Periodontal Disease: A Predictive Profile

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Received: 23/6/2022          Accepted: 5/10/2022          Published: 30/8/2023

Abstract

Periodontal diseases such as gingivitis and periodontitis are considered common diseases. This study is aimed to predicate these diseases using non-harmful specimens such as saliva throughout the detection of some parameters. In the beginning, a random 51 patients' saliva was collected from outpatients who suffered from different degrees of periodontal diseases. Concurrently, another 40 people who appeared to be healthy were collected, and both patients and healthy people were subjected to a questionnaire form and diagnosed by a specialist dentist. The results of this study revealed that there is no significant difference between males and females in infection with periodontal diseases. As well, smoking is not acting as the main factor causing this disease. Most patients' saliva pH was acidic. Immunological parameters such as toll-like receptor 2 (7.384 ± 5.313, 4.031 ± 3.106 ng/ml) and α-amylase (2.444 ± 1.870, 1.041 ± 1.044 ng/ml) are both increased significantly in patients than in the control group respectively, with no significant differences for CRP between both experimental groups, which may have been used both TLR2 and alpha-amylase with acidic pH as biomarkers to detect periodontal diseases.

Keywords: gingivitis, periodontitis, TLR2, α-amylase, CRP

أمراض اللثة: لمحة تنظيمية

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الخلاصة

تعتبر أمراض اللثة مثل التهاب اللثة والتهاب دواعم السن من الأمراض الشائعة. تهدف هذه الدراسة إلى إثبات إمكانية تشخيص هذه الأمراض باستخدام عينات غير ضارة كاللعاب من خلال الكشف عن بعض الدلائل. في البداية، تم جمع لعاب عشوائي لـ 51 مريضاً من مرضى العيادات الخارجية الذين يعانون من درجات مختلفة من أمراض اللثة. في الوقت نفسه، تم جمع 40 شخصًا آخرًا كانوا يتمتعون بصحة فم جيدة، وضاعف كل من المرضى والأشخاص الأصحاء لاستعمار استباناً وتشخيصاً من قبل طبيب أسنان متخصص. أظهرت نتائج هذه الدراسة عدم وجود فروق ذات دلالة إحصائية بين التكاثر والإنتاج في الإصابة بأمراض اللثة بالإضافة إلى أن التدخين لا يعمل كعامل رئيسي لحدوث أمراض اللثة. كما أن الورك اليدودي للعاب لمعظم المرضى ومجموعة البيطرة كان حاضنياً، تم الكشف عن بعض المعلمات المناعية مثل TLR2 (7.384 ± 5.313, 4.031 ± 3.106 نانوغرام / مل) والزئبق الالمينز (2.444 ± 1.870, 1.044 ± 1.044)

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1. Introduction

Periodontal disease (PD): One of the most prevalent infectious diseases in humans. It's a bacterial infection of the gums and periodontal tissues that surround the teeth [1]. The two most common types of periodontal disease were gingivitis and periodontitis [2]. Gingivitis is an inflammation of the gingival tissues induced by localized irritation of a substance created by plaque (microbial) deposition on the tooth surface, with no obvious lack of contact with connective tissue or bone [3]. If left untreated, Gingivitis can progress to apical inflammation and periodontitis, a more serious form of the illness [4]. Periodontitis is a chronic bacterial infection of the gingiva that results in the loss of attachment to the jaw bone and tooth [5].

Saliva is the best fluid biology to serve as the diagnostic tool for periodontal disease. Saliva collection is a safe, non-invasive, and uncomplicated procedure that can be repeated with minimal discomfort to the patient [6]. Saliva is a complex fluid composed of 99.5% water and 0.5% solid material that includes both organic and inorganic components [7]. The salivary gland, an exocrine gland that includes major and minor glands, secretes saliva. [8]. Saliva has a variety of functions that are essential for the maintenance of oral and systemic health [9].

