Salivary Immune Parameters as A Predictor of Oral Diseases

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Abstract

Diabetes mellitus (DM) is a metabolic and hormonal disorder in which the body does not produce sufficient or respond ordinarily to insulin, leading to an increase in blood sugar (glucose) levels. This disordered has many side effects on body health, one of them being oral health. This study aimed to find these effects on several oral immune parameters included (IL6, CRP, and alpha-amylase) and the possible use of these parameters in the prediction of oral health and further risk sequel. A total of 91 specimens including 51 DM patients and 40 apparently healthy individuals were enrolled in this study which was carried out from November/2021 to February/2022. The results revealed that, abnormal increase of both IL6 and CRP in the saliva of both experimental groups, with no significant differences between them. Simultaneously, alpha-amylase shows significant increases in the saliva of the patient's group than in the control. Receiver operating characteristic (ROC) analysis with Area under the curve (AUC) confirmed that alpha-amylase can be used as a predictor parameter reflected oral and body health.

Keywords: DM, salivary IL6, salivary CRP, salivary alpha-amylase

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العوامل المناعية اللعابية كمؤشر لأمراض الفم

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الخلاصة

مرض السكري هو اضطراب في التمثيل الغذائي والهرموني حيث لا يتمكن الجسم من انتاج ما يكفي من الأنسولين أو لا يستجيب عادة للأنسولين ، مما يؤدي إلى زيادة مستويات السكر في الدم (الجلوكوز). لهذا الاضطراب العديد من الآثار الجانبية على صحة الجسم ، من بينها صحة الفم. هدفت هذه الدراسة إلى إيجاد هذه التأثيرات على العديد من معايير المناعة الفموية المتضمنة (انترلوكين 6 ، بروتين سي التفاعلي و الالفا اميلز) والاستخدام المحتمل لهذه المعلمات في التنبؤ بصحة الفم و المخاطر المحتملة المستقبلية. تم جمع إجمالي 91 عينة شملت 51 مريضًا من مرضى السكري و 40 فردًا يبدو أنهم يتمتعون بصحة جيدة تم تسجيلهم في هذه الدراسة التي أجريت في الفترة من تشرين الثاني / 2021 إلى شباط / 2022. أظهرت النتائج زيادة غير طبيعية في كل من انترلوكين 6 و بروتين سي التفاعلي في لعاب المجموعتين التجريبيتين مع

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عدم وجود فروق ذات دلالة إحصائية بينهما. في نفس الوقت ، يُظهر عامل الالفا اميليز زيادات كبيرة في
لعاب مجموعة المرضى مقارنةً بالاشخاص غير المصابين بمرض السكر. أكد تحليل خصائص تشغيل
المستقبل و المنطقة الواقعة تحت المنحنى أنه يمكن استخدام انزيم الالفا اميليز كمعامل تنبؤ يعكس الحالة
الصحية للفم والجسم.

1. Introduction

Diabetes mellitus (DM) is a chronic disease that remains one of the most global health diseases that increases the risk of dying at an early age [1]. DM is characterized by hyperglycemia that can occur in both fasting and postprandial conditions [2] and happens when the body does not properly utilise insulin or when the pancreas does not create enough insulin[3]. which can lead to some diseases, including neuropathy, microvascular diseases, impaired wound healing, and increased ability for periodontal disease [4]. May also cause salivary gland activity to be disrupted, resulting in a decrease in salivary flow and alterations in the content of saliva [5].

