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OriginalResearch

Candida prevalence in the saliva of controlled and uncontrolled diabetic patients – A clinical study

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ABSTRACT:
Introduction: Diabetes mellitus is one of the major health concerns that has been growing in a faster rate globally.Diabetes mellitus is a metabolic disease with an abnormal elevated level of blood glucose along with down-regulation in the protein, carbohydrate and lipid metabolism.These patients are more susceptible for developing opportunistic infections especially caused by Candida species. The present study aimed to evaluate the prevalence of Candida in diabetic patients and nondiabetics patients.**Materials and Methodology:**A total of 90 patients were randomly divided into three categories based on glycated haemoglobin level (according to the American Diabetes Association Glycaemic Targets: Standards of Medical Care in Diabetes – 2018) into Group I, Group II, and Group III, each comprising of a total of 30 patients.**Results:**Out of 30 saliva samples collected from Group I (controlled diabetic patients), 14 samples showed the presence of candidial growth and out of 30 samples collected from Group II (uncontrolled diabetic patients), 25 samples showed the presence of candidial species growth. Out of 30 samples obtained from Group III (non- diabetic patients) showed no presence of candidial growth.The CFU mean values obtained from Student's t-test in Group II were found to be 40.28, which were shown to be significantly increased than the mean value of Group I which was 1.92. The computed data were found to be statistically significant. **Conclusion:**Diabetes mellitus makes the victimised patients to many opportunistic oral infections such as oral candidiasis. It usually begins as a benign asymptomatic colonization, these organisms with a favourable habitat could progress to a pathological overgrowth. The dentists are in a state of aware of various factors that could predispose patients with type 2 Diabetes mellitus and provide a holistic care that cater to the dental needs and also at the same time in order to prevent the occurrence of such infections.
Keywords:oral candida, colony forming unit, diabetes mellitus

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INTRODUCTION

Diabetes mellitus is one of the global health problems and is considered as a main source of mortality and morbidity rate. [1,2] Diabetes mellitus is a metabolic disease with an abnormal elevated level of blood glucose along with down-regulation in the protein, carbohydrate and lipid metabolism. [3] It's been found that the glycosylation process of Ig's (immunoglobulins) in case of uncontrolled diabetic patientsmore prone to infections with increased blood sugar levels (hyperglycaemia) which is the primary cause for continuity of diabetes.[4] These patients are more susceptible for developing opportunistic infections especially caused by Candida species. [5] The number of candidial species has been found to be higher in diabetes mellitus than in non-diabetic individuals. Though saliva function as an efficient antibacterial and antifungal, the mucins and other

agglutinins tend to cause the multiplication of candida microorganisms.[6] Various factors like salivary flow and the presence of enzymes tend to increase the colonization of the microorganisms. Those individuals with reduced salivary flow rates and in patients with a poor glycaemic control are at risk.[7]According to *Stehr* et al.,[8]the presence of a unique gene expression (LIP) has been found in oral candidiasis whichplay a significant role in the localization of infection with its maximum exploit found at a level of pH 7 and exceeding growth rate at a duration of 72 h. Numerous virulent factors that stump up to pathogenicity are the hydrolytic enzyme, such as secreted aspartic proteinase, which is generated from Candida species. The present study aimed to evaluate the prevalence of Candida in diabetic patients and nondiabetics patients.

MATERIALS AND METHODOLOGY

The study was conducted after following approval from the Institutional Ethics Committee of the respective institution.The outpatients visiting the Department, aged from 35–65 years with previous history of diabetes for the past 5 to 8 years and those who are under medication for diabetes, were included in the study. Those patients with a known history of other systemic illness or conditions or with mouth ulcerations, previous history of antibiotic/steroid medication, usage of any antiseptic mouthwashes during theduration of the study, and denture wearers, history of smoking, and alcoholics were excluded from the study. An Informed consent with all the details and self-explained format with regard to the study was obtained from all the patients.

A total of 90 patients were randomly divided into three categories based on glycated haemoglobin level (according to the American Diabetes Association Glycaemic Targets: Standards of Medical Care in Diabetes – 2018) into Group I, Group II, and Group III, each comprising of a total of 30 patients.

Group I – 30 patients: Diabetic patients with <6% value of glycosylated haemoglobin level (controlled diabetic patients)

Group II – 30 patients: Diabetic patients with more than 6% value of glycosylated haemoglobin level (uncontrolled diabetic patients)

Group III – 30 patients: Control group: Non-diabetic participants with a normal range of blood glucose level (postprandial blood glucose)

COLLECTION OF BLOOD SAMPLES

For the estimation of postprandial blood sugar level (PPBS), 1.5 ml of venous blood was withdrawn 2 hours after a meal in test tubes containing ethylenediaminetetraacetic acid (EDTA) under sterile condition.

For the estimation of glycosylated haemoglobin, the ion-exchange resin method was used. Around 1.5 ml of venous blood wasdrawn and collected in EDTA containing test tubes under sterile condition and were estimated according to the following formula:

% Glycohemoglobin (GHb) (% haemoglobin A) = Absorbance GHb/absorbance total Hb × factor (4.61). Conversion chart is used for obtaining values from glycosylated haemoglobin A1% to glycosylated haemoglobin A1c (HbA1c) %.

