

Comparison between normal oral bacterial flora in healthy and diabetic patients.

دراسة المقارنة بين زراعة البكتريا بين الاصحاء ومرضى داء السكري

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Abstract

The oral cavity contains some of the most varied and vast flora in the entire human body, several diseases involves the gastrointestinal systems and manifest in the oral cavity.

Materials and Methods. forty five diabetic patients (12 males and 33 females, age range between 23-68 years) complained of signs and symptoms of diabetes mellitus, the fasting blood sugar was above the normal (100 mg/d l). The second group included fifteen persons (13 males and 2 females) who presented with no signs and symptoms of diabetes and HbA1c was within normal range (5.5-6.5). Swabs from different site of teeth.

Results. Bacterial micro-organism was isolated from the all site of oral cavity, in normal person, *Staph. Epidermidis* and *Strepto. Mutanes* were high in number and *E.Coli.* and *Strepto. Sangious* were less number. *Strepto.Mutanes*, *Klibsielia Pneumonia* , *Staph . epidermidis* and *Strepto. Sangious* were high number bacterial micro-organisms which was inhabitant In uncontrolled diabetic patients , and *Strepto. proteus* was less number bacterial micro-organism was isolated from the all site of oral cavity.

Discussion. A variety of organisms in the microenvironment of the oral cavity adhere to the teeth, the gingival sulcus, the tongue, and the buccal mucosa. The normal flora in healthy individuals was differed than in un controlled diabetic patients may be due to dietary changes combined with poor oral hygiene especially in old age uncontrolled diabetic patient, or may be due to changes of the PH of the oral cavity and or decrease in salivary flow due to atrophy of aging.

Key words: bacterial oral cavity, healthy and uncontrolled diabetic patients.

ملخص البحث

تجوييف الفم يحتوي على اكثر انواع ومنتسح من البكتريا في كل انحاء الجسم امراض كثيرة تشمل جهازالمعدة و الامعاء تظهر في تجوييف الفم.المواد و الخطط. خمسة واربعون من مرضى السكري (اثنا عشر ذكر و ثلاث و ثلاثون انثى) تتراوح اعمارهم بين ثلاث و عشرون – ثمان و ستون سنة اجريت لهم فحص قياس نسبة السكر في الدم بعد الصوم و كانت قراتهم اعلى من الطبيعي اعلى من 100 ملغرام دسي لبيتر . اما المجموعة الثانية تتكون من خمسة عشر (ثلاث و كانت نتائجهم بين 5.5- 6.5 و اخذت عينات من مختلف Hb A1c عشر ذكر و اثنا عشر انثى) اجري لهم فحص اجزاء الفم و الاسنان و اللسان النتائج اظهرت و جود مختلف البكتريا في كل اجزاء السن ، بطانة الفم ، اللسان، في باعداد كثيرة *Staph.*

Epidermidis and *Strepto. Mutanes* الاشخاص الاصحاء اظهرت النتائج وجود

باعداد قليلة. اما البكتريا الموجودة في مرضى السكري الغير مسيطر عليه *E.Coli.* و *Strepto. Sangious*

الاكثر عدد. *Strepto.Mutanes*, و *Klibsielia Pneumonia* و *Staph . epidermidis* و *Strepto. Sangious*

. كانت الاقل عددا *Strepto.proteus* و

. المناقشة وجود انواع متعددة من البكتريا تتعايش في تجوييف الفم ملتصقة على الاسنان ، دهليز اللثة ، اللسان و بطانة الفم هناك تباين بين انواع و اعداد البكتريا بين الاشخاص الاصحاء و بين مرضى السكري الغير مسيطر عليه ويمكن ان نعزي ذلك لاسباب منها تغير انواع الاكل و رداءة صحة الفم خاصة لدى كبار السن من مرضى السكري او بسبب تغير درجة الحامضية لتجوييف الفم او قلة كمية اللعاب بسبب ضمور الغدة بسبب العمر.

