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Estimation of TNF- α and LDH in Chronic Periodontitis Patients in Mosul

Heba F. Hamodat[a], Mahmoud Y. M. Taha[b*]

[a]*Assist. lecturer, Department of Dental Basic Sciences, College of Dentistry, Mosul University*

[b]*Professor, Department of Dental Basic Sciences, College of Dentistry, Mosul University, Mosul, Iraq*

[a]*Email: mhhhsd1976@gmail.com*

[b]*Email: tahadent04@yahoo.com*

Abstract

The objective of the study is to estimate salivary concentration of TNF-α and LDH level in chronic periodontitis and control group, and correlate them with periodontal parameters. The study group consisted of 44 patients suffering from chronic periodontitis, aging between ≤16-45 years old and 40 control samples collected from healthy individuals ranged between 16-40 years old .Chronic periodontitis was assessed on the basis of several periodontal parameters, including probing pocket depth (PPD), clinical attachment loss (CAL), bleeding on probing (BOP) and plaque index (PI). 5ml of unstimulated saliva was collected from patients and control groups to measure salivary biomarkers by ELISA technique. the mean concentrations of TNF-α in the study and control groups were (6.9pg/ml) and (5.6 pg/ml) respectively، data analysis showed no significant difference between the two groups (P >0.05), and the mean LDH level in chronic periodontitis was (7.7855) and in control group the mean was (0.5555) and the difference was highly significant ($p \leq 0.000$). Salivary TNF-α and LDH showed positive correlation with clinical periodontal parameters.

***Keywords*:** Chronic periodontitis; TNF-α, LDH; periodontal parameters.

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* Corresponding author.

1. Introduction

Chronic periodontitis has been defined by the American Academy of Periodontology (AAP) as "an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss, and bone loss " [1]. Although microorganism (mainly anaerobic bacteria) is considered as instigating agents, the disease progression is influenced by the host response together with environmental and behavioral factors [2]. Chronic periodontitis was further classified as localized disease where in ≤ 30% of evaluated sites demonstrate attachment and bone loss, or as a generalized disease where in >30% of sites are affected [3]. The disease may also be classified according to the severity of the disease as: slight when there is 1-2 mm of CAL, moderate 3-4mm of CAL or sever 5-6mm of CAL [4]. Saliva is an important biological fluid that contains both local and systemically derived biochemical substances used for detecting periodontal disorders, the advantages of using saliva as a source of biomarkers include its easy of collection, relatively cheap technology as compared to other tests, painless, patient suffers no discomfort and little anxiety in the collection process, can be used to study special population where blood sampling is problem like (children, anxious, handicap, elderly patients), safer for health professionals than blood tests, saliva is cheaper to store and ship compared with blood and urine, in addition saliva does not clot and can manipulated more easily than blood [5]. One of these biomarkers, is lactate dehydrogenase which has been used for diagnosis of periodontal disease [6]. LDH is an enzyme found in cytoplasm of almost every human cell, which becomes extracellular following cell necrosis, cell death and tissue breakdown [7], so that salivary LDH level may be a feasible and useful parameter for screening periodontal disease [8]. TNF-α is the most important cytokine response of the host against the active component of Gram-negative bacteria (endotoxin). It is mostly generated by mononuclear phagocytes, T lymphocytes, natural killer cells (NK), and activated mast cells [9]. By destruction arachidonic acid, this cytokine causes increment of prostaglandin E2 concentration, and then activation of osteoclasts. Consequently, along with IL-1, this cause bone resorption, resulting in release of the MMPs and destruction of the extracellular matrix ([10].

2. Materials and Methods

2.1. Subject groups

The subjects attended to Periodontal Clinic in the College of Dentistry/Mosul University from January 2019 to May 2019, consisted of 44 patients with chronic periodontitis aging between 16 - 45 years old. Women, smokers, and individuals with either acute or chronic medical illness or on oral medication for the last three months were excluded, while control group consisted of 40 clinically healthy individuals ranged between 16-40 years old. This group had no signs of any systemic disease, with clinically healthy periodontium . All patients with the control group underwent an oral examination.

