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A Comparative Correlation between the Oral Microbiome of Diabetes Mellitus and Healthy Individuals and their Relation with Some Demographic Parameters

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Abstract

Diabetes Mellitus (DM) is a chronic disease distributed worldwide and dominantly related to different types of diseases, especially microbial infections. This study aimed to find the relationship between the DM mouth microbiome and some demographic factors. Sixty saliva specimens and bacterial oral swabs were collected from randomly selected DM patients, including 29 females and 31 males enrolled in this study, which was obtained from the Al-Mustansiriya University national diabetes center in Baghdad. Other 40 apparently healthy people's specimens and swabs were collected from 25 females and 15 males as a control group for the period starting November 2021 to February 2022.. The results revealed that the most prevalent bacterial genera in the patients' group were *Staphylococcus* spp. 57(38.26%), *Enterobacteriaceae* spp. 55(36.91%), *Pseudomonas* spp. 13(8.72%), *Streptococcus* spp. and *Acinetobacter* spp. each was 8(5.37%), and then, *Corynebacterium* spp. 5(3.36%), and each *Neisseria* spp., and *H. influenza* were 2(1.34%). These percentages were significantly different from those in the control group which were *Staphylococcus* spp. 33(43.4%)(*S. aureus* 34.21% and *S. epidermidis* 9.22%), *Enterobacteriaceae* spp. 32(42.11%), *Bacillus* spp. 4(5.2%), *Acinetobacter* spp. 3(3.9%), and each *Pseudomonas* spp. and *Streptococcus* spp. were 2(2.7%). In the same context, the results showed there is no significant difference between smokers for patients /control which were 25(17%)/15(19.74%), as well as, non-smokers 105 (71.43%) / 55 (72.37%) and ex-smokers 17(11.57%)/6(7.89%) in bacterial isolates foundation in the oral cavity of DM patients and controls. Acidic oral pH is the predominant pH among patients and control individuals, in spite of there being no significant differences among different oral pH levels. As well, as the results revealed that gender does not affect the types of the oral microbiome.

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Keywords: DM, gender, pH, smoking habit, mouth microbiota.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic condition that affects the body's capacity to use energy obtained from the diet [1]. Is characterized by hyperglycemia brought on either by insulin resistance or insulin insufficiency [2; 3]. It is the basis of several organ infections and other diseases [4]. DM is regarded as a major cause of death most recently, particularly in Iraq [2; 5]. Uncontrolled hyperglycemia is linked to several hazardous consequences, including renal failure, cardiovascular conditions, and oral diseases [6].

The oral cavity is a complex ecosystem that includes the tongue, soft and hard palate, teeth, buccal mucosa, and other structures that together make up a rich habitat for microbial life [7]. The mouth is home to a variety of microorganisms, including bacteria, fungi, and viruses.

The majority of living things in the mouth are bacteria, which are mostly Firmicutes, Proteobacteria, and Actinomycetes [8]. The majority of oral microbes are not harmful to humans, but opportunistic commensals fight against harmful pathogens and maintain oral health [9]. There are many factors such as age, pH, smoking, and gender that contribute to microbial proliferation in the mouth, which leads to oral illnesses [10]. Ageing influences the dialogue with inherent microorganisms. The risk of oral diseases increases dramatically through the course of life, to which it is argued that microorganisms contribute [11]. Low-pH saliva can cause changes in diabetics' mouths over time and contribute to tooth damage from acid-producing bacteria [12]. Smoking affects the microbial signatures of oral communities, with a decline in the commensal population and a corresponding rise in pathogens, according to findings from the nasopharyngeal environment [13]. Smoking even affects immune parameters in oral saliva. Also, saliva enzyme activity and quantity could influence personal mouth perception and consumption of dietary starch and can affect overall nutritional status [14] and types of mouth microbiome [15]. Moreover, studies have exposed that the form of the oral mucosa, roles of salivary glands, and salivary structures all alternate with age; these changes are increased dramatically with DM [16]. Therefore, this study aimed to find the relationship between the DM mouth microbiome and some demographic factors.

