



Assessment of Oral Candida Carrier Status in A Dental Hospital

KEYWORDS

Mean Candidal Carriage Score, Hypothyroidism, Hypertension, Blood group

Seema Patil

Patil Seema, MDS, Reader, Dept of Oral Medicine & Radiology D.A.P.M R.V Dental College

Asha R Iyengar

Iyengar Asha R, MDS, Professor & Head, Dept of Oral Medicine & Radiology D.A.P.M R.V Dental College

Sonali Ghai Choudhury

Choudhary Sonali G, Post Graduate, Dept of Oral Medicine & Radiology

Subash B Vasudeva

Vasudeva B Subash, MDS, Reader, Dept of Oral Medicine & Radiology D.A.P.M R.V Dental College

Sankarsan Choudhury

Choudhury Sankarsan, MDS, Dept of Oral and Maxillofacial Surgery, Mahaveer Cancer Sansthan, Patna, Bihar

ABSTRACT

The present study aimed to assess the candidal carriage status amongst patients visiting the dental outpatient department particularly in subjects with hypertension, hypothyroidism, tobacco habits and blood group secretor status. A total of 191 subjects were selected for the study and grouped into four categories based on tobacco habits, medically compromised conditions (diabetes, bronchial asthma, hypertension, and hypothyroidism) potentially malignant conditions and denture wearers. The fifth group included subjects who had a combination of any of the above. Cytological smears were obtained from the oral cavity in all subjects and examined for candidal hyphae. Based on the candidal load, a candidal carriage score was assigned for each subject. This was followed by the calculation of mean candidal carriage score (MCCS) for each group. It was noted that subjects in fifth group (subjects with multiple predisposing factors) had the highest MCCS(1.47). Amongst subjects with a single predisposing factor, denture wearers had the highest MCCS(1.31) followed by medically compromised conditions(1.21). Subjects with tobacco habits and potentially malignant conditions had a lower MCCS(0.91). The difference between the five groups was found to be statistically significant (p value - 0.0066). Individuals with Blood group O had higher MCCS although the difference was not statistically significant. It may be inferred from the present study that hypertension, hypothyroidism, tobacco habits and blood group secretor status do not influence the candidal carriage status to a large extent.

INTRODUCTION

Candidal organisms are common inhabitants of the oral cavity and have been detected in 17-75% of healthy individuals. They can however become pathogenic and cause opportunistic infections. A number of local and systemic predisposing factors influence both the carriage and infectious potential of candidal organisms. The local predisposing factors include denture wearing, smoking, inhalation steroids, hyperkeratosis, imbalance of the oral microflora etc. Immunosuppressive drugs, radiotherapy, chemotherapy, HIV infection, endocrine disorders (diabetes mellitus) and haematinic deficiencies are some of the systemic predisposing factors.¹

The role of a few predisposing factors such as prolonged use of antibiotics, topical steroids, denture wearing and uncontrolled diabetes with candidiasis is well established.² Whereas, the role of smoking and blood group secretor status in the pathogenesis of candidiasis is still inconclusive warranting further investigation. Moreover, very few studies have assessed oral candidal carriage in betel quid chewers and hence definitive conclusions cannot be arrived at. Furthermore, hypothyroidism and hypertension may be associated with hyposalivation predisposing individuals to increased candidal carriage. This association has not been investigated so far to the best of our knowledge suggesting a need for further research.

With the above background, the present study was designed to determine the oral candidal carriage in subjects visiting the dental outpatient department. These included subjects with tobacco habits, medically compromised con-

ditions (diabetes, bronchial asthma, hypertension, hypothyroidism) potentially malignant conditions (leukoplakia, Oral submucous fibrosis and Oral Lichen planus) and denture wearers. The association between the blood group secretor status and candidal carriage was also assessed.