COLLECTIONOF SALIVARY SAMPLE

Saliva was collected from all three category patients (n=90). Each individual was allowed to rinse their mouth using distilled water in order to remove the presence of food debris. After rinsing the mouth thoroughly for about 60 seconds, the patient was asked to spit out/ expel around 3 ml of saliva into a sterile container. Then the salivary sampleswere collected between 9 and 11 am and were transported to the lab within a period of 1 hour.

ESTIMATION OF CANDIDA SPECIES

A 3.26-mm internal diameter inoculating loop is used in order to which holds a drop of saliva for the estimation of Candida species a Sabouraud's dextrose agar plate is used. A drop of saliva is spread in a line along the entireSabouraud's dextrose agar plate in order to produce numerous times of isolated colonies. The agar plates were then incubated at a temperature of 37°C for about 48 hours. After 48 hours of incubation, the growth of Candida species was spotted by the growth of smooth, white or creamy coloured buttery colonies [Figure 1]. After the growth of the presence of number of candidial colonies on each agar plate was counted and recorded.

The data obtained from the three categories were calculated using the Chi-square test to assess the nature of candidial growth between the diabetic, non-diabetic and control group. To evaluate the gender predilection with respect to age, PPBS, HbA1c values, and candidial growth in saliva Student’s t-test.

RESULTS

The results from the total of 90 patients included in the study, a total of 30 saliva samples had shown the presence of candidial growth. Out of 30 saliva samples collected from Group I (controlled diabetic patients), 14 samples showed the presence of candidial growth and out of 30 samples collected from Group II (uncontrolled diabetic patients), 25 samples showed the presence of candidial species growth. Out of 30 samples obtained from Group III (non- diabetic patients) showed no presence of candidial growth.The values obtained from the study groups were subjected to the Chi-square test to assess the nature of candidial species growth between the three study categories. A significant difference in the nature of candidial growth between the uncontrolled diabetic and controlled diabetic group and control participants ($P < 0.001$) as shown in Table 1. The

nature of candidial growth was also assessed between the uncontrolled and controlled group and was found to be statistically significant with P < 0.021.

Table - 1: Comparison of candidal colony forming units among three groups

GROUP	No growth, n (%)	Growth, n (%)	Total, n (%)	*P*
Controlled diabetes	16 (17.8)	14 (15.5)	30	< 0.01
Uncontrolled diabetes	7 (8.8)	23 (25.5)	30	
Control	30 (33.3)	0	30	
Total	53	37	90	

Among 90 participants Student's t-test was used to evaluate the gender predilection with regard to age, PPBS value, HbA1c value, and candidial species growth in the saliva of uncontrolled and controlled diabetes individuals. InGroup I with 30 controlled diabetic patientsno statistical significance was observed in any of the above parameters, the PPBS and HbA1c had showeda higher mean value in male patients than female patients. The colony-forming units (CFU) mean value was found to be higher in females (2.90) than that of males (1.20). In Group II with 30 uncontrolled diabetic patients, no statistical significance was observed with regard to age to any of the above-mentioned parameters. The PPBS mean value for female patients was found to slightly higher than that of males while the males patientsexhibited a higher HbA1c % level when compared to female patients. The mean value of CFU was found to be increased in males (41.93) when compared to females (37.50).

The CFU mean values obtained from Student's t-test in Group II were found to be 40.28, which were shown to be significantly increased than the mean value of Group I which was 1.92. The computed data were found to be statistically significant.

Table 2: Comparison of CFU with glycosylated haemoglobin in controlled and uncontrolled diabetes mellitus

CFU	GROUP	n	Mean	SD	SE	*P*
	Controlleddiabetes	30	1.92	4.121	0.827	<0.001
	Uncontrolled diabetes	30	40.28	46.932	9.422	

DISCUSSION

Almost over 700 microbial species including species of bacteria, virus and fungi majorly constitute the normal oral flora. The Candida species might also form the normal commensal fungi component and its routine activity is normally regulated by various intrinsic and extrinsic factors. The pathogenic nature of Candida could easilybe corroborated with many systemic conditions, which in certainaspect, affect the immunity of the patients. Additionally, the enzymatic activity which has been obtained fromisolates of C. albicans species obtained from type 2 diabetes is much higher than normal patients.[9]When the considering the favourable conditions, candida species tends to attainenough growth and activity and it is for this reason it is considered as an effective among all the opportunistic infections.[10,11] Diabetic patients are usually predisposed to higher candidialload in oral cavity becauseof their poor glycaemic control. Various methods are applied in screening and assessing the glycaemic control, among which glycosylated haemoglobin HbA1c which has proved to be one of the reliable and recommended diagnostic methods as HbA1c is directly related to the rise in blood glucose over an interval of time. Another advantage is HbA1c values are not influenced by diet, therapy, physical activities, meals and patient co-operation at the time of testing of diabetes. Blood glucose of 120 mg/dl is almost effectively equivalent to 6% of HbA1c value which elaborates an efficient long-term glucose control. Any value of blood glucose above 120 mg/dl could directly correlates a fair to poor glycaemic control. HbA1c is not used as a diagnostic study for diabetes mellitusdue to the lack of standardization among test procedures and overlapping values between normal and diabetic patients and proposes solely to access glycaemic control over 6–12-week periods. In the present study, the patients were screened for postprandial blood glucose in order to analyse the current blood glucose level which serve as a diagnostic test. The glycosylated haemoglobin HbA1c was used to assess the glycaemic control over 3 months to analyse the association between glycaemic control and oral candidial carriage among diabetic patients.[12]