2. Material and method

60 specimens of randomly selected DM patients, including 29 females and 31 males, were enrolled in this study, most of them type 2 diabetes, and only two patients had type 1 diabetes, which was obtained from the Al-Mustansiriya University national diabetes center in Baghdad for the period starting November 2021 to February 2022. The type of diabetes is determined based on a medical diagnosis by consultant doctors and the patient’s medical treatment history. Patients' ages ranged from 18 to 74 years. 40 apparently healthy specimens were collected from 25 females and 15 males, with approximately the same age range as the patients' participants. A questionnaire format was specified in the datasheets to be filled out according to age, sex, drug administration, smoking, chronic disease, and workplace of each patient and control subject..

2.1 Specimens collection

At the beginning, all participants were instructed to fast for at least 1 or 2 hours before saliva collection. The mouths of each patient and control subject must be rinsed using sterilized water and waited for 10 minutes before collecting at least 2 ml of unstimulated saliva in a sterile cup. Saliva pH was measured directly using a pH strip (CYBOW, China). After that, the saliva specimens were centrifuged at 10,000 rpm for 15 min. to eliminate any insoluble material or cellular debris, and the supernatants were collected and stored in a freezer at -20 [17].

The major part of the oral infection test is based on swabs (Cito swabs, China). A sterile cotton swab is rubbed frequently between soft and hard mouth tissues in order to investigate the type of bacterial colonization in the mouth of diabetic patients and for comparison with the control group.

2.2 Bacterial isolates identification

A volume of 2 ml of sterile Brain Heart Infusion Broth (Himedia-India) was added to each swab tube and incubated at 37°C for 24 hours. A loopful of activated growth was streaked into Brain Heart Infusion Agar, MacConkey Agar, Mannitol Salt Agar, Chocolate Agar, and Blood Agar (Himedia-India. Subsequently, incubate all media aerobically at 37°C for 24 hours. These media were used to primary distinguish and obtain pure colonies [18]. Then, using biochemical tests to diagnose bacteria [19].

2.3 Statistical Analysis

The data were analyzed using the following software: Microsoft Excel, Minitab v17, and IBM SPSS A V26. Z-test was used to compare two proportions. Probability values less than 0.05 were considered significantly different [20].

3. Results and Discussion

Diabetes patients are more likely to develop bacterial infections as well as other types of oral infections. Reduced salivary flow, weakened defenses, and poor metabolic regulation might all contribute to the development of infection. Oral problems in DM patients are regarded as serious disease complications and might negatively affect the patients' quality of life [21].

Different bacterial species, including gram-positive and gram-negative bacteria, were isolated from the mouths of both patients and control subjects. The bacteria were isolated from the mouth (teeth and gingiva). The present results in **Table 1** show the most prevalent bacterial genera in the patients' group, which are listed in ascending order according to the percentages: *Staphylococcus* spp. 57(38.26%), *Enterobacteriaceae* spp. 55(36.91%), *Pseudomonas* spp. 13(8.72%), *Streptococcus* spp. and *Acinetobacter* spp. each were 8(5.37%), *Corynebacterium* spp. 5(3.36%), and each *Neisseria* spp., and *H. influenza* were 2(1.34%). These percentages were different from those in the control group, which were *Staphylococcus* spp. 33(43.4%), *Enterobacteriaceae* spp. 32(42.11%), *Bacillus* spp. 4(5.2%), *Acinetobacter* spp. 3(3.9%), and each *Pseudomonas* spp. and *Streptococcus* spp. were 2(2.7%). The characteristic thing each *Corynebacterium* spp., *Neisseria* spp., and *H. influenza* were not found in the control group, while *Bacillus* was not found completely in patients. Besides *Corynebacterium* spp., *Pseudomonas* spp., and *Bacillus* spp. recorded a significant difference between experimental groups ($p \leq 0.05$). There is a noticeable increase in the number of bacteria in DM patients compared to healthy ones.

