Study of Oral Manifestations & Isolation of Candida Albicans from Subgingival Plaque of Patients with or without Diabetes Mellitus Using Staining and Culture Method

Priya Shirish Joshi[1], Ravikumar Gedam[2], Shirish Govind Joshi[3], Bhagyalaxmi Praveen Hongal[4]

1- Prof. & Head, Department of Oral Pathology & Microbiology,Vasantdada Patil Dental College & Hospital, Kavalapur, Sangli, Maharashtra, India.
2- Postgraduate student, Department of Oral Pathology & Microbiology,Vasantdada Patil Dental College & Hospital, Kavalapur, Sangli, Maharashtra, India.
3- MD, Microbiology, Reader, Vasantdada Patil Dental College & Hospital, Sangli, Maharashtra, India.
4- Senior lecturer, Dept of Oral &Maxillofacial Pathology & Microbiology, Vasantdada Patil Dental College & Hospital, Sangli, Maharashtra, India.

Correspondence to:
Dr. Priya Shirish Joshi,
Prof. & Head, Department of Oral Pathology & Microbiology,Vasantdada Patil Dental College & Hospital, Kavalapur, Sangli, Maharashtra, India.
Contact Us : editor@ijdmr.com
Submit Manuscript : submissions@ijdmr.com
www.ijdmr.com

ABSTRACT

BACKGROUND: DM represents major chronic health problems facing the world today. There are varied oral manifestations in patients with DM and the frequent occurrence of Candida infections in these patients has been recognized. Hyperglycemia promotes yeast adhesion and diminishes its phagocytosis. Subgingival plaque in patients with periodontitis has been associated with various systemic diseases. Microbiological analysis of subgingival plaque can be used as quick and cost effective routine investigative procedure for management of refractory periodontitis. **INTRODUCTION:** To study the oral manifestations in patients with DM & isolation of C.albicans from subgingival plaque of patients with or without DM. **MATERIALS AND METHOD:** Total 80 cases of age group between 40-60 years were selected randomly which included 40 cases of known DM (Group I) and 40 control cases of patients with chronic periodontitis but without DM (Group II). In Group I Glycosylated haemoglobin& fasting blood glucose was estimated and in Group II random blood glucose estimation was done. Microbiological analysis of sub gingival plaque sample was done by Gram's stain and culture on Sabouraud's Dextrose Agar with chloramphenicol and Cornmeal agar. Germ tube test and demonstration of chlamydospores from culture was done for confirmation of C. albicans. Yeats corrected Chi-square test and student't' test was used. **RESULTS:** Oral manifestations seen in patients with DM were Gingivitis, Chronic Generalized Periodontitis and Xerostomia. Candidal Carriage assessment revealed statistically significant difference between the Diabetic (90%) and control group (7.50%) and there was a significant correlation between the Candidal Carriage rate (90%) and Russell's periodontal index score in group I. **CONCLUSION:** Oral Candidal Carriage assessment from subgingival plaque can be used as a quick routine investigative procedure in management of chronic periodontitis patients with or without DM.

KEYWORDS: Candida albicans, Chronic periodontitis, Diabetes mellitus, Oral manifestations, Subgingival plaque.

INTRODUCTION

Diabetes Mellitus(DM) represents one of the major chronic health problem facing the world today. Rising obesity rates due to alterations in dietary patterns as well as in life styles have led to increased prevalence of DM. The prevalence of diabetes is rapidly rising all over the globe at an alarming rate, estimated to be 2.8% in 2000 and 4.4% in 2030 for all age-groups worldwide.[1] It is important to note that the rise in prevalence is seen in all six inhabited continents of the globe.[2] India leads the world with a prevalence of 14% and 7% in urban and rural

How to cite this article:
Joshi PR, Gedam R, Joshi SG, Hongal BP. Study of Oral Manifestations & Isolation of Candida Albicans from Subgingival Plaque of Patients with or without Diabetes Mellitus Using Staining and Culture Method. Int J Dent Med Res 2014;1(4):6-16

populations respectively and incidence of nearly 2% thus earning it the dubious distinction of being termed the "Diabetes capital of the world".[3] Several studies on migrant Indians across the globe have shown that Asian Indians have an increased risk for developing type 2 diabetes and related metabolic abnormalities compared to other ethnic groups. Although the exact reasons are still not clear, certain unique clinical and biochemical characteristics of this ethnic group collectively called as the "Asian Indian phenotype" is considered to be one of the major factors contributing to the increased predilection towards diabetes.

There is a plethora of oral manifestations in patients with DM, many of which are related to the degree of glycemic control.[4] They include periodontal disease, angular cheilitis, taste alterations, lichen planus, mucosal conditions like burning mouth syndrome, altered wound healing, increased incidence of infection and Candidal infection (particularly acute pseudomembranous Candidiasis of the tongue, buccal mucosa, and gingiva), xerostomia and bilateral salivary gland enlargement or sialadenosis.[5]

One biological mechanism proposed to explain the increased incidence and severity of periodontal disease in individuals with diabetes is the finding of elevated levels of inflammatory mediators in the gingival crevicular fluid from periodontal pockets of patients with diabetes with poor glycemic control as compared with those with diabetes who are well controlled or those without diabetes. Those with poor glycemic control have considerable periodontal destruction with an equivalent bacterial challenge. The pro inflammatory cytokine TNF-α produced in large quantities by fat cells plays a significant role in this process and also plays a major role in insulin resistance. Periodontitis has also been associated with increased levels of TNF-α. Elevated levels of TNF-α may lead to greater bone loss by killing cells that repair damaged connective tissue or bone.[6]

Dental plaque is a dynamic oral bio film formed by the ordered succession of > 700 bacterial species. Most periodontopathogens are commensals in the oral cavity and express their virulence only in a susceptible host or when changes occur in their ecosystem. Dental plaque is known to be the cause of caries, gingivitis, and periodontitis. Recent studies have also explored the association between subgingival plaque in patients with periodontitis and various systemic diseases of cardiovascular or respiratory origin, DM and adverse pregnancy outcomes.

