

ORIGINAL ARTICLE**Salivary Interleukin-6 levels in periodontitis patients in Diyala – Iraq****Dhuha Adnan Neamah**

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ABSTRACT

Periodontitis (PT) is an inflammation of the tissues that support the teeth that causes bone loss due to increasing attachment loss. It is a chronic inflammatory illness that raises the level of the cytokine IL-6, which is detected in saliva and is a key biomarker for PT patients. The study aimed to comparing the levels of salivary IL-6 in patients and the control group, the effect of the impact of gender, age and smoking on the levels of IL-6 in the saliva of patients. The study included 64 samples, of which 42 were saliva samples from PT patients (15 to 61 years old; 16 males and 26 females); 22 were saliva samples from healthy people; and 5 ml of saliva was taken and stored until the ELISA test to determine the concentration of salivary IL-6. Were saliva samples from the patient and control groups had mean and standard deviations for IL-6 that were, respectively, 12.85 1.45 and 41.73 2.72 pg/ml. Additionally, the study's findings included a comparison of the salivary IL-6 levels in male and female patients, which were (42.704.87) pg/mL and (41.143.29) pg/mL, respectively. In comparison of its levels in two age groups (More than 40 Years and Less than 40 Years) results were respective values of (40.70 3.51, 44.31 3.80) pg/ml. While compared patients hoes smokers to nonsmokers had greater levels of (41.86 2.96, 41.37 6.45) pg/ml respectively. Therefore, the study came to the conclusion that sick samples had higher amounts of salivary IL-6 than healthy control samples. The percentage of female patients who were aroused by men rises with advancing age. IL-6 was decreases in patients who smoke, though.

Keywords: Salivary Interleukin-6, Periodontitis (PT), inflammation

Received 10.10.2022

Revised 12.11.2022

Accepted 30.11.2022

How to cite this article:

Dhuha Adnan Neamah. Salivary Interleukin-6 levels in periodontitis patients in Diyala –Iraq. Adv. Biores. Vol 13 [6] November 2022: 209-213.

INTRODUCTION

Inflamed gingival, bleeding during probing, deeper probing pockets, clinical attachment loss, pus discharge, and resorption of alveolar bone are all symptoms of periodontitis (PT), an infection of the tissues supporting the teeth that finally results in tooth loss [1]. Gram-negative bacteria are the primary cause of this anaerobic inflammation, which is multifactorial in origin. Additionally, in vulnerable people, an imbalance between the inflammatory and immunological pathways results in chronic inflammation, tissue degradation, and PT. This route involves several cytokine cascades, molecular, and cellular activities [2]. Environmental, behavioral, and systemic factors can also affect PT's onset, progression, and severity as well as be used to forecast how well it will respond to treatment [3]. Arrays of cytokines operate in concert to cause an inflammatory response in the periodontal tissue in response to the microbial onslaught [4]. Cytokines and chemokines are important in maintaining pro- and anti-inflammatory balance, but if they are released in large amounts, they can also result in periodontal tissue disease [5]. The creation of the gingival pocket and apical migration of the junction epithelium, which causes an accumulation of cells that cause an inflammatory response in the gingival tissues, are the two most important steps. These cells have been linked to alveolar bone loss, impaired periodontal tissue attachment, and inflammatory diseases [6, 7]. Long-term, repetitive physical therapy affects the bone and other supporting components by increasing levels of cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1, IL-4, IL-6, and IL-10 and causing low-grade systemic inflammation [8]. IL-6 and IL-1 have been acknowledged as significant biomarkers with accuracy sufficient for PT diagnosis [9]. Given that IL-6 is increased in refractory PT sites compared to stable sites, it is possible that IL-6 could be used as a diagnostic marker for areas where active periodontal disease is present [10].

