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## Original Research Article

## A study to evaluate etiology of denture stomatitis and to determine interrelationship with trauma and candida albicans count

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## ABSTRACT

**Background:** Denture stomatitis (DS) is a disease characterized by inflammation and erythema of the oral mucosa areas covered by the denture. Multifactorial etiological factors contribute to denture stomatitis. The purpose of this study is to identify the etiology of denture stomatitis and to establish the role of trauma and fungal infection in denture stomatitis.

**Materials and Methods:** Subjects wearing previously fabricated removable partial or complete denture who were diagnosed with denture stomatitis were included. It is a cross sectional questionnaire-based study, for mycological study swab sample was smeared, Sabouraud Dextrose Agar (SDA) was used as culture media. Germ tube test was used to identify *Candida albicans*. To test significance between different variables Kruskal-Wallis test was used.

**Result:** Out of 195 subjects' slight stomatitis was present in 52.80% patients, followed by moderate stomatitis in 32.31% and no stomatitis in 2.56% subjects. *Candida* was present in 25.64% patients. *Candida* significantly more common in moderate stomatitis than others.

**Conclusion:** This study has suggested that there is a significant relation in denture stomatitis and *Candida albicans* in north Indian population. No significant role of trauma was noticed. Strong correlation has been found in Denture hygiene, cleaning habits and Denture Stomatitis.

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### 1. Introduction

Insertion of complete dentures is not the final step in treatment of edentulous patients. When edentulous patients are delivered complete dentures, the important phase of oral and denture aftercare begins.<sup>1</sup> Initial recommendations to patients include need for periodical visits to the dentist for maintenance purposes and explaining denture cleaning and wearing habit.<sup>2,3</sup>

Studies have shown that most denture wearers with poor denture cleanliness, poor oral hygiene, or those who wear dentures at night have the accumulation of a

characteristic biofilm, which could damage adjacent mucosa cause denture stomatitis or systemic diseases.<sup>4-7</sup> Denture Stomatitis (DS) is a common oral disease in denture wearers and it is associated with multiple etiological and predisposing factors. It is divided into three types: Type I shows localized inflammation or pinpoint hyperemia; Type II shows more diffused erythema and Type III is a non-neo-plastic papillary hyperplasia with inflammation to a varying degree. Type I DS may be due to trauma from the denture (occlusion, undercuts, stability etc.) to the denture bearing area, whereas Type II and III may be associated with infection together with mechanical trauma. The prevalence of the three types of DS in different groups of elderly

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population has been reported to vary in the range of 10–65%.<sup>8,9</sup>

Adherence of *Candida albicans* to the acrylic surfaces of denture may be because of local or systemic factors that has been associated with pathology of DS. But, none of the factor has been found to be confirmed.<sup>10</sup> So this study was planned to ruled out the association of local as well as systemic factor with DS along with role of trauma among North Indian population.

## 2. Materials and Methods

This study was conducted on 195 subjects suffering from denture stomatitis visiting the Department of Prosthodontics, Faculty of Dental sciences, K G Medical University, Lucknow.

The study design was a cross sectional questionnaire-based study with fungal count testing, denture cleanliness scores and testing of trauma by erythema score in patients with denture stomatitis. Ethical clearance was taken from University and study was completed in 1 year Subjects wearing previously fabricated removable partial or complete dentures attending the Department of Prosthodontics, KGMU, UP who were diagnosed with denture stomatitis were included in the study. A diagnosis of denture stomatitis is made when erythema/hyperemia/hyperplasia is seen in relation to the denture base.

Demographic data of patients was recorded that includes name, age, gender, education and socioeconomic status. Subjects were asked about duration of wearing denture and whether they removed their denture at night and if yes then how frequently, also subjects were enquired about history of smoking, tobacco chewing, alcohol and any allergy, medication, preexisting diseases.

After recording their consent, a close-ended questionnaire<sup>11,12</sup> previously used in various studies covering multiple factors linked with denture stomatitis was given to the subjects in their language of preference (English/Hindi). Their responses were correlated with presence and severity of denture stomatitis. Also, scores given by a team of 3 dentists (majority opinion /consensus) on the presence and severity of denture stomatitis, presence /absence of trauma, fungal absence/presence and denture hygiene score was used to identify etiological and associated factors of denture stomatitis. The investigation was carried out at mid-morning at least 2 hours after drinking, eating or any oral hygiene procedure to avoid diurnal variations. (Tables 1 and 2)

For mycological study, swab sample was taken by rolling sterile cotton swab across the site of denture stomatitis and was immediately smeared on to the surface of culture medium with Saubauroud's dextrose agar (SDA - with chloramphenicol).