2.2. Data Source

Case sheet was performed especially for the purpose of examination.

2.3. Saliva collection

Unstimulated saliva was collected from patients and control groups via passive drooling into a sterilized disposable collector cup. Saliva was centrifuged at 3000 rpm for 20min. The clear supernatant fraction was then separated and dispensed in Eppendorff tubes and stored at -70˚C until required for analysis [11]. The parameters tested included probing pocket depth (PPD), clinical attachment level (CAL), bleeding on probing (BOP), and plaque index (PI). PPD represent the distance from the gingival margin to bottom of the pocket. The distance was calculated with a WHO probe , the pocket depth was measured at four sites of each tooth. CAL represent the distance between the bases of the pocket to the cemento-enamel junction , while BOP was calculated using the formula score 0= no bleeding after running periodontal probe, score 1= immediate bleeding or bleeding within 10 seconds of running periodontal probe. pI was determined using different scores , score 0= no plaque on the tooth surface, score 1= film layer of plaque, no visualization, only by probe, score 2= plaque seen by visual inspection and by running probe, and score 3= abundance amount of plaque exceed cervical third of crown.

2.4. Materials and kit Used for Salivary Serological Study

1. Human LDH test kit, R&D System. USA.

2.Human TNF alpha ELISA test kit, Abcam. UK.

2.5. Statistical Analysis

T-Test, Enova test ,correlations, Mean, Std Deviation, and Std Error.

3. Results

3.1. Comparison between study and control groups in relation to periodontal indices

The comparison between study and control groups using periodontal parameters (PPD, BOP, CAL, and PI), showed increasing in periodontal parameters in the study group in comparing with control group, and the difference was highly significant ($P \leq 0.001$) .

3.2. Comparison between study and control groups in relation to salivary parameters

The comparison between study and control groups using salivary parameters (TNF-α, and LDH), showed the mean enzyme activity of LDH in the study group was (7.7855), while in the control group was (0.5555). and the difference was highly significant ($P \leq 0.001$), while TNF- α its mean concentration in the study group was (6.9 pg/ml), and the mean concentration in the control group was (5.6 pg/ml), and the difference was not significant ($P > 0.05$) (Table 1).

Table 1: Student's t–test Comparison between Study and Control Groups

Salivary biomarkers	Group	No	Mean	DD SD	SS+	*t-t-value* *Significant*		*P-value*
LDH	Study	44	7.785	4.247		-7.544	0.000*	significant
	Control	40	0.555	0.577				
TNF-α	Study	44	6.9	0.034		1.421	0.163	non
	Control	40	5.6	0.163				

3.3. Relationships between salivary parameter concentrations and periodontal pocket depth in the study group

The comparison between salivary parameters (LDH, and TNF-α) levels and their relation with periodontal pocket depth within study group showed positive correlation between LDH, TNF-α and PPD, there was significant difference in TNF-α concentration in PPD 4mm compared to PPD 5 and 6mm (p= 0.012), while there was no significant difference in LDH enzyme activity in patients with different PPD levels. (Figure 1, Table 2).