Introduction

The normal oral flora is hence in a balance between pathogens and commensally that requires regular cleaning to be maintained. A decrease in oral hygiene is quickly followed by the build-up of oral biofilms on tooth surfaces and, if left untreated, will progress to gingival inflammation and possibly periodontitis, alveolar bone loss and loss of teeth. It is likely that differences in host-defense mechanisms, including antimicrobial protein profiles, determine whether bacterial colonization progresses to overt disease (1). The oral cavity contains some of the most varied and vast flora in the entire human body, several diseases involves he gastrointestinal systems and manifest in the oral cavity. In addition, a specific pathologic condition, such as periodontitis (i.e., inflammation of the periodontal attachment of the teeth and the alveolar bone), may be present in the oral cavity. These specific conditions in the oral cavity may create foci of infection that can affect many other vital systems, such as the cardiovascular and renal systems (2,3). Foci of infection in the oral cavity arising from chronic periodontitis or chronic periapical i.e., inflammation and abscess of the tissue attached to the apex of the root) may lead to sub acute bacterial endocarditis (BE) and glomerulonephritis (GN). The human body is home to microbial ecosystems (microbiotas) whose structure and function differ between different sites in the body (4).

A variety of organisms in the microenvironment of the oral cavity adhere to the teeth, the gingival sulcus, the tongue, and the buccal mucosa. Each site has a unique way of allowing the organisms to establish their residency (2,5). The normal flora in healthy individuals maintains similar patterns. The oral cavity is comprised of many surfaces, each coated with a plethora of bacteria, the proverbial bacterial biofilm. Some of these bacteria have been implicated in oral diseases such as caries and periodontitis, which are among the most common bacterial infections in humans. For example, it has been estimated that at least 35% of dentate U.S. adults aged 30 to 90 years have periodontitis (6). In addition, specific oral bacterial species have been implicated in several systemic diseases, such as bacterial endocarditis (7), aspiration pneumonia (8), osteomyelitis in children (9), preterm low birth weight (10), and cardiovascular disease (11). Periodontal disease has been associated with atherosclerosis, suggesting that bacteria from the oral cavity may contribute to the development of atherosclerosis and cardiovascular disease (12), human oral, gut, and plaque microbiota in patients with atherosclerosis (13). Surprisingly, little is known about the microflora of the healthy oral cavity. Although, etiology for 70-80% of oral cancers has been majorly linked to betel quid (tobacco) chewing, smoking and alcohol consumption (14), and certain bacteria (15). Of particular interest is the implementation of *Streptococcus anginosus* in the process of carcinogenesis, both because of its association with tumor and its ability to induce inflammation. It has been reported that the oral bacterium *S. anginosus* is associated with esophageal, gastric, and pharyngeal cancer tissues (16,17) with significant salivary specificity toward three bacteria, namely, *Capnocytophage gingivalis*, *Prevotella melaninogenica*, and *Streptococcus mitis*, as seen in oral cancers. It may be ideal not only in diagnosing the presence of a malignancy but also in executing the appropriate therapy, thus forming the signature of oral cancers (18). However, the bacterial flora associated with diabetic patients has not so far been investigated in Najaf.

The aim of this study was to evaluate bacterial flora, which was inhabitant in the oral cavity and comparison between healthy and diabetic patients.

Materials and Methods

Study population and sample types

The present study was conducted in the Al-Najaf Center for diabetic Patients in Al-Sadder Teaching Hospital, during the period from March 2012 to 2013. Swabs from surface of anterior lower teeth, posterior lower teeth, gum, dorsum of tongue and buccal mucosa were collected from the following groups; forty five diabetic patients (12 males and 33 females, age range between 23-68 years) complained of signs and symptoms of diabetes mellitus, the fasting blood sugar was

above the normal (100 mg/d l). The second group included fifteen persons (13 males and 2 females) who presented with no signs and symptoms of diabetes and HbA1c was within normal range (5.5-6.5).

