ORAL FINDINGS AND MICROFLORA IN TYPE II DIABETES MELLITUS IN SULAIMANI CITY

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ABSTRACT

Background

The effects of diabetes mellitus on human include long term dysfunction and failure of various organs. A number of oral diseases and disorders have been associated with diabetes mellitus. The susceptibility to periodontal disease often called the "sixth complication of diabetes mellitus" is the most common oral complication of diabetes. The oral cavity is comprised of many surfaces, each coated with a plethora of different bacteria, some of which have been implicated in oral diseases such as caries and periodontitis

Objective

This study was conducted to determine the most common microorganisms inhabiting the oral cavity of diabetic individuals in comparison to non diabetics, to determine the relation of oral microflora to oral conditions in diabetes and determine the susceptibility of oral microflora to common antimicrobial agents.

Methods

A case control study on two hundred persons with type II diabetes mellitus and fifty control cases. Oral findings were documented based on physical examination, microbial identification was based on culture methods and various identification tests. Antimicrobial susceptibility was performed according to Kirby-Bauer method.

Results

The oral findings in persons with type II diabetes mellitus showed higher occurrence of gingivitis, periodontitis, dental pain, xerostomia, taste disturbance, palatal ulceration and oral candidiasis in comparison with person without diabetes. Microorganisms studied by culture-dependent methods showed more bacterial isolates in diabetic groups of many stains such as of Bacillus species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, Viridans streptococci, *Branhamella catarrhalis*, *Escherichia coli*, *Stomatococcus*, *Veillonella* and *Candida albicans*. The bacterial isolate susceptibility to antimicrobial agents showed wide resistance to several commonly used antimicrobial agents in dental practice and intermediate response was shown to be arising to some antimicrobial agents.

Conclusion

Various oral and dental problems such as periodontitis and dental loss was observed in diabetics, also more microbial isolates were documented from diabetics with a notable shift to more virulent species. Most of the isolated organism were resistant to several commonly antimicrobial agents in use. Oral problems may reflect on the increasing oral population of certain bacterial species and this will further complicate their oral problems.

***Keywords*:** *Oral flora, Type II diabetes mellitus, Iraq, Sulaimani.*

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INTRODUCTION

The prevalence of diabetes for all age groups worldwide was estimated at 2.8% in 2000 and to be 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. The prevalence of diabetes is higher in men than women, but there are more women with diabetes than men. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people over 65 years of age [(1)]. Diabetes mellitus is highly prevalent among both sexes in member states of the Eastern Mediterranean region. Its prevalence ranges from 3.5% and 30.0% and it is highest among member countries of the gulf cooperation council (GCC) at a rate of 11.5% to 30.0% . Many countries in the region are now reporting the onset of type 2 diabetes mellitus at an increasingly young age. This is due to increasingly sedentary lifestyles and obesity [(2, 3)]. Within Europe, Type 2 diabetes affects 10–30% of subjects above pensionable age and in the USA about 40% of all those with diabetes fall into this category [(4)].

The long term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, neuropathy with risk of foot ulcers, amputation, and features of autonomic dysfunction including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular diseases [(5)].

A number of oral diseases and disorders have been associated with diabetes mellitus. The susceptibility to periodontal disease often called the "sixth complication of diabetes mellitus" is the most common oral complication of diabetes [(6)]. In adults with diabetes, gingival inflammation may occur at higher rates than those in adults without diabetes [(7)]. Patients with poorly controlled diabetes are at greater risk of developing periodontal disease. This will start with gingivitis and then, with poor glycemic control, will progress to advanced periodontal disease [(8)]. The subgingival microflora in patients with periodontitis who have diabetes mellitus generally is equivalent to that observed in patients with periodontitis who do not have diabetes [(9)]. Diabetic patients are more liable for the followings [(10)]; greater periodontal disease, greater susceptibility of oral tissues to trauma, more opportunistic infections (for example, candidiasis), greater accumulation of plaque, greater risk of caries, delayed wound healing, xerostomia, oral burning mouth or tongue paresthesia and altered taste sensation. Moore and co-workers [(11)] stated that diabetic patients are five times more likely to be partially edentulous than non diabetic patients.

Like the vast majority of microbial populations in nature, microbial inhabitants of the oral cavity are predominantly found in surface-attached multi-species communities. However, the warm, moist character of the mouth combined with the relatively open access to the wider environment presents a unique niche for microorganisms. In fact, the oral cavity is a heterogeneous environment that contains numerous different surfaces for bacterial colonization, including gums, teeth, cheeks, tongue, and palate. In total, more than 700 phylotypes of bacteria have been detected in the human mouth, representing at least 300 different species. A relatively small number of fungi and viruses are found in oral biofilms, of these only *Candida* spp. have been consistently reported [(12)]. The main habitats for micro-organisms are the teeth, the buccal mucosa, the tongue and the gingival crevice. Each of these provides an environment differing in term of nutrient, oxygen content, and pH. One of the unique features of the oral cavity is that it contains the only non shedding surface in the body, thus teeth enabling bacteria to accumulate to form dens aggregates or biofilms known as dental plaques. These biofilms typically contain as many as 10^{11} bacteria per gram weight. Bacteria in the oral cavity are subjected to powerful removal forces including the constant flow of saliva, swallowing, tongue movement and chewing [(13)].