Oral Candidiasis is a common opportunistic infection of the oral cavity due to overgrowth of Candida species and its incidence varies depending on age and certain predisposing factors.[7] In the oral cavity, yeasts commonly colonize the tongue, palate, and buccal mucosa and may occur in subgingival plaque of adults with severe perodontitis. C. albicans, have been recovered from periodontal pocket in a large number of patients with chronic periodontitis.C. albicans may contribute to the development of necrotizing periodontal diseases in HIV-infected patients. Although the role of yeasts in chronic periodontitis is largely unclear there is evidence to suggest that yeasts can be implicated in the pathogenesis of the tissue destructive periodontal diseases process.[8] The frequent occurrence of Candida infections in patients with DM has been recognized for many years. Hyperglycemia promotes yeast adhesion and diminishes its phagocytosis.[9]

The use of plaque for diagnosis has recently been promoted. Obtaining plaque is advantageous for patients, especially children and diabetic subjects, since the procedure is non-invasive, stress-free and allows multiple samplings. Keeping all the above factors in mind this study was attempted to observe the oral manifestations in DM & to isolate Candida albicans from subgingival plaque of patients with or without DM.

AIMS AND OBJECTIVES

To assess oral manifestations in patients with controlled & uncontrolled DM and to ascertain whether Candida albicans can be isolated from subgingival plaque of patient's with or without DM.

MATERIALS AND METHODS

The Study was conducted on total 80 cases selected randomly from amongst the patients attending the

OPD of the institution and included 40 cases of known DM and 40 control cases of patients with chronic periodontitis but without DM. Informed written consent was recorded from all subjects before collection of sample. A detailed general physical and oral examination was carried out and recorded. The duration of diabetes and medications for control were noted for each patient.

Inclusion criteria: Age group of 40-60 years.

Exclusion criteria: Patients with systemic diseases and immune-compromised conditions other than DM.

All the cases were evaluated for periodontal health by scoring on basis of Russell's Periodontal Index.[10]

Collection of blood sample: In Group I Glycosylated haemoglobin & fasting blood glucose was estimated and in Group II random blood glucose estimation was done. Glycosylated haemoglobin level in diabetic patients was estimated using ion exchange method (Kit- Coral system). Following scoring criteria was used to assess the control of DM --Very good (4.4-5.9), Good (6.0-6.7), Fair (6.8-7.4) and Poor (<7.5).[11]

Plaque collection and microbiological investigations: Sub gingival plaque samples were obtained from labial surfaces of lower anterior teeth with the help of Universal Gracey curette. Part of the sample was smeared on a sterile glass slide and stained by Gram's stain. Rest of the sample was inoculated with a sterile inoculation loop with internal circular tip diameter 2mm calibrated to 0.005ml (Nichrome loop-D-2, HIMEDIA lab, Mumbai) on Sabouraud Dextrose Agar (SDA) with chloramphenicol and incubated aerobically at 25-37˚c for a period of 1-7 days. Smears were then prepared from colony cultures and stained again with Gram's stain to identify yeast cells. Germ tube test for confirmation of C. albicans was performed on small portion of a pure colony grown on SDA with chloramphenicol.Germ tube formation was identified on wet mount. Part of colony from SDA with chloramphenicol was inoculated into Cornmeal agar and incubated at 25-37˚c for a period of 1-7 days. Wet mount of the colony in normal saline was observed under high power for the presence of chlamydospore. ATCC 90020 strain of C. albicans was procured for validation of our results.

Statistical Analysis: Yeats corrected Chi-square test and student't' test was used to statistically analyze the obtained data. Level of significance $p< 0.05$ was considered to be statistically significant.

RESULTS

This randomized case control study was conducted on total 80 cases in which Group included 40 patients with DM and Group II included 40 control patients without DM but with Chronic Generalized Periodontitis.

Clinical Data Analysis: In group I, DM was found to be more prevalent in age group of 51-60 years with 30 (75%) cases being in the range of 51-60 years,6 (15%) cases in the range of 41-50 years and 4 (10%) cases below the age of 40 years (Table1). DM was detected more commonly in males (n=27; 67.50%) than in females (n=13; 32.50%) amongst the cases of group I (Table 2). The commonest oral manifestations seen in patients of Group I were gingivitis (100%) and chronic generalized periodontitis (Figure 1a) (100%) followed by xerostomia (37.50%) and leukoplakia (Figure 1e) in 12.50% cases. Lichen Planus (Figure 1f) and fissured tongue (Figure 1b) were observed in 3 (7.50%) cases each. Gingival enlargement (Figure 1d) was observed in 2 (5 %) cases. Angular cheilitis, racial pigmentation & traumatic ulcer (Figure 1c) was observed in only 1 (2.50 %) case. (Table3).