Microbiological methods

The oral samples streaked on blood agar (5% v/v) and on MacConkey agar and incubated at 37°C for 24 hrs. Bacterial isolates were identified by the routine methodology in use at each laboratory. Confirmation of species identification was performed by biochemical conventional tests according to standard method described by Holt *et al.* 1994 (19), Collee *et al.* 1996 (20) and MacFaddin 2000 (21).

Results

In the control group, the study revealed that bacterial isolates, which was inhabitant in the surface of anterior lower teeth were *Streptococcus mutans* 4(0.071%), *Staphylococcus epidermidis* (%) and *Staphylococcus aureus* 1(0.017%). While bacteria Isolates, which was presented in the surface of posterior lower teeth, were *Staphylococcus epidermidis* 4(0.071%), *Streptococcus mutans* 3(0.053%) , *Staphylococcus aureus* 1(0.017%) and *Klebsiella pneumoniae* 1(0.017%) . From the gum , bacteria isolates were *Streptococcus mutans* 6(0.107%) , *Staphylococcus epidermidis* 3(0.053%) , *Streptococcus sanguis* 1(0.017%) , *Staphylococcus aureus* 1(0.017%) and *Klebsiella pneumoniae* 1(0.017%) . Bacteria isolated from dorsum of tongue were *Streptococcus mutans* 5(0.071%) , *Staphylococcus epidermidis* 5(0.071%) , *Staphylococcus aureus* 1(0.017%) , *Klebsiella pneumoniae* 1(0.017%) , *Escherichia coli* 1(0.017%) . Finally , from buccal mucosa the isolates bacteria were *Staphylococcus epidermidis* 7(0.125%) , *Streptococcus mutans* 3(0.053%) , *Streptococcus salivarius* 2(0.035%) and *Staphylococcus aureus* 1(0.017%) as shown in Table (1).

Our results revealed that bacteria isolated from dorsum of tongue and buccal mucosa of the oral cavity was the most common site in the control group patients, *Escherichia coli* was isolated from the dorsum of tongue. Our results reveals that *Staph. epidermidis* and *Strepto. mutans* was the most common type of bacteria was inhabitant in the different site of oral cavity, and In addition to *Staph aureus* was isolated in all site (surface of anterior lower teeth, surface of Post. lower teeth, from the gum, dorsum of tongue and from buccal mucosa).

in un control diabetic group our results revealed that micro-organism which was inhabitant in the surface of anterior lower teeth patients reveals *Streptococcus mutans*, *Klebsiella Pneumoniae*, *Strepto. sangious*, *Staph aureus*, *Staph. epidermidis*, *E. coli*. *Entrobacter aggulmerus*, *Strepto. Proteus* *Strepto. salivarius*.

while bacterial micro-organism which was inhabitant in the surface of posterior lower teeth was *Strepto. Mutanes*, *Strepto. Sangious*, *Klebsiella Pneumonia*, *Staph. epidermidis*, *Staph. Aureus* *Strepto. proteus*, *Strepto. salivarius*, *E. coli*, *Entrobacter aggulmerus*.

while micro-organism which was inhabitant in the gum was *Strepto. mutanes*, *Klebsiella Pneumonia*, *Staph. epidermidis*, *Strepto. sangious*, *Staph aureus*, *Strepto. Proteus*, *E. coli*, *Entrobacter aggulmerus*.

while micro-organism which was inhabitant in the dorsum of tongue was *strepto. mutanes* *Klebsiella Pneumonia*, *Staph aureus*, *Strepto. sangious*, *Staph. epidermidis*, *E. coli*, *Entrobacter aggulmerus*, *Strepto. salivarius*. while micro-organism which was inhabitant in the buccal mucosa was *strepto. mutanes*, *Klebsiella Pneumonia*, *Staph. epidermidis*, *Staph aureus*, *Strepto. sangious*, *Entrobacter aggulmerus*, *E. coli*. *Strepto. proteus*, *Strepto. salivarius*.