In both acute and chronic inflammatory reactions, IL-6 plays a pro- and anti-inflammatory role. It binds to the soluble IL-6R and the soluble glycoprotein 130, two different types of receptors (gp130)[11]. However, it is crucial to clarify that IL-6 is a component of a potentially complicated network of biomarkers because it is one of a wide range of cytokines involved in the inflammatory response. Numerous studies have found that IL6 is a crucial biomarker for identifying PT patients [12, 13]. When there is tissue damage, Monocytes, lymphocytes, or endothelial cells release IL-6, which causes the release of neutrophils and platelets from the bone marrow into the bloodstream [14]. Leukocyte and T-cell activation in response to inflammation, as well as their recruitment and apoptosis, are all involved. By either increasing the level of RANKL or by directly promoting the development of Osteoclasts, IL-6 and its soluble receptor cause bone resorption [15].

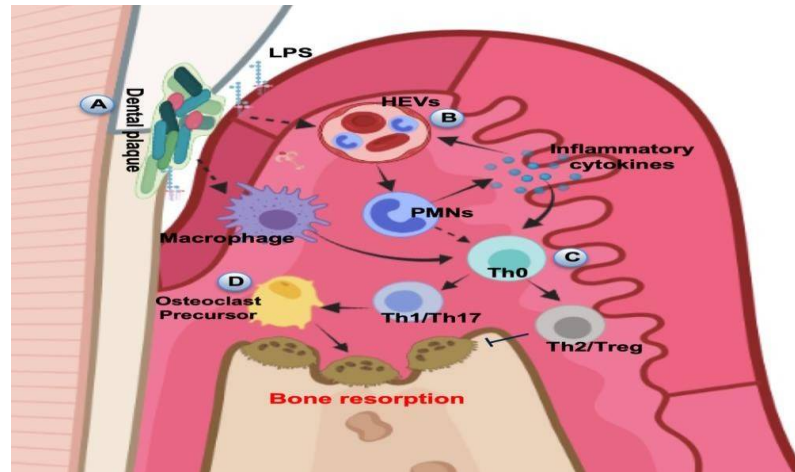


Figure 1 Pathogenesis of Periodontitis

Saliva is a biological fluid that, according to recent studies, represents numerous systemic changes in the body as well as general health problems. Along with the secretions of the major and minor salivary glands, it also includes a number of substances that are not derived from salivary glands. Blood and serum derivatives, expectorated bronchial and nasal secretions, viruses, fungi, bacteria, and bacterial products, as well as different cells, electrolytes, immunoglobulin's, proteins, and enzymes, as well as food debris and a small amount of gastro-esophageal reflux, are all examples of gingival reticular fluid. She becomes a valuable diagnostic tool as a result[17].

Salivary levels of inflammatory IL-6 rise as a result of PT, which are chronic inflammatory disorders [18]. It is stated that systemic saliva acts as a "mirror of the body". It serves as a sign of overall health, not just in the mouth cavity. Water makes up around 99.5% of its total volume, with the remaining 0.5% consisting of various ions, biochemicals, and numerous other significant biomarkers[19]. Due to its origin, composition that is similar to serum and interactions with other organs, salivary analysis has developed into a valuable tool for assessing both the general health of an individual and the severity of a disease. Therefore, using saliva offers many benefits, including an easy, painless method of collection and in expensive storage [20]. Saliva analysis has caught the interest of periodontitis in recent years since it is a crucial laboratory test for the diagnosis of numerous salivary disorders, including PT. Saliva is used as a biofluid for illness risk factor early detection, prognosis, and therapy response monitoring. Salivary biomarker detection is a minimally invasive laboratory test for the early diagnosis of PT[21].

This study's objectives included comparing salivary IL-6 levels between PT patients and a control group, as well as investigating the effects of gender, age, and smoking on these levels in infected individuals.