Toll-like receptors (TLRs) are pattern recognition receptors (PRRs), [10] that can be used to utilize pathogenic microorganisms that include conservative antigen molecules, such as lipopolysaccharide (LPS), lipoproteins, and other parts of the bacteria cell wall, leading the host to produce of inflammatory cytokines and chemokines [11]. TLRs are a group of leucine-rich repeat proteins that are expressed on the cell surface or in inflammatory cells' internal compartments [12]. TLRs are found on gingival fibroblasts, osteoclasts, osteoblasts, cementoblasts, and periodontal ligament fibroblasts, among other periodontal cells [13]. TLRs are a group of 13 receptors that have been discovered, with 10 of them being functionally expressed in humans [14]. Studied was observed TLR-2 expression in human inflammatory gingival fibroblasts was shown to be greater than in the healthy group [15]. The pentameric protein known as C-reactive protein (CRP) is produced by the liver and its level rises in response to inflammation. CRP is an acute-phase reactant protein that is mostly produced during the acute phase of an inflammatory/infectious process as a result of the action of IL-6 on the gene responsible for CRP transcription. Both pro-inflammatory and anti-inflammatory characteristics are found in CRP. It has an impact on the detection and removal of invading infections and damaged cells. It can activate phagocytic cells as well as the conventional complement system [16,17]. only a few studies have looked into the link between salivary CRP levels and periodontal disease. The study found no link between salivary CRP levels and gingivitis [18]. One important protein that makes up 60% of all the proteins generated by the salivary gland is the enzyme salivary a-amylase (SAA) [19]. Which of the following functions of saliva is responsible for breaking down high molecular weight carbohydrates into lower molecular weight sugars (i.e., glucose). In addition, amylase is also important in maintaining mucosal immunity [20]. Numerous investigations have revealed that people with periodontal disease have altered amounts of certain salivary proteins such as α-amylase, [21,22,23]. This study is aimed to find some predictive parameters to diagnose periodontal diseases using non-harmful specimens.

Materials and Methods
This study included Fifty-one patients (28 male and 23 female) with Periodontal disease their age range between 13-70 years and 40 (15 male and 25 female). The Participants were from attendants seeking treatment in the Amriya Specialized Dental Center and Almaamon Specialized Dental Center in Baghdad during the period from November 2021 to February 2022. They were subjected to a questionnaire, including; name, age, gender, medications, chronic disease, smoking, case of social and last treatment of antibiotics.

Unstimulated whole saliva approximately 2ml was collected for analysis from each patient and control individual, after being informed that must not have eaten or drunk at least 2h prior to sample collection. Depending on procedure instruction [24], the patients were instructed to rinse their mouths many times with sterile water and then excrete outside, then the saliva specimens were collected. After collecting the saliva, the pH of the saliva was determined using a pH Test Strip (cybow, china) by immersing the strip in saliva for about 2 seconds until color changes occurred, then comparing the results to the colored standard indicator. Each saliva sample was centrifuged for 10 minutes at 10000 rpm, then the clear supernatant was collected with a micropipette into Eppendorf tubes and stored at (-20c) until used. Assessment of TLR-2, CRP, and α-amylase in saliva was performed by ELISA according to manufacturer's protocol of instruction (BT LAB, china and Sunlong Biotech, china respectively). The diagnosis of periodontal disease including Gingivitis and periodontitis were diagnosed by a professional Dentist, based on the clinical findings compared to the control group.

**Statistical Analysis**

The following programs were used to analyse the data: IBM SPSS V26, Minitab v17, and Microsoft Excel. The outcomes of this investigation were presented as mean SD, and two proportions were compared using the Z-test. The Pearson correlation evaluates the linear relationship between two continuous variables, and if the categorical variable has two levels, the point-biserial correlation was used. Significant differences were defined as probability values less than 0.05.