The microbial population on the dorsal surface of the tongue consists a high proportion of Gram-negative anaerobic bacteria, including many species associated with periodontal disease such as *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Porphyromonas gingivalis*, and *Treponema denticola* [(12)].

The microflora of dental plaque is extremely complex and varies with the nature of anatomical site and also changes with time. Nevertheless, some generalization can be made. The numerically dominant organisms are invariably Streptococci and *Actinomyces* spp. and with members of the following genera usually being present in smaller numbers; *Veillonella*,

Hemophilus, *Branhamella*, *Fusobacterium* and *Propionibacterium*. Tooth surface above the gingival margin (supragingival) may harbour accumulations of bacteria, mainly Gram-positive filaments and Streptococcal species (*S. sanguis, S. mitis, S. gordonii, S. intermedius*, S. *anginosus, S. constellatus, S. oralis,* and *S. vestibularis*). These Streptococcal species are not present in the oral cavity until after the eruption of teeth, reflecting their high degree of adaptation for tooth surface [(14)].

Saliva has a very high content of bacteria (approximately 10^8 per milliliter) but these are derived mainly from the teeth and oral mucosal surfaces as a result of mechanical abrasion caused by chewing, talking and swallowing [(15)].

The cheek epithelium is colonized predominantly by *Streptococcal* species, mainly *S. mitis* and *S. sanguis*. Candida are found on the posterior dorsum of the tongue of between 40 - 60% of healthy humans. Under certain conditions, the yeast mainly *Candida albicans*, cause oral diseases which are collectively known as candidiasis. Other Gram-negative bacteria present in the oral cavity include *Eikenella corrodens*, a facultatively anaerobic rod which pits the surface of agar plates, *Haemophilus* and *Neisseria* species. Spirochetes belonging to genus *Treponema* (e.g. *T. denticola, T. macrodentum, T. orale, T. socranskii* and *T. vincentii*) are present in the gingival crevice [(14)].

MATERIALS AND METHODS

A case control study was performed on patients attending the diabetic centre in Sulaimani city during a period of 9 months, from May 2nd 2009 to February 1st 2010. Diabetic cases were devoid of other medically compromised conditions. Non diabetic control cases were collected from an outpatient clinic and were devoid of any systemic disorders. After obtaining permission, information were collected. Clinical examination was performed by examining the oral cavity, including teeth and gingiva for sings of diseases or abnormalities. Significant changes and findings were reported in patient's dental records. Swab samples for microflora were obtained from saliva or from existing infection if present and processed. Cases who had recent antimicrobial use (72 hours) were not included in the study. A total of 200 type 2 diabetic patients (137 females and 63 males) and 50 control cases (33 females and 17 males) were included in this study.

Culture media (Accumix, Tulip diagnostic (p) Ltd. and Rashmi Diagnostic, Pvt. Ltd. India) and diagnostic reagents were prepared according to manufactures' recommendations. For primary bacterial isolation of microorganism, cotton swab of each sample was inoculated in thioglycolate broth and then incubated for 18-24 hours at 37 °C. From the broth culture, each sample was cultured on different agar media (blood agar, chocolate agar, MacConkey agar and Sabouraud's agar) by direct streaking method. The cultures were incubated both aerobically and anaerobically using AnaeroGen ™ compact (Oxoid Ltd., UK) at 37 °C for 18-24 hours. further culturing were used until pure cultures were obtained for individual isolates.

Microorganism were identified based on culture characteristics, Gram staining properties [(16)] and various biochemical tests and diagnostic test such as slide coagulase test [(16, 17)] catalase test [(18)], various biochemical tests for identification of Gram negative organisms [(16, 19, 20)] and starch hydrolysis [(21)]. Microbial identification discs of Bacitracin, Novobiocin, Optochin and Trimethoprim/Sulphamethoxazole (Bioanalyse Ltd., Turkey) were used according to Brown [(18)].

