Mustansiria Dental Journal
DOI: 10.32828/mdj.v19i1.989

E- ISSN: 2618-0944
P- ISSN: 1813-8500

Research Article **Open Access** **Volume: 19, Issue: 1, 2023**

Assessment of Salivary TNF-α Level in Patients with Different Severities of Periodontitis

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Received: 5 March 2023; Accepted: 28 May 2023; Published: 28 June 2023

Abstract

Aim of the study: To assess the salivary levels of TNF-α in patients with different severities (stages I-IV) of periodontitis in comparison to healthy control.

Material and method: 171 individuals both men and women were inducted into this study. There were five groups established for them: clinically healthy periodontium control group (19 subjects), Stage I periodontitis (38 subjects), Stage II periodontitis group (38 subjects), Stage III periodontitis (38 subjects), and, Stage IV (38 subjects). The clinical periodontal parameters were examined after collecting whole unstimulated salivary samples from all individuals. TNF-α levels in saliva samples were estimated using the enzyme-linked immunosorbent assay (ELISA) method.

Results: The results revealed the mean level of salivary TNF-α was the highest in the stage IV periodontitis group (200.634±50.232), followed by stage III periodontitis group (159.315±36.473), then stage II periodontitis group (128.054±32.651), then stage I (102.798±21.685), and then the control group (51.454±10.446) in which their salivary levels were the lowest with significant differences among the groups ($p< 0.01$).

Conclusion: It may be suggested that TNF-α is related to periodontitis. Also, their salivary levels may have diagnostic potential.

Keywords: TNF-α, periodontitis, saliva.

Introduction

Periodontal diseases are progressive, destructive, and inflammatory conditions of the tissues that support the teeth [1,2]. Periodontitis is thought to be initiated by dental biofilms that accumulate below and at the gingival edge[3]. A variety of chemokines, cytokines, and inflammatory mediators are produced by the immune and inflammatory cells that assault diseased periodontal tissues [4].

The clinical periodontal parameters are the best diagnostic tools now available; however, they could only evaluate the disease's current severity and extent. No information could be obtained regarding future disease activity [5]. To overcome the limitations outlined above, various tools have been developed and introduced to detect periodontitis [6].

Saliva has long been thought to be a reflection of the overall health of the body since it includes the serum elements which are evaluated in standard blood tests for the disease and health monitoring, saliva as a diagnostic fluid provides a lot of advantages over serum for disease diagnosis [7]. saliva collection is not intrusive, consequently simpler, safer, and less expensive than withdrawing blood, Unlike blood, saliva collection is possible without the aid of skilled individuals [8].

Tumor necrosis factor-alpha (TNF-α) is a proinflammatory cytokine [9] with a broad range of actions, inclusive of the destruction of the tissues and migration of the cells, TNF-α affects cell migration by upregulating adhesion molecules, which promotes neutrophil rolling and adherence to the

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vascular wall, resulting in extravasation [10]. It has been observed that TNF-α activates matrix metalloproteinases (MMPs), which are responsible for the destruction of periodontal connective tissue during the progression of periodontitis[11]. Furthermore, TNF-α promotes the release of MMPs and Receptor activator of nuclear factor-kappa B ligand (RANKL), which is related to extracellular matrix breakdown and bone resorption[12],[13]. Therefore, the purpose of this study was to assess the salivary levels of TNF-α in patients with different severities of periodontitis in comparison to healthy control.

Materials And Methods

Study design

A total of 171 participants, males, and females, between the ages of 30 and 55 were inducted into this observational case-control study. Sample collection started from January 2022 to April 2022 at the Dental centers in AL-Najaf City and the Department of Periodontics in the College of Dentistry, University of Kufa. The study followed the principles of the Declaration of Helsinki. The ethical committee of the College of Dentistry, University of Baghdad, approved this study (Ref. number: 650).

Following a detailed explanation of the study's goals and objectives, all subjects voluntarily agreed to participate and were given informed consent forms. An oral examination and a thorough medical and dental history were obtained for each participant. All participants were systematically healthy and had at least twenty teeth.