Saliva is composed of 99 percent water and 1% electrolytes, immunoglobulin, proteins, enzymes, and nitrogenous compounds such as urea and ammonia, which are important for oral cavity protection [6]. Impaired salivary secretion (hypersalivation) increases the danger of oral disease [7]. Saliva aids in the creation of the acquired enamel pellicle and mucosal pellicle, which cover the mouth and soft tissues, and so serve to influence microbe adherence and colonization, and also determine the makeup of the resident oral microbiota. Saliva not only aids in the removal of microbes and food carbohydrates from the mouth cavity, but it also provides nutrition to colonizing bacteria via the breakdown of dietary starch, lipids, and proteins [8]. Due to its versatility as a non-invasive diagnostic tool, saliva is an essential specimen in dentistry research and oral physiology. which is used to identify several illnesses such as diabetes, heart disease, dental caries, and other oral problems [9]. Salivary alpha-Amylase is one of the most abundant proteins in saliva [10] it belongs to the glycoside hydrolase family and is generated topically in the mouth cavity by salivary glands, primarily the parotid gland [11] accounting for 10–20 percent of total protein content, and is known to facilitate digestion [10]. The important function of alpha-Amylase is to break down starch into maltose and dextrin. Furthermore, salivary alpha-amylase has been found to decrease bacterial adhesion and growth, allowing for bacterial clearing from the mouth. As a result, it is vital for oral mucosal immunity. The salivary gland and pancreas secrete alpha-amylase, which is found in saliva and serum [12].

Some researchers have classified Periodontal disease (PD) as a frequent consequence of diabetes due to strong evidence of a link between DM and PD [13]. Patients with type 2 diabetes have a shift in monocyte/macrophage function, which leads to an overproduction of pro-inflammatory cytokines in response to periodontal pathogens [14]. In diabetic individuals, greater levels of cytokines such as interleukin-6 (IL-6) have been detected, and a higher level of serum IL-6 has been linked to diabetes [15]. IL-6 is a multifunctional cytokine generated by macrophages, neutrophils, and endothelial cells, among other cells [16]. The liver produces and releases C-reactive protein (CRP) in response to IL-6 [17]. CRP is considered a gold parameter for inflammation [18]. CRP is one of a group of serum proteins known as acute-phase proteins that prevent the spread of infectious microbial [17]. It is widely used clinically to monitor infections, particularly those caused by bacteria, inflammatory diseases, and both acute infections and chronic conditions [19]. This study aimed to find the correlation of DM with immune parameters of oral included (IL6, CRP, and alpha-Amylase)and if can use these parameters to predict oral health.

2. Material and method

A total of 91 participants in this study included; 51 patients (26 males,25 females) with diabetes mellitus aged between 18 and 74 years old as outpatients of Al-Mustansiriya University national diabetes center in Baghdad. Besides, 40 apparently healthy individuals as control (15 males,25 females) approximate with same age range as the patients' participants their ages between 16 and 75 years. Each individual receives a form questionnaire containing (age, sex, drugs, smoking, and workplace). The type of diabetes is determined based on the patient's medical treatment history, all of them were type2 except one was under type1. Dependent on [20] saliva was collected, at the beginning, all participants were instructed to fast for at least 1 or 2 hours before collecting saliva, then rinse their mouths with sterilized water and wait 10 minutes before collecting 2 ml of saliva in a sterile cup, then a saliva pH was measured directly using a pH strip(CYBOW, China). After that, the saliva samples were centrifuged (10,000× g; 15 min) to eliminate any insoluble material or cellular debris, and the supernatants were collected and stored in a freezer at -20. At the time of use, the saliva is allowed to thaw at room temperature and according to the company's instructions, 3 ELISA kits were used to estimate salivary alpha –Amylase kit (SUNLONG, China), IL-6, and CRP (BT LAB, China) kits.

3. Statistical Analysis

The data were analyzed using the following software, Microsoft Excel, Minitab v17, and IBM SPSS V26. The results reported in this study were expressed as mean ± SD. The median test (non-parametric test) is used to test whether two (or more) independent groups differ in central tendency. Receiver operating characteristic (ROC) analysis was used to determine the best predictor of the diabetic patient. Probability values less than 0.05 were considered significantly different.

4. Results and discussion

A total of 91 Iraqi individuals including 51 DM patients with an age mean of 52.63 ± 12.76 including 26 (50.98%) males and 25 (49.02%)females. Other 40 apparently healthy subjects with an age mean of 45.73 ±13.37 including 15 (37.5%) males and 25 (62.5%)females were enrolled in this experiment which was focused on some oral immune parameters in saliva that may use as a predictable parameter related to DM. Most of the patients 50(98.04%) were T2DM and only 1(1.96%) was T1DM. Three main immune parameters (IL6, CRP, and alpha-amylase) are selected and were associated directly with inflammation. The results in table 1 revealed that IL6 is very high in both experimental groups, and no significant difference was recorded between them with a bias toward the patient's group, this result was fortunately strange, as we know IL6 is one of the pro-inflammatory cytokines releases as a signal of acute inflammation. The salivary IL-6 median and interquartile range for the control subject is 10.8 (0.5 - 34.3) Pg/ml as Thayalan et al.,[21] documented which was incompatible with the present study Figure 1.