Pure cultures subjected to antimicrobial susceptibility test according to Kirby-Bauer method. To prepare an inoculum, 3 to 5 colonies were transferred to a tube of 5 ml of nutrient broth to obtain a culture with 1.5 $\times 10^8$ CFU/ml by adjusting to turbidity of standard McFarland tube number (0.5) using sterile broth. Plates of Muller-Hinton agar medium were inoculated using swabs from the inoculum by rubbing the swab all over the surface of the medium. After the plates were left to dry for few minutes at room temperature with the lid closed, antimicrobial discs were applied gently. The plates were incubated within 30 minutes for 18-24 hours at 37°C in an inverted position [(22)]. The following antimicrobial discs were used; Ampicillin (10 Mcg), Amoxicillin-/clavulanic acid (20/10 Mcg), Ceftriaxone (10 Mcg), Ciprofloxacin (10 Mcg), Cloxacillin (1 Mcg), Erythromycin (15 Mcg), Lincomycin (2 Mcg), Metronidazole (5 Mcg), Tetracycline (30 Mcg) and Trimethoprim-/sulphamethoxazole (12.5/23.75 Mcg). The inhibition zone diameter was calculated according to National Committee for Clinical Laboratory Standards (NCCLS) [(23)]. Fasting level of blood sugar, serum cholesterol and serum triglyceride were estimated by the

laboratory department/diabetic centre using 5 ml of venous blood.

RESULTS

A total of 200 diabetic cases and 50 control cases were included in a comparative study. The age for the diabetic group was ranged from 31-70 years. The mean age was 50.83 years and SD was 9.011. The age for the control group was ranged from 31-70 years, mean age 41.96 years and SD deviation was 10.496. The age distribution is shown in table 1.

Taking accounts of occupation and educational achievement, both groups showed nearly similar data for occupation while it was found that the education achievement in diabetic group was below that of the control group (data not shown). For categorizing group study into diabetics and non-diabetic control group, fasting blood sugar (FBS) was estimated and it was found that in diabetic group, 160 (80%) cases had FBS between 127-450 mg/dl, this represents that their blood glucose level was not controlled. The FBS of 26 (13%) diabetic cases was between 111-126 mg/dl which could be regarded as moderately controlled diabetes, and finally in only 14 (7%) cases the FBS was between 80-110 mg/dl. In the control group, the FBS was between 60-110 mg/dl. Concerning serum cholesterol and serum triglyceride, both group showed nearly similar figures (data not shown).

Clinical oral examination revealed that both groups can be categorized, for purpose of comparison of the prevalence and severity of their oral manifestation (Table 2). As shown most of the diabetic cases showed many abnormal oral findings. During periodontal examination gingival bleeding was considered as a sign of periodontitis. Gingivitis in the diabetic patients was more than the normal cases majority of them were bleeding during tooth brushing. One hundred twenty (60%) of all the diabetic cases had gingivitis, while only 5 (10%) of the control cases had gingivitis. From the 200 diabetic cases, 100 (50%) had bleeding during tooth brushing, while in the control cases only 5 (10%) of them had bleeding during tooth brushing. Hundred cases (50%) of the diabetic group had periodontitis while only 5 (10%) of the control group had periodontitis. Prevalence of periodontitis was statistically significant among the diabetic group, the chi square test was performed (P value=0.000).

Table 1. Frequency distribution of age and gender in diabetic and control groups.

Age group	Diabetic group N=200		Control group N=50	
	Female	Male	Female	Male
31-40	32	5	16	11
41-50	39	21	8	2
51-60	55	26	9	3
61-70	11	11	0	1
Total No.(%)	137 (68.5)	63 (31.5)	33 (66)	17 (34)

Table 2. Oral manifestation in diabetic and control groups,*P value calculated using Chi square test.

	Diabetic group		Control group		*P value
Oral findings	**No.**	**%**	**No.**	**%**	
Gingivitis	120	60	5	10	0.000
Bleeding during tooth brushing	100	50	5	10	0.000
Periodontitis	100	50	5	10	0.000
Xerostomia	143	71.5	7	14	0.000
Taste disturbance	142	71	4	8	0.000
Dental pain	67	33.5	4	8	0.03
Tooth mobility	50	25	3	6	0.000
Oral candidiasis	32	16	1	2	0.026
Palatal ulceration	20	10	2	4	0.000

Concerning oral candidiasis, cases were complaining from white patches on the tongue, on oral mucosa, smooth red area on dorsal tongue, burning or painful mouth area, change in taste sensation, sensitivity to spicy food and decreased appetite. Oral candidiasis based on physical examination was found in 32 cases (16%) of the diabetic group, while in the control group only 1 case (2%) was documented for oral candidiasis.

During physical oral examination, dental caries, filled and missed teeth were reported. In the diabetic group, 418 teeth were decayed; this means 2.09 teeth per each case. Seventy teeth had been filled; this means 0.35 teeth per each case and only 16 cases had filled teeth, and from the diabetic cases, the number of lost teeth was 1390 meaning 6.95 teeth per each case. In the control group, the number of decayed teeth was 95 this means 1.9 teeth per each case. Filled teeth were 70, this means 1.4 teeth per each case, and the number of missed teeth was 134 which means 2.68 teeth per each case (Table 3). In the diabetic group, 31 cases had complete upper and lower denture (15.5%), and only 20 cases had partial denture (10%) while in the control group, only 4 cases had denture.