MATERIAL AND METHODS

In this study was 64 samples saliva in collected at the Specialized Dental Center in Diyala Governorate. Their stomatognathic system and periodontal status for the patient samples were assessed by the specialist doctor. As the number of males was 16 and the number of females was 26 within our study age range between (15-61) years, and samples was collected 22 as samples saliva of from apparently healthy people (Control group) of both sexes. Saliva samples collection was from the mouth, as 5 ml of saliva was with take by using a sterile use collection tube. The take was divided into equal amounts of 250 µl in small tubes (Eppendorf) and stored at a temperature of (-20°C) until use. While each section of the preserved saliva was used once to avoid repeated thawing and freezing of the sample.

A questionnaire has been created, filled with information regarding the sex, age and smoking status of the patient, for the purpose of studying these factors have an effect on the PT (on the levels of salivary IL-6), and measuring the levels of salivary IL-6 were by the direct enzyme-linked immunosorbent assay (ELISA) technique using a readymade kit for this purpose[23].

Statistical Analysis

Statistical analysis was carried out using the Statistical Package for Social Sciences version 22 with regard to numerical variables; they were described using the mean and standard deviation of the mean, and the comparison between the descriptive variables. Totals were performed using a t-test between two groups[22].

RESULTS

Measurement of IL-6 concentration in saliva of patients and healthy groups:-

In the current study, there were 64 participants: 24 healthy individuals (controls) and 42 PT patients. The results of Table (1) showed that the salivary IL-6 concentration in patients' is higher than that of in the control group, with significant differences ($p < 0.000$), with as the mean \pm standard deviation ($12.85 \pm 1.45, 41.73 \pm 2.72$) pg/mL respectively.

Table 1. Measurement of salivary IL-6 concentration of patients and Control groups.

Parameters	Control Mean \pm SE N=22	Patients Mean \pm SE N=42	t-test P-value
IL-6	12.85 \pm 1.45	41.73 \pm 2.72	* <0.000

*=Significant differences ($p < 0.5$), SE=Standard Error, N=Number

Comparison of salivary IL-6 concentrations between male and female patients:-

The results of Table (2) included a comparison of salivary IL-6 levels between females ($n = 26$) and males ($n = 16$) PT in patients, as it was noted that the mean \pm standard deviation of males is higher of the females ($41.14 \pm 3.29, 42.70 \pm 4.87$) pg/mL, respectively with higher significant differences ($P = 0.784$).

Table 2. Comparison of salivary IL-6 concentration between male and female patients.

Parameters	Female Mean \pm SE N=26	Male Mean \pm SE N=16	t-test P-value
IL-6	41.14 \pm 3.29	42.70 \pm 4.87	0.784**

**=High significant differences ($p > 0.5$), SE=Standard Error, N=Number

Comparison of the concentration of salivary IL-6 between the two groups of smokers and non-smokers patients:-

Table (3) shows the evaluation of IL-6 levels in the saliva of smokers ($n=11$) and non-smokers ($n=31$), in PT patients, the results of indicate that there are high significant differences ($p = 0.937$) in the levels of salivary IL-6 between smokers and non-smokers patients, the value of the mean \pm standard deviation ($41.37 \pm 6.45, 41.86 \pm 2.96$) pg/mL, respectively. As shown in the table below.

Table 3. Comparison of salivary IL-6 concentration between smokers and non-smokers patients.

Parameters	smokers Mean \pm SEN=11	non-smokers Mean \pm SEN=31	t-test P-value
IL-6	41.37 \pm 6.45	41.86 \pm 2.96	**0.937

**=high significant differences ($p > 0.5$), SE=Standard Error, N=Number

The current study showed that the effect of the smoking factor did not appear on PT due to the interaction of other factors, so we note in Table (3) that the concentration of salivary IL-6 in non-smokers is higher than its concentration in smokers.