METHODOLOGY :

A total of 191 subjects were included in the study from the outpatient department of Oral Medicine and Radiology. The study group was divided into five groups as follows:

- Group I: 64 subjects with the habit of using tobacco (smoking/ smokeless)
- Group II: 54 subjects with medically compromised conditions such as hypothyroidism hypertension, diabetes mellitus and bronchial asthma
- Group III: 27 subjects with potentially malignant conditions
- Group IV: 18 denture wearers (partial/complete)
- Group V: 28 subjects with combinations of any of the above

Pregnant women and subjects on anti-fungal therapy were excluded from the study. Prior to conducting the study, an informed consent from the study subjects and an ethical clearance from the institutional review board were obtained. The relevant history was

followed by an examination of the oral cavity for the presence of any candidal lesions. Smears were obtained from the sites presenting with pseudomembranous or atrophic forms of candidiasis. Cytological smears were further obtained from the dorsum of the tongue, buccal mucosa (bi-

laterally) and hard palate. The Olsen and Stenderup criterion³ was followed for obtaining smears. The smears were made with the help of sterile wooden spatulas under aseptic conditions. The material so obtained was fixed immediately with 1:1 ratio of ether and alcohol and then dried. The specimens were appropriately labeled and handled with care using universal precautions as they were considered as potential biohazards. The slides were stained with Periodic acid Schiff reagent and observed under light microscope (40 X high power view) for pseudohyphae. The presence/load of candidal hyphae was categorised either as low, moderate, heavy or very heavy as follows:

Load Of Candida	No. Of Candida Seen/HPF	Length Of Pseudohyphae	Clusters Present/Absent
Absence Of Candida	Nil	NA	NA
Low Load	Less Than 2 Hpf(May Or May Not Be Present In Every Hpf)	Smaller Than Epithelial Cell	Absent
Moderate Load	2-3/Hpf	Smaller Than Epithelial Cell	Absent
Heavy Load	4-10/Hpf	May/Maynot Be Longer Than Epithelial Cell	1-2 Clusters In The Entire Smear
Very Heavy Load	More Than 10/ Hpf	Longer Than Epithelial Cell	Many Clusters Seen

Further each of these categories was assigned a score as per the load of candida. Insignificant load was given a 0 score, low load score-1, moderate load -2, heavy load-3, very heavy load-4.

Mean candidal score was obtained for each subject by adding the candidal scores obtained for all sites and dividing it by the total number of sites. This was followed by calculation of the mean candidal carriage score (MCCS) in each individual group by taking an average of the candidal scores of all subjects in the group. The MCCS was compared between the five groups. The data was tabulated and statistically analysed.

RESULTS:

Of the 191 subjects in the study, 137 were males and 54 were females. Of these 137 males, highest numbers of males (60) were present in group I (subjects with tobacco habits) and the lowest was noted in group IV (denture wearers). (table1)

Table 1 : Distribution of the subjects in five study groups based on gender

Groups	Male	%	Female	%	Total	%
Group I (Tobacco habits)	60	43.80	4	7.41	64	33.51
Group II (Medically compromised)	22	16.06	32	59.26	54	28.27
Group III (Potentially malignant Conditions)	20	14.60	7	12.96	27	14.14
Group IV (Denture wearers)	12	8.76	6	11.11	18	9.42
Group V (combination of any of the above)	23	16.79	5	9.26	28	14.66
Total	137	100.00	54	100.00	191	100.00
Chi square=42.6734		p=0.000001,				

Of the 54 females, highest numbers of females (32) were noted in group II (medically compromised subjects) and lowest in group I (subjects with tobacco habits).

The mean age was highest (61 years) in group V, followed by group IV (58 years) and the least (31 years) was in group I. (table 2)

Table 2: Mean age and SD of subjects in the study groups

Groups	Age	
	Mean	SD
Group I	31.64	11.15
Group II	51.43	15.94
Group III	40.89	15.62
Group IV	58.67	14.36
Group V	61.93	14.03
Total	45.53	18.05
F-value	32.4411	
P-value	0.00001*	

The MCCS was highest (1.47) in group V, followed by group III(1.31) and lowest in groups I (0.91)(table3)

Table 3: Comparison of Mean candidal carriage score (MCCS) amongst the five study groups by Kruskal Wallis ANOVA test