Bacterial identification from oral samples was based on schematic process including culture characteristics, morphology and response to various tests (17, 20). Twelve different microorganism were isolated from both groups, including fungus species *Candida albicans*, table 4.

In the diabetic group a total isolate of 755 organisms (3.77 organism per case) was documented. These were including 506 aerobic organism (67% of total 755 isolates), 174 anaerobic organism (23.04% of total 755 isolates) and 75 fungal isolates (9.93 of total 755 isolates), while in control group 76 (1.52 organism per case) organism were isolated and this included 58 aerobic organism (76.31% of total 76 isolates), 16 anaerobic organism (21.05% of total 76 isolates) and two fungal isolates (2.63 of total 76 isolates).

To find relation of flora with blood glucose level, isolated microorganism from the three groups of diabetics according to blood glucose level was shown (Table 5). As shown in table 5, 79.73 % of isolated were from the uncontrolled groups (blood glucose 127-450 mg/dl) but as this group which include 160 (80%) of the diabetic case, the number of isolate per cases were 3.76 and this showed to significant difference among the three groups.

Table 3. Teeth status in diabetic and control groups

Teeth status	Diabetic group	Control group
Number of decayed teeth	418	95
Number of decayed teeth per case	2.09	1.9
Number of filled teeth	70	70
Number of filled teeth per case	0.35	1.4
Number of missing teeth	1390	34
Number of missing teeth per case	6.96	2.68

Table 4. Microorganisms isolated from diabetic (N=200) and control group (N=50. The percentage is calculated from the total cases of diabetes and control group. * P value is calculated using Chi square test.

Isolated microorganisms			Diabetic group		Control group		P* value
			No.	%	No.	%	
Aerobic bacteria	**Gram- positive**	*Streptococcus pyogenes*	100	50	8	16	0
		Viridans streptococci	86	43	12	24	0.014
		Bacillus species	67	33.5	8	16	0.16
		Staphylococcus epidermidis	56	28	16	32	0.576
		Streptococcus pneumoniae	53	26.5	2	4	0.013
		Staphylococcus aureus	31	15.5	2	4	0.3
		Staphylococcus saprophyticus	28	14	4	8	0.256
	Gram-negative	*Branhamella catarrhalis*	56	28	4	8	0.003
		Escherichia coli	29	14.5	2	4	0.044
Anaerobic bacteria	**Gram- positive**	*Stomatococcus*	100	50	8	16	0.000
	Gram - negative	*Veillonella*	74	37	8	16	0.005
Fungi		*Candida albicans*	75	37.5	2	4	0
Total isolates			755		76		

Table 5. Microorganisms isolated from diabetic diabetic group (N=200) categorized according to their blood glucose level.

Isolated microorganisms	Blood glucose level (mg/dl)					
	6-110		111-126		127-450	
	No.	%	No.	%	No.	%
Streptococcus pyogenes	3	0.39	14	1.85	83	10.99
Viridans streptococci	7	0.92	13	1.72	66	8.74
Bacillus species	9	1.19	10	1.32	48	6.35
Staphylococcus epidermidis	6	0.79	10	1.32	40	5.29
Streptococcus pneumoniae	2	0.26	4	0.52	47	6.22
Staphylococcus aureus	3	0.39	5	0.66	23	3.04
Staphylococcus saprophyticus	2	0.26	1	0.13	25	3.31
Branhamella catarrhalis	6	0.79	6	0.79	44	5.82
Escherichia coli	1	0.13	3	0.39	25	3.31
Stomatococcus	9	1.19	12	1.58	79	10.46
Veillonella	3	0.39	10	1.32	61	8.07
Candida albicans	3	0.39	11	1.45	61	8.07
Total isolates	54	7.15	99	13.11	602	79.73
Isolates per case	3.85		3.88		3.76	

Susceptibility of aerobic bacterial isolates to ten antimicrobial agents was categorised in to susceptible, intermediate response and resistant according to the diameter of inhibition zone [(23)]. Table 6. shows the various response of each bacteria tested for. Resistance to antimicrobial were more among diabetic isolates than the control group. Resistance to many antimicrobials were observed in many species. For examples *Streptococcus pyogenes* showed wide resistance to several agent ranging from 56% for Amoxiclave, 59% for Ciprofloxacin, 73% for both Cloxacillin and Ceftriaxone to reach 96% resistance to Ampicillin. Generally resistance response were more than susceptibility among all the isolated species while it was observed that intermediate response developing to some agents such as Erythromycin for *S. saprophyticus* (Table 6).

Regardless the bacterial isolate and according to the antimicrobial agent, table 7 shows various response in number and percentage of isolates to individual antimicrobial agent. In the diabetic group average susceptibility to antimicrobial was 23.54% compared to 78.18% in control group. While intermediate response to antimicrobial was observed to some antimicrobials.