Table 1: The median levels of salivary factors across groups

| Variables	patients N=51		Control N=40		P-value¥
	Median	(IQR)©	Median	(IQR)	
IL-6 ng/l	71300	(17280 – 140000)	61100	(15727– 111390)	0.906
CRP mg/l	1.095	(0.879 – 1.252)	1.022	(0.828 – 1.268)	0.89
alpha-Amylase ng/ml	4.900	(3.410 – 7.25)	1.806	(0.850 – 2.332)	0.001**

©: **IQR: Inter Quartile Range. ¥ Nonparametric Tests (chi-square)**
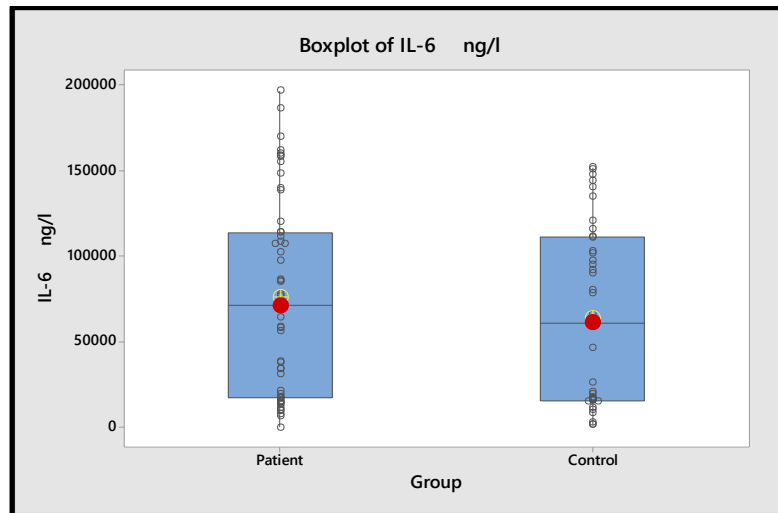
Figure 1: A comparable median boxplot of IL6 between patients and control groups

Goel et al.,[22] reported that, the increase of IL-6 has been related to multidrug resistance protein expression by keratinocytes that up-regulate this protein expression seen in some oral lesions as Dreuw et al.,[23] mentioned. As well, the levels of IL-6 increase in the serum of patients with malignant lesions[24]. This interleukin has the ability to inactivate the p53 tumor suppressor gene [25], Thus promoting tumor cell propagation [26], and then increasing the growth of some cancers [27], such as oral squamous cell carcinoma [28]. Also, this cytokine has other Immunological activities including B-cell differentiation and induction of IgG secretion, T cell differentiation, and development [29], and they found the level of IL6 in serum is proportional to that in saliva. Therefore, it can be suggested that levels of IL-6 in the saliva are considered reliable indicators of its level in serum, and may be used saliva as an alternative diagnostic tool to blood.

