Association between The Cytokine IL-23 in Saliva with Periodontal Health and Disease

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ABSTRACT

Background: In the term of the prevalence of chronic inflammatory conditions manipulating human beings, periodontal diseases were the predominant. Cytokines are proteins of low molecular weight responsible for the inception of inflammatory response and further stages of it in which they coordinate the extent and the duration of the response. IL-23 is a member of a heterodimeric IL-12 cytokine family, generated from macrophages and dendritic cells after the exposure to bacterial pathogens. Saliva is a hypotonic solution formed by the salivary glands, gingival crevicular fluid, and oral mucosal exudate.

Objective: this study aimed to find the association of salivary concentration of IL-23 with periodontal health and disease by examining its' level in individuals with healthy periodontium, with dental biofilm-induced gingivitis and periodontitis (localized and generalized).

Patient and method: Saliva were collected from 90 participants with age ranged from 20-60 years. All of them were systemically healthy. 25 cases enrolled under the dental biofilm-induced gingivitis group, 25 cases under the localized periodontitis group and 25 cases under the generalized periodontitis group, with 15 subjects under healthy periodontium (control) group. Detection of IL-23 in saliva was done by Enzyme Linked Immunosorbent Assay (ELISA).

Results: Our data revealed that the salivary IL-23 level was significantly ($P<0.05$) reduced in disease groups in comparison to healthy (control) group with a significant difference between dental biofilm-induced gingivitis and localized periodontitis groups.

Conclusion: Salivary IL-23 has a significant and negative relationship with all disease groups as compared to healthy subjects.

Keywords: Cytokine IL-23, Saliva, Periodontal health, Disease.

INTRODUCTION

In the term of the prevalence of chronic inflammatory conditions manipulating human beings, periodontal diseases were the predominant. Usually caused by a combination of factors include bacterial factors, environmental factors and compelling host involvement that is accountable for greater soft and hard tissue devastation. This could sacrifice the tooth in severe illness [1].

Two variety of periodontal diseases affecting the periodontium. The first is dental biofilm-induced gingivitis that is an inflammatory condition that is restricted only to the gingiva and doesn't involve the rest of the supporting attachment (cementum, periodontal ligament and alveolar bone). Commonly caused by an interplay between dental plaque biofilm and the host immune response. Such disease doesn't go beyond the muco-gingival line and the tissue can return back to the clinically healthy condition by the removal of bacterial deposit at and apical to the gingival line [2]. This disease entity could lead to periodontitis if left untreated, therefore early diagnosis is efficient in reducing the risk of periodontitis development [3].

The second one is periodontitis, which is represented by periodontal attachment loss caused by microbial and host mediation of inflammation. A circumferential assessment of the whole erupted dentition by a special standard periodontal probe, which can detect that clinical attachment loss in relation to the cemento-enamel junction [4]. By the extent, periodontitis can be divided into three categories [5]:

- ➢ If the bone loss due to periodontitis is limited to molars/incisors named "Molar/incisor pattern'.
- ➢ If the bone loss due to periodontitis affect number of teeth less than or equal 30% the pattern is "Localized periodontitis".
- ➢ If the bone loss due to periodontitis affect number of teeth more than 30% the pattern is "Generalized periodontitis".

For the purpose of quality and quantity of pertinent clinical information objectively, efforts have been made to shift the periodontal investigation from the conventional ways into using biomarkers [6]. Biomarker is a property used to indicate normal biological and pathological processes that can be calculated and estimated objectively. Also, it is considered to monitor pharmacological feedback to therapeutic interference" declared by the National Institutes of Health Biomarkers Definitions Working Group [7].