Table 6 . The susceptibility of aerobic bacterial isolate in both diabetic and control group to antimicrobial agents. * Antimicrobial used respectively were Ampicillin(Amp.), Amoxicillin/clavulanic acid (Amc.), Ceftriaxone (Cef.), Ciprofloxacin (Cip.), Cloxacillin (Clo.), Erythromycin (Ery.), Lincomycin (Lin.), Metronidazole (Met.), Tetracycline (Tet.) and Trimethoprim/sulphamethoxazole (Tri). ** Susceptible (S), Resistant (R), and Intermediate response.

Isolated organisms	Study Group	Amp*. S/R/I**	Amc. S/R/I	Cef. S/R/I	Cip. S/R/I	Clo. S/R/I	Ery. S/R/I	Lin. S/R/I	Met. S/R/I	Tet. S/R/I	Tri. S/R/I	Total isolates
Bacillus sp.	Diabetic	10/57/0	32/35/0	32/35/0	22/35/10	12/55/0	38/29/0	32/35/0	0/67/0	20/35/12	10/57/0	67
	Control	1/7/0	4/4/0	4/4/0	2/4/2	0/8/0	4/4/0	8/0/0	8/0/0	3/4/1	2/6/0	8
E. coli	Diabetic	9/20/0	24/2/3	22/7/0	18/2/9	9/20/0	9/13/7	15/10/4	13/16/0	4/22/3	7/19/3	29
	Control	2/0/0	2/0/0	2/0/0	0/0/2	2/0/0	0/2/0	2/0/0	2/0/0	1/0/1	2/0/0	2
B. catarrhalis	Diabetic	22/34/0	20/33/3	16/40/0	10/39/7	14/42/0	6/42/8	16/33/7	8/48/0	6/47/3	15/41/0	56
	Control	4/0/0	4/0/0	4/0/0	3/0/1	0/4/0	0/3/1	3/1/0	2/2/0	1/2/1	2/2/0	4
S. aureus	Diabetic	0/31/0	13/14/4	5/26/0	7/10/14	11/20/0	4/17/10	10/20/1	0/31/0	6/23/2	4/27/0	31
	Control	1/1/0	1/1/0	2/0/0	0/1/1	0/2/0	0/1/1	0/2/0	0/2/0	1/1/0	0/1/1	2
S. epidermidis	Diabetic	2/54/0	16/30/10	18/38/0	17/36/3	17/39/0	2/36/18	11/39/6	5/51/0	7/41/8	10/46/0	56
	Control	16/0/0	16/0/0	6/10/0	16/0/0	16/0/0	16/0/0	14/2/0	16/0/0	16/0/0	16/0/0	16
S. pneumoniae	Diabetic	3/50/0	17/30/6	15/38/0	16/36/1	10/43/0	2/42/9	4/44/5	2/51/0	6/41/6	1/52/0	53
	Control	2/0/0	2/0/0	2/0/0	2/0/0	2/0/0	2/0/0	2/0/0	2/0/0	2/0/0	2/0/0	2
S. pyogenes	Diabetic	4/96/0	30/56/14	27/73/0	35/59/6	27/73/0	9/69/22	17/73/10	11/89/0	12/75/13	7/93/0	100
	Control	8/0/0	8/0/0	8/0/0	8/0/0	8/0/0	8/0/0	8/0/0	8/0/0	8/0/0	8/0/0	8
S. saprophyticus	Diabetic	1/27/0	14/6/8	15/13/0	13/12/3	7/21/0	2/10/16	12/12/4	3/25/0	11/11/6	9/19/0	28
	Control	0/4/0	1/3/0	3/1/0	0/3/1	0/4/0	0/3/1	3/1/0	3/1/0	1/3/0	0/3/1	4
Viridans strep.	Diabetic	8/78/0	34/43/9	32/54/0	24/49/13	25/61/0	16/53/17	31/51/4	8/78/0	8/65/13	27/59/0	86
	Control	11/1/0	12/0/0	12/0/0	9/0/3	11/1/0	9/1/2	10/2/0	8/4/0	7/3/2	9/3/0	12

Table 7 . The number and percentage of overall susceptibility of aerobic bacterial isolate in both diabetic (n=506) and control group (n=66) to antimicrobial agents. * Antimicrobial used respectively were Ampicillin(Amp.), Amoxicillin/clavulanic acid (Amc.), Ceftriaxone (Cef.), Ciprofloxacin (Cip.), Cloxacillin (Clo.), Erythromycin (Ery.), Lincomycin (Lin.), Metronidazole (Met.), Tetracycline (Tet.) and Trimethoprim/sulphamethoxazole (Tri).

