COMPARATIVE EVALUATION OF SALIVARY INTERLEUKIN-6 IN DIABETIC AND NON-DIABETIC PATIENTS WITH CHRONIC PERIODONTITIS

Dr. Tasneem Fatima[1*], Dr. Sanjay Gupta[2], Dr. NeeluVerma[3], Dr. Zeba Rahman Siddiqui[4], Dr. Deepti Chandra[5], Dr. Diksha Maurya[6]

Abstract-

Aim- To evaluate salivary interleukin 6 in diabetic and non-diabetic patients with chronic periodontitis using the newer technique ECLIA. Materials and Method- For present study a total of 80 (both males and females) were selected and divided into four groups. Pre-treatment records were taken (HbA1c and random blood sugar) and periodontal assessment using clinical parameters (PI, GI, PPD and CAL) was done. Non-stimulated whole expectorated saliva sample (approx 3ml) was collected into sterile container according to the method described by Navajesh.[8] Saliva sample container was immediately placed in the ice bag and transported to the laboratory and centrifuged at 3500 rpm for 10 minutes and stored at (-20^{o} C) until subsequent IL-6 analysis. Salivary concentration of interleukin-6 is determined by using Cobas E Analyzer (Roche made in Germany), based on electroche miluminescene (ECLIA). Results- IL-6 was detected (pg/ml) in all four groups, maximum in Group IV followed by Group II, Group III and Group I. Conclusion- In the present study, there was a significantly elevated level of IL-6 in type 2 diabetes mellitus with chronic periodontitis group than chronic periodontitis group, followed by diabetes mellitus depicting the host modulatory role of IL-6 in these patients.

[1*]Post graduate student 3rd Year, Career postgraduate institute of dental sciences & Hospital, Lucknow, fatimatasneem858@gmail.com
[2]Professor and Head of the department, Career postgraduate institute of dental sciences & Hospital, Lucknow, sanru17@rediffmail.com
[3]Reader, Career postgraduate institute of dental sciences & Hospital, Lucknow, drneeluraja@gmail.com
[4]Reader, Career postgraduate institute of dental sciences & Hospital, Lucknow, zeba.rahman1@gmail.com
[5]Reader, Career postgraduate institute of dental sciences & Hospital, Lucknow deeptichandra27@gmail.com
[6]Pg-1st Year , Dept of Periodontics, Career postgraduate institute of dental sciences & Hospital, Lucknow

***Corresponding Author:** Dr. Tasneem Fatima
*Post graduate student 3rd Year, Career postgraduate institute of dental sciences & Hospital, Lucknow, Fatimatasneem858@gmail.com

DOI: - 10.48047/ecb/2023.12.si5a.0419

INTRODUCTION

Periodontal disease is a chronic microbial and inflammatory process characterized by the presence of pathogenic bacteria, impaired host immune response and destruction of supporting structures of the teeth, including gingiva, periodontal ligament and supporting alveolar bone.[1]

There are many systemic conditions that can modify the host's susceptibility to periodontitis. Although more than 50 different systemic conditions have been associated with periodontal diseases such as cardiovascular disease, diabetes mellitus, osteoporosis, respiratory illness and adverse pregnancy outcomes.[2]

There is a close link between periodontal disease and diabetes mellitus (DM). In other words, patients with type II diabetes are more likely at high risk (2.81 times) of clinical attachment loss than healthy individuals.[3]An epidemiological link between diabetes and periodontitis was established in 1960, and the interaction is classified by age and type of diabetes in most studies. Findings from the **Third National Health and Nutrition Examination Survey (NHANES III)** in the US indicatedthe prevalence of diabetes among people with periodontal disease was about two-fold higher than periodontally healthy diabetic subjects.[4]

Diabetes mellitus (DM) is a term applied to a heterogeneous group of disorders that develops as a result of either deficient production of insulin or impaired use of insulin.[5] The mechanism behind inter-relationship of diabetes and periodontal diseases is a change in monocyte/macrophage of patients with type II diabetes, which results in the overproduction of proinflammatory cytokines in response to periodontal pathogens, which could exacerbate the pathogenesis of periodontal disease. Interleukin-6 (IL-6) is one of the main proinflammatory cytokines which decisively involved in the development of insulin resistance and T2DM through the involvement of various pathways.[6]

Saliva has the potential to be used as a diagnostic fluid due to its different biochemical components and provide different advantages, including easy collection and non-invasive sampling method. As traditional periodontal diagnostic aids like PCR and ELISA, are accurate and effective for evaluation of salivary interleukin 6 level, but are greatly limited because of complexity and time-consuming.