Age groups	Controls	%	Diabetics	%
≤40 years	1	2.50	4	10
41-50years	21	52.50	6	15
51-60years	18	45	30	75
Mean age	51.30		54.40	
Standard Deviation	5.67		7.48	
Total	40	100	40	100

Table 1: Age wise distribution of cases in control and diabetic group

Sex	Controls	%	Diabetics	%
Male	26	65	27	67.50
Female	14	35	13	32.50

Table: 2 Gender wise distribution of cases in control and diabetic group

	Oral manifestations	Percentage (%)
	Gingivitis	40(100)
	Generalized Periodontitis	40(100)
	Xerostomia	15(37.50)
	Leukoplakia	5(12.50)
	Lichen planus	3(7.50)
	Fissured tongue	3(7.50)
	Gingival hyperplasia	2(5.00)
	Racial pigmentation	1(2.50)
	Traumatic Ulcer	1(2.50)
	Angular Cheilitis	1(2.50)

Table: 3 Prevalence of oral manifestations in diabetic mellitus group

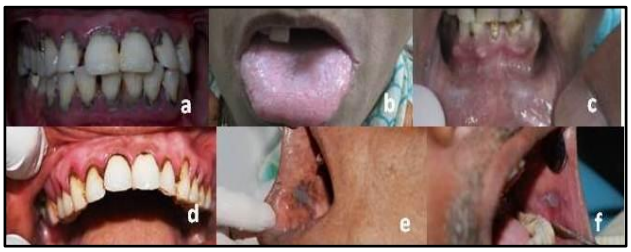

Figure 1- a) Chronic generalized periodontitis, b) fissured tongue, c) traumatic ulcer, d) Gingival enlargment, e) Leukoplakia, f) Lichen Planus

Statistical analysis done to compare the Russell's periodontal index score between group I and group II with student't' test showed significant difference between the two groups [p value =0.0033 (p< 0.05)] (Table 4).

Glycosylated Hemoglobin levels provide information about the degree of blood glucose control. We found 19 (47.50) cases in Good control followed by 9 (22.50 %) cases of Very Good & Fair control each and 3 (7.50%) cases of Poor control (Table 5). Analysis of the duration of DM revealed that 27 (67.50 %) cases were suffering from the disease for more than 6 years, 12 (30%) cases were suffering for last 2-5 years and only 1 (2.50%) case was newly detected diabetes of less than 1 year. Three out of 40 cases from Group II were detected with increased random blood sugar above 210 mg/dl.

	Mean	SD	P-value
Diabetic group I (40)	6.1775	0.4312	0.0033*
Control group II (40)	5.7775	0.7141	

Table: 4 Comparison of Russell's periodontal index score in group I & II

Glycosylated Hemoglobin levels (HbA1c %)	No&% of cases
Very good (4.4-5.9)	9 (22.50)
Good (6.0-6.7)	19 (47.50)
Fair (6.8-7.4)	9 (22.50)
Poor (<7.5)	3 (7.50)
Total	40 (100)

Table: 5 Glycosylated Hemoglobin levels in Group I (HbA1c %)

Microbiological Analysis: Gram's staining of subgingival plaque smears revealed large quantity of Gram positive cocci in chains and clusters, few Gram negative bacilli , filamentous Grampositive actinomycetes, epithelial cells, pus cells and organic debris.In addtion Gram positive Candidal pseudohyphae along with budding yeast cells were seen in 36(90%) cases of Group I &in 3 (7.50%) cases of group II (Figure 2). This finding was statistically significant (p<0.001) (Table 6)

After culture on SDA with Chloramphenicol, colonies of Candida appeared within 3-4 days as white to cream coloured, smooth, pasty with yeasty odour. In some cases the growth was observed within an overnight incubation. All the suspected, 36 positive smears for Candida in group I and 3 positive smears in group II showed growth as described above on SDA with Chloramphenicol

(Figure 3).Statistical analysis was done with Yates corrected Chi-square test. The difference in growth on SDA with Chloramphenicol was significantly higher in Group I compared to GroupII.($p< 0.001$) (Table 6).For all positive cases subsequent Gram's staining of colony smear revealed Gram positive budding yeast cells as spherical, smooth surfaced structures(Figure 4).All positive cases also showed germ tube formation within 2 hours on incubation with human serum at 37^{o}c(Figure 5).All the positive samples showed a growth on culture with cornmeal agar after incubation at 25-37^{0}c within 3-4 days. The growth appeared as white to cream coloured colonies with smooth, pasty surface and a yeasty odour. In some cases the growth was observed within an overnight incubation (Figure 6). All the positive samples also showed chlamydospore formation. Chlamydospores appeared as spherical, doubled walled bodies at terminal, sub-terminal, calary & intercalary regions over the pseudohyphae (Figure 7).