Cytokines are proteins of low molecular weight responsible for the inception of inflammatory response and further stages of it in which they coordinate the extent and the duration of the response [8]. IL-23 is a member of a heterodimeric IL-12 cytokine family [9]. Within few hours after the introduction of lipopolysaccharide and other bacterial products to dendritic cells (DCs) and macrophages, they produce

IL-23. Macrophages, DCs, natural killer T (NKT) cells, Memory T cells, and naive T cell populations have IL-23R (IL-23 receptor) [10]. This cytokine considered as a link between nonspecific and specific immunity [11]. IL-23 has many biologic functions like the ability to prompt IL-17 production clarifying its importance in the expression of early immune response against pathogens [12]. Thus IL-23 help in instant neutrophil infiltration to the target organs [13]. Due to the role of IL-23 in constancy and expansion of Th17 response, any defect in the IL-23 production lead to a powerful polarization of Th17 cells and continued generation of IL-17. This imbalance is a leading cause of the chronic inflammation damaging the periodontal tissue [14].

A study has done by **Luo et al.** [15] and **Wang et al.** [16] suggest that in the term of pathogenesis of periodontium destruction, IL-23 could play a turn due to its up-regulation in diseased periodontal lesion. Another study by **Liukkonen et al.** [17] have suggested that IL-23 is a pro-inflammatory cytokine secreted after adaptive immunity has activated. IL-23 has elevated at the initial phases of periodontitis. IL-23 has a role in autoimmune diseases as an example its role in the etiology and pathogenesis of ulcerative colitis and Crohn's disease [18]. Saliva is a hypotonic solution formed by the salivary glands, gingival crevicular fluid, and oral mucosal exudate [19]. Saliva has a significant turn in health maintenance of the mouth [20].

The goal behind employing saliva as a bio-fluid is that it is easy to collect, inexpensive and non-invasive [21]. The aim of this research was to find the association of salivary concentration of IL-23 with periodontal health and disease by examining its level in individuals with healthy periodontium, dental biofilm-induced gingivitis and periodontitis (localized and generalized).

2. MATERIAL AND METHOD
2.1 Study design and ethics: This study was a case-control study that took place from January 2022 to June 2022 at the Teaching Clinics of the Periodontics Department, College of Dentistry, University of Baghdad. For the goal of obtaining salivary samples, 90 participants were examined and separated into the following four groups:

- Group A: Healthy periodontium as a control group involved 15 subject who had BOP < 10%, PPD ≤3 mm on intact periodontium (no clinical attachment loss) [2].

- Group B: Dental biofilm-induced gingivitis included 25 cases who had generalized gingivitis BOP >30%, PPD ≤ 3 on intact periodontium (no clinical attachment loss) [2].

- Group C: Localized periodontitis where the number of teeth affected by periodontitis related bone loss was ≤ 30% [5], included 25 cases unstable periodontitis with PPD ≥5mm or PPD at ≥4mm and BOP [4].

- Group D: Genralized periodontitis where the number of teeth affected by periodontitis-related bone loss was > 30% [5], included 25 cases unstable periodontitis with PPD ≥5mm or PPD at ≥4mm and BOP [4].

All cases of periodontitis were characterized as [4]: Interdentally detectable CAL at ≥ 2 non-adjacent teeth or CAL present at ≥ 3 mm on the buccal (facial) or lingual/palatal surfaces in conjunction with pocketing > 3 mm at ≥ 2 teeth.
Salivary samples were used to compare the salivary levels of IL-23 in patients with dental biofilm-induced gingivitis and periodontitis to healthy controls. Assessing clinical parameters and relating them to the concentration of the chosen biomarker.

Inclusion criteria: In our study we needed systemically healthy patients have at least 20 teeth and were not taking any medications for the previous 3 months.

Excluson criteria: Individuals with systemic diseases, subjects with any previous extensive periodontal therapy or presently subjected to periodontal therapy, subjects taking antibiotic or immunosuppressant therapy within the previous 3 months, subjects presented with necrotizing ulcerative gingivitis, aphthous ulcer or any lesion not related to the disease, subjects who smoke or take alcohol, subjects who were receiving orthodontic or dental implant, pregnant or lactating mothers and women taking contraceptive pills.

