INTERLEUKIN-6 LEVELS AND CLINICAL PARAMETERS IN CHRONIC PERIODONTITIS PATIENTS WITH AND WITHOUT TYPE 2 DIABETES MELLITUS

JEVIN FEBRY TANDIAN¹, IRMA ERVINA², DHARMA LINDARTO³, AMETA PRIMASARI⁴,
AINI H. NASUTION⁵

^{1,2,5}Department of Periodontics, Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia.

³Division of Endocrinology-Metabolism, Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara, Medan / H. Adam Malik Hospital, Medan, Indonesia

⁴Department of Oral Biology, Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia.

ABSTRACT

Epidemiological studies have established that patients with diabetes have an increased prevalence and severity of periodontal disease. Interleukin (IL)-6 is a multifunctional cytokine that participates in normal functions of the immune system, haematopoiesis, metabolism, as well as in the pathogenesis of metabolic diseases such as diabetes. IL-6 also plays a role in the tissue inflammation that characterizes periodontal disease. This study was designed to compare IL-6 levels in saliva and gingival crevicular fluid (GCF) with clinical parameter levels of chronic periodontitis patients with and without type 2 diabetes mellitus (T2DM).

Saliva and GCF samples and clinical parameters were obtained from 33 periodontitis patients with T2DM and 30 periodontitis patients without T2DM. IL-6 levels were assessed in the saliva and GCF by enzyme-linked immunosorbent assay (ELISA) analysis. The clinical parameters and saliva & GCF IL-6 levels were compared between the two groups. The results showed higher clinical parameters and IL-6 levels in periodontitis patients with T2DM than periodontitis patients without T2DM.

Within the limits of this study, it was concluded that T2DM was associated with increased expression of IL-6 in saliva and GCF of periodontitis patients with T2DM, relative to periodontitis patients without T2DM, and that such over expression may be involved in the mechanism by which T2DM enhances periodontal destruction.

KEYWORDS: Periodontitis, Interleukin – 6, Type 2 Diabetes Mellitus

Received: Jun 06, 2020; **Accepted:** Jun 26, 2020; **Published:** Jun 30, 2020; **Paper Id.:** IJMPERDJUN2020143

INTRODUCTION

Periodontitis is a chronic inflammatory disease of the mouth that involves the gingiva, teeth, and supporting bone¹. The initial presentation of the disease is localized to the gingival sulcus and coronal connective tissue². However, the disease process, if allowed to progress, can result in periodontal attachment loss and bone destruction^{1,2}. Severity of periodontitis can be affected by systemic disease such as diabetes mellitus (DM)³.

Diabetes mellitus is a complex metabolism failure that is characterized by appearance of chronic hyperglycemia⁴. Several studies stated that diabetes mellitus can affect periodontal condition, and causing severe gingival inflammation, deep pocket depth, and bone loss.³ Those conditions can be worsened if the patient with DM does not control their blood sugar level.⁴

Among the two types of DM, the one that shows higher frequency in Indonesia is type 2 diabetes mellitus

(T2DM), with 57% population exposed in 2012 and increased by 2,1% in 2013⁵. T2DM, also known as Non-Insulin Dependent Diabetes Mellitus, which usually affects the individual in adult age, and it is caused by imbalance between insulin secretion and glucose from the heart^{4,5}. The relationship between periodontal disease and DM is two way, meaning both can influence the severity of the other.⁶ Both diabetes and periodontitis stimulate the release of proinflammatory cytokines.^{5,6}

The microbial challenge provokes an inflammatory response in the periodontal tissue that involves a network of cytokines functioning in synergy.⁶ Interleukin-6 (IL-6) is secreted by macrophages in response to inflammation and is involved in recruitment and apoptosis of leukocyte and T-cell activation. IL-6 and its soluble receptor induce bone resorption, either by increasing the receptor activator of nuclear factor κ ligand (RANKL) or by directly inducing the formation of osteoclast.⁷

There are many evidence from the literature to substantiate the elevated levels of oral fluids (saliva and GCF) IL-6 at the periodontally infected sites from diabetic patients proving the systemic influence of diabetes on periodontium.⁸ Thus, by evaluating the salivary and GCF IL-6 level, the risk and severity of periodontitis in type 2 diabetic patients can be predicted. In the future, the salivary and GCF IL-6 levels can be used as an important biomarker for the diagnosis, prognosis, and to predict the treatment outcomes of periodontitis. Hence, this study aims to evaluate the clinical parameter and salivary and GCF levels of IL-6 in T2DM and periodontitis patients.

MATERIAL AND METHOD

The study was a cross-sectional research. Ethical clearance was given by the ethical committee, Faculty of Medicine, University of Sumatera Utara, Indonesia. The inclusion criteria for this study were chronic periodontitis with or without T2DM and age ≥ 35 years old. The exclusion criteria were gingivitis, aggressive periodontitis, consuming vitamin, antibiotics or anti-inflammatory drugs within 1 month, smoking, alcoholic and patients undergoing periodontal treatment within three month. Thirty three (33) periodontitis patients with T2DM and 30 periodontitis without T2DM were included in this study.

