Depth (PPD), Gingival Recession (GR), Clinical Attachment Level (CAL) with the help of mouth mirror and UNC -15 graduated periodontal probe.

Results: Result showed that the Chronic Generalized Periodontitis patients with and without Diabetes Mellitus exhibited higher concentrations of salivary IL-6 than the control group and diabetes groups. Further, the salivary IL-6 was correlated with glycosylated hemoglobin A levels in patient with diabetes. This is due to the fact that both Diabetes & Periodontitis are chronic inflammatory disease that significantly increase the expression of IL-6 which cause insulin resistance in adipocyte and thereby affecting HbA1c levels. Therefore, both can affect severity of each other. Salivary concentration of IL- 6 was determined using an Human IL-6 (Interleukin- 6) ELISA Kit. Kruskal Wallis test was applied to compare any statistical difference between groups for clinical parameters, HbA1c and IL-6. Spearman correlation test was used to find any relation between HbA1c and IL -6 among all the groups. A significance level of 5% was set ($P<0.05$).

Conclusion: The salivary concentration of IL-6 was elevated in patients with periodontitis with and without diabetes. Therefore, levels of salivary IL-6 can be considered as an important biomarker in the diagnosis of periodontitis and diabetes.

Keywords : Diabetes Mellitus ; Hemoglobin Aglycosylated; Interleukin -6 ; Chronic generalized periodontitis.

Introduction:

The oral cavity provides a continuous source of infectious agents and its condition often reflects progression of systemic pathologies. Periodontitis is a chronic inflammatory disease that results from an imbalance in the interactions between periodontal pathogens and the host response.[1] This imbalance causes the over expression of proinflammatory cytokines and the subsequent destruction of supporting connective tissue attachment and alveolar bone.[2] Periodontitis is multifactorial in origin. Environmental, behavioral, and systemic factors also influence the onset, progression, and severity of periodontitis.

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Diabetes mellitus(DM), a metabolic disorder characterized by hyperglycemia resulting From defects in insulin secretion or action.[3] is considered a risk factor for periodontitis.[4] Type I, insulin dependent diabetes mellitus, is characterized by abrupt onset at any age, due to destruction of 80 to 90% of the

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pancreatic islet cells that produce insulin.Type 2, non-insulin dependent diabetes mellitus, may develop over a period of time and is often called adult onset diabetes. Type 2 diabetics have a reduction in insulin production and control their blood sugar by diet and hypoglycemic agents, or by diet alone.[5] The disease is characterized by an increased susceptibility to infection, poor wound healing, and increased morbidity and mortality associated with disease progression.

The relationship between periodontal disease and Diabetes Mellitus is two way, meaning both can influence the severity of the other.[6] An explanation may be that persistent hyperglycemia in patients with diabetes creates an imbalance between periodontopathogens and the host response, thereby causing over expression of proinflammatory cytokines, formation of advanced glycation end products, dysfunction of polymorphonuclear leukocytes, and ultimately break down of supporting connective tissue attachment and alveolar bone.[7]

Interleukin (IL)-6 is an inflammatory mediator that stimulates osteoclast activity and bone resorption in periodontitis.[8] It is secreted by macrophages in response to inflammation and is involved in recruitment and apoptosis of leukocyte and T-cell activation. The elevated levels of salivary IL-6 at the periodontally infected sites from diabetic patients proving the systemic influence of Diabetes on periodontium.

Saliva is one of the most important body fluids, and serve as a potential source to measure biomarkers released during disease initiation and progression, it has significant association with inflammatory, connective tissue destruction and bone remodeling phases of periodontal disease. Subsequently, saliva has been used in the diagnosis of periodontal disease and monitor response to treatment.[9] According to Pellegrini et al.[10] and Ito et al.[11] quantifying biomarkers in saliva may serve as a useful tool to predict an individual's susceptibility to periodontitis, to provide information for periodontal activity, and to monitor the effectiveness of periodontal therapy. Thus, by evaluating the salivary IL-6 level in saliva, the risk and severity of periodontitis in type 2 diabetic patients can be predicted.