Table 1. Groups test for Bacterial isolates

Bacteria	**Group**				**P-value**
	Patient (60)		**Control (40)**		
	N	**%**	**N**	**%**	
Staphylococcus spp.	**57**	**38.26%**	**33**	**43.42%**	**0.457**
Bacillus spp.	**0**	**0.0%**	**4**	**5.26%**	**0.040***
Streptococcus spp.	**7**	**4.67%**	**2**	**2.63 %**	**0.413**
Neisseria spp.	**2**	**1.34%**	**0**	**0.0%**	**0.155**
Enterobacteriaceae spp.	**55**	**36.91%**	**32**	**42.11%**	**0.452**
Corynebacterium spp.	**5**	**3.36%**	**0**	**0.0%**	**0.023***
Acinetobacter spp.	**8**	**5.37%**	**3**	**3.95%**	**0.624**
Pseudomonas spp.	**13**	**8.72%**	**2**	**2.63%**	**0.039***
H. influenza	**2**	**1.34%**	**0**	**0.0%**	**0.155**
Total	**149**	**100.0%**	**76**	**100.0%**	

Despite the fact that the majority of bacterial isolates are thought to be part of the normal oral flora, some of them have the potential to become pathogenic, particularly in people with impaired immune systems or because of other factors like diabetes. According to [22], many species of *Staphylococcus*, *Enterococcus*, and *Streptococcus* are present in the mouth of healthy individuals but become more prevalent in diabetes patients.

While [23] found that *Streptococcus* and *Neisseria* species were also discovered as subgingival flora of T2DM individuals with chronic periodontitis. Also, *Neisseria mucosa* was detected in the saliva of patients with T2DM but not in non-diabetics [24]. Moreover, [25] clarified that patients without gingival bleeding had higher levels of *Actinobacteria spp.*, those with diabetes or pre-diabetes with gingival hemorrhage had higher levels of *Bacteroidetes*.

Whereas, [26] found that the *Actinomyces* and *Selenomonas* taxa were substantially more prevalent in the DM group. [27] found a high prevalence of *Bacillus cereus* in non-diabetic individuals, which was confirmed by the present study.

On another hand, the relationship between bacteria and smoking habit results was shown in **Table 2**. The results showed there was no significant difference between smokers for patients and controls, which were 25(17%)/15(19.74%), as well as non-smokers, 105 (71.43%) / 55 (72.37%) and ex-smokers 17(11.57%)/6(7.89%) in bacterial isolates foundation in the oral cavity of DM patients and control for all bacterial isolates except *Corynebacterium* spp. in non-smoking and *Pseudomonas* spp. in smoking patients recorded a significant variation. It is suggested that bacteria are found in the oral cavity in either DM patients or healthy individuals; this may be related to mouth hygiene and some eating and drinking habits. The resulting match with [28] result showed there are no differences in oral bacteria between smokers and non-smokers in diabetes patients. While [29] found that diabetic smokers often have a more varied microbiome and harmful species than non-smokers, [30] concluded that diabetic smokers have a higher chance of developing periodontal disease than non-smoking patients, which is another reason why people with DM should be persuaded not to smoke.

