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 Joshi¹, Ravikumar Gedam², Shirish Govind Joshi³, Bhagyalaxmi Praveen Hongal⁴

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. Yeasts corrected Chi-square test and student’s 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 (75%) 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.

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.
populations respectively and incidence of nearly 2% thus earning it the dubious distinction of being termed the “Diabetes capital of the world”.

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. 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.

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.

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. In the oral cavity, yeasts commonly colonize the tongue, palate, and buccal mucosa and may occur in subgingival plaque of adults with severe periodontitis. 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. The frequent occurrence of Candida infections in patients with DM has been recognized for many years. Hyperglycemia promotes yeast adhesion and diminishes its phagocytosis.

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.\(^\text{10}\)

**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).\(^\text{11}\)

**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\(^\circ\)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\(^\circ\)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:** Yates 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).

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Controls</th>
<th>Diabetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤40 years</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>41-50 years</td>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td>51-60 years</td>
<td>18</td>
<td>30</td>
</tr>
<tr>
<td>Mean age</td>
<td>51.30</td>
<td>54.40</td>
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<tr>
<td>Standard Deviation</td>
<td>5.67</td>
<td>7.48</td>
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</table>

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

<table>
<thead>
<tr>
<th>Oral manifestations</th>
<th>Percentage (%)</th>
</tr>
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<tbody>
<tr>
<td>Gingivitis</td>
<td>40(100)</td>
</tr>
<tr>
<td>Generalized Periodontitis</td>
<td>40(100)</td>
</tr>
<tr>
<td>Xerostomia</td>
<td>15(37.50)</td>
</tr>
<tr>
<td>Leukoplakia</td>
<td>5(12.50)</td>
</tr>
<tr>
<td>Lichen planus</td>
<td>3(7.50)</td>
</tr>
<tr>
<td>Fissured tongue</td>
<td>3(7.50)</td>
</tr>
<tr>
<td>Gingival hyperplasia</td>
<td>2(5.00)</td>
</tr>
<tr>
<td>Racial pigmentation</td>
<td>1(2.50)</td>
</tr>
<tr>
<td>Traumatic Ulcer</td>
<td>1(2.50)</td>
</tr>
<tr>
<td>Angular Cheilitis</td>
<td>1(2.50)</td>
</tr>
</tbody>
</table>

Table: 3 Prevalence of oral manifestations in diabetic mellitus group

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic group I</td>
<td>6.1775</td>
<td>0.4312</td>
<td>0.0033*</td>
</tr>
<tr>
<td>(40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group II</td>
<td>5.7775</td>
<td>0.7141</td>
<td></td>
</tr>
<tr>
<td>(40)</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Glycosylated Hemoglobin levels (HbA1c %)</th>
<th>No&amp;% of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very good (4.4-5.9)</td>
<td>9 (22.50)</td>
</tr>
<tr>
<td>Good (6.0-6.7)</td>
<td>19 (47.50)</td>
</tr>
<tr>
<td>Fair (6.8-7.4)</td>
<td>9 (22.50)</td>
</tr>
<tr>
<td>Poor (&lt;7.5)</td>
<td>3 (7.50)</td>
</tr>
<tr>
<td>Total</td>
<td>40 (100)</td>
</tr>
</tbody>
</table>

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 Gram positive actinomyces, epithelial cells, pus cells and organic debris. In addition Gram positive Candidal pseudohyphae along with budding yeast cells were seen in 36(90%) cases of Group I and 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 $37^\circ C$ (Figure 5). All the positive samples showed a growth on culture with cornmeal agar after incubation at 25-37$^\circ C$ 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. Chlamydoospores appeared as spherical, doubled walled bodies at terminal, sub-terminal, calary & intercalary regions over the pseudohyphae (Figure 7).