	Controls	%	Diabetics	%	Total	%	Yates corrected Chi-square	P-value
Plaque smear Gram's stain for Candida								
Positive	3	7.5	36	90	39	48.75	51.2320	0.0000*
Negative	37	92.5	4	10	41	51.25		
Sabouraud's Dextrose Agar with Chloramphenicol Culture for Candida Albicans								
Positive	3	7.5	36	90	39	48.75	51.2320	0.0000*
Negative	37	92.5	4	10	41	51.25		
Colony smear Gram's stain for Candida Albicans								
Positive	3	7.5	36	90	39	48.75	51.2320	0.0000*
Negative	37	92.5	4	10	41	51.25		
Germ tube formation For Candida Albicans								
Positive	3	7.5	36	90	39	48.75	51.2320	0.0000*
Negative	37	92.5	4	10	41	51.25		
Cornmeal Agar culture for chlamydospores								
Positive	3	7.5	36	90	39	48.75	51.2320	0.0000*
Negative	37	92.5	4	10	41	51.25		
Total	40	100	40	100	80	100		

*p<0.05

Table: 6 Microbiological analysis in diabetic & control group

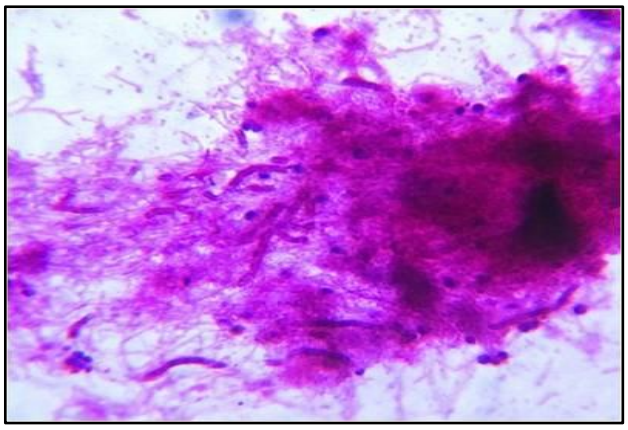

Figure 2- Gram's staining showing pesudohyphae in subgingival plaque smear (40 X)

Figure 3-Culture growth on SDA with Chloramphenicol

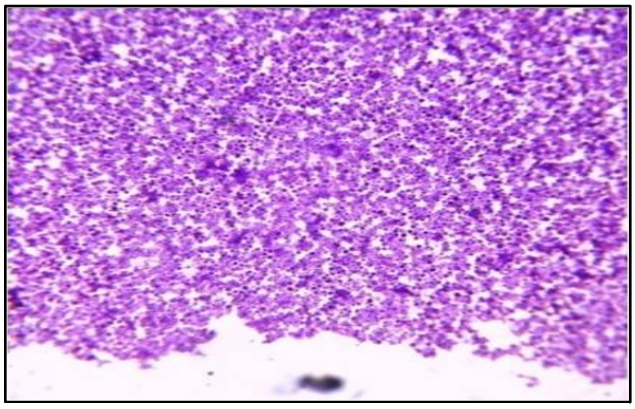

Figure 4-Gram's staining of colony smear showing yeast cells (10 X)

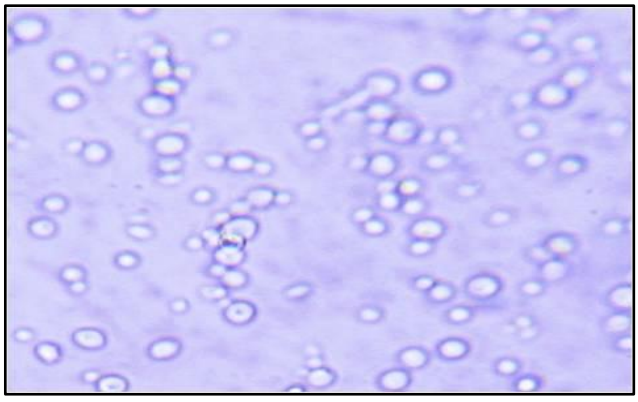

Figure 5-Germ tube formation (40 X)

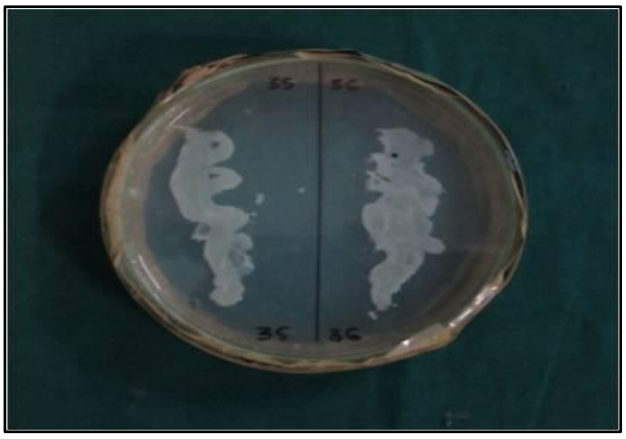

Figure 6- Culture growth on Cornmeal Agar

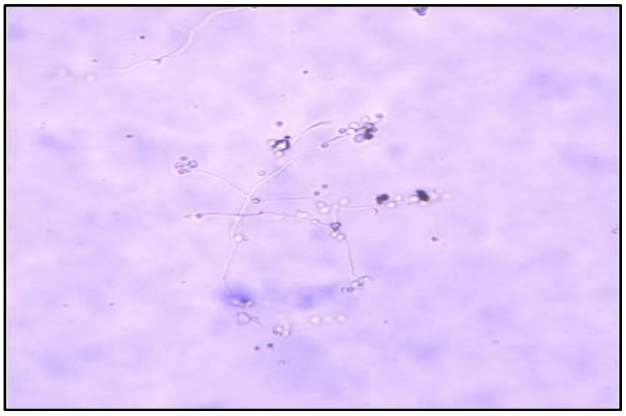

Figure 7-Chlamydospores formation (40 X)

Candidal carriage	Diabetic group (%)	Control group (%)	Total	%
Present	36 (90)	3 (7.50)	39	48.75
Absent	4 (10)	37 (92.50)	41	51.25
Total	40	40	80	

Yates corrected chi-square= 51.2302 P = 0.0000**p<0.0

Table: 7 Prevalence of candidal carriage in diabetic and control group

Validation: The control ATCC 90020 strain of C. albicans which was run parallel for all cases in group I & II yielded results like formation of Germ tube, chlamydospores and Gram's positive yeast cells on smears. The results were similar to the strain of C. albicans which we isolated.