Table 2. Relation of oral bacterial isolates with smoking habit

Bacteria	**Smoking habits**								
	Smoking			**Nonsmoking**			**Ex-smoking**		
	Patient n=10	**Control n=7**	**p-value**	**Patient n=44**	**Control n=30**	**p-value**	**Patient n=6**	**Control n=3**	**p-value**
	N **%**	**N** **%**		**N** **%**	**N** **%**		**N** **%**	**N** **%**	
Staphylococcus spp.	8 32.0%	4 26.66%	0.718	43 40.95%	26 47.27%	0.599	6 31.58%	3 50.0%	0.621
Bacillus spp.	0 0.0%	3 20.0%	0.053	0 0.0%	1 1.81%	0.313	0 0.0%	0 0.0%	1.00
Streptococcus spp.	1 4.0%	1 6.67%	0.724	4 3.80%	1 1.81%	0.400	2 10.52%	0 0.0%	0.135
Neisseria spp.	0 0.0%	0 0.0%	1.00	2 1.90%	0 0.0%	0.153	0 0.0%	0 0.0%	1.00
Enterobacteriaceae spp.	9 36.0%	6 40.0%	0.801	40 38.09%	23 41.81%	0.476	6 31.58%	3 50.0%	0.621
Corynebacterium spp.	0 0.0%	0 0.0%	1.00	4 3.80%	0 0.0%	0.041*	1 5.26%	0 0.0%	0.304
Acinetobacter spp.	3 12.0%	1 6.67%	0.560	3 2.86%	2 3.63%	0.498	2 10.52%	0 0.0%	0.135
Pseudomonas spp.	4 16.0%	0 0.0%	0.029*	8 7.62%	2 3.63%	0.227	1 5.26	0 0.0%	0.304
H .influenzae	0 0.0%	0 0.0%	1.00	1 1.95%	0 0.0%	0.315	1 5.26%	0 0.0%	0.304
Total percentage of patient/control	25 16.78%	15 19.74%	0.653	105 70.47%	55 72.37%	0.335	19 12.75%	6 7.89%	0.491

*mean p≤ 0.05

The results in **Table 3** show that the number of bacteria in DM patients with an acidic pH is higher than in neutral and alkaline patients because diabetes increases the acidity in the oral cavity, which leads to the growth of bacteria. The result reveals a non-significant difference between patients and control groups. As [31] demonstrated, a reduction in salivary pH encourages the formation of acidic bacteria, which further permits the growth of acidogenic bacteria, providing an unfavorable environment for bacteria that protect the oral cavity. Therefore, the balance of the oral environment might shift in favor of harmful bacteria, which then lower salivary pH and keep the cycle going. The acidic pH in DM patients is related to microbial activity or a decline in bicarbonate with the rate of salivary flow [32].

While a small number of bacteria appear in alkaline pH, only *Staphylococcus* spp. and *Enterobacteriaceae* appear in all patients with alkaline pH because they are tolerant to alkalinity and can grow in pH from 5 to 10 [33; 34]. In the same context, some oral normal flora, such as *Streptococcus salivarius*, have the capability to prevent inflammation. The members of the bacterial community also help to adjust the oral acidity; people without caries have bacterial species that are capable of converting arginine or urea in the diet to pH-balancing ammonia. Since any disturbances in the number of such bacterial species may lead to the increased alkalinity of saliva [35], Therefore, it can be suggested that the oral microbiome increases in acidic pH more than other pHs; this shift in bacterial number may be a decline in several oral microbiomes due to using an excessive agent to manage oral hygiene.

Table 3. Relation of bacterial isolates with saliva pH.

