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Effect of Some Demographic Changes on Some Oral Immune Aspects in Periodontal Disease Patients

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Abstract

Periodontal diseases (PD) are worldwide diseases of humans, either in childhood or as adults. The present study aimed to find a correlation between some demographic and saliva immunological factors, including the determination of saliva TLR-2, IL6, CRP, and α-amylase in patients with periodontal diseases. For this purpose, 60 patients, out of which 33 were males and 27 were females, participated in this study from different Dental treatment Centers (Amirya Specialized Dental Center and Almaamon Specialized Dental Center) in Baghdad, Iraq, for the period starting from November / 2021 to February 2022. Both age ranges for patients and control are (13-70) years, and patients' mean ages are 34.29±15.01. Additionally, the control specimens were collected randomly from 40 apparently healthy people. The results of some demographic parameters revealed that there were no significant differences between males (55.0%) and females (45.0%) in PD. As well, periodontal disease is not directly associated with rising smoking among patients or non-smoking individuals with PD. Acidic oral pH is not significantly found in most PD patients (47.1%) and control subjects (66.7%); simultaneously, smoking may not be directly associated with acidic oral saliva. Other tests in concern with PD patients are considered to be very important in many aspects. First, interleukin interleukin (IL6) recorded high non-significant mean occurrence results in both patients' 50161± 63869 ng/l and control 52087 ± 62756 ng/l groups. Secondly, C-reactive protein (CRP) as IL6 recorded a non-significant increase in the saliva of PD patients at 0.537 ± 0.607 mg/l and control at 0.607 ± 0.266 mg/l. Toll-like receptor-2 (TLR-2) is the third immune parameter measured in saliva, which recorded significant differences between PD patients (7.384 ± 4.031 ng/ml) and controls (5.313 ± 3.106 ng/ml) simultaneously. Then the fourth saliva parameter is α-amylase. The results recorded a significant difference between PD patients (2.444 ± 1.870 ng/ml) and controls (1.041

± 1.044 ng/ml. Also, the results showed that there was no correlation between the measured demographic and immunological parameters.

Keywords: Periodontal disease, pH saliva, Smoking, TLR-2, a-amlyase.

1. Introduction

Periodontal disease (PD), which encompasses gingivitis and periodontitis, is the major cause of tooth loss in adults worldwide, accounting for 10–60% of all cases [1]. Gingivitis is a kind of periodontal disease that is reversible since the inflammation is restricted to the gingiva and no supporting tissues are damaged. Periodontitis, on the other hand, is described as the persistent degradation of the periodontium's deeper components, which results in connective tissue attachment and alveolar bone loss, periodontal pocket formation, tooth movement, and, eventually, tooth loss [2, 3]. Periodontal disease prevalence rises due to a variety of variables, including age, gender, and smoking habits [4].

Saliva is a complex mouth fluid composed of water, cells, organic and inorganic components, and other compounds. The salivary glands secrete between 500 - 700 ml of saliva per day, with the three major salivary glands secreting 90% and the smaller salivary glands secreting the remainder [5]. Saliva's activities include cleansing, antimicrobials, buffering, pH management, lubricating the oral cavity, and protecting against various pathogens [6]. Salivary pH might be regarded as a significant element in maintaining dental health [7]. Furthermore, variations in salivary pH are regulated by the duration of smoking and the nicotine amount labeled on the cigarette [8].

Toll-like receptor 2 (TLR-2) is a transmembrane receptor that belongs to the pattern recognition receptor family. TLRs predominantly respond to pathogen-associated molecular patterns (PAMPs). They play a crucial role in the innate system in recognizing infections and initiating actions that aid in their clearance [9]. Professional first-line phagocytes like macrophages, neutrophils, and dendritic cells typically express TLRs [10].

Interleukin-6 is a pro-inflammatory cytokine that immune cells, fibroblasts, keratinocytes, adipose tissue, and muscles produce. IL-6 is engaged in inflammation and infection responses, modulates host response to bacterial infections, and contributes to periodontitis pathogenesis through its association with molecular mediators of active periodontal degradation and periodontitis development [11].

CRP is a pentraxin-family protein that is mostly made by hepatocytes during the acute phase when IL-6 and IL-1 are increased. Its levels are increased in situations involving inflammation, injury, or infection [12].

Alpha-amylase (AA) consists of 50–60% of salivary protein [13]. Salivary AA is mostly generated by the serous cells of the parotid gland, although other salivary glands also produce this protein [14]. One of the functions of AA in saliva is to directly limit the development of specific bacteria, as well as to bind to the bacterial surface structure and to bacterial toxin, both of which trigger tissue-damaging inflammatory reactions. Periodontal disease affects the amounts of certain salivary proteins, such as AA. As a result, it appears that certain salivary

elements may have a role in the etiology of this illness [15]. Therefore, this study aimed to find the correlation between some demographic and salivary immunological factors in patients with periodontal diseases.