		Amp.*	Amo.	Cef.	Cip.	Clo.	Ery.	Lin.	Met.	Tet.	Tri.	Totals
Diabetic group	**Susceptible**	59/11.6	200/39.5	182/35.9	162/32	132/26	88/17.3	148/29.3	50/9.8	80/15.8	90/17.7	1191/23.54
	Resistant	447/88.3	249/48.2	324/64	278/54.9	374/73.9	311/61.4	317/62.6	456/90.1	360/71.1	413/81.6	3529/69.74
	Intermediate response	0/0	57/11.2	0/0	66/13	0/0	107/21.1	41/8.1	0/0	66/13	3/0.5	340/6.72
Control group	**Susceptible**	53/80.3	58/87.8	51/77.2	48/72.7	47/71.2	47/71.2	58/87.8	57/86.3	48/72.7	49/74.2	516/78.18
	Resistant	13/19.6	8/12.1	15/22.7	8/12.1	19/28.7	14/21.2	8/12.1	9/13.6	13/19.6	15/22.7	13/19.6
	Intermediate response	0/0	0/0	0/0	10/15.1	0/0	5/7.5	0/0	0/0	5/7.5	2/3.0	22/3.33

DISCUSSION

The oral cavity is an ever changing dynamic environment which is affected by many factors. These factors my potentiate or counteract the effect of each other. Finding non biased study groups may be difficult when many variable factors come into action. However, trying to find the effect of diabetes on oral cavity can be to some extend verified by comparing diabetics and non diabetics with same age range or by following the diabetics for a long time in relation to their diabetes control, oral condition and their oral flora. In this study, the age and the gender factors of both groups were within the same range with the mean age of diabetics was more than the control group while concerning occupation and education achievements, both groups showed minor difference (data not shown).

According to the results of oral findings (Table 2), it is obvious that in the diabetic group, there are more gingivitis, bleeding during tooth brushing, both showed to be a significant finding. Also the results showed that periodontitis was more among the diabetic group. Hundred cases (50%) of the diabetic group had periodontitis while only 5 cases (10%) of the control group had periodontitis these were also statistically significant difference. Our study samples were showed to be middle age to old aged type 2 diabetics that had bad oral hygiene, improper oral care and as an example, sixty percent were not practicing regular tooth brushing. Previous studies found that diabetes increases the risk for periodontal diseases [24, 25] although, some found that certain subgroups of type 2 diabetic patients are more prone to periodontal diseases when compared with normal cases [26]. Patients with poorly controlled diabetes are at greater risk of developing periodontal diseases. This starts with gingivitis and then, with poor glycemic control, progresses to advanced periodontal disease [27]. Periodontitis is caused by microorganisms that adhere to and grow on the tooth's surfaces, along with an overly aggressive immune response against the microorganisms [28]. A strong consistent relationship has been postulated between hyperglycemia and the incidence and progression of periodontal disease in type 2 [29]. In contrast to this study, a study showed no significant improvement in glycemic control with periodontal disease [30]. In this study, periodontitis was more in uncontrolled diabetes as 79 of uncontrolled diabetics had periodontitis, while only 5 cases with normal fasting blood sugar levels had periodontitis, and in those with impaired fasting blood sugar levels, 16 cases showed feature of periodontitis. Our study is in support to the former study but not with the latter and this may suggest a multifactor aetiology of periodontitis.

Aging is also associated with an increased incidence of periodontal disease [31, 32]. However, it has been suggested that the increased level of periodontal destruction observed with aging is the result of cumulative destruction rather than a result of increased rates of destruction. Thus aging is not a risk factor [33]. In our study, samples were ranged from middle aged and old aged individuals with type 2 diabetes and non diabetic control cases. Periodontitis was found in both groups, but higher incidence was in the diabetic group with the note that the diabetics were showed more mean age.

In relation to smoking, thirty three (16.5%) diabetic cases were smokers, while in the control group only 3 (6%) cases were smokers, and all the cases from both groups were male smokers. The percentage of smoking was higher among the diabetic group than the control group. From the diabetic smoker group, 22 (11%) of them had periodontitis and 11 (5.5%) of them had no periodontitis. We could not conclude a significant relation between smoking and periodontitis among the diabetic cases due to the small size of the smoking groups .

The prevalence of dental pain was statistically significant among the diabetic group (Table 2), but from the hundred diabetics with periodontitis, 46 cases had dental pain and 54 cases had no dental pain. It was suggested that dental pain could be multifactorial; it might be due to carious cavity, periodontal diseases, lowered resistance and a longer healing process, and bad oral hygiene [34].

Another statistically significant finding was xerostomia (Table 2), xerostomia may be due to polyuria, an underlying metabolic or endocrine problem [35]. Further to this, xerostomia and hyposalivation were prevalent in patients with type 2 DM and were associated with higher numbers of oral pathogens in the saliva [36]. Both these findings support our results as more bacteria were isolated among the diabetic xerostomia cases. The washing effect of saliva is a clear mechanism to decrease bacterial population in the mouth [37].