In the same context, CRP is one of the acute-phase proteins secreted from the liver as a result of IL6 secretion [30]. Gauri and Suresh [31] elucidated the normal range of CRP in saliva was 0.05 to 64.3 μg/L, which was significantly lower compared to plasma CRP concentrations (0.14 to 31.1 mg/L). The present result in Table 1, also recorded an increasing CRP level in both experimental groups with no significant difference between them, Figure 2. This result in fact is proportional directly to the level of IL6 as mentioned before.
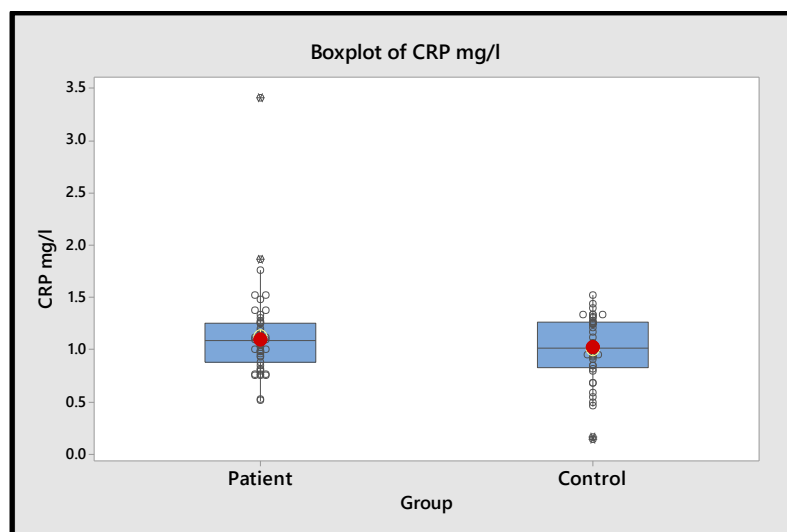
Figure 2: A comparable median boxplot of CRP between patients and control groups

On another hand, alpha-amylase is a digestive enzyme involved in the analysis of starch. Salivary glands secret this enzyme mostly in response to beta-adrenergic stimuli [32]. studies have shown that alpha-amylase activity and quantity could influence personal mouth perception and consumption of dietary starch, and can advance affect overall nutritional status [33,34]. Moreover, studies have exposed that the form of the oral mucosa, roles of salivary glands, and saliva structures all alternated with age [35]. As shown in Table 1, the results recorded a significant increase in DM patients than that in the control group figure 3, this result was expected, because DM patients were susceptible to different types of diseases that alpha - amylase acts as an indicator for them as Yazid et al., [36] reported an early detection of alpha-amylase in saliva act as a biomarker can help the clinicians to detect early risk of caries occurrence before irreversible damage occurs. Also, Jung et al., [37] indicated that increasing alpha-amylase may have been associated with psychological stress and has been shown to trigger systemic lupus erythematosus (SLE).
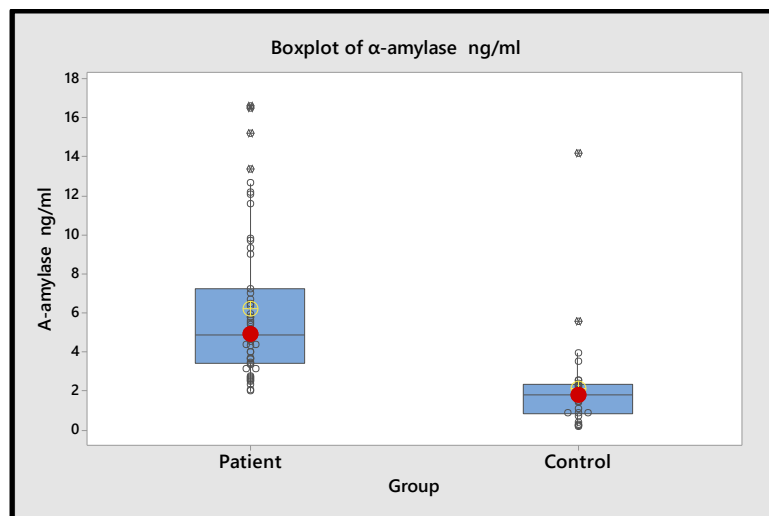
Figure 3: A comparable median boxplot of Alpha-amylase between patients and control groups.

Additionally, previous results of the present study are not consistent with other reports that have revealed higher IL6 and CRP in saliva of both patients and control, and can be suggesting that these parameters may reflect a systemic inflammatory response and complications, especially after a coronavirus outbreak.