The **new quantitative Electrochemilumine scence Immunuassay (ECLIA)** biosensors combine the advantages of both electrochemical and photoluminescence analysis and are suitable for high sensitivity and simple pathogenic bacteria detection and also showed good reproducibility, linearity and functional sensitivity.[7]

Therefore, salivary interleukin 6 level can be used as an important biomarker for the diagnosis, prognosis, and to predict the treatment outcomes of periodontitis. Hence, this study aims to comparative evaluation of salivary interleukin 6 in diabetic and non-diabetic patients with chronic periodontitis using the newer technique ECLIA.

MATERIALS & METHOD-

This cross-sectional observational study was conducted in the Department of Periodontology, Career Post Graduate Institute of Dental Sciences and Hospital, Lucknow, U.P., India. The study design and protocol was approved by, Institutional Human Ethical Committee Ref. No. (CPGIDSH/22/299) and written informed consent was obtained from all participants. For present study a total of 80 (both males and females) were selected from the Outpatient Department of Periodontology based on following inclusion and exclusion criteria.

Inclusion criteria-

1. Patients within the age group 30-55 years.
2. Presence of a minimum 20 natural teeth (excluding third molars) in the oral cavity.
3. Patients with chronic periodontitis having probing pocket depth ≥ 5mm found in minimum four teeth and clinical attachment loss(CAL) ≥ 2mm.
4. Glycemic patients should be previously diagnosed with Diabetes Mellitus with confirmed glycosylated Hb (HbA1C) ≥ 7% and Random blood sugar >200mg/dl with no major diabetic complications since at least 1 year and under oral hypoglycemic drugs/insulin.

Exclusion criteria-

1. History of periodontal therapy (non-surgical and surgical), antibiotics, corticoid or immunosuppressive therapy within the six months.
2. The existence of any systemic illness other than diabetes mellitus.
3. Pregnant and lactating women, chronic smokers, alcoholics and tobacco users.

4. Patients with malignancy, autoimmunity, infectious disease, xerostomia or traumatic ulcer.

Study Design-

The study comprises of eighty participants (80) including healthy volunteers and patients visiting the Career Post Graduate Institute of Dental Sciences and Hospital, Lucknow. The selected participants were divided into four groups consisting 20 participants in each group. (Table 1)

Group I (Healthy Control)	Systemically and periodontally healthy.
Group II (Chronic periodontitis only)	Non diabetic and systemically healthy and diagnosed with chronic generalized periodontitis
Group III (Type 2 Diabetes Mellitus)	Type 2 Diabetes mellitus with no evidence of chronic generalized periodontitis
Group IV (Type 2 Diabetes Mellitus with Chronic periodontitis)	Type 2 Diabetes mellitus with chronic periodontitis.

Table

Methodology-

Pre-treatment records were taken (HbA1c and random blood sugar) and periodontal assessment during clinical parameters (PI, GI, PPD and CAL) was done. Non-stimulated whole expectorated saliva sample (approx 3ml) was collected into sterile container according to the method described by Navajesh.[8] Saliva sample container was immediately placed in the ice bag and transported to the laboratory and centrifuged at 3500 rpm for 10 minutes and stored at (-20^{o} C) until subsequent IL-6 analysis. Salivary concentration of interleukin-6 is determined by using **Cobas E Analyzer** (Roche made in Germany), based on **electrochemiluminescene (ECLIA).**

Test principle of ECLIA (As manufacturer's instructions)

Sandwich principle- Total duration of assay: 18 minutes

- **1st incubation:** 30 µL of sample are incubated with a biotinylated monoclonal IL-6- specific antibody.
- **2nd incubation:** After addition of a monoclonal IL-6-specific antibody labeled with a ruthenium complexa) and streptavidin-coated microparticles, the antibodies form a sandwich complex with the antigen of the sample.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Application of a voltage to the electrode then induces chemilumine scent emission which is measured by a photomultiplier.

Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

Reagents –

M- Streptavidin-coated microparticles (transpa rent cap), 1 bottle, 6.5 mL: Streptavidin coated microparticles 0.72 mg/mL; preservative.

R1- Anti-IL-6-Ab~biotin (gray cap), 1 bottle, 9 mL: Biotinylated monoclonal anti-IL-6 antibody (mouse) 0.9 µg/mL; phosphate buffer 95 mmol/L, pH 7.3; preservative.

R2- Anti-IL-6-Ab~Ru(bpy) (black cap), 1 bottle, 9 mL: Monoclonal anti-IL-6 antibody (mouse) labeled with ruthenium complex 1.5 µg/mL; phosphate buffer 95 mmol/L, pH 7.3; preservative.

Precautions and warnings

- For in vitro diagnostic use.
- Exercise the normal precautions required for handling all laboratory reagents.
- Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).
- Do not test samples from patients who have indicated or whose clinical status or history would indicate they are currently taking high doses of biotin (> 10 mg per day).

Sample stability: Saliva 6 hours at 20-25 °C, 2 days at 2-8 °C 24 months at -20 °C (± 5 °C)

Calculation The analyzer automatically calculates the concentration of each sample in pg/mL.

PARAMETRES-

Clinical parameters-

1. Plaque Index (Silness and Loe 1964)
2. Gingival Index (Loe and Silness 1963)
3. Pocket Probing Depth (in mm)
4. Clinical Attachment Level (in mm)

Systemic parameters

1. Random Blood Sugar (RBS)- ⩾200 mg/dl (⩾11.1 mmol/l)

2. Glycated Hemoglobin (HbA1C)- HbA1c ≥ 7% (48 mmol/mol) as a diagnostic criterion.

Statistical analysis

Continuous data were summarized in Mean ± SE (standard error of the mean) whereas discrete (categorical) in number (no) and percentage (%). Continuous four independent groups were compared by one factor analysis of variance (ANOVA) and the significance of mean difference between (inter) the groups was done by Tukey's HSD (honestly significant difference) post hoc test after ascertaining normality by Shapiro-Wilk's test and homogeneity of variance between groups by Levene's test. Categorical groups were compared by chi-square ($\chi 2$) test. A two-tailed (α=2) $P < 0.05$ was considered statistically significant. Analysis was performed on SPSS software (Windows version 22.0).

RESULT-

In the present study, IL-6 was detected (pg/ml) in all four groups. The mean IL-6 level in control group (Group I) is 4.43 ± 0.42, in Group II is 27.73 ± 0.30, in Group III is 11.65 ± 0.23 and in Group IV is 37.08 ± 0.36. The mean IL-6 show similar trend as of clinical parameters (PI, GI, PPD and CAL), maximum in Group IV followed by Group II, Group III and Group I, the minimum [Group I (4.43 ± 0.42) < Group III (11.65 ± 0.23) < Group II (27.73 ± 0.30) < Group IV (37.08 ± 0.36). Comparing the mean IL-6 in all four groups, it showed significantly different IL-6 among the groups as group I, group II, group III and group IV ($F=1984.00$, $P < 0.001$).

DISCUSSION-

Chronic periodontitis is characterized by destruction of periodontal connective tissues and tooth supporting bone accompanied by apical migration of the epithelial attachment.[9]This process is guided by the host immune-inflammatory reaction in response to putative pathogenic bacteria.[10] It is now widely accepted that chronic periodontitis is one of the classical complications of diabetes. There is, however, contradictory evidence about the effect of type 2 diabetes on dental plaque microbiota**. Casarin RC et al (2013)** have reported significant differences in the bacterial composition of dental plaque between individuals with and without type 2 diabetes,[11] while **Taylor JJ (2013)** failed to detect any difference. [12]

The relationship between the mechanism of DM and chronic periodontitis has been described as bidirectional, in which DM negatively affects the periodontal condition and periodontal disease negatively influencing glycemic control, increasing the risk of complications in diabetic patients.[13]