A statistically significant relation was also found for taste disturbance, this was reported in 142 (71%) of the diabetics compared to 4 (8%) among the controls. It is suggested that taste impairment may provide a good indicator to the course of some diseases such as diabetes mellitus, or it could be secondary to a variety of the causes including lesions of lingual epithelium, neurological impairment or due to pharmacological effect of some drugs [38]. The higher number of those with taste disturbance in our study may not be a true figure as this is a subjective feeling and may be difficult to document truly but putting the oral condition of the study group in consideration, the condition of the diabetic mouth and bacterial population may indicate high taste disturbance in diabetics.

Palatal ulceration was found in 20 (10%) cases of the diabetic group, while in the control group only 2 (4%) cases were documented for palatal ulceration. Evidence supports that unclean dentures and poor hygiene care are major predisposing factors for palatal lesions because healing of the lesions is often seen after meticulous oral and denture hygiene is instituted [39]. In our study prevalence of palatal ulceration was statistically significant among the diabetic group, but we did not find any relation between palatal ulceration and those wearing dentures.

The long cumulative effects of oral problems in the diabetic group (gingivitis, periodontitis, xerostomia and others) have reflection on the dental conditions. As shown, 31 cases (15.5%) of the diabetics had complete upper and lower dentures, and 20 cases (10%), had partial denture while in the control group, only 4 cases had dentures indicating missing teeth were more among the diabetic group (6.96 missing teeth per case) than the control group (2.64 missing teeth per case), table 3. Dental caries was slightly higher among the diabetic group, 2.09 teeth per case compare to 1.9 teeth per case in the control while filled tooth was higher in the control group. It was suggested that there is higher percentage of dental caries among diabetic patients, and also higher decayed teeth per patient in comparison with non diabetics [40]. A local study on oral manifestation in diabetes showed that the incidence of dental caries was higher among the diabetics [41] while others suggested no relation [42]. Our results showed agreement with the two former studies but not with the latter and this may be due to poor knowledge of our population about dental care and ignoring their oral problems.

Oral candidiasis was found by physical examination in 32 (16%) of the diabetics but in only one control case and most of these diabetics (18 cases) had upper denture. Oral candidiasis is an opportunistic fungal infection commonly associated with hyperglycemia and is thus a frequent occurrence of marginally controlled or uncontrolled diabetes [43]. Oral candidiasis appears to occur more frequently among people with diabetes, including those who wear dentures and it was also found that diminished salivary flow causes an increase in salivary glucose levels which creates an attractive environment for fungal infections such as thrush. All these factors were seen among many of the diabetic cases, so the results are expected and more *Candida albicans* even were isolated by using culture method (75 isolates in diabetic and two isolates in control) rather than depending on physical features of oral thrush [44].

In current study we isolated several microbial species in both groups (Table 4). Clearly, the total number of isolates were generally more among diabetics than the control with the note that only *Staphylococcus epidermidis* was higher in the control group. Among the anaerobic isolates were Stomatococcus and Veillonella, both are anaerobic normal inhabitant of oral cavity that rarely were documented as pathogens [45]. The mouth harbours many microorganisms such as *S. aureus*, *S. epidermidis*, in addition, the teeth and surrounding gingival tissue are colonized by their own particular species, such as *Streptococcus mutans* [46]. These microorganism can be altered in disease conditions such as gingivitis, periodontitis and dental caries [47]. In a previous study of the periodontal flora, they found similar microorganisms in both diabetic and non diabetics people and suggest that alteration in host responses to periodontal pathogens account for differences in periodontal destruction [48]. We found the same microorganisms in both groups, although we could not conclude a big difference but our result showed differences in isolate numbers among the two groups, and this shows agreement with Mandell and co-workers suggesting that diabetes may result in impairment of neutrophil adherence, chemotaxis, and phagocytosis, which may facilitate bacterial persistence in the periodontal pocket and significantly increase periodontal destruction [49].

As a dynamic environment, the oral flora show continuous alternation of their inhabitant, also the sampling method will affect the isolated species.

We have identified a scope of aerobics, anaerobe, Gram positive and negative microorganisms that range from low virulence Viridanse streptococci and Branhamella to more virulent species such as *Staphylococcus aureus* and *Streptococcus pyogenes*.

Comparing the two groups in consideration with Streptococci, a clear difference can be observed as higher numbers of *S. pyogenes* that were found in diabetics while in the control group a higher numbers of Viridans streptococci was isolated. This difference between the pathogenic *S. pyogenes* and Viridans streptococci may be related to factors such as diabetes, oral disease, xerostomia and immunity of the diabetes. Whatever the cause, the increase in these pathogenic Streptococci will definitely cause more problems to the host orally or causing other diseases.