Periodontal parameters and clinical examination
Except for the wisdom teeth, all other teeth were subjected to periodontal examination. This involved full mouth plaque score (FMPS), gingival bleeding on probing (BOP), probing pocket depth (PPD), clinical attachment level (CAL) using a periodontal probe (Michigan O probe) marking at 1, 2, 3, 5, 7, 8, 9, and 10 mm at six places per tooth except for the full mouth score (FMPS) which covered four surfaces of the tooth. The staging of periodontitis was assessed where the tooth with the worst clinical attachment loss was present. The extent is determined by dividing the number of teeth with clinical attachment loss by the total number of teeth.

Salivary sample collection
Unstimulated whole saliva was collected into a sterile test tube from all participants [22]. The volume of saliva collected was 3 ml per participant on average. The collected samples were placed directly on the ice then they were centrifuged at 3000 rpm for 5 minutes by centrifuge machine (80-1 Electronic Centrifuge, China) to eliminate any cellular debris from the salivary samples. Following the manufacturer's instructions and using (HumanReader HS, HUMAN Society for Biochemical and Diagnostic mbH, Wiesbaden, Germany), saliva samples then were analyzed for the

protein level of IL-23 by using commercially available ELISA kits that were ordered from BioSource in California, USA. The concentrations were then stored and transferred to spread sheets to be analyzed.

Ethical approval:

The study was approved by the research ethics committee of the college of Dentistry, University of Baghdad. An informed written consent was taken from each participant after full explanation and total information about the participation in the study.

Statistical analysis

The continuous data were expressed as mean, SD, while for categorical variables, frequency and percentage were applied. The data distribution was investigated utilizing the Shapiro-Wilk test. Continuous variables were subjected to ANOVA test, which was followed by post-hoc test. The Kruskal-Wallis test served as an alternative for non-parametric data. Mann Whitney test was also applied. If clinical and biochemical parametric variables were correlated, it was determined using Spearman's or Pearson's correlation test (relying on the distribution of the data). All data were processed using SPSS (version 25). $p \leq 0.05$ was considered significant.

RESULTS

Age of the participants ranged between 20 and 60 y with a mean of 33.733 ± 11.987. Gender distribution throughout the sample showed that the females had the higher percentage. Descriptive statistics of clinical parameters showed that (PI) data comparison among groups showed a significant difference between them (P=0.000) with the highest values in disease groups in comparison with the healthy (control) group. Regarding BOP, also the disease groups had significantly the peak values as compared to the healthy control group with significant difference between localized and generalized periodontitis groups. For PPD & CAL, there were a significant difference when comparing them between periodontitis groups with the generalized periodontitis group had the highest value.

The distribution of the participants according to gender, age and clinical periodontal parameters in each group were illustrated in table (1). ANOVA test results showed significant downregulation of IL-23 level among groups in comparison with the healthy (control) group. Also, there was a significant difference between dental biofilm-induced gingivitis and localized periodontitis group (Figure 1). When we correlated salivary IL-23 concentration with clinical periodontal parameters, results displayed that IL-23 was significantly and negatively correlated with all periodontal parameters with correlation coefficients ® for the four parameters (PI, BOP, PPD, CAL) were -0.328, -0.427, -0.316, -0.464 respectively (Table 2).

Table (1): Descriptive statistics of gender, age and clinical periodontal parameters among groups.

Gender	Descriptive statistics	Group A n =15	Group B n = 25	Group C n = 25	Group D n =25	Total n =90
Male	Frequency (n)	2	15	7	12	36
	Percentage %	13.3 %	60 %	28 %	48 %	40 %
Female	Frequency (n)	13	10	18	13	54
	Percentage %	86.7 %	40 %	72 %	52 %	60 %
Age	Mean± SD	23.600± 2.746	24.440± 5.575	36.240± 8.847	46.600± 9.273	33.733±11.98
Clinical periodontal parameters						
PI	Mean± SD	7.567± 1.731	84.444± 19.448	75.438± 2.513	79.279± 23.850	
BOP	Mean± SD	5.050± 1.151	53.846± 11.220	44.436± 9.165	56.910± 17.206	
PPD	Mean± SD	0.000± 0.000	0.000± 0.000	1.837± 2.323	3.970± 1.865	
CAL	Mean± SD	0.000± 0.000	0.000± 0.000	2.489± 0.785	3.766± 0.815	