![Figure 2](image2.png)

**Figure 2** - Gram’s staining showing pseudohyphae in subgingival plaque smear (40 X)

![Figure 3](image3.png)

**Figure 3** - Culture growth on SDA with Chloramphenicol

![Figure 4](image4.png)

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

![Figure 5](image5.png)

**Figure 5** - Germ tube formation (40 X)

<table>
<thead>
<tr>
<th>Controls</th>
<th>% Diabetics</th>
<th>% Total</th>
<th>Yates corrected Chi-square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque smear Gram’s stain for Candida</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>7.5</td>
<td>36</td>
<td>90</td>
</tr>
<tr>
<td>Negative</td>
<td>37</td>
<td>92.5</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Sabouraud’s Dextrose Agar with Chloramphenicol Culture for Candida Albicans</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>7.5</td>
<td>36</td>
<td>90</td>
</tr>
<tr>
<td>Negative</td>
<td>37</td>
<td>92.5</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Colony smear Gram’s stain for Candida Albicans</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>7.5</td>
<td>36</td>
<td>90</td>
</tr>
<tr>
<td>Negative</td>
<td>37</td>
<td>92.5</td>
<td>4</td>
<td>10</td>
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<tr>
<td>Germ tube formation For Candida Albicans</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>7.5</td>
<td>36</td>
<td>90</td>
</tr>
<tr>
<td>Negative</td>
<td>37</td>
<td>92.5</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Cornmeal Agar culture for chlamydospores</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>7.5</td>
<td>36</td>
<td>90</td>
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<td>Negative</td>
<td>37</td>
<td>92.5</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>10</td>
<td>40</td>
<td>10</td>
</tr>
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</table>

* $p<0.05$
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)]

DISCUSSION

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.\(^\text{14}\) 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\(^\text{5}\), Chris E. Ekpenyong et al\(^\text{14}\) and Narumol Sirsaphum et al.\(^\text{15}\)

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.\(^\text{14}\) 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.\(^\text{14}\) This is in contradiction to the findings of the studies conducted by Belmiro Cavalcanti do Egito Vasconcelos et al\(^\text{5}\) and Narumol Sirsaphum\(^\text{15}\), 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.\(^\text{14}\) Out of 27 diabetic male patients 19 had central and general obesity while out of 13 diabetic 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.\(^\text{16}\)

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.\(^\text{17}\). 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\(^\text{18}\) 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\(^\text{19}\), Stegeman et al\(^\text{20}\), and Mealey BL et al.\(^\text{21}\) Studies conducted by Kinane et al\(^\text{18}\) 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 monocyte secretion of inflammatory mediators in response to Gram-negative bacterial lipopolysaccharide (LPS) challenge - a MÖ 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 MÖ 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 TNFα, may be important in expression of disease severity in the presence of infectious challenge, such as periodontal disease.\(^\text{22}\)

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%)\textsuperscript{23}, Cheng–Chieh-Lin et al\textsuperscript{24}, Gun.E.Sandberg et al\textsuperscript{25} (53.5%), Jonathan A. Ship et al\textsuperscript{26} and Aren G et al\textsuperscript{27} who demonstrated that diabetics have a decreased salivary flow rate. But Miralles et al\textsuperscript{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.\textsuperscript{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.\textsuperscript{26} But contradictory results have been documented by Maria Rozeli et al.\textsuperscript{23} They opine that diabetics have no significant mucosal lesions when compared to the healthy controls. Study conducted by Dikshit R et al\textsuperscript{23} 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.\textsuperscript{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\textsuperscript{30} and Bajaj S et al.\textsuperscript{31}

Glycosylated haemoglobin has been the most widely used and accepted test for monitoring the glycemic 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\textsuperscript{32} to 49% by Hill et al\textsuperscript{33}, 54% by A.M.G Darwazeh et al\textsuperscript{34}, 81.8% by Khaled H Abu-Elteen et al\textsuperscript{9} and 68% by M. Bharathi et al.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.  

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.  

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 and 69.2% 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 hydrodases, 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.  

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 reassociation, 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.

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