The number of isolated bacteria and fungi in our study were more in those cases who had periodontitis than those cases who did not have periodontitis. Periodontal disease involves a shift in the oral/dental flora from the normal, gram-positive aerobic and anaerobic bacteria to predominantly gram-negative aerobic and anaerobic bacteria [(50)]. We could not conclude a relation between dental pain or xerostomia and bacterial isolates indicating that a relation could not be deduced by including a single factor alone and rather it is a mutlifactorial relation in the oral flora that will affect its inhabitants.

Oral candidiasis was assumed to occurs with increased frequency in patients with diabetes mellitus [(44)]. For evaluating this, we compared the frequency of oral Candida colonization. Overall, a significant difference in *Candida albicans* colonization was found between cases with diabetes in which 75 (37.5%) *Candida albicans* isolates were identified, while in the control group 2 (4%) *C. albicans* isolates were documented, but from these cases with candidal isolates only 16% diabetic cases had oral candidiasis and 2% of the control group had oral candidiasis. It was suggested that *C. albicans* with putative effects of some oral flora may play important roles in periodontitis in type 2 DM in subjects with poorly controlled DM [(51)].

The antimicrobial susceptibility test was performed for total 564 isolates excluding *Candida albicans* and anaerobic isolates. In general and regardless to the antimicrobial agent used, a resistance response was more clearly prevalent than susceptibility and this was more in the diabetic isolates, this figure was revered in the control isolates while to lesser extent, an intermediate response were observed among both group isolates. The results of antimicrobial susceptibility showed that the Amoxiclave in the diabetic isolates was more effective, followed by Ceftriaxone. However, the control group isolates results showed that Ceftriaxone was more effective followed by Amoxiclave. Unlike in the diabetic group, isolated bacteria showed high susceptibility rate to Metronidazole in the control group.

The susceptibility pattern for *E. coli* in the diabetic group showed that it possesses a high sensitivity rate to antimicrobial agents more than the other isolated bacteria, while in the control group *S. epidermidis* was highly susceptible to antimicrobial agents, more than the other isolated species. *S. aureus* in the control group was 100% susceptible to Ceftriaxone and less susceptible to other antimicrobial agents. Isolated bacteria showed higher resistance response to Ampicillin especially Staphylococci in both diabetic and control groups.

The isolated bacteria showed resistance to most antimicrobial agents in both diabetic and control groups, this resistance could be due to a successful mutations [(52)] as a results of increased use of antibiotics in our population. The incidence resistance to β-lactams antibiotic is obvious in. This resistance could be due to the ability of isolated bacteria to produce β-lactamase enzyme [(53)].

We have tested Metronidazole on aerobic micro-organisms and found that the majority of isolates were resistant to it in the diabetic group but not in the control isolates, this drug was found to be benefited in treatment of infections caused by anaerobic oral microorganisms [(54)]. Tetracycline is used commonly in our dental clinics as mouth wash especially for diabetic cases. In our study there was high incidence of intermediate response to Tetracycline, this indicates shifting toward resistance rather than susceptibility as it was concluded that Tetracycline probably caused a reduction in the periodontal infection and inflammation and over usage of this agent may be responsible for developing resistance [(55)].

In current study *E. coli* was highly susceptible to most antibiotics in both diabetic and control groups. This has been shown in a study suggested that *E. coli* was highly susceptible to most

antibiotics in both diabetic and control groups and Trimethoprim was the most effective against *E. coli* bacteria, also Tetracycline was the most effective true antibiotic tested against *E. coli*. [56].

Erythromycin in the diabetic group showed higher intermediate response on total isolated bacteria, followed by Tetracycline and Trimethoprim. This may reflect a shift to resistance to these agents in the future.

The developing resistance is a noticeable problem worldwide; this is attributed to a variety of factors such as increased use of antimicrobials and factors related to the bacteria itself. Our results showed that diabetic isolates showed more resistance than the control isolates. Diabetics may complain from many systemic or oral problems, so there is more chance of antimicrobials usage and this will increases the possibility of resistance development. With these current figures, the blind antimicrobial therapy for oral problem may have limited benefits in general dental practice. Ampicillin figures showed the ineffectiveness of this drug while other antimicrobials such as third generation antimicrobials should be prescribed in isolated cases when the patients are in actual need for the drugs.

In conclusion, oral diseases were common among diabetics, this includes gingivitis, periodontitis, taste disturbance, dental pain, xerostomia, palatal ulceration, tooth mobility, oral candidiasis and eventually tooth loss. The number of oral bacterial and candidal isolates was greater among diabetics with a shift to more virulent bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes*. The higher incidence of oral diseases among the diabetic group could be due to dense oral bacterial population of virulent species, bad oral hygiene, and these diseases may promote more bacterial colonization by bacteria. The developing resistance is noticeable among many bacteria, irrational antimicrobial use is mostly unbeneficial, some strains are still sensitive to Amoxiclave and to third generation of cephalosporin. These antimicrobials must be kept for actual needs and discouraging irrational use of antimicrobials may limit this problem.

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