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Investigation of the Association of Oral Infections with Diabetes Mellitus

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Abstract

Diabetes mellitus (DM) is a chronic metabolic disease that is considered a major worldwide healthcare problem. Multiple studies have revealed that people with DM are more likely to acquire oral problems, such as periodontal diseases, because the oral microbiota plays a major role in oral health and may affect the saliva composition. This study aimed to characterize the oral microbiota of a sample of DM patients and its association with some demographic factors, such as smoking habits and gender. 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. Whole saliva was collected in a sterile tube, and oral swabs were obtained from both patients and the control groups. The results of the present study show there was no significant difference between both genders in DM hits. As well, a smoking habit is considered a predisposing habit that may increase the risk of oral diseases in DM patients. The acidic pH of saliva recorded higher values between patients and control subjects than other pH items. On the other hand, the most prevalent bacterial isolates found in oral DM patients were *Staphylococcus spp.* (37.12%), *E.coli (*12.9%), *Klebsiella spp.* (10.60%), *Pseudomonas spp.* (9.84%), *Enterobacter* (8.33%), both *Streptococcus spp.* and *Acinetobacter spp.* (5.30%), *Corynebacterium spp.* and *Proteus spp.* (3.8%), *Neisseria spp. and Haemophilus Influenza* was 1.51%. These percentages were significantly different from those in the control group, which were *Staphylococcus spp. (*43.4%), *Klebsiella spp. (*25.0%), *Enterobacter* (7.89%), *E.coli* (6.58%)**,** *Bacillus spp. (*5.2%*), Acinetobacter spp. (*3.9%*), Pseudomonas spp., Streptococcus spp.*, and *Proteus spp.* (2.7%).

Keywords: Diabetes mellitus, pH, smoking habit, mouth microbiota

التحقق من علاقة التهابات الفم بمرض السكري

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الخلاصة

مرض السكري هو مرض ايضي مزمن يعتبر مشكلة رئيسية للرعاية الصحية في جميع أنحاء العالم. كشفت دراسات متعددة أن الأشخاص المصابين بمرض السكري أكثر اكتسابًا لمشاكل الفم ، مثل أمراض اللثة لأن الميكروبات الفموية تلعب دورًا رئيسيًا في صحة الفم والتي قد تتأثر بمستوى مكونات اللعاب. هدفت هذه الدراسة إلى توصيف الميكروبات الفموية لعينة من مرضى السكري وارتباطها ببعض العوامل الديموغرافية مثل عادات

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التدخين ونوع الجنس. تظهر نتيجة الدراسة الحالية عدم وجود فرق معنوي بين كلا الجنسين في الإصابة بمرض السكري، كذلك تعتبر عادة التدخين عادة مهيئة قد تزيد من مخاطر الإصابة بأمراض الفم لدى مرضى السكري. سجل الأس الهيدروجيني الحمضي للعاب قيمًا عالية بين المرضى والأشخاص في مجموعة السيطرة مقارنة بعناصر الأس الهيدروجيني الأخرى. من ناحية أخرى ، كانت الأجناس البكتيرية الأكثر انتشارًا في الفم لمرضى السكري هي ؛ *Staphylococcus spp.* (37.12%) , *Pseudomonas spp.* , *E.coli* (12.9%), *Klebsellia spp.* (10.60%), *Streptococcus spp.* (9.84%) , *Enterobacter* (8.33%), *Acinetobacter spp.* و *Proteus* (5.30%), *Corynebacterium spp.* (3.8%) ثم *Neisseria spp.* و *Haemophilus.* (1.51%) و*spp. Influenza* كانت هذه النسب مختلفة بشكل كبير عن تلك الموجودة في مجموعة السيطرة والتي كانت *Staphylococcus spp.* (43.4%) *E.coli*,(7.89%) *Klebsellia, Bacillusspp.* (6.58%) *Enterobacter* (25.0%), *Acinetobacter spp.* (5.2%) *Pseudomonas spp.*, *Streptococcus spp.*, و*Proteus spp.* (2.7%)

1. Introduction

The oral cavity contains more than 650 bacterial species and other microorganisms like fungi and viruses [1]. The importance of understanding the ecology of the oral cavity and identifying the conditions that cause the oral microbiota to change from a commensal to a pathogenic interaction with the host [2], which causes a variety of problems in the oral cavity, such as decay, periodontal disease, and tooth loss, but also involves cardiovascular disease, cancer, and diabetes, which are systemic diseases [3].