DISCUSSION

The results of study [10] confirmed that the concentration of Interleukin-T increases in patients with PT compared to its concentration in healthy subjects, and this is consistent with the results of the current study. The results also are in agreement with the results of the study [3]. The results of the study also [23] showed that there were statistically significant differences in the concentration of salivary IL-6 between patients with PT and the control group, as the average levels of IL-6 in saliva in stage IV

patients (22.18±5.96 pg/ml) were much higher than it is in the healthy groups (2.23±2.17pg/ml). He studied [24]) three groups (healthy people only), Their stomatognathic system and periodontal status were assessed, salivary IL-6 levels were lower in G 3 (n=20) than in G 2 (n=24) and G 1 (n=20), (p<0.001). Patients in the PT group had significantly higher median salivary IL-6 levels [195.4 (184.6–205.9 pg/ml)] compared to the HS group 101.9 (89.5–115.4 pg/ml) (p<0.001) [25]. Concentration of salivary IL-6 was elevated in patients with PT with and without diabetes. Thus, salivary IL-6 levels can be considered as an important biomarker in the diagnosis of PT and diabetes [26].

As for the gender factor and its effect on the concentration of salivary IL-6, the results of the study [23] showed a high significant difference, between the test group that consisted of males (n = 14) and females (n = 14) patients, and the group Controller (n = 22), 10 males and 12 females, P values for male-female ratio in study groups were p=0.7242 for male patients, and p=0.7230 for female patients. Another study found that salivary concentrations of pro-inflammatory cytokines declined with age in healthy volunteers, with no statistically significant variations in levels of salivary cytokines (IL-1, IL-6, and TNF) observed on sex in groups three. G1 has n=20, G2 has n=24, and G3 has n=20. [24].

Periodontitis ranks eleventh among the most common diseases in the world and is considered more prevalent than cardiovascular diseases, Gum disease increases as people age and rises rapidly in those aged 50-60 years [27]. The results of the study showed that the Old ages increased salivary IL-6 significantly, also showed that Salivary IL-6 is one of the biomarkers of periodontitis in that the elderly [28]. The study also agrees with the current study, which indicates that people with PT over the age of 50 years constitute a higher percentage of about 30% of the world's population compared to adults aged (20 to 50 years) at a rate of 15% [10]. The results of [23] patients 18-50 years old, the mean age of patients in the test group was 46±4.1 and for the control group 39±11.3 (p=0.0069). Salivary concentration of pro-inflammatory cytokines decreased with age in healthy subjects. It could be influenced by ageing-related changes [24].

The association between the periodontal conditions and smoking is attracting increasing attention, the results indicate suggest that smoking is an important risk factor for in Periodontitis patients demonstrated by clinical measurements and IL-6 and TNF- α gene expression. [29]. Note that the levels of cytokines are higher in the saliva of smokers compared to non-smokers, so smoking is considered a significant risk factor in Periodontitis. This reflects the impact of smoking on immune response and its role in the pathogenesis of periodontal disease [26]. The results of the study [23] showed a significant difference (p = 0.4788) between the test group (15 patients with periodontitis smokers), and 14 smokers within the control group.

CONCLUSION

It can be concluded from the current study that saliva contains many substances (IL-6), which increased its concentration in the saliva of patients with PT compared to the control group, so it could be an important diagnostic marker for PT. It can also be concluded that the levels of salivary IL-6 are affected by the factor of sex and age, as they increase in male patients compared to females, and also rise significantly with age. while their levels decrease in the saliva of non-smoking patients compared to smokers.