Materials and Methods

In the present study, a total of 64 patients were selected from the outpatient Department of Periodontology, Peoples College of Dental Sciences and Research Centre, Bhanpur, Bhopal. The study protocol was approved by the institutional ethical committee with the institutional ethical clearance no. Ec201814. It was mandatory for all participants to read and sign the consent form before inclusion in the present investigation. Un-stimulated whole saliva sample will be collected into sterile tubes according to Navazesh method.[12] Patients were refrained from eating, drinking, and oral hygiene for at least one hour before saliva collection. Saliva samples were placed on ice box & send to the laboratory in Centre for Scientific Research and Development (CSRD)& aliquoted before freezing at -20°C. Avoid repeated freeze-thaw cycles. After collection of saliva, Salivary concentration of IL-6 was determined using an Human IL-6 (Interleukin 6) Elisa Kit (fig. 1).

Fig 1: Elisa Kit

A. Reagents for estimating interleukin-6:

Reagents used were antihuman IL-6 wash buffer (25X), 2 vials of recombinant human IL-6, 20 ml of sample/standard Dilution Buffer, 60µlof concentrated Biotin-labeled Antibody, 5ml of Antibody Dilution Buffer, 2 vials of biotinylated antihuman IL-6 hp streptavidin concentrate, 60µl of concentrated horseradish peroxidase-conjugate streptavidin, 3,3′,5,5′ tetramethylbenzidine (TMB) one-step substrate reagent, 10 ml of TMB in buffered solution, and 10 ml of 2M sulfuric acid as stop solution. All reagents stored at 2-8°C.

B. Enzyme-linked immunosorbent assay procedure:

All reagents, samples, and standards were brought to room temperature before use. All samples were diluted with Sample

Dilution Buffer. Plates were washed 2 times before adding standard, sample and control. 100 µl of standard or sample was added into appropriate wells and covered to incubate for 90 minutes at 37°C temperature. Plates was washed & aspirated 2 times. Then add 100 µl of prepared Biotin - labeled antibody working solution to each well and incubation done for 1 h at room temperature. Plates was washed & aspirated 3 times. Then add 100 µl prepared Streptavidin solution and incubated for 30min at room temperature. Plates was washed & aspirated 5 times. Then add 90µl of TMB one step substrate agent to each well.

c. Glycosylated hemoglobin A (HbA1c) measurement:

HbA1c levels were analyzed for the metabolic assessment in all the four groups. HbA1c was measured and expressed as percentages. Normal range of HbA1c test was <6%.

d. Study Population:

The study population was 34 males and 30 females aged 25-60 years and presented a minimum of 20 natural teeth excluding third molars & with Probing pocket depth 4mm, clinical attachment level 4mm & bleeding on Probing. The other inclusion criteria were (i) Patients having moderate to severe periodontitis affecting more than 30% of sites of test groups, (ii) Subjects are systemically healthy except patient with type 2 diabetes mellitus & Glycated Hemoglobin (HbA1c) test > 6%. The exclusion criteria were: (i) Smokers, (ii) Pregnant women and lactating females, (iii) Patients with Aggressive Periodontitis, (iv) Subjects taking antibiotic or periodontal treatment in the previous 6 months except for oral hypoglycemic agents, insulin therapy or both, (v) Uncontrolled Diabetes with Major Diabetic Complication such as (Retinopathy, Nephropathy, Neuropathy, Atherosclerosis).

A total of 64 subjects was divided in four groups: 1) control group (n = 16) = systemically and periodontally healthy subjects; 2) PD group (n = 16) = subjects who were systemically healthy and clinically diagnosed with chronic generalized periodontitis;3) DM group (n = 16) = subjects with type 2 diabetes mellitus and a healthy periodontium; and 4) PD + DM group (n = 16) = subjects with type 2 diabetes mellitus and clinically diagnosed with chronic generalized periodontitis.

e. Clinical Parameters:

Clinical parameters were recorded like Gingival Index (GI), Oral Hygiene Index (OHI-S), Probing Pocket Depth (PPD), Gingival Recession (GR), Clinical Attachment Level (CAL) with the help of mouth mirror and UNC -15 graduated periodontal probe after collection of an un- stimulated saliva sample.

f. Biochemical analysis:

Biochemical analysis was carried out at Centre for Scientific Research and Development (CSRD), Bhopal.

The data so collected was entered into MS excel and analyzed using SPSS version 20.0 (IBM; Chicago). Descriptive data was presented as mean ± S.D. Kruskal Wallis test was applied to compare any statistical difference between groups for clinical parameters, HbA1C and IL-6. Spearman correlation test was used to find any relation between HbA1c and IL -6 among all the groups. A significance level of 5% was set ($P<0.05$)

Results:

Sixty four patients in this study were divided into four groups: control group who were periodontally & systemically healthy(C), (PD) group who were systemically healthy & clinically diagnosed with chronic generalized periodontitis, Type 2 diabetes mellitus (DM) patient with a healthy periodontium, and PD + DM group subjects with chronic generalized periodontitis & type 2 diabetes mellitus. Clinical examination was done after collection of saliva and salivary concentrations of samples were assessed by ELISA Kit.