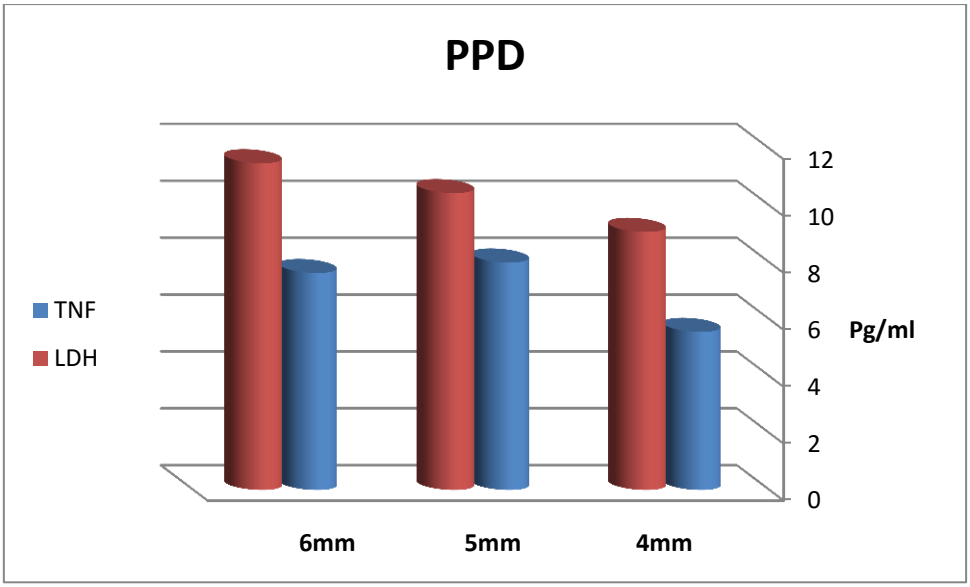

Figure 1: Relationships between salivary parameters concentrations and periodontal pocket depth in the study group

3.4. Relationships between salivary parameter concentrations and bleeding on probing index in the study group

The comparison between salivary parameter (LDH, and TNF-α) levels and their relation with BOP within study group showed positive correlation with BOP, with no significant difference. (Figures 2, Table 2).

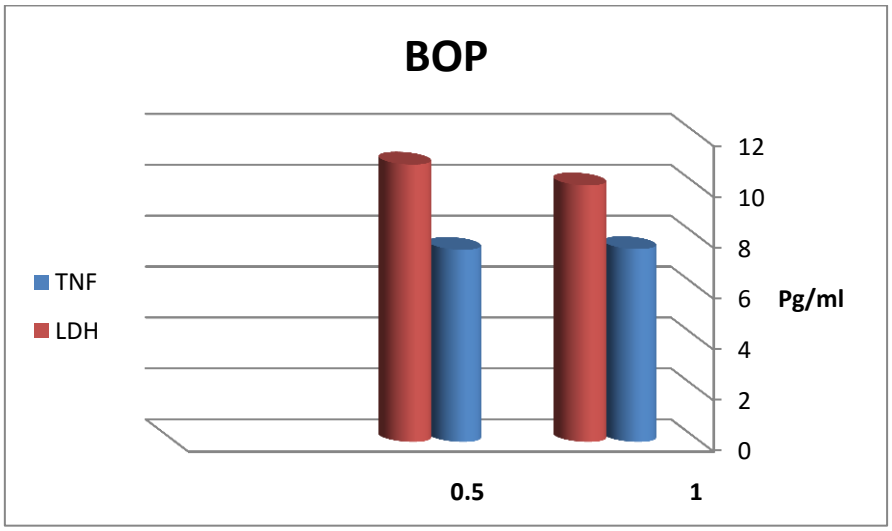

Figure 2: Relationships between salivary parameter concentrations and bleeding probing in study group

3.5. Relationships between salivary parameters concentration and clinical attachment level in study group

There was a positive correlation between (LDH, TNF-α) and CAL , comparison between salivary parameter (LDH, and TNF-α) levels in relation with different levels of CAL study group, TNF-α showed significant difference in CAL 2 mm from CAL 3mm and 4mm (*p*=0.012) , while there was no significant difference in LDH in patients with different CAL levels(Figures3,Table2).