Table (1): Distribution of bacterial isolates in oral cavity of healthy persons (n=15)

Type of bacteria	No. (%) of bacteria isolated from:					Total No. (%)
	Surface of ant. Lower Teeth	Surface of Post. Lower Teeth	From the Gum	Dorsum of tongue	From buccal mucosa	
Strepto. Mutanes	4(0.071%)	3(0.053%)	6(0.1o7%)	5(0.071%)	3(0.053%)	21(0.375%)
Strepto. Salivarius	0(0%)	0(0%)	0(0%)	0(0%)	2(0.035%)	2(0.035%)
Strepto. Sangious	0(0%)	0(0%)	1(0.017%)	0(0%)	0(0%)	1(0.017%)
Staph. Aureus	1(0.017%)	1(0.017%)	1(0.017%)	1(0.017%)	1(0.017%)	5(0.071%)
Staph. epidermidis	4(0.071%)	4(0.071%)	3(0.053%)	5(0.071%)	7(0.125%)	23(0.410%)
Klibsielia Pneumonia	0(0%)	1(0.017%)	1(0.017%)	1(0.017%)	0(0%)	3(0.053%)
E. Coli.	0(0%)	0(0%)	0(0%)	1(0.017%)	0(0%)	1(0.017%)
Total	9(0.160%)	9(0.160%)	12(0.214%)	13(0.232%)	13(0.232%)	56(100%)

Chi- square is inappropriate because there is expected frequency less than 1, from any observed value.

Our results revealed that (nine) type of micro-organism was inhabitant in the all site of oral cavity of un control diabetic patients , Strepto.Mutanes and Klibsielia Pneumonia was the most common micro-organism in un control diabetic patients (table 2).

Table (2): Distribution of bacterial isolates in oral cavity of un control diabetic patients (n=45).

Type of bacteria	Surface of ant. Lower Teeth	Surface of Post. Lower Teeth	From the Gum	Dorsum of tongue	From buccal mucosa	Total
Strepto.Mutanes	13(0.057%)	15(0.066%)	12(0.053%)	11(0.048%)	12(0.053%)	63(0.28%)
Strepto. Salivarius	1(0.004%)	1(0.004%)	0(0%)	1(0.004%)	1(0.004%)	4(0.017)
Strepto. Sangious	5(0.022%)	9(0.04%)	4(0.017%)	4(0.017%)	3(0.013%)	25(0.111%)
Strepto. proteus	2(0.008%)	2(0.008%)	2(0.008%)	0(0%)	2(0.008%)	8(0.035%)
Staph Aureus	4(0.017%)	3(0.013%)	3(0.013%)	5(0.022%)	5(0.022%)	20(0.088%)
Staph . epidermidis	4(0.017%)	5(0.022%)	5(0.022%)	4(0.017%)	8(0.035%)	26(0.115%)
Klibsielia Pneumonia	13(0.057%)	11(0.048%)	12(0.053%)	11(0.048%)	11(0.048%)	58(0.257%)
E. Coli.	2(0.008%)	1(0.004%)	2(0.008%)	4(0.017%)	2(0.008%)	11(0.048%)
Entrobacter aggulmerus	2(0.008%)	1(0.004%)	1(0.004%)	3(0.013%)	3(0.013%)	10(0.044%)
Total	46(0.204%)	48(0.213%)	41(0.182%)	43(0.191%)	47(0.208%)	225(100%)

Chi -square =14.8 df =23 p= 0.9959 which was not significance.