In thiscurrent study, the candidialload present in the saliva of patients reported with uncontrolled diabetic were found to be higher when compared to the controlled group of diabetic and non-diabetic patients and same findings were observed by other studies.[13] The mechanism that has been proposed for increased candidial colony could be attained to the quantitative and qualitative production of saliva. Hyposalivation observed in diabetic patients could possibly related to polyuria and also the replacement of normal functioning gland tissue by adipose tissue in almost all the major salivary glands. Hence, growth of candida species in the oral cavity is easily identified due to reduced production of saliva and the

consequentially reduced immunological activity of saliva. While in another suggested mechanism for the prevalence of oral candida in uncontrolled diabetic patients when compared to controlled diabetic patients, the accumulation of sugar in the tissues could easily favours the growth and proliferation of fungus and was seen in the coated tongue of uncontrolled diabetic patients in their study.[14] The increased level of salivary glucose could increase candidial adherence to the buccal epithelial cells which form the products of glycosylation with the proteins present in tissues.[15] Thus, the episodes of hyperglycaemic in uncontrolled diabetic patients can easily lead to the accumulation of glycosylation products which thereby help in increasing the receptor for Candida. Various other factors that might influence the candidial growth are opted to be reduced activity of neutrophils and decreased salivary flow rate as observed in diabetic patients. In the present study, identical findings were observed in the nature of candidial growth in the saliva of uncontrolled diabetic patients favoured significant higher candidial growth when compared to controlled diabetic patients. In the current study, the age, postprandial glucose values, HbA1c values and CFUs did not show any significant relation with respect to gender in controlled and uncontrolled diabetic patients. The same findings were seenin several other studies.[16]The results showed that there was no significant correlation between CFU and gender in both the diabetic groups, the candidial carriage was found to be highamong females of controlled group while males favoured the predilection for candidial growth in uncontrolled diabetic patient's group. Taken into account, saliva is a unique fluid and could be used as an effective diagnostic medium. The analysis of saliva same as blood-based analyses, has served two purposes: the first is to screenthe individuals with disease and the second is to follow the progress of the affected individual who are under treatment.[17]

The condition diabetes mellitus is a growing public health concern and a common metabolic disease globally. Diabetes mellitus can easily predispose patients to many opportunistic infections and is related with either directly or indirectly to the glycaemic control. When considering the oral fluid, often called the "mirror of the body," is a perfect medium to be natured for health and disease surveillance. The oral biofluid is readily available through non-invasive collection which is helpful to monitor systemic diseases and conditions. The advantage of taking saliva is its easy collection, storage, transport, low cost, sufficient and quantities for analysis, reduces anxiety and discomfort, inability to clot, minimally invasive, less expensive, reduced risk and procurement of many repeated samples makes the saliva superior in diagnostics.[18]Various discrete lesions could actually begin as benign colonization as a result of increased candidial carriage which in time can easily progress to pathological overgrowth.

In sustained diabetic patients, there is increase in the deposition of advanced glycation end products which could lessen the normal homeostatic transport across the membrane thus resulting in increased production of vascular endothelial growth factor, but adds on to the microvascular complications of diabetes. Oral manifestations of diabetes mellitus which directly predicts the poorly controlled glycemic status of the individual.[19]Oral mucosal disorders like candidiasis mainly occur due to chronic immunosuppression and diabetes associated hyposalivation. There is a significant increase in the incidence of oral candidiasis with increased blood glucose level (hyperglycemia) observed in case of uncontrolled diabetes patients. The commensal organismpresent on the surface of oral mucosa are maintained in a state of haemostasis. The haemostasisbetween the virulent potential of microorganisms and the inflammatory and phagocytic activity of neutrophils, lymphocytes, and macrophages.[20]The increased fungal load exceeds the immunomodulatory threshold of the oral mucosa and its contributory factors like dentures which serves as a reservoir of candidial species. There is a maximum limit of the amount of C. albicans that is well tolerated by the host defence mechanism and the invasion of the Candida species from superficial lining of the mucosa to the underlying layers has extensively utilized for detection by receptors which are present on the surface of microbes like the toll-like receptors, the C-type lectin receptors, and the nod-like receptors.

CONCLUSION

Diabetes mellitus makes the victimised patients to many opportunistic oral infections such as oral candidiasis. It usually begins as a benign asymptomatic colonization, these organisms with a favourable habitat could progress to a pathological overgrowth. The dentists are in a state of aware of various factors that could predispose patients with type 2 Diabetes mellitus and provide a holistic care that cater to the dental needs and also at the same time in order to prevent the occurrence of such infections.

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