**Results and Discussion**

Gingivitis is a periodontal disease, that occurred commonly among people, and may depend on different parameters such as age, eating, the route of teeth, cleaning habits, smoking, chronic diseases...etc [25]. This research was focused on several demographic and immunological parameters that may be predicated on to cause of gingivitis in Iraqi individuals. The results of collected data reveal that there was no significant difference between male 28(54.9%) and female 23(45.1%) patients (Table 1). These results were inconsistent with some epidemiologic studies, which show that males are more likely than females to develop severe periodontal diseases [26] found further revealed that males were a free risk factor for gingival bleeding in late adolescents in Japan and that gingival health status has been linked to oral hygiene practices [27].

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percent</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>28</td>
<td>54.9</td>
<td>0.320</td>
</tr>
<tr>
<td>Female</td>
<td>23</td>
<td>45.1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

\*Z-test was used to test two proportions*

Simultaneously, smoking is another parameter that was checked, the results in Table 2 revealed that there is a substantial difference in both experimental groups between smoking and non-
smoking. All subjects in this experiment were randomly selected and most of them are non-smoking.

### Table 2: Group test for smoking

<table>
<thead>
<tr>
<th>Smoking habits</th>
<th>Group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient</td>
<td>Control</td>
</tr>
<tr>
<td>Smoking</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>14</td>
<td>27.5%</td>
<td>3</td>
</tr>
<tr>
<td>Non-smoking</td>
<td>37</td>
<td>72.5%</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>100.0%</td>
</tr>
<tr>
<td>Chi-Square Tests</td>
<td>P-value</td>
<td>0.015 *</td>
</tr>
</tbody>
</table>

Despite the fact that smoking is linked to the development of several oral disorders, it also increases the number and complexity of periodontal pockets, as well as the loss of periodontal ligaments. Tobacco can increase gingival collapse and alteration in the oral mucosa and subsequent loss of tissue strength brought on by toxic substances. [28]. Smokers have both severe and moderate periodontitis more frequently than non-smokers. Periodontitis is two to twenty times higher in smokers than in non-smokers as [29] mentioned. Whereas, the Iraqi patients in this study show that gingivitis is not correlated directly with increasing smoking among patients.

On another hand, several studies correlated saliva pH as a biomarker for oral disease occurrence, thus this study was also conducted with the saliva of patients and controlled individual pH.

### Table 3: Group test for pH of saliva

<table>
<thead>
<tr>
<th>pH saliva</th>
<th>Group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient</td>
<td>Control</td>
</tr>
<tr>
<td>Acidic</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>33</td>
<td>64.7%</td>
<td>23</td>
</tr>
<tr>
<td>Neutral</td>
<td>13</td>
<td>25.5%</td>
</tr>
<tr>
<td>Alkaline</td>
<td>5</td>
<td>9.8</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>100.0%</td>
</tr>
<tr>
<td>Chi-Square Tests</td>
<td>P-value</td>
<td>0.549 N.S</td>
</tr>
</tbody>
</table>

The results in Table 3 reveal no significant difference between saliva pH for both patients and control groups. As shown in Table 3 most of the participants either patients or control were acidic saliva pH. Ranjith and coworkers [30] documented that the saliva pH for healthy gingiva ranged from 6.8 to 7.2, while in the case of gingivitis was ranged from 6.5 to 6.7, through influences of calculus formation and periodontal illness. With a multitude of biomarkers and challenges in their determination, the salivary pH may be utilized as a fast test as [31] documented. Although, the results in this study indicated there are no significant changes in the acidic pH between patients and control groups, this may have reflected the susceptibility of normal subjects to gingivitis.

Saliva includes several components that aid in the defense of the host. Early studies on biomarkers for periodontal diseases fixated on the evolution of gingival fluid for inflammatory molecules [32]. However, in the past, considerable efforts have been made in identifying markers in saliva, an easily accessible bio-specimen that is acquiescent for painless and frequent
collection [33]. Different classes of molecules such as cytokines, receptors, and matrix metalloproteinases in saliva, have been evaluated as biomarkers for periodontal diseases [34].