Patients that were not included in this study were: patients who have been or are currently undergoing significant periodontal therapy, patients who have taken anti-inflammatory or antibiotic medications in the last three months, patients suffering from chronic systemic diseases, immunocompromised patients, smoking, women who take contraceptive pills, post-menopausal women, breastfeeding mothers and pregnant, smokers, patients with orthodontic appliances, removable dentures, implants, crowns, and bridges, patients with soft tissue lesions like an aphthous ulcer or lichen planus.

Following the collection of saliva, a thorough periodontal examination was performed including Plaque Index (PLI) [14], Bleeding on Probing (BOP) [15], Probing Pocket Depth (PPD), and Clinical Attachment Loss (CAL). A periodontal probe (the university of Michigan O probe with Williams marking) was utilized to measure all clinical periodontal parameters. Six surfaces (four for plaque assessment) of every tooth excluding the wisdom teeth were examined. The clinical examination was done by the same examiner for all participants.

The control (periodontal health) group was defined as patients who had no CAL, PPD ≤3 mm, BOP <10% [16]. The periodontitis groups were defined as Interproximal CAL was observed at two non-adjacent teeth. or buccal or oral CAL≥3 mm with pocketing >3mm was observed at two teeth [17].

According to the new classification for periodontal and peri-implant diseases and conditions, periodontitis cases were split into stages I, II, III, and IV [17]. All periodontitis cases were generalized and unstable status (PPD ≥5mm or PPD ≥4mm with BOP) with no risk factors (diabetes mellitus [DM] and/or smoking). each group consists of 38 patients, except the control group which consists of 19 patients.

Calibration

the periodontal parameters (PLI, BOP, PPD, and CAL) for 10 subjects were measured for both inter and intra-calibration by the researcher and skilled supervisor for

DOI: 10.32828/mdj.v19i1.989

inter-calibration while for intra-calibration the researcher measured the periodontal parameters with a two hours interval between the two measurements and using Intraclass correlation Coefficient (ICC) which is the acceptable limit is >0.75.

Saliva collection

Patients' whole unstimulated saliva samples were collected prior to the investigation of clinical periodontal parameters between 9 to 11 A.M. The patients must not consume anything except water one hour before sample collection. The patients were instructed to rinse their mouths properly to remove any debris and to wait for (1-2) minutes for the water to clear. Saliva passively drooled over the lower lip to the plain tube. Samples that contain blood were discarded after collection[18], 3ml of saliva was collected, tube was labeled with the subject's number that had been previously recorded on the case sheet. The saliva was centrifuged for 20 minutes at 3000 rpm, and the resulting supernatant was aspirated into Eppendorf tubes with a micropipette and then stored at -80 °C in the laboratory's freezer until the day of analysis.

Quantitative analysis of Salivary TNF-α

The concentrations of TNF-α were determined in the saliva of periodontitis groups and control group by using (ELISA) kit (My BioSource) in accordance with the manufacturer instructions. The absorbance was determined utilizing a spectrophotometer plate reader (HumaReader HS, Germany).

sample size calculation

To calculate sample size, the salivary concentration of the biomarker (TNF-α) was used as a primary outcome of the study. The saliva concentration of the biomarker during health is estimated to be equal to (2.15pg/ml) whereas during periodontitis is unregulated as up as (12.92pg/ml) (Varghese, 2015)[19]. This yields an expected odds ratio of (6) between periodontal health and periodontitis which was used to calculate sample size using http://riskcalc.org:3838/samplesize/ at 95% confidence interval and 5% error margin. The calculated total sample size was 171 subjects divided into five groups, each group will consist of 38 subjects except for healthy periodontium control which will include 19 subjects.

Statistical analysis

Statistical Package for Social Science (SPSS) version 21 (Chicago, USA, Illinois) was used to describe, analyze, and present the data. Statistical analyses are divided into two types: 1) descriptive analysis included frequency and percentages, mean and standard deviation (SD).2) inferential analysis included Shapiro Wilk test, ANOVA test, Games-Howell test and, Pearson correlation (r)test. Level of significance as: Not Significant (NS) $P>0.05$, Significant (S) $P<0.05$ and $P<0.01$.