All the above findings point to the fact that Candidal Carriage and isolation of C. albicans was significantly higher in the group I (92.50 %) as compared to group II (7.50 %) and this finding was statistically significant. [Yates corrected chi-square test. ($p< 0.001$). (Table 7)

DISCUSSSION

Diabetes epidemic is more pronounced in India than in any other country, as the World Health Organization (WHO) reports show that 32 million people had diabetes in the year 2000. The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40 million in India and this is further set to rise to 69 million by the year 2025.[3]

Oral health is an integral part of general health. It promotes self confidence and improves the quality of life. Oral cavity frequently undergoes changes that are related to the diabetic condition, and oral infections may adversely affect metabolic control of the diabetic state.[12] Oral manifestations of DM were first described more than 100 years ago. Several studies show that patient's age at onset of DM, duration and degree of metabolic control may exert a greater influence on oral and systemic complications than the type of DM present. A number of oral disorders have been associated with DM such as fungal & bacterial infections, alteration in the function of salivary glands and composition of salivary chemistry, increased incidence of periodontal infection, high incidence of dental caries & xerostomia.[5] Candida is a yeast-like fungus that normally inhabits the oral cavity, gastrointestinal tract, other mucous membranes and skin. They have been reported to be present in 40% to 50% of population. In most humans, they are in a commensal relationship. When changes in the host environment cause imbalance of the flora or a decrease in resistance, Candida becomes an opportunistic pathogen.[13] Present study was undertaken to investigate the oral manifestations in patients with controlled & uncontrolled DM and to ascertain whether C. albicans can be isolated from subgingival plaque of patients with or without DM. For this purpose 80 patients were selected from amongst those attending the OPD of the institution. Patients were divided in two group, Group I (DM) and Group II (control patients with chronic periodontitis but without DM.)

Age and sex are globally identified risk factors for DM. The greatest numbers of people with diabetes are aged between 40 and 59 years. The worsening of insulin resistance with age, increased inactivity and longevity of diabetes patients due to improved care are the reasons given for the rising prevalence of Type 2 DM with age. Worth noting is the fact that age related increase in insulin resistance is not a universal finding, and the reasons for the discrepant results probably include general health, physical activity, changes in liver size and delay in carbohydrate absorption.[14] Our study reinforced this observation and we found that prevalence of DM was highest in the age group of 40 to 60 years. This finding is in accordance with the studies conducted by Belmiro Cavalcanti do Egito Vasconcelos et al [5], Chris E. Ekpenyong et al[14] and Narumol Sirsaphum et al.[15]

The worldwide diabetes prevalence is similar in men and women, but it is slightly higher in men greater than 60 years of age and women of older ages.[14] In our study prevalence of diabetes was 67.50% (27) in men and 32.50% (13) in women. This finding is in accordance with the studies conducted by Chris E. Ekpenyong et al.[14] This is in contradiction to the findings of the studies conducted by Belmiro Cavalcanti do Egito Vasconcelos et al[5]and Narumol Sirsaphum[15]wherein they have noted a female prevalence of 64-70%. The difference could be due to causes like genetic, environmental or other confounders. We found a positive family history in 9 out of 40 (22.50) diabetic patients. Our finding is in agreement with previous study conducted by Chris E. Ekpenyong et al.[14] Out of 27diabetic male patients 19 had central and general obesity while out of 13diabetic female patients 6 had central and general obesity. Thus we can state that old age, positive family history, poor oral hygiene and obesity are more significant risk factors for developing DM. Our findings are consistent with studies conducted by Grant JF et al.[16]

Increased periodontal disease severity has been reported to occur in diabetic patients, particularly in those with long duration as reported in the studies conducted by Hugoson et al.[17] In our study, in group I there was no correlation between severity of periodontal disease and duration of DM. All 40 patient's in this group had severe advanced periodontal involvement irrespective of the duration of DM. Kinane et al [18]in his review has also concluded that the duration of DM does not influence severity of periodontitis. We analyzed the periodontal status of all subjects in both groups with the help of Russell's Periodontal Index. On Comparison of results in Group I (DM) and Group II (Control) there was a statistically significant difference in the involvement of periodontitis in diabetics group. (p=0.0033*) The increased incidence of periodontitis in diabetics is in accordance with the review done by Ionescu et al[19], Stegeman et al[20], and Mealey BL et al.[21] Studies conducted by Kinane et al[18] have shown that the risk of periodontal disease is 3 times greater in diabetic patients than in the general population.