Plates were aerobically incubated at 37 °C for 48-72 hrs. Culture growth which was negative for candida growth was

left on the bench for another 72hrs and examined again, before been discarded as negative. Number of candida colonies were determined by counting colony forming units on SDA plates. Preliminary identification for candida was carried out after the growth of characteristic creamy, convex yeast colonies on SDA. Smear will be made for gram staining and was examined under microscope to recognize gram positive budding cells. Yeast was identified as *Candida albicans* on the basis of positive germ tube test.<sup>13</sup>

Examination of denture cleanliness was made using a plaque detector (proflavine-monosulphate in a 0.3% aqueous solution) to disclose plaque on intaglio surface of the denture. According to quantity of plaque on the denture base, patients were divided into three groups by using Budtz-Jorgensen's Index (Budtz-Jorgensen & Bertram, 1970) of denture cleanliness:<sup>13</sup>

1. Excellent: None or only few spots of plaque
2. Fair: More extended plaque, less than half of the denture base covered by plaque
3. Poor: More than half of denture base covered by plaque

Association of gender, age, partial/complete denture wearer, duration of wear ,how old the denture is, systemic disease, allergy ,oral denture hygiene, tobacco/alcohol use, presence/absence of trauma and presence/absence of fungi was correlated with presence and extent of denture stomatitis. The null hypothesis was that denture stomatitis occurs independent of all above mentioned factors.

An evaluation of collected data was made with the help of standard tests such as mean, standard deviation, T test and Chi-square test. P value of 0.05 was considered as statistically significant. Statistical analysis was made using the statistical software SPSS 10.0 for Windows. Descriptive statistics was made and the normality of distribution was tested by the one-way Kolmogorov-Smirnov test. To test significance between different variables the Kruskal-Wallis test was used if distribution of data is not normal, and if distribution is normal ANOVA test was used.

## 3. Results

A total of 195 patients were included in the study, out of which 62.1% were male and 37.9% were female.

Mean Age of studied subjects were 56.76 year (S.D. = 12.39). Median Age was 56.0 year. Most of the patients were between 41 – 50 age groups. 60 % of patients chew tobacco and out of which 23 % chew daily. 22.56 % of patient's drinks alcohol. 26.15% patients having systemic disease. Hypertension was most common systemic 54.9%. Patients had no history of allergy. 89.23% patients remove their denture at night.73.33% patients remove the denture every day at night and 13.33% patients remove alternate days, 82.56 %. Brushing with tooth paste was most common method of cleaning (44.10%) than hand soaping, rising

(36.41%) and rinsing and soaking in water is minimum (19.49%).

**Table 1:** Questionnaire of Oral Denture Hygiene

Oral & Denture Hygiene	
1.	<b>Do you remove your dentures at night?</b>
a)	Yes
b)	No
c)	Not answered
2.	<b>If Yes, how often?</b>
a)	Every day
b)	Alternative days
c)	Once a week
d)	2 times / week
3.	<b>How do you clean your dentures?</b>
a)	Brush with toothpaste
b)	Rinsing or soaking in water
c)	Soaking in a solution (denture cleaner, sodium hypochlorite)
d)	Hand Soaping and rinsing
e)	Don't clean
4.	<b>When (how frequently) do you clean your dentures?</b>
a)	Twice a day
b)	Every day
c)	Alternative day
d)	Once a week
e)	2 times/week
f)	Never
5.	<b>How do you maintain oral hygiene?</b>
a)	Brush twice a day
b)	Brush once a day
c)	Rinse mouth after meals
d)	Do nothing
6.	<b>Has a dentist or his staff ever shown you or told you how to clean your dentures?</b>
a)	Yes
b)	No

Everyday cleaning of denture was most common in 71.80% subjects followed by alternate day (16.41%) and once a week was (2.56%) minimum. Denture cleanliness/hygiene score in 51.28 % subjects was fair, excellent in 21.03% and poor in 27.69 % patients. (Tables 3 and 4) Candida was more with poor denture cleanliness condition. Candida was present in 25.64% (n=50) patients. One large colony was most common in 22% of subjects followed by two large colony (20%). (Table 5)

Only 5 subjects (2.56%) showed trauma from denture (Table 6). The candida was significantly more in the subjects who and does not remove the denture at night. (Table 7) Candida significantly more occurred in the subjects who have cleaned their denture once a week. Candida was maximum in patients rinse mouth after meals then others and significant. (Tables 8 and 9)

Out of 195 patients's slight stomatitis was present in 52.80% patients, followed by moderate stomatitis 32.31% and no stomatitis was 2.56% (Minimum). Candida