* SD: standard deviation
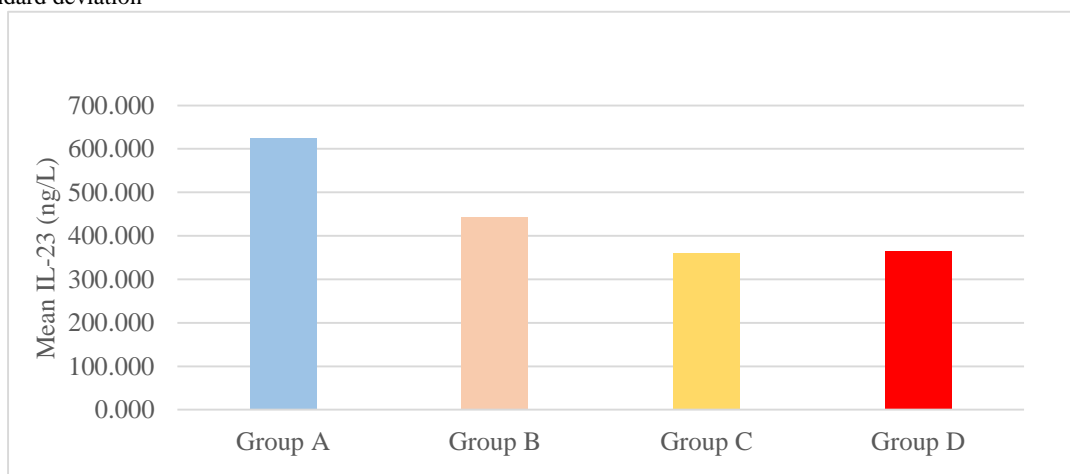
Figure (1): Salivary IL-23 level among study groups.

Table (2): Correlation between salivary IL-23 and clinical periodontal parameters

		PI	BOP	PPD	CAL
IL-23	Significance	0.002	0.000	0.002	0.000
	Correlation coefficient	-0.328**	-0.427**	-0.316**	-0.464**

Correlation is significant at the 0.05 level (2-tailed) *
Correlation is significant at the 0.01 level (2-tailed) **

DISCUSSION

According to our findings the severity of periodontal diseases rose with rising age. Increased exposure to the irritant (bacterial plaque) with time increases the possibility to get more sever periodontal diseases and deterioration of the supporting tissue and it is considered as a reflection of human oral cumulative history [23].

Gender distribution throughout the sample showed that the females had the higher percentage. These findings agree with these studies **Mohammed et al.** [24], **Al-dhaher et al.** [25]. The reason behind that because female was more prone to incipience and advancement of gingivitis and periodontal diseases that their sex hormones causes alteration in bacterial flora [26]. Descriptive statistics of clinical parameter (PI) findings agree with other studies [27, 28]. This may result from inadequate oral hygiene measures leading to the aggregation of bacterial deposit (plaque biofilm) that represent the main etiologic factor for periodontal disease leading to initiation of periodontium inflammation [29].

Regarding BOP findings, they agree with another study [30]. The BOP index results showed that inflamed periodontal tissue experience higher pathophysiological activity than does clinically healthy periodontal tissue due to the effect of plaque deposition on blood flow. Additionally, the degree of the inflammation determines the severity of the bleeding and the impact of its cause [31].

For PPD & CAL, findings agree with the study accomplished by **Mousa and Saliem** [28]. Sulcular and junctional epithelium extermination and the following bone loss in periodontitis may be due to amount of the plaque and accompanied increased bacterial invasion [32]. Results showed significant downregulation of IL-23 level among case groups as compared to the healthy control group. Our findings coincide with the only study that was done by **Sadeghi et al.** [33] where GCF level of IL-23 concentration was greater in the healthy control group than disease groups. Our results disagree with the study done by **Liukkonen et al.** [17] where salivary level of IL-23 was higher in the localized periodontitis group in comparison with healthy control group then reduced after that in the generalized periodontitis group as compared to the control group. As an explanation for our data, this can be attributed to the presence of other biomarkers like IL-10 that acts as anti-inflammatory