Since, this study was assessed several parameters including; TLR2, CRP, and α- amylase may have used as indicators for gingivitis.

TLR2 is one of the pattern recognition receptors (PRRs) that bind conserved molecular characteristics that are shared by a wide range of microorganisms. It is included two forms cellular surface bounded and soluble form [35]. In this study, soluble TLR2 was assessed which has been on the rise in recent years. The result of TLR2 in this study revealed a significant increase in patients $7.384 \pm 4.031$ than in the control group $5.313 \pm 3.106$. Several studies found decreasing in the soluble TLR2 in the case of periodontal disease [36], which was incompatible with the present study result. While, Al-Ghurabi, [37] found increasing in these soluble bio-molecules in the saliva of the patient with periodontal diseases. Increasing TLR2 was indicated indirectly to the bacterial infection especially with gram-negative ones because bacterial peptidoglycan is considered aligned for this receptor [36].

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients N=51</th>
<th>Control N=40</th>
<th>P-value&lt;sup&gt;+&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR-2 ng/ml</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>0.009**</td>
</tr>
<tr>
<td></td>
<td>7.384 ± 4.031</td>
<td>5.313 ± 3.106</td>
<td></td>
</tr>
<tr>
<td>CRP mg/l</td>
<td>0.537 ± 0.607</td>
<td>0.607 ± 0.266</td>
<td>0.053</td>
</tr>
<tr>
<td>α-Amylase ng/ml</td>
<td>2.444 ± 1.870</td>
<td>1.041 ± 1.044</td>
<td>0.001**</td>
</tr>
</tbody>
</table>

<sup>+</sup> Independent t-test was used to test between groups

As well, increasing of α- amylase in the saliva of patients in this study is considered another indicator of gingivitis as [38] documented that a crucial protein called α- amylase is secreted in the saliva as a part of oral host immunological response to periodontal disease. Additionally, the action of salivary α- amylase is to recruit digestion of carbohydrate process. Additionally, demonstrating the inhibitory effect against microbes plays a significant part in controlling bacterial adhesion and growth on intraoral surfaces. Hence, the recorded salivary α- amylase parameter increased, which demonstrated that periodontal disease's inflammatory state increases. Many studies have compared salivary α- amylase levels in health and chronic periodontal diseases; and found a significant increase in salivary α- amylase in diseased conditions [39]. Therefore, both TLR2 and salivary α- amylase may have been used as a biomarker for the diagnosis of gingivitis.

C-reactive protein is a factor in the acute inflammation phase. In some cases, this protein is used for the prediction and early detection of periodontal disease [40]. The results in Table 4 revealed no significant difference between patients $0.537 \pm 0.607$ mg/l and the control group $0.607 \pm 0.266$ mg/l with a bias toward the control group. These results are considered high in comparison with the normal range which ranged from 0.00005-0.0643 mg/l, simultaneously this range is lower than that found in the blood which was 0.14 to 31.1 mg/l [41]. As mentioned, increasing CRP is dependent on the severity of oral diseases, then in the case of gingivitis and normal healthy subject, this marker does not increase significantly, while it increases significantly in the case of periodontitis. Thus, increasing of CRP in both experimental groups
may reflect an abnormal condition that may be correlated with the late marker of coronavirus disease residue [42]

Conclusion
It can be concluded that most patients in the present study were with gingivitis than periodontitis. Additionally, the possibility of using saliva as an alternative human specimen reflected the body's health condition.

Ethical Clearance
The Biotechnology Department's local ethics committee approved the trials in this thesis and all of the volunteers signed off on them by hand. Patients were also given information and benefits based on the research.

Conflict of Interest
There are no conflicts of interest between authors. And the research depends on Special financial support for the authors only.

References