**Table 2:** Questionnaire of Clinical Denture Examination

Clinical Denture Examination	
1.	Degree of erythema score:
0:	No Stomatitis
1:	Slight Stomatitis (Localized slight hyperaemia)
2:	Moderate Stomatitis (Diffuse hyperaemia)
3:	Severe Stomatitis (Diffuse and papillary hyperplasia)
2.	Denture cleanliness or hygiene score:
Excellent	None or only few spots of plaque
(1):	
Fair(2):	More extended plaque, less than half of the denture base covered by plaque
Poor(3):	More than half of denture base covered by plaque
3.	Test for Candida albicans presence
	Absent/Present
	If present count:
4.	Signs of trauma from denture
	Yes/No
	If yes ,due to
a.	Occlusion
b.	Stability
c.	Extension
d.	Undercuts

**Table 3:** Denture Cleanliness or Hygiene score

Denture cleanliness	Percentage %	Percentage%
Excellent	41	21.03
Fair	100	51.28
Poor	54	27.69
<b>Total</b>	<b>195</b>	<b>100</b>

significantly more common in moderate than others. (Table 10)

#### 4. Discussion

Candida albicans is a dimorphic yeast strongly gram positive able to live as normal commensal organism in the oral cavity of healthy people and more frequently isolated in the oral cavity. Presence of denture is a predisposing factor for the onset of pathologies related to C. albicans. Clinical studies have shown that C. albicans is not only able to adhere to the mucous surfaces, but also to stick to the acrylic resins of the dental prostheses. Both the plaque accumulated on the denture and the poor oral hygiene contributes to the virulence of Candida, offering the clinical picture of Candida-associated denture stomatitis.<sup>14</sup> Multifactorial etiological factors contribute to DS, but it seems that Candida plays the key role.<sup>15</sup>

Several methods for sampling (mouth rinsing, denture imprint and swabbing) are available to detect oral yeast carriage; swabbing is most commonly used particularly easy in elderly subjects.<sup>15</sup>

**Table 4: Candida and Hygiene score**

Hygiene score Candida	Excellent %		Fair %		Poor %	
Absent	36	87.8	73	33	36	66.7
Present	5	12.2	27	27	18	33.3
<b>Total</b>	<b>41</b>	<b>100</b>	<b>100</b>	<b>60</b>	<b>54</b>	<b>100</b>

$\chi^2 = 5.66$  'p'=0.05(Sig.)

**Table 5: Candida albicans colony count**

Option	N	Percentage
One large colony 20 – 30	6	12.00
One large colony	11	22.00
Two candid colony	5	10.00
Two colony of candida	6	12.00
Two large colony	10	20.00
Three large colony	6	12.00
Three large colony	6	12.000
<b>Total</b>	<b>50</b>	<b>100.00</b>

**Table 6: Candida and trauma**

Candida	No		Yes	
	N	Percentage	N	Percentage
Absent	139	73.45	6	100.00
Present	50	26.55	0	0.0
<b>Total</b>	<b>189</b>	<b>100.00</b>	<b>6</b>	<b>100.00</b>

$\chi^2 = 2.14$  'p'=0.14 (NS) No significant relation of trauma.

**Table 7: Correlation of Candida with removal of denture at night**

	Candida	No		Yes		
		N	Percentage	N	Percentage	
Removal of Denture at night	Absent	11	52.40	134	77.00	$\chi^2 = 5.902$ 'p'=0.015
	Present	10	47.60	40	23.0	
	<b>Total</b>	<b>121</b>	<b>100.00</b>	<b>74</b>	<b>100.00</b>	

**Table 8: Correlation with Candida with frequency of cleaning**

Candida	Twice a day		Everyday		Alternate day		Once a week	
	N	%	N	%	N	%	N	%
Absent	12	66.70	111	79.30	22	68.80	0	0.00
Present	6	33.30	29	20.70	10	31.20	5	50.00
<b>Total</b>	<b>18</b>	<b>100.0</b>	<b>140</b>	<b>100.0</b>	<b>32</b>	<b>100.0</b>	<b>5</b>	<b>100.0</b>

$\chi^2 = 17.37$  'p'=0.001 (Sig.)

**Table 9: Correlation of Candida with maintenance of oral hygiene**

Candida	Brush twice a day		Brush once a day		Rinse mouth after meal		Used of powder	
	N	%	N	%	N	%	N	%
Absent	12	100.0	100	89.3	27	41.54	6	100.0
Present	0	0.0	12	10.7	38	58.46	0	0.0
<b>Total</b>	<b>12</b>	<b>100.0</b>	<b>112</b>	<b>100.0</b>	<b>65</b>	<b>100.0</b>	<b>6</b>	<b>100.0</b>

$\chi^2 = 56.02$  'p'=0.000 (Sig.)