Discussion

Recent data estimate that the oral cavity may contain up to 19000 bacterial phylo-type (22), but each individual will only have a rate of the total numbers of pathogens. Indeed, there is a substantial diversity in the content of the microflora between individuals (23) and between different oral sites within the same individual (24, 25). A variety of organisms in the microenvironment of the oral cavity adhere to the teeth, the gingival sulcus, the tongue, and the buccal mucosa. Each site has a unique way of allowing the organisms to establish their residency. A variety of organisms in the microenvironment of the oral cavity adhere to the teeth, the gingival sulcus, the tongue, and the buccal mucosa. Each site has a unique way of allowing the organisms to establish their residency.

The normal flora in healthy individuals maintains similar patterns(2,6,26). Our results revealed that bacterial flora which was isolated from healthy mouth was *Staph. Epidermidis* presented in all site and high number especially in buccal mucosa then *Strepto. Mutanes* and especially in the gum, while *E. Coli*, *Strepto. Sangious*, *Strepto. Salivarius* were seen only in one site. while bacterial flora which was isolated from uncontrolled diabetic mouth was *Strepto.Mutanes* inhabitant in all site of oral cavity and high number then *Klibsielia Pneumonia* was isolated from all site and high number, *Strepto. Salivarius* *Strepto. Proteus* were not isolated from all site and low number of patients in comparison with other bacterial microorganism. the dysbiosis in the composition of the oral bacteria between healthy and uncontrolled diabetic may be due to dietary changes combined with poor oral hygiene especially in old age uncontrolled diabetic patient. our results was in agreement with Avila M et al 2009 (25) they reported that dietary changes combined with poor hygiene can cause a shift in the composition of the oral bacteria, in agreement with Al-Ahmad A et al (27), Francesco Carinci et al 2013 (1), Berezow AB et al 2001 reported that oral micro biome changes as humans age (28).

Or may be due to changes of the PH of the oral cavity and or decrease in salivary flow due to trophy of aging and or due to diabetes mellitus or increase urate in oral cavity or may be decrease in immunoglobulin which was secreted with saliva or systemic effect of immunsystem. Aside from the production of saliva, the large salivary glands also help protect us from the numerous microbes that we are constantly exposed to through our mouth. Plasma B cells reside in the salivary glands and produce IgA antibody which is then secreted in the saliva. IgA binds the mucus layer that covers the epithelia lining of the oral cavity, thus providing a barrier against potentially dangerous pathogens (29,30,31,32) .or may be pregnant with bad oral hygiene , in agreement with Talib Najjar et al 2014 (2) they reported that The microenvironment of the oral cavity changes with the age of the patient, the eruption or loss of teeth, and the appearance of disease states (eg, caries, periodontal disease). Systemic changes, such as pregnancy or drug intake, also alter the number and proportion of flora. These changes are due to alterations in the flow and composition of salivary fluid and in the levels and activity of defense components (eg, immunoglobulins, cytokines) in the saliva.

Conclusion.

1. Bacterial micro-organism was isolated from the all site of oral cavity, surface of anterior and posterior lower teeth, from the gum, dorsum of the tongue and from buccal mucosa.
2. in normal person, *Staph. Epidermidis* and *Strepto. Mutanes* were high in number bacterial micro-organism was isolated from the all site of oral cavity and *E.Coli*.and *Strepto. Sangious* were less number .
3. There was difference between type and number of bacterial micro-organisms which was inhabitant in normal person and uncontrolled diabetic patients and in the different site at the same patient.
4. *Strepto.Mutanes*, *Klibsielia Pneumonia* , *Staph . epidermidis* and *Strepto. Sangious* were high number bacterial micro-organisms which was inhabitant In uncontrolled diabetic patients , and *Strepto. proteus* was less number bacterial micro-organism was isolated from the all site of oral cavity.
5. *Staph. Epidermidis* and *Strepto. Mutanes* were high in number bacterial micro-organism in normal person, . *Strepto.Mutanes*, *Klibsielia Pneumonia* were high in number bacterial micro-organism in uncontrol diabetic patients.

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