The results of our study depict that mean gingival index, simplified oral hygiene index score, probing pocket depth, clinical attachment level, gingival recession was more in chronic generalized periodontitis & type 2 diabetes mellitus (PD+DM) patients along with systemically healthy & clinically diagnosed with chronic generalized periodontitis (PD) than patients who were periodontally & systemically healthy controls (C) & patients with type 2 diabetes mellitus

(DM) & a healthyperiodontium.

The mean salivary biomarker (IL-6) was higher in chronic generalized periodontitis & type 2 diabetes mellitus (PD+DM), patient who were systemically healthy & clinically diagnosed with chronic generalized periodontitis (PD), patients with type 2 diabetes mellitus (DM) & a healthy periodontium than patients who were periodontally & systemically healthy controls(C).

The mean HbA1c was higher in patients with chronic generalized periodontitis & type 2 diabetes mellitus (PD+DM) & Patients with type 2 diabetes mellitus (DM) & a healthy periodontium than patient who were systemically healthy & clinically diagnosed with chronic generalized periodontitis (PD) & patients who were periodontally & systemically healthy controls (C).

Table 1: Clinical and biochemical parameters in Group 1 – Healthy individuals

Variable	Mean + S.D	Minimum	Maximum
Age	30.00 ± 9.50	23.00	59.00
GI	0.68 ± 0.13	0.50	1.00
OHI	1.21 ±0 .19	1.00	1.60
PD	2.09 ± 0.44	1.48	2.87
GR	0.38 ± 0.31	0.01	1.10
CAL	2.12 ± 0.33	1.78	2.75
HbA1c	5.26 ± 0.30	5	6
IL – 6	0.42 ± .05	0.34	0.53

Table 2: Clinical and biochemical parameters in Group 2 – Periodontitis patient

Variable	Mean + S.D	Minimum	Maximum
Age	35.81 ± 13.49	23.00	65.00
GI	1.45 ± 0.24	1.10	1.90
OHI	1.80 ±0 .40	1.30	2.50
PD	3.26 ± 0.61	2.08	4.01
GR	0.56 ± .60	0.01	2.14
CAL	3.73 ± 0.36	3.02	4.22
HbA1c	5.07 ± 0.61	4	6
IL - 6	1.71 ± .060	1.62	1.84

Table 3: Clinical and biochemical parameters in Group 3 – Diabetes mellitus patients

Variable	Mean + S.D	Minimum	Maximum
Age	46.06 ± 10.99	25.00	61.00
GI	0.72 ± 0.09	1.10	1.90
OHI	1.20 ±0 .94	0.60	1.20
PD	2.07 ± 0.59	1.15	3.45
GR	0.44 ± .36	0.01	1.10
CAL	2.26 ± 0.44	1.76	3.01
HbA1c	8.50 ± 1.73	6	12
IL – 6	1.46 ± .056	1.34	1.55

Figure 2: Mean HbA1c concentration across all groups

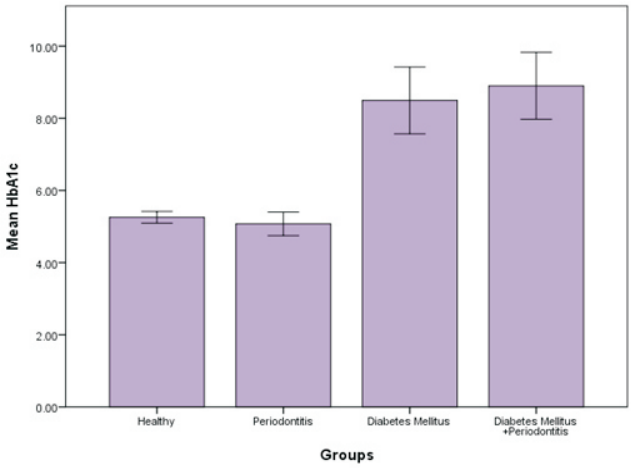
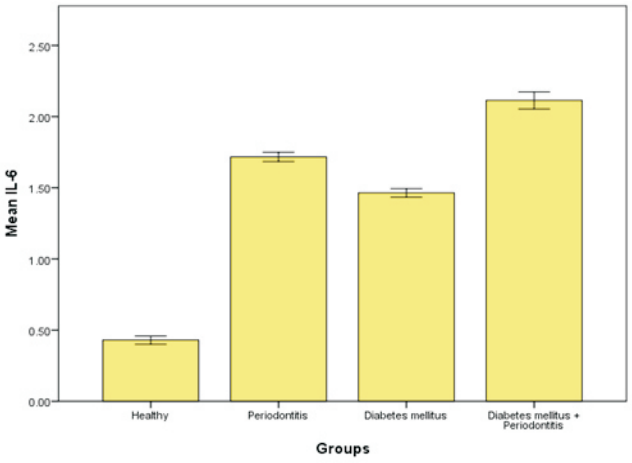