Several studies support the concept that certain "high risk" patients have an abnormal monocytic secretion of inflammatory mediators in response to Gram-negative bacterial lipopolysaccharide (LPS) challenge - a MØ trait –has been associated with increased susceptibility to severe forms of periodontal disease. Patients with refractory, early onset and IDDM associated periodontal disease have been suggested to possess the MØ phenotype. This increase in monocytic inflammatory mediator secretion as a response to LPS challenge has been proposed to be regulated by genes in the HLA-DR and -DQ regions. Furthermore, the polymorphism in the promoter region of cytokine genes, such as the involvement of transcription factor(s) in LPS-induced human TNFα gene regulation has been postulated to be of importance in modulating the magnitude of the LPS stimulated TNFα secretory response. This suggests to us that genetic polymorphism in the promoter region of certain key inflammatory mediators including TNF α, maybe important in expression of disease severity in the presence of infectious challenge, such as periodontal disease.[22]

In present study, xerostomia was seen in 15(37.50%) patients of group I who were also suffering from chronic periodontitis, thus revealing

a strong correlation between decreased salivary flow rate which creates a favorable environment for the growth of organisms and initiation of periodontal disease. This finding is similar to the studies conducted by Maria Rozeli et al (68.6%)[23], Cheng–Chieh-Lin et al[24], Gun.E.Sandberg et al[25] (53.5%) , Jonathan A. Ship et al[26] and Aren G et al[27]who demonstrated that diabetics have a decreased salivary flow rate. But Miralles et al[23] in their study of xerostomia in diabetics have suggested that there is no difference between the diabetics and healthy subjects in relation to salivary flow. In group I, we had 5 cases (12.50%) of leukoplakia and 3 cases (7.50 %) of Lichen Planus. This result is in accordance with the study conducted by Maria Albrecht et al.[28] They have noted that 6.2% of diabetics had Leukoplakia and 1 % of diabetics had Lichen planus. Similar results are also noted in the studies conducted by Jonathan A. Ship et al.[26] But contradictory results have been documented by Maria Rozeli et al.[23] They opine that diabetics have no significant mucosal lesions when compared to the healthy controls. Study conducted by Dikshit R et al[29] has stated that diabetes is an independent risk factor for oral leukoplakia and erythroplakia.

Many possible mechanisms for an association between diabetes and pre-malignant oral lesions may be proposed. Diabetes (type II) is usually associated with insulin resistance and increased pancreatic secretion. Chronically increased level of insulin resulting in hyperinsulinaemia has been associated with colon cancer pathogenesis and with cancer of breast, pancreas and endometrium. Insulin promotes the synthesis and biological activity of insulin like growth factors 1 (IGF1) which help in cell proliferation and inhibits apoptosis. Prospective and retrospective studies have demonstrated that serum IGF-1 levels are associated with increased risk of various epithelial tumors including prostate, breast, colorectal, lung and esophagus. Positive association between IGF1 in primary oral cancer and stage of disease has also been observed. Limited evidence suggests that the effect of IGF-1 might also be related to p53 mutations, which are quite common in head and neck tumours. Certain type of p53 mutation will lead to p53 over expression, which in turn will up-regulate IGF receptor I (IGF-IR) expression.[29] A positive association between diabetes mellitus and premalignant lesion in our study may be due to other confounding risk factors like tobacco chewing, use of mishri, smoking and alcohol consumption. We found 3 cases (7.50%) of Fissured tongue and one case each of Traumatic Ulcer and Angular Cheilitis. This result is in accordance with the study conducted by Saini R et al[30] and Bajaj S et al.[31]

Glycosylated haemoglobin has been the most widely used and accepted test for monitoring the glycaemic control in individuals with diabetes. In our study, irrespective of glycemic control, all patients were suffering from chronic periodontitis. Analysis of 3 cases having poor glycemic control revealed that they shared common factors like long duration of diabetes (8-10 years), advanced age, insulin resistance in 2 cases of insulin dependent diabetes mellitus (IDDM), in adequate insulin doses, overweight, irregularity in consuming medication and uncontrolled diet. It is a known fact that advanced periodontitis worsens the glycemic control and vice versa. In our study the Candidal Carriage was found to be 90%in Group I, but the results of rate of candidal carriage in the literature review are found to be varying from 23% in studies done by James et al[32] to49%by Hill et al[33], 54% by A.M.G Darwazeh et al[34], 81.8% by Khaled H Abu-Elteen et al[9]and 68% by M. Bharathi et al.[35]

The frequent occurrence of Candidal infections in patients with DM has been recognized for many years and oral Candidiasis in particular is thought to be more prevalent among these individuals.[36] The adhesion of micro-organisms to host mucosal surfaces is a necessary pre-requisite for successful microbial colonization and infection and the role of adhesion in the pathogenesis of many fungal infections is widely appreciated**.** The genetically determined inability to secrete the water-soluble glycoprotein forms of the ABO blood group antigens in saliva has been described in association with increased incidence of Candida infections.[34] Oral epithelium in diabetic individuals favours adhesion, colonization of Candida unlike in non-diabetic subjects. It is possible that there may be intrinsic qualitative changes on the cell surface

receptors modulating Candida adhesion in diabetic subjects. Hyperglycemia could contribute to the risk of oral Candidal infection by increasing salivary glucose levels, which promotes overgrowth of Candida.[36]

Although the role of yeasts in chronic periodontitis is largely unclear there is evidence to suggest that yeasts can be implicated in the pathogenesis of the tissue destructive periodontal diseases process.[8] Extensive studies on Candidal Carriage in patients with periodontitis have been documented. The results of Candidal isolation in the previous studies have been shown to vary from 17% by Dodds et al[37], 30% by Canabarro A et al[38] and69.2% by Urzua B et al.[39] In the present study Candidal Carriage in patients with periodontitis was found to be 7.5%. Our results are in accordance with the study conducted by Dahlen G et al[40] (7.3%).