REFERENCES

1. Seoane, T., Bullon, B., Fernandez-Riejos, P., Garcia-Rubira, J. C., Garcia-Gonzalez, N., Villar-Calle, P., & Bullon, P. (2022). Periodontitis and Other Risk Factors Related to Myocardial Infarction and Its Follow-Up. *Journal of Clinical Medicine*, 11(9), 2618.
2. Rezvani, G., Taleghani, F., & Valizadeh, M. (2022). Effect of Green Tea on the Level of Salivary Interleukin-1 Beta in Patients with Chronic Periodontitis: A Randomized Clinical Trial. *International Journal of Dentistry*, 20, 23-29
3. Rodríguez-Montaña, R., Ruiz-Gutiérrez, A. D. C., Martínez-Rodríguez, V. M. D. C., Gómez-Sandoval, J. R., Guzmán-Flores, J. M., Becerra-Ruiz, J. S., ... & Guerrero-Velázquez, C. (2022). Levels of IL-23/IL-17 Axis in Plasma and Gingival Tissue of Periodontitis Patients According to the New Classification. *Applied Sciences*, 12(16), 8051.
4. Mancini, L., Americo, L. M., Pizzolante, T., Donati, R., & Marchetti, E. (2022). Impact of COVID-19 on Periodontitis and Peri-Implantitis: A Narrative Review. *Frontiers in oral health*, 3, 822824.
5. Liu, X., & Li, H. (2022). A systematic review and meta-analysis on multiple cytokine gene polymorphisms in the pathogenesis of periodontitis. *Frontiers in immunology*, 12, 3474.
6. Miron, M. I., Barcutean, M., Luca, R. E., Todea, C. D., Tudor, A., & Ogodescu, E. (2022). The Effect of Changing the Toothbrush on the Marginal Gingiva Microcirculation in the Adolescent Population—A Laser Doppler Flowmetry Assessment. *Diagnostics*, 12(8), 1830.
7. Kuralt, M., Cmok Kučić, A., Gašperšič, R., Grošelj, J., Knez, M., & Fidler, A. (2022). Gingival shape analysis using surface curvature estimation of the intraoral scans. *BMC oral health*, 22(1), 1-11.
8. Shazam, H., Shaikh, F., Hussain, Z., Majeed, M. M., Khan, S., & Khurshid, Z. (2020). Evaluation of osteocalcin levels in saliva of periodontitis patients and their correlation with the disease severity: cross-sectional study.