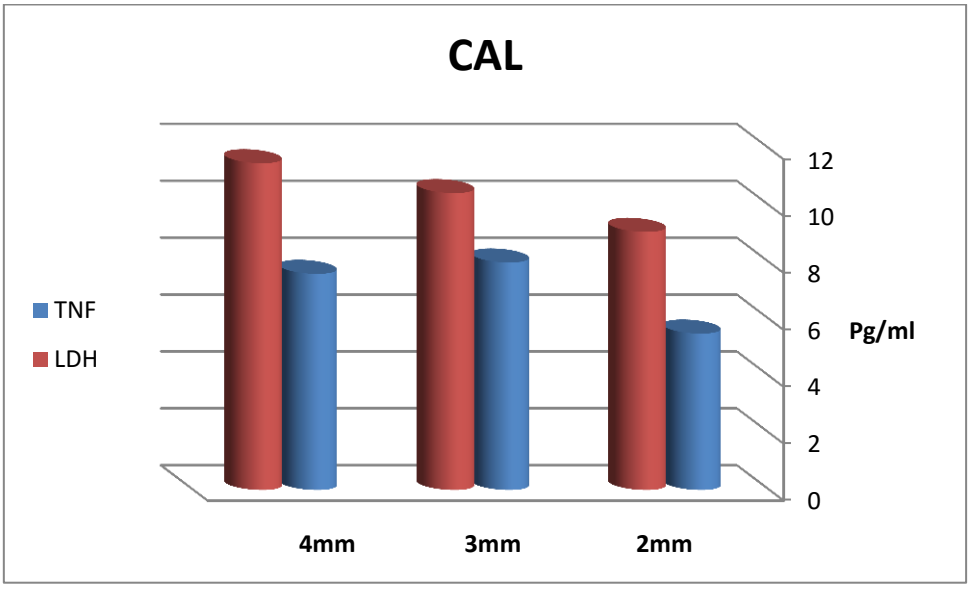

Figure 3: Relationships between salivary parameters concentrations and clinical attachment level in the study group

3.6. Relationships between salivary parameter concentrations and plaque index in the study group

There was a positive correlation between (LDH, TNF-α) and PI, with no significant difference in LDH, and TNF-α in patients with different PI values. (Figures 4, Table 2).

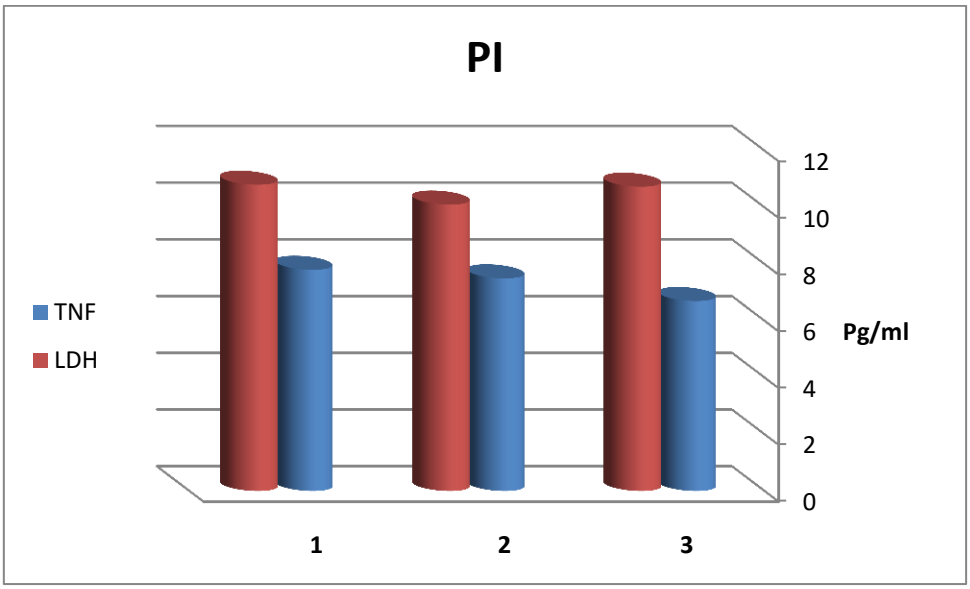

Figure 4: Relationships between salivary parameters concentrations and PI value in the study group