Figure 3: Mean IL - 6 concentration across all groups



Discussion:

Saliva is used as a diagnostic tool in dentistry because it is easy to collect and comprises both microbial and host response mediators. Quantifying biomarkers in saliva serve as an important tool to predict an individual's susceptibility to periodontitis. Costa et al.

The present study was conducted to evaluate the salivary concentration of proteins associated with periodontal diseases & the results demonstrate, the differentially expressed IL-6 level in all the four groups, showing the influence of diabetes on periodontium.

The age of patient with respect to all four groups ranges from 25-60 years.(Table 1,2,3 & 4), Sheridan[13] found that periodontal disease increases in prevalence and severity with age of the patient. This is because altered collagen metabolism in diabetics would be expected to contribute to the progression of periodontal disease as stated by Oliver and Tervonen.[14]

The gingival index (GI) score and simplified oral hygiene index (OHI-S) shows a significant difference between the groups (Table 1, 2, 3 & 4), but on comparing between the groups, Group D(PD+DM) shows higher value along with Group B(PD) as compared to Group A (Control) & Group C (DM), which was statistically highly significant with p value < 0.001. This is due to less amount of plaque in healthy patients which increased gradually with the progress of the periodontal disease. The Mean gingival score was 0.68, 1.45, 0.72 & 2.45 while simplified oral hygiene index score was 1.21, 1.80, 1.20 & 3.84 for Group A ,Group B, Group C & Group D respectively. These results are consistent with studies done by Javier Enrique Botero et al.[15] which shows that diabetes could aggravate periodontal disease and affect the systemic health of individuals.

The Probing pocket depth (PPD) & Clinical attachment level (CAL) shows a significant difference between the groups (Table 1,2, 3 & 4), but on comparing between the groups, Group D(PD+DM) shows higher value along with Group B (PD) as compared to Group A (Control) & Group C (DM), which was statistically highly significant with p value .001. The Mean probing pocket depth score was 2.09, 3.26, 2.07 & 3.65 while clinical attachment level (CAL) was 2.12, 3.73, 2.26 & 3.98 for Group A ,Group B, Group C & Group D respectively. These differences between the groups are consistent with studies by Morita et al.[16] which concluded that the elevated levels of HbA1c were associated with developing periodontal pockets of more than 4 mm.

The Mean Gingival recession shows a significant difference between the groups (Table 1, 2 ,3 & 4), but on comparing between the groups, Group D (PD+DM) shows higher value along with Group B (PD) as compared to Group A (Control) & Group C (DM) ,which was statistically non-significant with p value > 0.001.The Mean Gingival recession at baseline for Group A, Group B, Group C & Group D was 0.38, 0.56, 0.44 & 0.59 respectively. These results were consistent with studies done by Adriana monea et al.[17] suggesting that diabetic subjects presented distortion in periodontal attachment, with changes in both epithelial and connective tissues, when compared to the healthy controls, suggesting that diabetes mellitus has an independent effect on periodontal tissue. Therefore, Group D (PD+DM) shows higher value along with Group B (PD) as compared to Group A (Control) & Group C(DM).

The Mean HbA1c score shows a significant difference between the groups (Table 1,2,3 & 4), but on comparing between the groups, Group D (PD+DM) shows higher value along with Group C (DM) as compared to Group A (Control) & Group B (PD) , which was statistically highly significant with p value <0.001.This is because IL-6 secretion in periodontitis cause immunoregulatory action which affect glucose homeostasis & metabolism directly or indirectly by acting on adipocytes and pancreatic beta cells which cause hyperglycemia and thereby affect HbA1c level as stated by Masataka Nakamura et al.[18] Therefore, in our study Hb A1c was higher in patient with diabetes & periodontitis. There by, indicating a bidirectional relationship between Diabetes Mellitus (DM) and Periodontal diseases (PD). The Mean HbA1c score at baseline for Group A, Group B, Group C & Group D was 5.26, 5.07, 8.50 & 8.90 respectively.