Mean candidal carriage score (MCCS)				
Groups	Means	Std.Dev.	Median	Sum of ranks
Group I	0.91	0.61	0.75	5037.00
Group II	1.21	0.81	1.00	5341.50
Group III	1.06	0.53	1.00	2535.50
Group IV	1.31	0.77	1.40	1999.00
Group V	1.47	0.72	1.42	3423.00
Total	1.14	0.71	1.00	
H-value	14.2377			
P-value	0.0066*			
Pair wise comparison of five groups by Mann-Whitney U test				
Group I vs Group IV	P=0.0347*			
Group I vs Group V	P=0.0007*			
Group III vs Group V	P=0.0202*			

Medical Condition	MCCS
Diabetic	1.196
Hypertension	1.174
Hypothyroidism	1.104
Bronchial Asthma	1.575
Total	1.228

The difference in the MCCS between the five groups was analysed with the help of Kruskal Wallis, ANOVA test and it was found to be statistically significant. (p=0.0066, table 3). Further, a pair wise comparison of the five groups was done using the Mann-Whitney U test and there was a statistically significant difference between group I and group IV(p=0.0347), group I and group V(p=0.0007) and group III and group V(p=0.0202). (table3)

In subjects with tobacco habits, MCCS was highest in smokers (2.81), followed by Betel quid users (1.143) and gutkha chewers (0.715), but this difference was not statistically significant ($p= 0.118$, chi-square statistical test).

Amongst subjects with medically compromised conditions, the MCCS was highest in subjects with bronchial asthma and the least was noted in subjects with hypothyroidism (table 4). It was noted that the MCCS increased with the duration of asthma, whereas in subjects with hypothyroidism, the MCCS decreased with the duration of condition. In subjects with hypertension and diabetes, the MCCS were not influenced by the duration of the conditions (table 5). The MCCS was also correlated with the type of medication as elaborated in table 6.

Table 4: Type of medical condition and MCCS

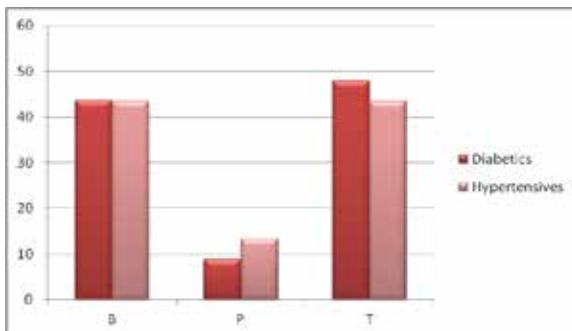
Table 5: Comparison of the duration of medical conditions with MCCS

Medical conditions	Duration	MCCS
Bronchial asthma	6mths-5 yrs	0.75
	6years-10yrs	0.91
	11-15yrs	1.80
	>16yrs	3.25
hypertension	6-10 yrs	1.00
	11-15yrs	1.08
	>16yrs	0.50
Diabetes	6mths-5 yrs	1.25
	6-10 yrs	1.25
	11-15 yrs	1.07
hypothyroidism	6mths-5 yrs	1.20
	6-10 yrs	0.91

Table 6: Comparison of the type of medication with MCCS

Medical condition	Type of medication	MCCS
Hypertension	ACE inhibitors	1.00
	Diuretics	0.75
	Calcium channel blockers	1.05
	Beta blockers	1.16
Bronchial asthma	Beta-2-agonists	1.25
	Inhaled corticosteroids	1.85
	Combination	0.50
Diabetes mellitus	Oral hypoglycaemic agent+diet	1.03
	Insulin+diet	0.75

In subjects with diabetes and hypertension, buccal mucosa and tongue presented with a heavy load of candida as compared to palate. (figure1)



Twenty seven subjects in the study presented with potentially malignant conditions. It was found that the

MCCS was highest for subjects with oral leukoplakia (1.25), followed by subjects with OSMF (1.071), and oral lichen planus (0.985).