In our study it is possible that confounding local factors were the reasons for isolation of C. albicans in three cases of chronic periodontitis of control group. Two of three positive cases from the control group had habits like alcohol consumption and tobacco chewing. All the three cases were accidentally discovered to be diabetic after random blood sugar estimations (>200 mg/dl). Also it was noted that these patients were on long term antibiotic therapy and were suspected cases of refractory periodontitis.

C. albicans express virulence factors that may have an important role in the pathogenesis of periodontal disease, such as the ability to adhere and penetrate the epithelium, inhibition of polymorphonuclear cells, lysis of monocytes, dimorphism, phenotypic switching, interference in host's immune system, production of hydrolases, and ability to respond to environmental changes. Moreover, Candida species is also relatively tolerant to innate and cell mediated immunity. Adherence is considered the first stage of the infection process for Candida species and an essential step for the expression of the persistence of the microorganism in the host, as the ability to adhere avoids microorganisms of being eliminated by saliva.[38] C. albicans is not uniformly distributed in mouth of healthy people. It is found that prevalence and density of C. albicans is low in region of anterior labial sulcus. So studies on isolation of C. albicans can be done more accurately by multiple sampling from different intraoral sites.
In our study subgingival plaque was sampled for isolation of C. albicans. Since plaque is a bio film containing mixed population of microorganisms, isolation of Gram positive and Gram negative organisms along with Candida could have yielded more specific results to compare their associations. This is a very exhaustive study and long term interventional studies in this direction can be encouraged in future. Quantitative studies for mycotic and bacterial count can also be done by the multiplex PCR methods. Hence quantitative studies in this direction can also be encouraged in future. In recent years DNA–DNA re-association, DNA fingerprinting, Southern hybridization with appropriate DNA probes, Gene polymorphism studies, peripheral monocyte culture studies have been reported to recognize Candida species in culture or in clinical materials. However genotypic methods have the disadvantage of being laborious and time consuming, and also require specialized equipment.[41] Studies involving larger sample size, patients with longer duration of DM should be encouraged in future to observe more specific association between diabetes and its oral manifestations.

CONCLUSIONS

The oral manifestations in patients with DM, are related to the degree of glycaemic control and includes gingivitis, periodontitis, angular cheilitis, xerostomia, taste alterations, lichen planus, leukoplakia, fissured tongue, racial pigmentation and increased incidence of Candidiasis. Oral manifestations in patients with diabetes of duration more than 15-20 years should be evaluated more accurately to study their associations. There was a statistically significant difference in Russell's periodontal index score between Diabetic and control group. The use of plaque for diagnosis is advantageous for patients and the procedure is non-invasive, stress-free and allows multiple samplings. Candidal Carriage assessment from subgingival plaque can be used as a quick and cost effective

routine investigative procedure in management of chronic refractory periodontitis patients with or without DM. We propose future studies involving large sample size in order to reveal more qualitative results.

REFERENCES

1. Wild S, Roglic G, Green A, Sicree R. King H. Global prevalence of diabetes. Estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27: 1047-53.
2. Chandramohan P, Mohan V. High Prevalence of Diabetes and Metabolic Syndrome among Policemen. JAPI 2008; 56: 837-38.
3. Mohan V, Deepa M, Anjana RM, Lanthom H, Deepa R. Secular trends in the prevalence of diabetes and impaired glucose tolerance in urban south India, The Chennai Urban Epidemiological Study (CURES-17). Diabetologia 2006; 49:1175-78.
4. Sunday O. Akintoye, Michel T. Collins, Jonathan A. Ship**.** Diabetes Mellitus & Endocrine Diseases, In: MS Greenberg, Michael Glick, Editors, Burkitt's Oral Medicine, Diagnosis and Treatment, 11th ed, BC Daker publication; 2008. 509-17.
5. Belmiro Cavalcanti do Egito Vasconcelos, Moacir Novaes, Francisco Aurelio Lucchesi Sandrini, Almir Walter de Albuquerque Maranbao Filbo, Leila Santana Coimbra. Prevalence of oral mucosa lesion in diabetic patients: a preliminary study. Rev Bras Otorrinolaringol.2008; 74; 423-28.
6. Gurenlian JAR. The role of dental plaque bioflim in oral Health. Annals of Periodontology. 1998; 3:257-61.
7. Akpan A**,** Morgan R. Oral candidiasis. Postgraduate Medical Journal. 2002; 78:455-59.
8. Jarvensivu A., Hietane J, Rautema R., Sorsa T. Candida yeasts in chronic periodontitis tissues and subgigival microbial bioflims in vivo. Oral diseases 2004; 10:106-12.
9. Edward J. Boyko and Benjamin AL Infection and Diabetes [online]; Available from: www.t1da.org/chapter22_Infection and Diabetes.
10. Soben Peter. Essential of preventive and community dentistry. 4th edition. Arya Medi Publishing House.2009: 326-29.
11. Skamagas M, Breen TL, LeRoith D. Update on diabetes mellitus: prevention, treatment, and association with oral diseases. Oral Diseases 2008; 14:105–14.
12. Boon NA, Colledge NR, Walker BR (editors) Davidson's Principles and Practice of Medicine 20th Edition, Philadelphia: Churchill Livingstone; 2006; 813-17.
13. Cannon RD, Holmes A, Mason AB & Monk BC. Oral Candida; clearance, colonization or candidiasis? J of Den Res 1995; 74: 1152-61.
14. Ekpenyong CE., Akpan UP. Gender and Age specific prevalence and associated risk factors of type 2 diabetes mellitus in UYO metropolis, south eastern Nigeria. Diabetologia Croatica 2012; 41:17-27.
15. Srisaphum N, Taneepanichskul S. Oral health status in Diabetic patients at Changhan District, ROI province Thailand. J Health Res 2010; 24:45-50.
16. Grant JF, Hicks N, North West Adelaide Health Study team. Gender- specific epidemiology of diabetes: a representative cross-sectional study. Int J Equity Health 2009; 8:9. Available at: http//www.equity health j.com/content/8/1/6.
17. Hugoson A, Thorstensson H, Falk H and Kuylenstierna. Conditions in insulin-dependent diabetics. J ClinPeriodontol 1989; 16: 215-23.
18. Denis F. Kinane.In 2000 reviewed the environmental factors that modify the periodontal diseases in a cross–sectional study of 1426 patients. The study showed that diabetes was the only associated disease in relation to attachment loss. J Periodontol 1998; 69:1426–34.
19. Ionescu O, Sonnet E. Oral manifestations of endocrine dysfunction. Annals of endocrinology 2005; 30:48-51.
20. Stegeman CA. Oral Manifestations of Diabetes. Home Health care Nurse 2005; 23:233-40.
21. Mealey BL, Oates TW. Diabetes mellitus & Periodontal disease. Periodontology 2006; 77:1289-1303.
22. Salvi G, Collins, John G Collins. Monocytic TNF alpha secretion patterns in IDDM patients with periodontal disease. Journal of clinical Periodontology 1997; 24:8-16.
23. Maria Rozeli S. Quirino.Oral manifestations of diabetes mellitus in controlled & uncontrolled patients. Brazilian Dental Journal 1995; 6:131-36.
24. Cheng CL**.** Impaired salivary functions in patients with non insulin dependent Diabetes Mellitus with xerostomia. Dept medical research 2001; 16:176-79.
25. Sandberg GE..A controlled study of oral self-care & self perceived oral health in type 2 diabetic