Saliva was taken by spitting method and collected in saliva pot. GCF was taken using a micropipette and put inside Eppendorf tubes, both samples was then stored at -80 C. The sample was tested using the enzyme-linked immunosorbent assay (ELISA kit IL-6, Elabscience, USA). The concentration of IL-6 was examined using ELISA assay kits, and the result was analyzed by a microplate reader. Clinical parameters such as oral hygiene index (OHIS), papillary bleeding index (PBI), gingival index, pocket depth and loss of attachment were recorded.

The numerical descriptive data were inputted into the computer and analyzed using SPSS19.0. Data Processing began with the descriptive statistical analysis and hypothesis testing. Descriptive statistics were used to present the data in the form of a frequency distribution of data centre size, such as the average value (the value of mean) and in the form of a data spread size, such as standard deviation (SD). Data distribution was tested using Saphiro-Wilk. Statistic was tested by T-test. Data that considerably deviated from the normal distribution were analyzed using the Mann-Whitney test. Correlation between parameters was tested using spearman test. A p-value less than .05 was considered statistically significant.

RESULTS

Data collection was conducted by clinical examination, questionnaires on oral hygiene habit, medication and smoking status, saliva and GCF sampling. The selection of samples was conducted by purposive sampling. 63 total samples which were grouped by chronic periodontitis (CP) with (33) and without T2DM (30).

Table 1: Clinical Parameters

Variable	CP with T2DM	CP without T2DM	P
OHIS	2.80 (1.5-4.8)	2.95 (1.3-4.7)	0.00
PBI	53 (3.0-79.0)	52.5 (33.0-75.0)	0.00
Gingival Index	2.10 (1.7-2.9)	1.85 (1.1-3.0)	0.00
Pocket Depth	5.40 (3.41-10.5)	4.20 (0.0-7.8)	0.00
Loss of Attachment	6.79 (3.85-84.0)	5.06 (0.0-9.3)	0.00

Table 1 show that all clinical parameter of chronic periodontitis with T2DM group was significantly higher than the group without T2DM.

Table 2: Salivary and GCF IL-6 Levels

Variable	CP with T2DM Median (Min-Max)	CP without T2DM Median (Min-Max)	p
Salivary IL-6 level (pg/ml)	128.71 (17.42 – 758.42)	67.66 (17.72 – 501.9)	0.382
GCF IL-6 level (pg/ml)	664.42 (463.29 – 912.54)	246.41 (199.4 – 450.86)	0.000

p<0,05

Salivary IL-6 level was higher in chronic periodontitis with T2DM group but statistical analysis shows no significant difference. In the other hand GCF IL-6 level of chronic periodontitis with T2DM group was almost thrice higher than the group without T2DM and was different significantly.

DISCUSSION

This study shows that clinical parameters of the group with chronic periodontitis with T2DM was significantly higher than the group without T2DM. These results are consistent with those of Javed et al.,⁸ and Abdul et al.⁹. Higher clinical parameters such as PBI and gingival index shows more severe inflammation in the T2DM group. Periodontitis patients with T2DM are systemically stressed by the bacteria in plaque, the increased in circulating inflammatory mediators, and an increased in insulin resistance which worsen the glycemic control.^{10,11}

This study shows that both salivary and GCF IL-6 levels was higher in the T2DM groups than without T2DM groups, which shows IL-6 maybe a good indicator of inflammation. However, even though the salivary IL-6 levels was higher in the T2DM groups, statistical analyses shows no significant difference between the groups (p = 0,382). This result is consistent with the finding of Javed et al which shows elevated IL-6 levels in T2DM groups but not significant.⁸ Another study by Monea et al., showed significantly higher salivary IL-6 levels in T2DM groups.¹² The difference in significance maybe caused by difference in sample size and reagent used for measurement.

This study also shows higher GCF IL-6 levels in T2DM group, This result is consistent with the finding of Camargo et al and Kardesler et al.^{13,14}; that is, The IL-6 levels in GCF was higher in periodontitis patient with T2DM than in the periodontitis patients without T2DM. In contrast with this study, Kurtis et al didn't find any significant difference of GCF IL-6 levels between periodontitis patient with or without T2DM.¹⁵ The difference may be caused by the severity of

inflammation in patient which affect the quantity of IL-6 in the samples. Moreover, the difference in sample collecting method may also influence the IL-6 in the samples.