Results

Table 1 shows that the mean values of PLI, BOP%, PPD, and CAL were increased from control till stage IV periodontitis with a significant difference ($p<0.01$), the mean value of PLI for control was (25.092±3.506), (58.920±7.947) for stage I. (63.371±5.837) for Stage II, (65.499±5.449) for Stage III, and (67.546±9.104) for Stage IV, the mean value of BOP% for control was (7.042±1.788), (48.669±5.374)for Stage I. (51.418±3.027) for Stage II, (53.564±4.514) for Stage III, and (54.385±6.124) for Stage IV, the mean value of PPD for Stage I (4.145±0.071), (4.671±0.186) for Stage II, (5.351±0.281) for Stage III, and (5.748±0.162) for Stage IV, the mean value of CAL for Stage I was (1.488±0.087), (2.362±0.451) for Stage II, (4.218±0.208) for Stage III, and

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(6.305±0.237) for Stage IV (ANOVA test was used).

Table 1: clinical periodontal parameters for control and periodontitis groups (ANOVA test used)

Groups	PLI		BOP %		PPD		CAL	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Control	25.092	3.506	7.042	1.788	-	-	-	-
Stage I	58.920	7.947	48.669	5.374	4.145	0.071	1.488	0.087
Stage II	63.371	5.837	51.418	3.027	4.671	0.186	2.362	0.451
Stage III	65.499	5.449	53.564	4.514	5.351	0.281	4.218	0.208
Stage IV	67.546	9.104	54.385	6.124	5.748	0.162	6.305	0.237
P-value	0.000 (S)		0.000 (S)		0.000 (S)		0.000 (S)	

(S): Significant

As shown in **(table 2)** the results showed that the mean level of TNF-α was increased from control till stage IV periodontitis with a significant difference ($p<0.01$), the mean value of TNF-α for control was (51.454pg/ml±10.446),(102.798pg/ml±21.685) for Stage I, (128.054pg/ml±32.651) for Stage II, (159.315pg/ml±36.473) for Stage III, and (200.634pg/ml±50.232) for Stage IV (ANOVA test was used).

Table 2: Salivary levels of TNF-α in study groups (ANOVA test used)

Groups	Mean	±SD	P value
Control	51.454	10.446	0.000 (S).
Stage I	102.798	21.685	
Stage II	128.054	32.651	
Stage III	159.315	36.473	
Stage IV	200.634	50.232	

(S): Significant

Following multiple pairwise comparisons (using Games-Howell test) among groups as seen in **(table 3)** all results were significant difference ($p<0.01$).

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Table 3: Multiple Comparisons of TNF-α (Games-Howell test used).

(I)Groups	(J) Groups	p value
Control	Stage I	0.000 (S)
	Stage II	0.000 (S)
	Stage III	0.000 (S)
	Stage IV	0.000 (S)
Stage I	Stage II	0.001 (S)
	Stage III	0.000 (S)
	Stage IV	0.000 (S)
Stage II	Stage III	0.001 (S)
	Stage IV	0.000 (S)
Stage III	Stage IV	0.001 (S)

(S): Significant

As seen in **(table 4)** there was a significant positive correlation between TNF- α and each of PLI, PPD, CAL in Stage III and Stage IV (r=0.410, p=0.011, r=0.449, p=0.005, r=0.511, p=0.001, r=0.397, p=0.013, r=0.679, p=0.000, and, r=0.668, p=0.000) respectively. (Pearson correlation (r)test was used).

Table 4: Correlation between periodontal parameters and salivary TNF-α by groups (Pearson correlation (r)test used)

Groups	PLI		BOP		PPD		CAL	
	r	P	r	P	r	P	r	P
Control	0.209	0.391(NS)	0.203	0.404(NS)	-	-	-	-
Stage I	0.296	0.071(NS)	0.269	0.103(NS)	0.203	0.221(NS)	0.301	0.066(NS)
Stage II	0.138	0.407(NS)	0.054	0.749(NS)	0.320	0.050(NS)	0.315	0.054(NS)