Santos VR et al (2010) have analyzed glycemic control and periodontal disease and found a positive relationship between poorly controlled type 2 DM and the severity of periodontal disease.[14] A number of inflammatory cytokines, such as interleukin IL-1, IL6, IL-8, TNF-α, prostaglandins and matrix metalloproteinases are involved in periodontal diseases. There is ample evidence from the study by **Salvi GE et al. (1997)** to substantiate the elevated levels of salivary IL-6 at the periodontally infected sites from diabetic patients proving the systemic influence of diabetes on periodontium.[15]

Biomarkers are often studied in three areas, including early diagnosis of disease, severity of disease, and effective therapeutic methods. The serum, GCF and saliva are appropriate sites for collecting biological samples.

Furthermore, biopsy from the gingival tissue is often used to determine the level of the biomarkers. Saliva is used as a diagnostic tool in medicine and dentistry due to the easy collection method and storage and containing locally-produced microbial and host response mediators.[16] According to **Miller et al. 2006,** many of these pro-inflammatory cytokines including IL-6 have been detected in oral fluids, which has allowed saliva to emerge as an important and easily accessible biological fluid that can provide important diagnostic information regarding oral health and disease.

Jaedicke et al. (2016) and Belstrom et al. (2017) also suggested that cytokines level in the saliva might be linked with the periodontal status of the patient. Thus, qualitative changes in the levels of these biomarkers could have diagnostic and therapeutic significance.[16]

Hence, salivary cytokine levels have the potential to reflect current activity, disease severity and possibly predict future disease progression, and make aware of immediate or future treatment needs.

Thus, by evaluating the salivary IL-6 level, the risk and severity of periodontitis in type 2 diabetic patients can be predicted. In the future, the salivary IL-6 levels can be used as an important biomarker for the diagnosis, prognosis, and to predict the treatment outcomes of periodontitis. In the present study, salivary IL-6 level was estimated by a newer technique **ECLIA (Electrochemilumine scent immunoassay)** using a ***Cobas E 601 analyzer (Roche diagnostic)*****. Prieto B (2010)** examined new quantitative electrochemilumine scence method (ECLIA) for interleukin-6 (IL-6) measurement and compared the ECLIA with semi quantitative immunoassay (MileniaBiotec GmbH, Gieben, Germany) and found the new quantitative ECLIA method showed good reproducibility, linearity and functional sensitivity.[17]

Bolton et al. (2020) demonstrated the superiority of the ECLIA based serological assay over the conventional ELISA. The two assays show strong quantitative agreement. However, because of the extremely wide linear range of the ECLIA, a simple single-point measurement is sufficient to determine antibody titers. By contrast, in the ELISA, multiple dilution points are necessary for each sample and then serial dilutions are required to create a titration curve from which a titer can be calculated. Furthermore, the ECLIA can be multiplexed to measure responses to multiple antigens simultaneously from a single sample. These characteristics make the ECLIA the preferred platform for immunoprofiling, which is crucial for the identification of biomarkers of exposure or correlates of immunity.[18]

Studying the effect of IL-6 is complicated by the fact that is multifunctional cytokine (as pro- and anti- inflammatory). It is involved in activation of osteoclast and Th-cells, induces the production of IL-1ra, thus contributes to the anti-inflammatory process. According to **Khosravi R (2013),** there is no evidence to support an association between increased level of IL-6 and destructive periodontal disease among individuals with hyperglycemia. However, **Javed et al. (2012)** reported that upregulation of IL-6 together with IL-1α could be associated with diabetes related periodontal tissue destruction.[19] The clinical importance of this study is that saliva IL-6 detection might serve as an indicator to predict the evolution of periodontal disease in subjects with type 2 diabetes mellitus. As a diagnostic fluid, saliva is yet insufficiently used in daily practice. It offers some advantages over serum, because of its non-invasive sampling method, which eliminates the need for clinicians' special training.

CONCLUSION-

In the present study, there was a significantly elevated level of IL-6 in type 2 diabetes mellitus with chronic periodontitis group than chronic periodontitis group, followed by diabetes mellitus depicting the host modulatory role of IL-6 in these patients.