Table 2: Correlation between salivary biomarkers and periodontal parameters within the study group

Salivary Biomarkers		PI	BOP	PI	CAL
TNF-α	Pearson correlation	0.117	0.074	0.016	0.016
Sig. (2-tailed) DH		0.472	0.652	0.924	0.924
LDH	Pearson correlation	0.317*	0.135	0.153	0.153
Sig. (2-tailed)		0.046	0.408	0.345	0.345

4. Discussion and conclusion

The present study showed that elevation of mean values of all clinical periodontal parameters (PI, CAL, BOP, and PPD) were highly significant ($P \leq 0.001$) among the study group compared with control group. These results

are concurrence with Khongkhunthian and his colleagues [12]. The increase mean value of PI explained the role of the pathogen in the development and severity of PD. This is clear since plaque is the essential etiological factor in PD and is supported by the fact that the microbial biofilm is considered the primary and the major etiological factor responsible for initiation of periodontal disease [13]. The highest PI mean was found in study group. This could be related to the abnormally shaped gingival recession and the periodontal pocket formation in this group which may increase the plaque accumulation. The result of BOP index indicated the effect of plaque accumulation on blood circulation and the actual pathophysiological process that happened more in inflamed periodontal tissue compared to the clinically healthy periodontal tissue. In addition, the severity of bleeding and the influence of its incitement depend on the intensity of the inflammation where more accumulation of plaque with increased number of active sites that occur with chronic periodontitis [14]. The results of PPD and CAL in the present study were expected in chronic periodontitis group which could be due to increase in the bacterial invasion and the amount of plaque that triggered destruction of the sulcular, junctional epithelium and collagen fibres resulted in apical migration of the clinical attachment level and increasing of probing depth [15]. If periodontal pathology starts, or its cells get damaged due to external or internal environment, or destruction of a cellular membrane, LDH which is intracellular enzyme is being released in great amount into saliva and it activity can be measured. This enzyme can be used as biochemical markers of the functional condition of periodontal tissues [16]. In this study the result indicated that LDH level was highly significant in the study group compared with control group similar finding was observed by Manoj and his colleagues [17]. This study showed a positive correlation between clinical periodontal parameters (BOP, PI, PD, and CAL) and the activity of LDH in saliva. Similar finding was achieved by Manoj study [18].

The activity of LDH enzyme was linear increasing, with the increasing value of gingival index, this increasing activity of LDH indicates the increasing probability of pathological changes in gingiva that leads to coincide with the initial stage of periodontal disease. In this study, salivary TNF-α levels in the study group were higher but not significant when compared with the control group. This study suggested that the salivary level of this cytokines cannot be appropriate factor to differentiate the periodontal disease from the healthy periodontium, similar finding obtained by Yousefimanesh and his colleagues [19].Kurtis and his colleagues [20], showed higher TNF-α in chronic periodontitis patients significantly different from control group.

A possible explanation for the discrepancy might be variation in disease severity between the study group, or may be due to change in salivary composition due to use stimulated saliva and discrepancy in case selection. Among the clinical parameters in the study group, there was a positive correlation between salivary TNF-α level and clinical periodontal parameters (PI, BOP, PD, and CAL), and this result was in agreement with Kurtis and his colleagues [21] who reported a significant positive correlation between GCF TNF-α levels with pocket depth in chronic periodontitis patients. While Sheeja and his colleagues [20]. showed no correlation with most of the clinical parameters possibly due to extensive dilution of these markers in saliva. Salivary biomarker level of LDH could be used as a marker in early diagnosis or detection of chronic periodontitis, so this can resist the disease progression, whereas TNF-α cannot be used as biomarker for detection of early periodontal disease . One of the limitation of the study was female, not included within the study because hormonal changes in female and this will effect on the result.

5. Recommendations

Estimation of other cytokines and matrix metalloproteinase as biomarkers for early prediction of chronic periodontitis.

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