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Stage III	0.410	0.011(S)	0.134	0.424(NS)	0.449	0.005(S)	0.511	0.001(S)
Stage IV	0.397	0.013(S)	0.218	0.188(NS)	0.679	0.000(S)	0.668	0.000(S)

(S): Significant, (NS): Non Significant

Discussion

According to the study's findings, the mean values of PLI and BOP were increased from the control group to stage IV group with a significant difference. These findings were in agreement with (Saliem, Mousa and Talib, Ahmed) [20,21]. These results were attributable to the fact that subjects in the control group had healthy periodontium and maintained proper oral hygiene and plaque control using toothbrushes and interdental aids. In addition, microbial biofilm is regarded as an essential etiological factor in the onset of periodontal diseases [22]. As for BOP, these findings demonstrated the influence of plaque accumulation on blood circulation as well as the actual pathophysiological process that occurred more in inflamed tissues, such as increased capillary fragility and permeability, and that the severity of bleeding and the ease with which it can be provoked dependent upon the intensity of the inflammation[23].

Also, the findings of this study demonstrated that both PPD and CAL mean values were increased significantly from stage I to stage IV. This may be attributed to increased bacterial invasion and plaque accumulation, which result in the destruction of sulcular epithelium, junctional epithelium, and alveolar bone in periodontitis. Those findings were in agreement with (Ali, Ahmed) [24] who demonstrated that both PPD and CAL mean values were elevated in periodontitis with increased severity.

Regarding the salivary level of TNF-α, the findings of this study demonstrated that the level of TNF-α was significantly higher in all periodontitis groups than in controls. These results were consistent with those of (Afacan, Atmaca İlhan and, Mahmood, Al-Ghurabi) [25,26] who observed that TNF-α levels were significantly higher in periodontitis patients than in control subjects, implying that the level of TNF-α in saliva may help in the detection, diagnosis, and management of the periodontal disease.

Another finding of this study is that there was a significant positive correlation between TNF-α with PLI, P.P.D, and CAL in stage III and IV periodontitis groups. This results in agreement with (Afacan, Atmaca İlhan) [25] who demonstrated that the correlation between the salivary level of TNF-α and clinical parameters such as BOP, PLI, P.P.D, and CAL was significantly positive. Also, the results were consistent with (Mahmood, Al-Ghurabi) [26] who revealed a significant positive correlation between the salivary level of TNF-α and clinical parameters like P.P.D and CAL.

These results may be due to the fact that PMNs will be attracted to the site of infection upon exposure to LPS from gram-negative bacteria, monocytes and activated macrophages produce cytokines like (TNF-α and IL-1b) that induce further tissue damage in response to endotoxin [27] in vitro study has shown that gingival epithelial cells release cytokines like TNF-α, IL-8, IL-6, and IL-1 when exposed to P.gingivalis [28,29]. In addition, TNF-α promotes connective tissue destruction and bone loss by inducing (MMPs)expression, stimulating the synthesis of (PGE2) and increasing osteoclast formation and activity [30].

Conclusion

The findings of this investigation showed that periodontitis groups' salivary levels

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of TNF-α were significantly higher than those of a healthy group; consequently, TNF-α may be an important factor in the pathogenesis of periodontitis, and its salivary levels may have the potential to be employed as diagnostic tools for periodontitis.

Suggestion

Longitudinal study to compare salivary TNF-α levels in patients with periodontitis before and after periodontal therapy, employ the same research strategy to patients suffering from systemic diseases such as osteoporosis, rheumatoid arthritis, and diabetes mellitus, and Investigate and contrast TNF-α levels in generalized and localized periodontitis.

Limitations of the study

The current study did not include patients suffering from periodontitis with other risk factors like smoking and diabetes. However, generalization of the results of this study is not advised without including a larger number of patients.

Conflicts of Interest

The authors reported that they have no conflicts of interest.

Funding

This research received no external funding.

Data Availability Statement

Data are available from the authors upon reasonable request.

Conflict of interest

The authors reported that they have no conflicts of interest.

Acknowledgments

The authors would like to thank College of Dentistry, Baghdad University, Department of Periodontics, Iraq, for their support in the present work.

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