patients. Oral surg oral med oral pathol oral radiol endod 2001;7:76-82
26. Jonathan A ship. Diabetes and Oral health. JADA 2003; 134:4S-10S.
27. Aren G, Sepet E. Periodontal health, salivary status and metabolic control in children with type 1 Diabetes Mellitus. Journal of Period ontology 2003; 74:1789-95.
28. Maria A. Occurrence of oral leukoplakia & lichen planus in Diabetes Mellitus. Oral Pathol and Med 1992; 21:364-66.
29. Rajesh P. Dikshit1, K. Ramadas, Mia Hashibe. Association between diabetes mellitus and pre-malignant oral diseases: A cross sectional study in Kerala, India. Int. J. Cancer 2006: 118, 453–57.
30. Saini R Sadeq Ali Al –Maweri, Saini D, Ismali NM. Oral Mucosal lesion in non oral habit diabetic patients and association of diabetes mellitus with oral precancerous lesions. Diabetes Research and Clinical Practice. 2010; 89:320-26.
31. Bajaj S, Prasad S, Gupta A, Singh V B. Oral manifestations in type-2 diabetes and related complications. Indian J Endocrinol Metab. 2012; 16: 777–79.
32. Guggenheimer J, Myers D, Weyant R. Insulin dependent diabetes mellitus and oral soft tissue pathologies .Prevalence and characteristics of Candida and candidal lesions. Journal of Diabetes and its complications 2003; 89:570-76.
33. Hill LV, Tan MH, Pereira LH, Embil JA. Association of oral candidiasis with diabetic control. J ClinPathol 1989;**42**:502-05
34. Darwazeh AMG, Lamey PJ, Samaranayake LP, Macfarlane TW, Fisher BM, Macrury SM and Maccuish AC. The relationship between colonisation, secretor status and in-vitro adhesion of Candida albicans to buccal epithelial cells from diabetics. J. Med Microbiol 1990; 33:43-49.
35. Bharathi M, Anaparthy U, Cautha SA Comparative Study of Carrier State of Candida and its Speciation in Oral Flora – among Healthy Individuals, Persons with DM and HIV Sero positive Individuals. Our Dermatol Online. 2012; 3: 102-06.
36. Lamey PJ, Darwaza A, Fisher BM, Samaranayake LP, Macfarlane TW, Frier BM. Secretor status, candidal carriage and candidal infection in patients with diabetes mellitus. J Oral Pathol 1988; 17:354-57.
37. Dodds MWJ, Yeh CK, Johnson. Salivary alterations in type 2 (non-insulin-dependent) diabetes mellitus and hypertension. Community Dentistry and Oral Epidemiology 2000; 28: 373–81.
38. Canabarro A, Valle C, Farias MR , Santos FB , Lazera M , Wanke B. Association of subgingival colonzation of Candida albicans and other yeasts with severity of chronic periodontitis. J of Periodontal R 2013;48 (4): 428–432
39. Urzua B, Hermosilla G, Gamonal J, Morales-Bozo I. Yeast diversity in the oral microbiota of subjects with periodontitis: Candida albicans and Candida dubliniensis colonize the periodontal pockets. J. Clin Periodontal 2008; 46:783-93.
40. Dahlen G, Wikstrom M. Occurrence of enteric rods, staphylococci and Candida in subgingival samples. Oral Microbiol Immunol 1995; 10:42-46.
41. Liguori G, Lucariello A, Colella G, Luca A De, Marinelli P. Rapid identification of Candida species in oral rinse solutions by PCR. Clin Pathol 2007; 60:1035–39.

Source of Support: Nil
Conflict of Interest: Nil