Receiver operating characteristic (ROC) analysis figure 4 shows that there are 3 variables examined (IL6, CRP, and alpha-amylase), only alpha-amylase could be considered an important biomarker in distinguishing between oral hygiene of DM patients and the control group.
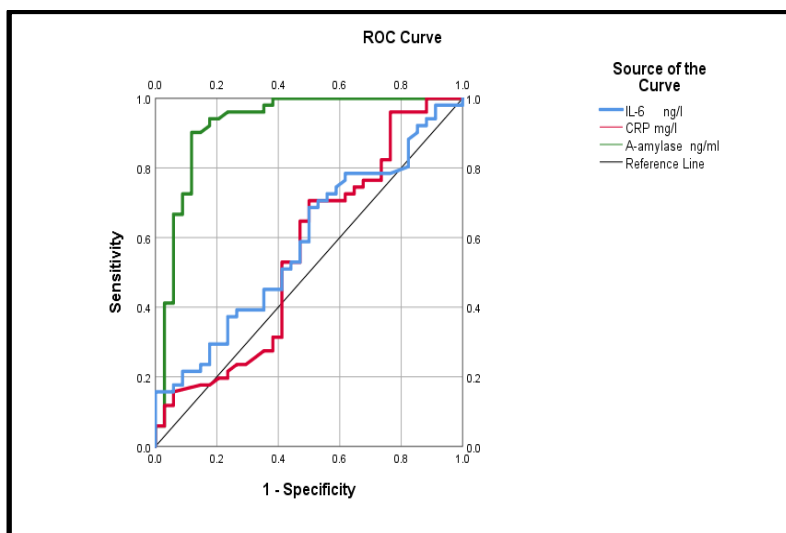
Figure 4: A comparable ROC curves of alpha-amylase, IL6, and CRP in patients

Also, ROC results were confirmed by estimating Area under the curve (AUC) in Table 2. The results revealed both IL6 and CRP were poor predictors throughout AUC ranged (0.463-0.708) and (0.429-0.668) respectively, whereas alpha -amylase ranged from (0.855-0.991) which is suggested as a good predictor of determining oral health and further sequels.

On another hand, the Cut-off value results in the analysis of IL6 using the area under the ROC curve (AUC) (0.585). The curve's optimum cutoff value has a sensitivity of 68.6 %with a specificity of 50.0 %, and a cut-off P-value was 0.184. The abnormal case is represented by a test value larger than 28742 ng/l, while the healthy case is illustrated by a number lower than 28742 ng/l. therefore, there was no significant difference between both patients and the control groups with pathogenic elevation in both.

As well, CRP analysis by AUC in ROC is 0.559, with a sensitivity of 64.7% and specificity of 53.0%, and a cut-off P-value of 0.363. The abnormal case is considered by a test value larger than 0.987mg/l, while the healthy case is represented by a number lower than 0.987mg/l. Thus, CRP has recorded pathogenic elevation values in both DM patients and the control groups.

Whereas, Alpha-Amylase AUC is recorded as a significant pathogenic value in DM patients than the control one. The Alpha-Amylase AUC was 0.923 with a sensitivity of 92.2% and specificity of 82.4%, a cut-off P-value of 0.001. The abnormal case is considered by a test value larger than 2.50 ng/l, while the healthy case is represented by a number lower than 2.50 ng/l.

Table 2: Predictive values of saliva levels of IL6, CRP, and alpha-amylase in detecting DM patients

Variable	AUC (95% CI)	P-Value	Cut-off value	Youden index	Sensitivity %	Specificity %
IL-6 ng/l	0.585 (0.463 – 0.708)	0.184	28742	0.186	68.6	50.0
CRP mg/l	0.559 (0.429 – 0.668)	0.363	0.987	0.177	64.7	53.0
Alpha-Amylase ng/ml	0.923 (0.855 – 0.991)	0.001**	2.50	0.746	92.2	82.4

5. Conclusion

The results of this study concluded that mouth hygiene act as a mirror for body health, and alpha-amylase is a good predictor for mouth diseases. Besides, extensive studies were needed to substitute blood with saliva as a good sampling agent.

6. Ethical Clearance

The Biotechnology Department's local committee agreed to the experiments mentioned in this thesis and all volunteers give oral consent details and advantages. The study was undertaken by the University of Baghdad team under the supervision of doctors in the Al-Mustansiriya University national diabetes center in Baghdad.

7. Conflict of Interest

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

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