cytokine to prevent over reactive immune response. Thus inhibits IL-23 to maintain equilibrium between pathogen resistance and injurious systemic inflammation [34]. Another possible reason is that IL-23 dominates the first inflammatory response in infected or wounded peripheral tissue. Only once the original danger signal has been processed, will the inflammatory response be replaced by suitable immunological effector activities, such as an influx of activated CD4 and CD8 T cells. The IL-12-IFN axis is anticipated to become the major route at this time. This outcome is consistent with Th1 response dominating the IL23-IL-17 immunological pathway [12]. When we correlated salivary IL-23 concentration with clinical periodontal parameters, results showed that IL-23 was significantly and negatively correlated. this could be attributed to the same possibilities mentioned above that could explain the reason behind the negative correlation of IL-23 level with elevated measurements of clinical periodontal parameters among the disease groups that refer to the severity of periodontal disease.

CONCLUSION

There is significant and negative association between salivary IL-23 level and periodontal diseases as compared to periodontal health where IL-23 level is reduced in disease groups as compared to healthy (control).

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REFERENCES
1. **Huang N, Gibson F (2014):** Immuno-pathogenesis of periodontal disease: current and emerging paradigms. Current oral health reports, 1 (8): 124-132.
2. **Chapple I, Mealey B, Van D et al. (2018):** Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. Journal of periodontology, 89: 74-84.
3. **Murakami S, Mealey B, Mariotti A, Chapple I (2018):** Dental plaque–induced gingival conditions. Journal of clinical periodontology, 45: 17-27.
4. **Tonetti M, Greenwell H, Kornman K (2018):** Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. Journal of periodontology, 89: 159-172.
5. **Milward M, Roberts A (2019):** Assessing periodontal health and the british society of periodontology implementation of the new classification of periodontal diseases 2017. Dental Update, 46 (2): 918-929.
6. **Taba M, Kinney J, Kim A, Giannobile W (2005):** Diagnostic biomarkers for oral and periodontal diseases. Dental Clinics, 49 (7): 551-571.

7. **Group B, Atkinson J, Colburn W, Degruttola V** *et al.* **(2001):** Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clinical pharmacology and therapeutics, 69 (7): 89-95.

8. **Handa T, Yoshimura A (2002):** Regulation of cytokine signaling and inflammation. Cytokine & growth factor reviews, 13 (8): 413-421.

9. **Tang C, Chen S, Qian H, Huang W (2012):** Interleukin-23: as a drug target for autoimmune inflammatory diseases. Immunology, 135 (3): 112-124.

10. **Parham C, Chirica M, Timans J, Vaisberg E** *et al.* **(2002):** A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rβ1 and a novel cytokine receptor subunit, IL-23R. The Journal of Immunology, 168 (3): 5699-5708.

11. **Langrish C, Chen Y, Blumenschein W** *et al.* **(2005):** IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. The Journal of experimental medicine, 201 **(7):** 233-240.

12. **Mckenzie B, Kastelein R, Cua D (2006):** Understanding the IL-23–IL-17 immune pathway. Trends in immunology, 27 (12): 17-23.

13. **Cheung P, Wong C, Lam C (2008):** Molecular mechanisms of cytokine and chemokine release from eosinophils activated by IL-17A, IL-17F, and IL-23: implication for Th17 lymphocytes-mediated allergic inflammation. The Journal of Immunology, 180 (11): 5625-5635.

14. **Tan Z, Bealgey K, Fang Y, Gong Y, Bao S (2009):** Interleukin-23: immunological roles and clinical implications. The international journal of biochemistry and cell biology, 41 (20)**:** 733-735.

15. **Luo Z, Wang H, Sun Z, Luo W, Wu Y (2013):** Expression of IL-22, IL-22R and IL-23 in the peri-implant soft tissues of patients with peri-implantitis. Archives of oral biology, 58 (12): 523-529.