Different species of staphylococcus, streptococcus, Bacteroides, and enterococcus are well-known bacteria found in the oral cavity of normal individuals, but in diabetic patients, their population increases. Staphylococcus has the ability to produce catalase enzymes and can ferment glucose, mostly residing in the oral cavity as normal flora, but is also considered a fatal pathogen that can cause multiple infections in humans [4].

Diabetes mellitus (DM) is a metabolic disease characterized by chronic hyperglycemia, caused by a decrease in glucose in the cells and an increase in blood sugar levels, and changes in fat, protein, and carbohydrate metabolism due to an absolute or relative decrease in insulin secretion or insulin resistance [5]. It is changing the way insulin functions in tissues and pancreatic cells to varying degrees [6, 7] and is considered a major risk factor for stroke, kidney failure, heart disease, and blindness [8]. The number of diabetics is steadily increasing in both developed and developing countries throughout the world [9] and has great morbidity and death rates [10]. DM affects the makeup and activities of the saliva [11] and is also associated with increased pathogen carriage in saliva [12]. Saliva is the fluid that coats the oral cavity's surfaces and contains biological elements necessary for maintaining oral homeostasis and protecting the oral cavity from pathogens, such as proteins and enzymes. Every day, around 0.5 liters of saliva are secreted [13, 14]. The changes in salivary components and the decrease in salivary flow rate are caused by parenchymal injury, changes in salivary gland microcirculation, dehydration, and glycemic contraction problems [15]. Many factors cause an overgrowth of bacteria in the mouth, which leads to oral diseases such as age, flow of saliva, [16] pH, and smoking. Smoking causes the loss of beneficial oral bacterial species. As a result, smoking can lead to pathogen colonization and, eventually, illness [17].

The first region of the body to come into contact with smoke is the oral cavity. Cigarette smoke contains toxicants that can disrupt mouth microbial ecology through antibiotic action and oxygen deprivation [18] and may cause oral dysbiosis, which impairs the variety and

functional capacity of the oral microbiota [3]. Also, the pH of saliva is vital for the oral bacteria's survival, development, and multiplication [19]. DM causes the pH levels in the body to become increasingly acidic, resulting in a condition called ketoacidosis [20]. When salivary pH is very low, the amount of acidophilic bacteria is elevated while the amount of acid-sensitive bacteria drops. A high risk of caries can be indicated by a higher concentration of acidophilic bacteria in the dental plaque [21]. pH has an impact on the environmental factors that are important for microbial growth and survival [22]. Some studies showed gender plays a role in relation to DM and oral health.

So periodontal disease is likely related to heredity, poor dental hygiene, and even diseases such as diabetes [23]. Diabetes occurs more often in men than women, especially in middle-aged populations. Males are more susceptible than females to acquiring obesity, insulin resistance, and hyperglycemia [24]. Therefore, this study aimed to find the correlation between DM and some demographic parameters (saliva pH, smoking habits, and gender) related to bacterial oral hygiene in Iraqi patients.

2. Materials and methods

A total of 91 specimens (oral swabs and saliva) were collected randomly from 51 patients suffering from diabetes mellitus. 26 males with a mean age of 53.96 and 25 females with a mean age of 50.2 were randomly collected from November 2021 to February 2022 from the Al-Mustansiriya University national diabetes center in Baghdad, and 40 apparently healthy individuals were used as controls (15 males and 25 females). All participants answered the questionnaires on age, gender, drugs, smoking, and workplace.

2.1 Specimen collection

All participants were asked to stop eating and drinking for at least two hours before swabs and saliva collection. After that, they rinsed their mouths with sterilized water and waited 10 minutes before collecting 2 mL of saliva in a sterile cup and measuring the pH by using a pH strip (CYBOW, China), centrifuged (10,000× g; 15 min), and the supernatant was collected in a sterile tube and frozen for further experiments [25]. while the swab was collected from each one to isolate and characterize oral bacteria by using sterile cotton swabs (Cito swabs, China).