This study shows that GCF IL-6 levels was higher than saliva IL-6 levels whether it was periodontitis with or without T2DM. Possible reason for the results is because GCF was originally secreted as exudate in inflamed site, therefore GCF contain much more IL-6 than saliva.¹⁶ Another reason is that inside saliva, there are mucin like protein and enzyme which may degrade the IL-6. This statement was supported by the study of Wozniak et al, which shows the declining levels of cytokine in saliva.¹⁷

CONCLUSIONS

Periodontitis with T2DM shows higher clinical parameter than periodontitis without T2DM, which shows the inflammation in periodontitis with T2DM is more severe than periodontitis without T2DM, more over IL-6 levels in both saliva and GCF were also higher in periodontitis with T2DM which shows IL-6 may be a good marker for inflammation. This study also shows GCF are much more reliable than saliva because there are many confounding factor inside saliva such as mucin and degradation enzyme which may alter the results of the assessment.

REFERENCES

1. Persson G R. *Diabetes and periodontal disease: an update for health care providers. Diabetes Spectrum* 2011; 24(4): 195-198.
2. Reddy B. *Interleukin - 6 levels in generalized and localized aggressive periodontitis patients. Thesis. Richmond, Virginia: Virginia Commonwealth University, 2004: 1-30.*
3. Ernawati T. *Periodontitis and diabetes mellitus. Stomatognathic* 2012; 9(3): 152-154.
4. Harsas N A, Lessang R, Soeroso Y, Putri G A. *Periodontal Status Differences between Chronic Periodontitis Patient with and Without Type 2 Diabetes Mellitus. JIDMR* 2019; 12 (1): 175-179.
5. Riskesdas. *Ministry of health of Indonesian Republic data bank and information. Jakarta* 2014: 1-7.
6. Balaji A, Chandrasekaran S C, Subramaniam D, Fernz A B. *Salivary Interleukin-6 - A pioneering marker for correlating diabetes and chronic periodontitis: A comparative study. IJDR* 2017; 28(2): 133-137.
7. Southerland J H, Taylor G W, Offenbacher S. *Diabetes and Periodontal infection: making the connection. Clinical diabetes* 2005; 23(4): 171-178.
8. Javed F, Ahmed H B, Saaed A, Mehmood A, Bain C. *Whole salivary interleukin-6 and matrix metalloproteinase-8 levels in patients with chronic periodontitis with and without prediabetes. J periodontal* 2014; 85: 130-135.
9. Alwan A H. *Determination of interleukin -1B (IL-1B) and interleukin -6 (IL-6) in gingival crevicular fluid in patients with chronic periodontitis. Journal of dent and Med Sciences* 2015; 14(11): 81-90
10. Aljehani Y A. *Risk factors of periodontal disease: Review of the literature. IJD* 2014; 2014: 1-9.
11. Tunes R S, Foss-Freitas M C, Nogueira-Filho G R. *Impact of periodontitis on the diabetes-related inflammatory status. J can Dent Assoc* 2010; 76(35): 1-7.
12. Monea A, Gruber R, Elod N, Beresescu G, Moldovan C, Monea M. *Saliva and serum levels of TNF-A and IL-6 in a sample of Romanian adult Subjects with type 2 diabetes mellitus and periodontal disease. ESJ* 2014; 10(9): 350-359.

13. Camargo G A, Lima A, Fortes T V, de Souza C S, de Jesus A M, de Almeida R P. Effect of periodontal therapy on metabolic control and levels of IL-6 in the gingival crevicular fluid in type 2 diabetes mellitus. *IJDR*2013; 24(1): 110-116.
14. Kardesler L, Buduneli N, Cetinkalp S, Lappin D, Kinane D F. Gingival crevicular fluid IL-6,tPA, PAI -2, albumin levels following initial periodontal treatment in chronic periodontitis patients with or without type 2 diabetes. *JIR* 2011; 60(2): 143-151.
15. Kurtis B, Develioglu H, Taner IL, Balos K, Tekin IO. IL-6 levels in gingival crevicular fluid (GCF) from patients with non-insulin dependant diabetes mellitus (NIDDM), adult periodontitis and healthy subjects. *JOS* 1999; 41(4): 163-167.
16. Taba M, Kinney J, Kim A S, Giannobile W V. Diagnostic biomarkers for oral and periodontal diseases. *Dent Clin North Am* 2005; 49(3): 551-556.
17. Wozniak K L, Arribas A, Leigh J E, Fidel P L Jr. Inhibitory effect of whole and parotid saliva on immunomodulators. *Oral MicrobiolImmunol* 2002; 17(2): 100-107.
18. Rao, Yeluri Seshagiri, and Vanamali Dharma Rao. "Prevalence of Periodontitis among patients with Type-2 Diabetes Mellitus." *International Journal of General Medicine and Pharmacy (IJGMP)*, 5 2, (2016): 15 22.
19. Pande, Sonali S., and Arun Chutani. "A Cross-Sectional Study of Pulmonary Function Tests in Type 2 Diabetes Mellitus and Correlation with Glycemic Index and Duration of Diabetes." *International Journal of Medicine and Pharmaceutical Science (IJMPS)* 9.6 (2019):39–46
20. Sur, Dipanshu. "A tale of genetic variation in the human Slc22a1 gene encoding OCT1 among type 2 diabetes mellitus population groups of West Bengal, India." *IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)* 2.5 (2014): 97-106.