**Table 10:** Candida and Degree of erythema score

Candida	No		Slight		Moderate		Severe	
	N	%	N	%	N	%	N	%
Absent	5	100.0	93	90.3	29	46.03	6	25.0
Present	0	0.0	10	9.70	34	53.97	18	75.0
<b>Total</b>	<b>5</b>	<b>100.0</b>	<b>103</b>	<b>100.0</b>	<b>63</b>	<b>100.0</b>	<b>24</b>	<b>100.0</b>

$\chi^2 = 55.25$  'p'=0.0 (Sig.)

Detection of Candida is important step. Several methods are available for this which can be broadly classified into microscopic examination {specific and non-specific staining/ FISH (Fluorescence in situ hybridization)} conventional detection methods (culture based, serological, nucleic acid based) and nonconventional techniques (Lab chip device based), culture media is still considered gold standard.<sup>16</sup> Common culture media for isolating clinical candida species are blood agar, potato dextrose agar (PDA) or broth (PDB), Sabouraud brain heart infusion agar, Yeast nitrogen base (YNB) etc.<sup>17</sup> In Present study Cultivable Candida species were then identified from the macroscopic morphology of colonies and from additional tests after subculture on Sabouraud agar with chloramphenicol and gentamycin, the latter involved namely germ tube formation in human serum.<sup>18</sup> Recently to distinguish various morphotypes TEM (Transmission electron microscopy), Phase contrast microscopy and SEM (scanning electron microscopy) are also used.<sup>19</sup> Colonies of candida were counted on SDA culture, and the most commonly found presentation was two candida colony in 42% cases followed by one large colony in 34% subjects and 24% subjects with 3 large colonies. Colony forming unit count was estimated by using the following formula: CFU/mL=1000×number of colonies/4.<sup>20</sup> So, in present study 500cfu/ml was most common whereas least one was 750cfu/ml.

Candida associated denture stomatitis represents as a condition affected by multiple factors that can be either systemic or local. In Local factors saliva play dual role in pathogenesis of denture stomatitis because mechanically saliva cleans the oral cavity and reduces the adhesion of Candida to the acrylic resin of dentures, but some studies states that salivary proteins like mucines and the statherins may act as adhesion receptors for Candida by interacting with its mannoproteins.<sup>21</sup>

In our study lack of oral hygiene has been found more frequently in patient with DS also habit of nocturnal denture wearing. These habits cause spontaneous remission of infection and increase the susceptibility to candidiasis.<sup>22–24</sup> According to **Ghalib et al** number of Candida species decreases significantly with increased cleaning frequencies as denture brushing frequencies increased, the numbers of Candida in the saliva decreased. Furthermore, cleaning frequencies were inversely proportional to the levels of Candida species in saliva.<sup>21</sup>

In the present study for the measurement of denture cleanliness we used Budtz-Jørgensen Index and mostly patients were found in fair category.<sup>25</sup>

Denture surface plays role in presence of candida, the micro-porosity on denture intaglio surface promotes the growth of yeast leading to stomatitis.<sup>26,27</sup> Trauma is also one of the local factors which can be due to ill fitted denture or due to unbalanced occlusion does not play a direct role but it acts as co-factor which helps in or increases candida adhesion to the tissue under denture.<sup>28</sup>

Besides local factors, systemic factors also showed association with denture stomatitis. Systemic illness like diabetes and Renal disease and xerostomia does not directly cause denture stomatitis but they play major role in decreasing host immunity and favorable environment for candida growth. One co-relating factor between candida growth and diabetes can be decrease in salivary secretion or xerostomia that promotes candida growth. With increasing age salivary secretion decreases. Besides diabetes and aging xerostomia also presents as a side effect of some drugs like antihypertensive, antipsychotic, antidepressant etc. which are common in old age population and promotes candida growth.<sup>29</sup>

## 5. Conclusion

Denture stomatitis is a disease with multifactorial etiologies and Candida albicans is as one of the causative factors for this in North Indian population. Though Several systemic and local factors play important role but presence of denture with poor oral as well as denture hygiene aggravate the condition. Candida-associated denture stomatitis, even if asymptomatic, should be treated as it may act as reservoir for infections more extensive and encourage the resorption of the alveolar bone. Topical therapy is the first line of treatment, use of clotrimazole or nystatin lozenges with the denture removed from the mouth, is recommended. Besides the pharmacological treatment maintenance of good oral hygiene as well as denture cleanliness plays equally important role.

## 6. Conflict of Interest

The authors declare that there are no conflicts of interest in this paper.

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None.

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