2.2 Bacterial isolate identification

Activation of bacterial isolates was done using Brain Heart Broth or Himedia-India and incubated at 37 °C for 24 hours. Then, primary characterization was carried out using selective and deferential media, including MacConkey agar, Mannitol salt agar, chocolate agar, and blood agar from Himedia, India. [26]. Then further secondary characterization was done using biochemical tests (Methyl Red, Voges-Proskauer, indole test, oxidase test, urease test, coagulase test, catalase test, and Simmons citrate test) and using CHROM agar and Himedia-India to certificate the diagnosis of bacterial isolates [27].

3. Results and Discussion

For diabetics, oral illness is still the most prevalent complication. Diabetes raises the risk of additional oral disorders and impairs the body's ability to fight infections. Besides, hyperglycemia promotes the growth of germs and bacteria in the mouth. High blood sugar is the relationship between diabetes and oral conditions. Oral health concerns are more likely to occur if blood sugar is inadequately managed [28].

The study focused on some demographic and bacteriological factors that may affect the mouth health of diabetic patients directly or indirectly. The results revealed that there was no significant difference between males and females in diabetic hits, as shown in Table 1.

Table 1: Test Two Proportions (Gender) in the Patient Group

Gender	Frequency	Percentage	P-value¥
Male	26	51.0	0.843
Female	25	49.0	
Total	51	100%	

This result was incompatible with Hongyan et al. [29], who documented that diabetes among females was more prevalent than among males. While Muhammad and his colleagues [30] reviewed the data, they found that male diabetics were observed more than females.

As well, the results in Table 2 recorded no significant difference between the patients and control groups, which reflected that smoking was not the main cause of oral diseases in diabetic patients.

Table 2: Groups test for smoking

Smoking habits	Group				P-value
	Patient		Control		
	N	%	N	%	
Smoking	**5**	**9.8%**	**7**	**17.5%**	**0.292** N.S
Non-smoking	**40**	**78.4%**	**30**	**75.0%**	**0.625** N.S
Ex-smoking	**6**	**11.8%**	**3**	**7.5%**	**0.262** N.S
Total	**51**	**100.0%**	**40**	**100.0%**	
N.S: non-significant					

Studies done by Sujaya et al. [31] concluded that periodontal diseases were significantly associated with smoking, diabetes, hypertension, and age. In the same context, Masuma et al. [32] reported that the incorporation of tobacco cessation within dental care for people with diabetes was considered viable and would provide good results in eliminating gum diseases. Microbial networks in smokers were narrow and mostly congeneric, but those in diabetics and diabetic smokers had significant inter-generic networks. Smoking and hyperglycemia have diverse effects on the subgingival microflora, and when these alarms collide, the synergistic impact is greater than the total of each effect, as Sukirth et al. [33] mentioned. Thus, it can be suggested that smoking is not the main factor that aids periodontal diseases but may act as a predisposing factor in healthy and diabetic subjects.

On the other hand, mouth pH is one of the effective parameters related directly to oral health. The results in Table 3 elucidate no significant difference between patients and control groups. In spite of this, the acidic pH was more dominant among participants in this experiment than other pH items.

Table 3: Groups test for saliva pH

pH saliva	Group				P-value
	Patient		Control		
	N	%	N	%	
Acidic	37	72.5%	22	55.0%	0.081 N.S
Neutral	11	21.6%	13	32.5%	0.244 N.S
Alkaline	3	5.9%	5	12.5%	0.284 N.S
Total	51	100.0%	40	100.0%	
N.S: non-significant					

Seethalakshmi et al. [34] clarified that there was a significant correlation between DM and the increased occurrence of dental caries and periodontal diseases, and the significant reduction in the salivary pH in diabetes mellitus patients compared to that of non-diabetic subjects was suggested as an indicator of oral diseases.