The Mean IL-6 score shows a significant difference between the groups (Table 1,2,3 & 4), but on comparing between the groups, Group D (PD+DM) shows higher value along with Group B (PD) as compared to Group A (Control) & Group C (DM) , which was statistically significant with p value < 0.001.The Mean IL-6 score at baseline for Group A, Group B, Group C & Group D was 0.42, 1.71, 1.46 & 2.11 respectively. These results were in accordance with studies conducted by Karjalainen and Knuuttila[19] & various other studies conducted by different authors such as Duarte et al.[20] and found higher IL-6 levels in patients with diabetes and periodontitis compared to non diabetics depicting the diabetes modulated expression of IL-6 in periodontal patients. Rodrigues DC[21] suggested that the formation of Advanced glycation end products (AGE) & its interactions with

Receptor for Advanced glycation end product (RAGE) play an important role in systemic hyper inflammatory state of patients with diabetes. On Contrary, in the present study, the DM group, in which the high formation of AGEs was expected, presented lower salivary IL-6 concentrations than the Group B (PD) and Group D (PD + DM) groups. This is because all patients in DM (Group C) group were treated with oral hypoglycemic agents, exogenous insulin, or both. This is because insulin activity seems to decrease the effects of IL -6 because these cytokines act as insulin antagonists. In addition patients were not periodontally compromised, therefore local inflammatory process was not influencing cytokine expression. Despite of receiving oral hypoglycemic drug in Group D (PD+DM), IL-6 was elevated since imbalance between periodontal pathogens and host response cause over expression of proinflammatory cytokines. Thereby, from various studies it was found that both diabetes & periodontitis are chronic inflammatory diseases that cause increase in inflammatory marker that is IL-6 which reflects in saliva. Nesse W et al.[22] showed a causal relationship between type 2 diabetes and periodontitis. On the contray, there were studies by Hasaan Gassim Mohamed et al.[23] indicating that type 2 diabetes has no significant influence on the prevalence of periodontitis.

Scannapieco et al[24] stated that HbA1c test is used to monitor the glycemic control in diabetes patients and it measures the amount of glucose irreversibly bound to hemoglobin molecule (Hb). Bachu L[25] in his study stated that HbA1c can be used as an effective screening tool at the community level than Fasting blood sugar (FBS) among Diabetics and Non-diabetics since it is unaffected by transient hyperglycemia from acute stress or illness & can measure prolong complications of diabetes.

In the present study, there was positive correlation between IL-6 concentration & HbA1c levels in the GroupD (PD+DM) & Group C (DM) groups since both Diabetes &Periodontitis are chronic inflammatory disease that significantly increase the expression of IL-6 which further exacerbate inflammatory response and cause tissue destruction. This result was consistent with studies by Lim et al[26] & showed a positive association between poor metabolic control & periodontitis. The dentist can play an important role in diabetic patients overall health care through recognition and treatment of their periodontal needs & understanding Periodontitis as “Sixth Complication of diabetes mellitus.”

Conclusion:

Within the limitation of present study such as small sample size, it can be concluded that the concentration of salivary IL-6 was significantly elevated in patients with periodontitis with or without diabetes, depicting the host modulatoryrole of IL-6 in these patients & confirming the hypothesis that the inflammation linked to periodontal disease is more severe in type 2 diabetic patients compared to the systemically healthy individuals. When the Hb A1c levels were assessed, we have found increased Hb A1c levels in diabetic patients with periodontic is than the diabetic patients alone, depicting the inflammatory role of IL-6 on glycemic control. Even though IL-6 is an inflammatory mediator that stimulates osteoclast activity and bone resorption in periodontitis, several other inflammatory and immune mediators which modulate the periodontal destruction should be considered in the future studies. Local and systemic conditions, periodontitis, and diabetes, respectively, may change the expression of salivary proteins and should be taken into account in the future studies. Thereby, the results of our study allow us to conclude that saliva was an adequate fluid to assess inflammatory mediators and it is an efficient and safe enough tool for diagnosis & evaluation of periodontal disease progression in type 2 diabetic patients.

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