- European Journal of Dentistry, 14(03), 352-359.
9. Nibali, L., Fedele, S., D'aiuto, F., & Donos, N. (2012). Interleukin-6 in oral diseases: a review. *Oral diseases*, 18(3), 236-243.
 10. Alwan, A. H., Taher, M. G., Getta, H. A., & Hussain, A. A. (2015). Estimation of the level of Salivary Interleukin 6 (IL-6) and its' correlation with the clinical parameters in patients with periodontal diseases. *IOSRJ Dent MedSci*, 14(9), 82-8.
 11. Yucel-Lindberg, T., & Båge, T. (2013). Inflammatory mediators in the pathogenesis of periodontitis. *Expert reviews in molecular medicine*, 15, 100-109
 12. Branco-de-Almeida, L. S., Cruz-Almeida, Y., Gonzalez-Marrero, Y., Huang, H., Aukhil, I., Harrison, P., Shaddox, L.M. (2017). Local and plasma biomarker profiles in localized aggressive periodontitis. *J D R Clinical & Translational Research*, 2(3), 258-268.
 13. Becerik, S., Öztürk, V. Ö., Atmaca, H., Atilla, G., & Emingil, G. (2012). Gingival crevicular fluid and plasma acute-phase cytokine levels in different periodontal diseases. *Journal of periodontology*, 83(10), 1304-1313.
 14. Bruno, A., Dolcetti, E., Azzolini, F., Moscatelli, A., Gambardella, S., Ferese, R., ... & Stampanoni Bassi, M. (2022). Interleukin 6 SNP rs1818879 Regulates Radiological and Inflammatory Activity in Multiple Sclerosis. *Genes*, 13(5), 897.
 15. Myojin, Y., Kodama, T., Sakamori, R., Maesaka, K., Matsumae, T., Sawai, Y., & Takehara, T. (2022). Interleukin-6 is a Circulating Prognostic Biomarker for Hepatocellular Carcinoma Patients Treated with Combined Immunotherapy. *Cancers*, 14(4), 883.
 16. Jiang, Q., Huang, X., Yu, W., Huang, R., Zhao, X., & Chen, C. (2022). mTOR Signaling in the Regulation of CD4+ T cell Subsets: Implications for the Prophylaxis and Treatment of Periodontal Diseases. *Frontiers in Immunology*, 361.
 17. Ntovas, P., Loumprinis, N., Maniatakos, P., Margaritidi, L., & Rahiotis, C. (2022). The Effects of Physical Exercise on Saliva Composition: A Comprehensive Review. *Dentistry Journal*, 10(1), 7.
 18. Han, Y., Huang, W., Meng, H., Zhan, Y., & Hou, J. (2021). Pro-inflammatory cytokine interleukin-6-induced hepcidin, a key mediator of periodontitis-related anemia of inflammation. *Journal of Periodontal Research*, 56(4), 690-701.
 19. Ntovas, P., Loumprinis, N., Maniatakos, P., Margaritidi, L., & Rahiotis, C. (2022). The Effects of Physical Exercise on Saliva Composition: A Comprehensive Review. *Dentistry Journal*, 10(1), 7-90
 20. Zhang, D., Wang, X., & Chen, J. (2022). Saliva: Properties and Functions in Food Oral Processing Biophysics, Food Microstructures and Health, 2022, pp. 1-24.
 21. Pawlukowska, W., Baumert, B., Meller, A., Dziewulska, A., Zawislak, A., Grocholewicz, K., & Masztalewicz, M. (2022). The Influence of Periodontal Diseases and the Stimulation of Saliva Secretion on the Course of the Acute Phase of Ischemic Stroke. *Journal of Clinical Medicine*, 11(15), 4321.
 22. Ajai S. Gaur and Sanjaya S. Gaur. (2009). *Statistical Methods for Practice and Research: A Guide to Data Analysis Using SPSS* Publisher: SAGE Publications India Pvt Ltd.
 23. Hadzic, Z., Pasic, E., Hukic, M., Vukelic, M. G., & Hadzic, S. (2021). Salivary Interleukin-6 Levels in patients with Periodontitis Stage IV. *Meandros Medical and Dental Journal*, 22(2), 140.
 24. Costantino, E., Castell, S. D., Harman, M. F., Pistoressi-Palencia, M. C., & Actis, A. B. (2022). Salivary Proinflammatory Cytokines IL-1 β , IL-6 and TNF Decrease With Age. *BioRxiv*, pp 1-14.
 25. Isola, G., Giudice, A. L., Polizzi, A., Alibrandi, A., Murabito, P., & Indelicato, F. (2021). Identification of the different salivary Interleukin-6 profiles in patients with periodontitis: a cross-sectional study. *Archives of Oral Biology*, 122, 104997.
 26. Balaji, A., Chandrasekaran, S. C., Subramaniam, D., & Fernz, A. B. (2017). Salivary Interleukin-6: A pioneering marker for correlating diabetes and chronic periodontitis: A comparative study. *Indian Journal of Dental Research*, 28(2), 133-137.
 27. Dolińska, E., Milewski, R., Pietruska, M. J., Gumińska, K., Prysak, N., Tarasewicz, T., & Pietruska, M. (2022). Periodontitis-Related Knowledge and Its Relationship with Oral Health Behavior among Adult Patients Seeking Professional Periodontal Care. *Journal of Clinical Medicine*, 11(6), 1517.
 28. Jawzali, J. I. (2018). Salivary Interleukin 6 and Sialic acid in Periodontitis. *Brazilian Journal of Oral Sciences*, 17, e18015-e18015.
 29. Dosseva-Panova, V., Pashova-Tasseva, Z., & Mlachkova, A. (2022). Relationship between smoking and periodontal clinical findings and gene expression of IL-6 and TNF- α in severe periodontitis (clinical and laboratory data). *Biotechnology & Biotechnological Equipment*, 36(1), 662-667.

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