However, there are some limitations of the present study such as- the study excluded the probability of developing hyperglycemia due to obesity because it is well known risk factor for periodontal disease and developing diabetes, no other pro and anti-inflammatory mediators were assessed in this study. Due to its cross sectional design, it is not possible to predict the systemic and periodontal impact of the observed salivary cytokine over the long term.

REFERENCES-

1. Barsotti O, Poulet PP. Les infections du parodonte. Microbiologie enodontosto matologie. 2006; 24(5)256-257.
2. Carranza FA, Elangovan S, Camargo PM. The Periodontal Pocket. Newman and Carranza's Clinical Periodontology. Elsevier Health Science. 2018;13:303-315
3. Taylor GW, Burt BA, Becker MP, Genco RJ, Shlossman M, Knowler WC et al. Severe periodontitis and risk for poor glycemic control in patients with non- insulin-dependent diabetes mellitus. J Periodontol. 1996;67: 1085-1093.
4. Soskolne WA, Klinger A. The relationship between periodontal diseases and diabetes: an overview. Ann Periodontol. 2001;6:918.
5. Nicki R. Davidson's principles and practice of medicine. Elsevier Health Sciences. 2006;24.
6. Frodge BD, Ebersole JL, Kryscio RJ, Thomas MV, Miller CS. Bone remodeling biomarkers of periodontal disease in saliva. J Periodontol. 2008;79(10):1913-1919.
7. Shen J, Zhou T, Huang R. Recent advances in electrochemiluminescence sensors for pathogenic bacteria detection. Micromachines. 2019; 10(8):532.
8. Navazesh M. Methods for collecting saliva. Ann N Y Acad Sci.1993;694(1):72-77.
9. Flemmig TF. Periodontitis. Ann Periodontol. 1999;4(1):32-37.
10. Preshaw PM, Taylor JJ. How has research into cytokine interactions and their role in driving immune responses impacted our understanding of periodontitis. J ClinPeriodontol. 2011; 38:60-84.
11. Casarin RC, Barbagallo A, Meulman T, Santos VR, Sallum EA, Nociti FH et al. Subgingival

biodiversity in subjects with uncontrolled type-2 diabetes and chronic periodontitis. J Periodontal Res. 2013;48(1):30-36.
12. Taylor JJ, Preshaw PM, Lalla E. A review of the evidence for pathogenic mechanisms that may link periodontitis and diabetes. J Clin Periodontol. 2013;40: 113-134.
13. Taylor GW, Borgnakke WS. Periodontal disease: associations with diabetes, glycemic control and complications. Oral Dis. 2008;14 (3):191-203.
14. Santos VR, Ribeiro FV, Lima JA, Napimoga MH, Bastos MF, Duarte PM. Cytokine levels in sites of chronic periodontitis of poorly controlled and well- controlled type 2 diabetic subjects. J ClinPeriodontol. 2010;37(12):1049-1058.
15. Salvi GE, Yalda B, Collins JG, Jones BH, Smith FW, Arnold RR et al. Inflammatory mediator response as a potential risk marker for periodontal diseases in insulin-dependent diabetes mellitus patients. J Periodontol. 1997; 68(2):127-135.
16. Jaedicke KM, Preshaw PM, Taylor JJ. Salivary cytokines as biomarkers of periodontal diseases. Periodontol 2000. 2016;70(1):164-183.
17. Prieto B, Miguel D, Costa M, Coto D, Álvarez FV. New quantitative electrochemilumine scence method (ECLIA) for interleukin-6 (IL-6) measurement. Clin Chem Lab Med. 2010; 48(6):835-838.
18. Bolton JS, Chaudhury S, Dutta S, Gregory S, Locke E, Pierson T et al. Comparison of ELISA with electro-chemiluminescence technology for the qualitative and quantitative assessment of serological responses to vaccination. Malaria J. 2020; 19(1):1-3.
19. Javed F, Ahmed HB, Saeed A, Mehmood A, Bain C. Whole salivary interleukin- 6 and matrix metalloproteinase-8 levels in patients with chronic periodontitis with and without prediabetes. J periodontol. 2014; 85(5):130-135.