2. Material and method

A total of 60 specimens of randomly selected patients (33 males and 27 females) participated in this study, which was obtained from different dental treatment Centers (AL-Amriya Specialized Dental Center and Almaamon Specialized Dental Center) in Baghdad, Iraq, for the period from November 2021 to February 2022. The diagnosis of periodontal disease was done by professional dentists based on the clinical findings. Patients' ages ranged from 13-70 years. Out of the control specimens, 40 apparently healthy people specimens were collected from 25 females and 15 males, with approximately the same age range as the patients' participants, approximately. Patient case histories are regulated throughout the questionnaire form (name, age, gender, medication, chronic disease, smoking, case of social, and last treatment of antibiotics).

2.1 Specimens Collection

Unstimulated whole saliva, approximately 2 ml, was collected for analysis from each participant after being informed that they must not have eaten or drunk at least 2 hours before sampling. Dependent on procedure instructions [16]. Briefly, they were instructed to rinse their mouth several times with sterilized water and eliminate it outside. In the meantime, the saliva specimens were collected. Using a pH Test Strip (Cybow/China), the salivary pH was determined after collection by dipping the strip into the saliva for approximately 2 seconds until a color change occurred and comparing it to the standard color indicator. Each sample of collected saliva was centrifuged at 10,000 rpm for 10 minutes, after which the clear supernatant was collected and kept at -20 °C until used.

2.2 Immunological assay

Assessment of TLR-2**,** CRP, IL-6, and α-amylase in saliva was performed by ELISA according to the manufacturer's protocol of instruction (BT LAB, China, and Sunlong Biotech, China, respectively) and measured in a microplate reader at a wavelength of 450nm.

2.3 Statistical Analysis

The data were analyzed using the following software: Microsoft Excel, Minitab v17, and IBM SPSS V26. A Z-test was used to compare two proportions. Probability values less than 0.05 were considered significantly different [17].

3.Results and discussion

Adults frequently experience periodontal disorders, which have a variety of risk factors, including smoking, poor dental hygiene, diabetes, medication, age, heredity, and stress [18]. The results of the collected specimens showed that there was no significant difference between male 33 (55.0%) and female 27 (45.0%) patients (**Table 1**). These results were consistent with [19]

and exhibited no significant association between gender and periodontal disease. Whereas, [20] found that males had a greater frequency of periodontal diseases due to worse oral hygiene than female subjects.

Table 1. Test Two Proportions (Gender) in Patients Group

Gender	Frequency	Percent	P-value¥
Male	33	55.0	0.271
Female	27	45.0	
Total	60	100.0	

In this study, the age means of periodontal patients was 34.29±15.01 and ranged from 13 to 70 years. [21] showed that gingivitis was most common in the late adolescent age group (17–25), whereas chronic periodontitis was most common in the early elderly age group (46–55).

According to the main role and great functions of saliva in the mouth's cavity, saliva pH is one of the important parameters in the oral cavity. Smoking is a bad habit that may lead to changes in the salivary pH. Thus, the study focused on finding the effect of smoking and its role in changing oral pH. The current result in **Table 2** demonstrated that there was no significant difference between saliva pH and smoking habits for patients and controls in all pH items.

Table 2. Comparison of saliva pH and Smoking habits in patients and controls

pH of saliva	Smoking habits					
	Smoking			Non-smoking		
	Patient	control	p-value	patient	control	p-value
	N (%)	N (%)		N (%)	N (%)	
Acidic	8 (47.1%)	2(66.7%)	0.510	31(72.1%)	21(56.8%)	0.149
Neutral	7(41.2%)	1(33.3%)	0.792	8(18.6%)	9(24.3%)	0.535
Alkaline	2(11.8%)	0(0.0%)	0.132	4(9.3%)	7(18.9%)	0.219
Total	17(100%)	3(100%)		43(100%)	37(100%)	

These results agree with a study by [22] that concluded that long-term use of tobacco smoking does not produce any alterations in salivary flow rate or pH and does not create any notable gingival changes. As well, [23] observed that smokers had lower baseline saliva pH levels than non-smokers; however, this was not statistically significant. Whereas, [24] found that smokers with periodontitis had a lower salivary pH (acidic) than non-smokers. In the same context, a study done by [25] documented that saliva from tobacco smokers with periodontitis had a lower pH value than saliva from non-smokers with periodontitis.

On the other hand, this study assessed several parameters, including IL-6, TLR2, CRP, and α-amylase, in the saliva of the patient and compared them with the control group.

Table 3. Groups test of immunological parameters in patients and control group.