16. **Wang H, Han Q, Luo Z** *et al.* **(2014):** Oral lichen planus may enhance the expression of Th17-associated cytokines in local lesions of chronic periodontitis. Clinical Oral Investigations, 18 (2): 1647-1654.

17. **Liukkonen J, Gürsoy U, Pussinen P, Suominen A, Könönen E (2016):** Salivary Concentrations of Interleukin (IL)-1β, IL-17A, and IL-23 Vary in Relation to Periodontal Status. Journal of periodontology, 87 (20): 1484-1491.

18. **Abdul-hussein S, Ali E, Alkhalidi N, Zaki N, Ad'hiah A (2021):** Roles of IL-17A and IL-23 in the Pathogenesis of Ulcerative Colitis and Crohn's Disease. Iraqi Journal of Science**,** 62(8): 2526-2535.

19. **Zhang C, Cheng X, Li J, Zhang P** *et al.* **(2016):** Saliva in the diagnosis of diseases. Int J Oral Sci, 8 (1): 133-7.

20. **Ahmed R, Al-jubouri R (2017):** Oral Health Status, Salivary MMP-8& Secretory Leukocyte Peptidase Inhibitor (SLPI) Among Uncontrolled Type-I Diabetes Mellitus In Iraqi Patients. Journal of baghdad college of dentistry, 29 (5): 65-70.

21. **Buczko P, Zalewska A, Szarmach I (2015):** Saliva and oxidative stress in oral cavity and in some systemic disorders. Journal of physiology and pharmacology: an official journal of the Polish Physiological Society, 66 (8): 3-9.

22. **Navazesh M (1993):** Methods for collecting saliva. Annals of the New York Academy of Sciences, 694 (31): 72-77.

23. **Aljehani Y (2014):** Risk factors of periodontal disease: review of the literature. International journal of dentistry, 20 (2):4-9.

24. **Mohammed H, Abdulkareem A, Zardawi F, Gul S (2022):** Determination of the accuracy of salivary biomarkers for periodontal diagnosis. *Diagnostics,* 12 (10)**,** 2485.

25. **ALDHAHER Z, AL-GHURABI B, ALWAN B (2018):** Serum levels of IL-22 and ACPA in patients with Rheumatoid Arthritis. *Journal of Pure and Applied Microbiology,* 12 (2)**:** 687-691.

26. **Boyapati R, Cherukuri S, Bodduru R, Kiranmaye A** (**2021):** Influence of Female Sex Hormones in Different Stages of Women on Periodontium. J. Midlife Health, 12 (4): 263- 266.

27. **Salman S (2015):** Measurement of salivary Immunoglobulin A of participants with a healthy, gingivitis and chronic periodontitis conditions. Journal of Baghdad College of Dentistry, 27 (6): 120-123.

28. **Mousa H, Saliem S (2016):** Assessment of alkaline phosphatase, salivary flow rate and salivary potential of hydrogen in relation to severity of chronic periodontitis. Journal of Baghdad College of Dentistry, 28 (4): 126-131.

29. **Strohm T, Alt K (1998):** Periodontal disease—etiology, classification and diagnosis. Dental anthropology, 40 (44): 32-78.

30. **Rai B, Kharb S, Jain R, Anand S (2008):** Biomarkers of periodontitis in oral fluids. Journal of oral science, 50 (5): 53-56.

31. **Carranza F (2009):** Clinical Periodontology. Philadelphia . https://www.researchgate.net/publication/299345706_C arranza's_Cl…

32. **Newman M, Takei H, Klokkevold P (2012):** Carranza's Clinical Periodontology. 11th edition St. Louis: Mosby**,** Pp: 414-418.

33. **Sadeghi R, Sattari M, Dehghan F, Akbari S (2018):** Interleukin-17 and interleukin-23 levels in gingival crevicular fluid of patients with chronic and aggressive periodontitis. Central European Journal of Immunology, 43 (7): 76-80.

34. **Lyakh L, Trinchieri G, Provezza L, Carra G, Gerosa F (2008):** Regulation of interleukin-12/interleukin-23 production and the T-helper 17 response in humans. Immunological reviews, 226 (1): 112-131.