DM is revealed by changing the salivary components and their functions. Altering the mouth environment recruits pathogenic bacteria, and the destructive cavity's hard and soft tissues lead to improved cariogenic activity and gum lesions. Since saliva has a preventive effect and can cause tooth cavities when salivary functions are clinically reduced [35], variations in the salivary pH are frequently reported in DM patients. There is a regular correlation between pH alterations in plaque and sugar clearance from saliva [36]. The declining or decreased salivary pH offers acidogenic hygiene for the growth of acid-uric bacteria, leading to dental caries and further decreasing salivary pH, creating a vicious cycle. DM encourages periodontal diseases through an overstated inflammatory response to the periodontal microbiome [37].

An increasing number of publications have reported a close relationship between DM and susceptibility to periodontal diseases [38], derived from disturbances of the oral microbiota equilibrium that increase the establishment of microbial pathogens. The present results in Table 4 show that the most prevalent bacterial genus in the patient groups was Staphylococcus spp. (37.12%), followed by E.coli (12.9%), Klebsellia spp. (10.60%), Pseudomonas spp. (9.84%), Enterobacter (8.33%), Streptococcus spp. and Acinetobacter spp. (5.30%), Corynebacterium spp. and Proteus spp. (3.8%), then each Neisseria spp. and Haemophilus Influenza was (1.51%), and these percentages were significantly different from those in the control group, which were Staphylococcus spp. (43.4%), Staphylococcus spp. (43.4%), Klebsiellia spp. (25.0%), Enterobacter (7.89%), Bacillus spp. (5.2%), Acinetobacter spp. (3.9%), each of Pseudomonas spp., Streptococcus spp., and Proteus spp. (2.7%), and the characteristics of each Corynebacterium spp., Neisseria spp., and H. influenza were not found in the control group with a significant difference in Corynebacterium and Pseudomonas between patients and control (p=0.023*) as seen in Table 4.

Table 4: Groups test for bacteria

Bacteria	Group				P-value
	Patient (51)		Control (40)		
	N	%	N	%	
Staphylococcus spp.	49	37.12%	33	43.42%	0.373
Bacillus spp.	0	0.0%	4	5.26%	0.040 *
Streptococcus spp.	7	5.30%	2	2.63 %	0.319
Neisseria spp.	2	1.51%	0	0.0%	0.154
Klebsellia spp.	14	10.60%	19	25.0%	0.011 *
E.coli	17	12.9%	5	6.58%	0.122
Enterobacter	11	8.33%	6	7.89%	0.911
Proteus spp.	5	3.79%	2	2.63 %	0.641
Corynebacterium spp.	5	3.79%	0	0.0%	0.023 *
Acinetobacter spp.	7	5.30%	3	3.95%	0.648
Pseudomonas spp.	13	9.84%	2	2.63%	0.023*
H. influenzae	2	1.51%	0	0.0%	0.154
Total	132	100%	76	100 %	
*mean p≤ 0.05					

There are around 700 kinds of bacteria in the oral cavity that act as microflora, one of the body's most diverse and active ecosystems [39]. The most dominant bacterial phyla are *Firmicutes, Actinobacteria, Fusobacteria, Proteobacteria*, and *Bacteroidetes* characterized within the oral microbiota [40]. These microorganisms normally proportion with their host due to coevolution; however, behavioral factors can lead to a dysbiosis of the oral ecosystem, such as poor oral hygiene and diet, genetics, medication, debilitated immune systems, and certain diseases [41]. This difference is normally related to the development of pathogenic microorganisms, which can lead to increased susceptibility to oral sickness [42]. Factors such as lifestyle, age, diet, denture wear, saliva flow, several diseases, medication, and a poor immune system tend to affect the microbiome composition [43].

4. Conclusions

It can be concluded that gender, smoking, and oral pH in Iraqi DM patients are not the main factors directly related to periodontal diseases in DM patients. The bacteriological results in the present study revealed that most microbiome profiles were found across diabetics and the control group, suggesting that diabetes may not be influencing the association between DM and oral microbiota; it may be more closely associated with either lifestyle or nutrition habits than diabetes.

5. Ethical Clearance

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

6. Conflict of interest

There are no conflicts of interest between the authors.

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