Variables	patients N=51	Control N=40	P-value¥
	Mean ± SD	Mean ± SD	
IL-6 ng/l	50161 ± 63869	52087 ± 62756	0.886
TLR-2 ng/ml	7.384 ± 4.031	5.313 ± 3.106	**0.009
CRP mg/l	0.537 ± 0.607	0.607 ± 0.266	0.053
a-Amylase ng/ml	2.444 ± 1.870	1.041 ± 1.044	0.001**

The results in **Table 3** showed that TLR2 has a significant increase in patients (7.384±4.031 ng/ml) compared to the control group (5.313±3.106 ng/ml). The present result matches [26], who noted that gingivitis had a significantly greater amount of salivary TLR-2 than the control. While [27] found a decrease in the soluble TLR2 in the case of periodontal disease, which contradicted the current study results,

As well, the increase of α-amylase in the saliva of patients in this study is considered another indicator of periodontal diseases. The present results were in accordance with [28], who reported salivary alpha-amylase (SAA) levels rising as the severity of periodontal disease increased. This enzyme level in saliva decreases following periodontal therapy. As a result, SAA levels can be utilized as a biomarker for gingivitis and periodontitis. In contrast to this study, [29] demonstrated there was no significant statistical difference in SAA activity in stimulated whole saliva among patients with aggressive and chronic periodontitis, as well as a healthy control group. Therefore, both TLR2 and salivary α-amylase may have been used as biomarkers for the diagnosis of periodontal diseases.

Another immunological parameter, IL-6, was evaluated in this study; the results do not significantly differ between the patient and control groups, according to the results in **Table 3**. This interleukin result was revealed to be a strange level in saliva; it is so high in both patients and control groups. These data are compatible with [30], who revealed that the levels of IL-6 in saliva were found to be the same in periodontal disease and periodontal health. In contrast to the present study, [31] showed that the chronic periodontitis group had greater levels of IL-6 than the gingivitis and control groups; this difference was statistically highly significant.

Accordingly, C-reactive protein has a role in acute inflammation. In certain cases, this protein is utilized to predict and detect periodontal disease [32]. The results in **Table 3** revealed no significant difference between patients (0.537 ± 0.607 mg/l) and the control group (0.607 ± 0.266 mg/l), with a bias toward the control group. These results are consistent with [33] results, which showed there was no difference in CRP levels in the saliva of patients and controls. While [34] found that CRP levels differ significantly between chronic periodontitis and healthy subjects,

Simultaneously, the study included the correlations between immune parameters and demographic factors. The present results in **Table 4** showed the salivary levels of IL-6, TLR-2, CRP, and α-amylase were not significantly correlated with age, gender, pH saliva, or smoking habits.

Table 4. Pearson correlation between parameters and demographic factors

Variable	Statistics	IL-6 ng/l	TLR-2 ng/ml	CRP mg/l	a-Amylase ng/ml
Age	r_s	-0.07	0.10	-0.13	0.18
	P-value	0.625	0.503	0.345	0.196
Gender	r_s	-0.06	0.21	0.00	0.09
	P-value	0.672	0.146	0.977	0.547
pH saliva	r_s	-0.16	-0.06	0.00	0.04
	P-value	0.251	0.668	0.991	0.802
smoking habits	r_s	0.14	0.14	-0.16	0.20
	P-value	0.311	0.312	0.260	0.164

These results are consistent with [35] elucidated that there were no variations in salivary IL-6 concentrations between male and female subjects, suggesting that there is no relationship between gender and the concentration of inflammatory indicators. Moreover, [36] discovered that smoking has no effect on salivary IL-1 and IL-6 concentrations. [37] indicated that the concentration of α-amylase decreases with age, although the difference is not significant. However, the mean salivary α-amylase level did not differ substantially between males and females [38]. Furthermore, [39] noted that smokers had greater salivary α-amylase levels than non-smokers, although there was no statistically significant difference between the two groups. Additionally, TLR2 expression levels have not been reported to change consistently with age; however, this might be tissue type-specific and stem from variances across cell types. TLR2 expression appears to be unaltered by aging, its function, and its activation capability [40]. Finally, [41] reported that there was no statistically significant correlation between age, gender, and CRP concentration.

Whereas, the present results are not compatible with [42] who supported the hypothesis that exposure to a pathogen in early life decreases inflammatory responses in adults. However, the defensive effects vary by gender and can be declined by growing obesity through life development, which raises risks for other inflammatory illnesses. Furthermore, elderly individuals (40–80 years) had substantially higher IL-6 and CRP levels. Smokers showed considerably greater salivary CRP levels than non-smokers [43]. In addition, [44] found that smoking increased the expression of toll-like receptor 2 (TLR2) in gingival tissue compared to nonsmokers.

4.Conclusion

Based on the findings of the current study, there is no big difference between males and females in the percentage of periodontal disease hits among Iraqi individuals. Smoking is not the main factor related to periodontal disease, but it may worsen the oral condition of individuals. Acidic oral saliva is more prevalent among Iraqi individuals, and smoking does not play a role in converting oral pH to acidic conditions. The dysregulated inflammatory status, assessed through pro-inflammatory markers in saliva (IL6 and CRP) in both experimental groups, was determined by up-regulated levels in saliva and may reflect systemic conditions.

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