Among the 25 denture wearers, 9 patients presented with candidal lesions and 19 did not present with any lesions. The MCCS was higher (2.06) in subjects with lesions as compared to subjects without lesions who had a MCCS of 1.331.

Group V comprised of 28 subjects with multiple predisposing factors for candidiasis. Of these, 9 subjects were medically compromised and tobacco users, 3 subjects were denture wearers and tobacco users and 16 subjects were medically compromised and were also denture wearers. It was noted that the subjects with medically compromised conditions and dentures had a high mean candidal score of 1.836, followed by subjects with medically compromised conditions and tobacco habits who had a MCCS of 1.028, and finally subjects with tobacco habits and denture had a MCCS of 1.011. The difference in the MCCS amongst the above three groups was found to be significant. ($p=0.008$, chisquare statistical test, table 7)

Table 7: Comparison of MCCS amongst subjects in Group V

Combinations	No of subjects	MCCS
Tobacco users + medically compromised conditions	9	1.028
Tobacco users + denture wearers	3	1.011
medically compromised conditions + dentures wearers	16	1.836

Out of 857 sites assessed, 37.34% had an insignificant load of candida, 27.07% had low load, 17.74% had moderate load, 10.50% had heavy load and 7.53% had very heavy load of candida. There was no significant correlation between the site and candidal load.

Out of 191 subjects, 59 subjects were unaware of their blood group type. The distribution of the remaining 132 subjects based on their blood group is depicted in table 8. The MCCS was found to be highest (1.29 ± 0.67) in blood group O, followed by blood group A (1.25 ± 0.90) and blood group B (1.02 ± 0.65). However, the difference in the candidal carriage scores between the groups was not found to be statistically significant (table 8). ($p=0.2417$, Kruskal Wallis ANOVA test)

Table 8: Comparison of MCCS in subjects with different blood groups using Kruskal Wallis ANOVA test

Blood groups	Mean	Std.Dev.
A+	1.25	0.90
A-	1.17	0.91
B+	1.02	0.65
B-	0.25	0.00
O+	1.29	0.67
O-	0.50	0.71
AB+	0.95	0.57
H-value	7.9517	
P-value	0.2417	

DISCUSSION:

The present study assessed the association of certain local and systemic predisposing factors with candidal carriage.

The association of tobacco habits and candidiasis is unclear. A candidal carriage rate of 30 to 70 % has been reported in smokers. It has been postulated that the epithelial alterations caused by the cigarette smoke favors candidal growth. Further, it has been suggested that the nicotine and polycyclic aromatic

hydrocarbons contained in the smoke provide nutrition for *Candida* species thereby facilitating their proliferation.^{1,4} On the contrary, a few researchers^{5,6} have found no association between smoking and oral colonization with *Candida* species in healthy subjects.

Gutkha chewers were found to have an increased candidal carriage in a study. This was attributed to the reduced salivation in gutkha chewers which alters the oral microflora thereby favouring candidal growth.⁷ However, a study conducted in 2011 in India opined that, the slaked lime content of gutkha creates an alkaline pH in the oral cavity which is unfavourable for candidal growth.⁸

Nicotine may act as a nutrient for *Candida* species, thus increasing the candidal load in betel quid chewers with tobacco.⁷ However, a recent study done in 2013 reported that chewing BQ (with or without tobacco) did not influence oral *Candidal* carriage.⁹ Similar results have been reported in the limited studies⁷ which assessed the oral *Candida* carriage in betel quid chewers.

In the present study, the M CCS was found to be the least in subjects with tobacco habits in comparison to the other groups. This suggests that tobacco probably does not influence candidal carriage to a large extent. Amongst the subjects with tobacco habits, the M CCS was higher in smokers (cigarettes/beedis) than betel quid chewers and gutkha chewers.