Bacteria	pH saliva								
	Acidic			Neutral			Alkaline		
	Patient n=46 N %	Control n=22 N %	p-value	Patient n=11 N %	Control n=13 N %	p-value	Patient n=3 N %	Control n=5 N %	p-value
Staphylococcus Spp.	44 39.64%	18 43.90%	0.637	10 32.25%	10 40.0%	0.648	3 42.86%	5 50.0 %	0.771
Bacillus Spp.	0 0.0%	1 2.44%	0.311	0 0.0%	3 12.0%	0.062	0 0.0%	0 0.0%	1.00
Streptococcus spp.	6 5.41%	1 2.44%	0.358	1 3.22%	1 4.0%	0.782	0 0.0%	0 0.0%	1.00
Neisseria spp.	2 1.80%	0 0.0%	0.154	0 0.0%	0 0.0%	1.00	0 0.0%	0 0.0%	1.00
Enterobacteriaceae	40 36.04%	18 43.90%	0.382	12 38.70%	9 36.0%	0.820	3 42.86%	5 50.0 %	0.771
Corynebacterium spp.	3 2.70%	0 0.0%	0.079	2 6.45%	0 0.0%	0.144	0 0.0%	0 0.0%	1.00
Acinetobacter spp.	5 4.51%	2 4.88%	0.924	3 9.67%	1 4.0%	0.387	0 0.0%	0 0.0%	1.00
Pseudomonas spp.	10 9.0%	1 2.44%	0.070	2 6.45%	1 4.0%	0.831	1 14.28%	0 0.0%	0.280
H .influenzae	1 0.90%	0 0.0%	0.315	1 3.22%	0 0.0%	0.310	0 0.0%	0 0.0%	1.00
Total of patients/control	111 74.5%	41 53.95%	0.358	31 20.8%	25 32.89%	0.553	7 4.7%	10 13.16%	0.869

*mean $p \leq 0.05$

Simultaneously, the results in **Tables 4, 5** recorded no significant difference between males and females in the microbiome types in both the patient and control groups. These results were confirmed by [36], who found the oral microbiome has no significant correlation with gender.

Table 4. Relation of bacterial isolates according to gender in patients group

Bacteria	Male (31)		Female (29)		p-value
	N	%	N	%	
Staphylococcus Spp.	30	37.04%	27	39.71%	0.739
Bacillus Spp.	0	0.0%	0	0.0%	1.00
Streptococcus spp.	5	6.17%	2	2.94%	0.337
Neisseria spp.	1	1.23%	1	1.47%	0.901
Enterobacteriaceae	28	34.6%	27	39.71%	0.518
Corynebacterium spp.	2	2.47%	3	4.41%	0.521
Acinetobacter spp.	5	6.17%	3	4.41%	0.630
Pseudomonas spp.	9	11.11%	4	5.88%	0.246
H .influenzae	1	1.23%	1	1.47%	0.901
Total of patients	81	100%	68	100%	

Whilet his result was incompatible with [37], who documented that in the fed condition, Porphyromonas and Capnocytophaga were more prevalent in the male salivary samples compared to female saliva, variations in the saliva microbiota between men and women were even more noticeable. Therefore, biological indicators and microbiota in saliva may serve as indicators of eating circumstances and gender identity. [38] clarify that overweight women or women with diabetes affect oral health, and 98% of them have *Acinetobacter* spp. and *Veillonella parvula* in their oral. Also **Table 5** exhibits no significant difference between gender in the oral microbiome for control.

Table 5. Relation of bacterial isolates according to gender in control group.

Bacteria	**Male (15)**		**Female (25)**		**p-value**
	N	**%**	**N**	**%**	
Staphylococcus Spp.	15	44.12%	18	42.86%	0.912
Bacillus Spp.	1	2.94%	3	7.14%	0.393
Streptococcus spp.	1	2.94%	1	2.38%	0.881
Neisseria spp.	0	0.0%	0	0.0%	1.00
Enterobacteriaceae	15	44.12%	17	40.48%	0.749
Corynebacterium spp.	0	0.0%	0	0.0%	1.00
Acinetobacter spp.	1	2.94%	2	4.76%	0.678
Pseudomonas spp.	1	2.94%	1	2.38%	0.881
H .influenzae	0	0.0%	0	0.0%	1.00
Total of patients	34	100%	42	100%	

4. Conclusion

The present study concluded that DM as a chronic disease has a significant effect on the number of bacterial spp. in the oral cavity. Acidic saliva pH is the most predominant among patients and controls and is more directly correlated with oral bacterial percentage than other pH parameters, which may reflect bacterial activity and oral hygiene management. Additionally, the present study showed that gender is not affected by the type of oral microbiome.

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