In the medically compromised group, moderate M CCS was obtained in comparison to other groups in the present study. Asthmatics had the highest M CCS,

followed by diabetics, hypertensive and hypothyroid subjects. Beta -2 agonists, a common drug used by asthmatics is known to reduce salivary flow which may predispose individuals to candidal infection.¹⁰ Inhalational corticosteroids, another drug used by asthmatics results in the local deposition of glucocorticoids which leads to a reduction in the number of circulating T cells in turn increasing the risk for oropharyngeal candidiasis.¹¹ In the present study, most of the asthmatics were either on beta -2 agonists or inhalational corticosteroids exclusively and one subject was on a combination of the two. It was noted that subjects using inhalational corticosteroids had the highest M CCS. It was also observed that the M CCS increased with the duration of asthma. All the subjects used dry powder inhalers (DPIs) and none used Metered Dose Inhalers (MDIs). It has been reported that DPIs significantly lower salivary pH when compared to MDIs thus favoring candidal growth.¹⁰ In the present study, as all the asthmatics used DPIs, it may be inferred that they are more prone to develop candidiasis.

Diabetes mellitus has been associated with increased candidal carriage for a number of reasons. In the presence of glucose, a sticky extracellular layer is formed around the candidal organism which increases their adherence. Glu-

cose also serves as a growth medium for *Candida*. Pyruvate, a product of glucose utilization reduces the salivary pH harbouring candidal growth.² A study conducted in 2005 in India reported a high incidence of candidal carriage in diabetics. They however concluded that age, sex and duration of diabetes did not influence the degree of candidal carriage and reported that anti-diabetic

therapy and glycaemic control in diabetes did not influence the carriage status.⁹ In the present study duration of diabetes did not seem to influence the M CCS. Further the M CCS was not influenced by the type of medication as no significant difference in candidal load was found between diabetics on oral hypoglycaemic agents (OHA) diabetics on insulin. This finding is in accordance with the above mentioned studies.

Antihypertensive medications such as angiotensin converting enzyme inhibitors, alpha and beta-adrenergic blockers, calcium channel blockers, diuretics are known to be xerogenic. Chronic usage of the above medications leads to reduced salivary flow.¹² In the present study, The M CCS did not correlate with duration of hypertension. Further, hypertensive subjects on beta blockers had a higher M CCS as compared to subjects taking ACE inhibitors, Calcium channel blockers and diuretics.

It has been suggested in literature that hypothyroidism causes atrophy of the salivary glands. It has also been demonstrated in that the salivary flow, pH and buffering capacity are low in hypothyroid patients as compared to healthy subjects. Hypothyroid state also reduces the cellular immunity. Thus it may be hypothesised that alterations in the saliva and depressed cellular immunity predispose hypothyroid subjects to candidiasis. Furthermore, it has been reported that attainment of euthyroid state results in complete cure of candidal lesions.¹³ This may be attributed to the improved salivary flow secondary to administration of thyroxine. In the

present study also, the M CCS was found to decrease as the duration of treatment increased.

Potentially malignant conditions are associated with alterations in thickness of the epithelium. Atrophy, hyperplasia or dysplasia affect the barrier mechanism of the mucosa and predispose to candidal colonization.^{2,14} Studies have revealed a higher candidal prevalence in OSMF patients¹⁵, Oral lichen planus and leukoplakia when compared to a control group.^{14,16} Further, a high incidence of oral dysplasia and squamous cell carcinoma has been noted in potentially malignant conditions associated with *Candida*.¹⁴

In the present study, the M CCS was found to be quite less in comparison to all the other groups except the group I with tobacco habits. M CCS was highest in the leukoplakia group, followed by OSMF and lichen planus groups. This is in accordance with another Indian study which reported similar findings.¹⁴

In vitro studies illustrate that *Candida albicans* attaches readily to various resins, glass, and metal surfaces due to its attractive hydrophobic and repulsive electrostatic forces. It has been seen that Denture stomatitis is frequent among denture wearers and the prevalence ranges from¹⁷ 10% to 75%. In the present study, M CCS in denture wearers was found to be higher than most other groups except the group V ranking it the most important single predisposing factor for candidal carriage. Further, M CCS was found to

be higher in patients clinically diagnosed as denture stomatitis than in patients with no candidal lesions.

The Group V consisted of subjects with the highest MCCS suggesting that subjects with multiple predisposing factors have a higher probability of candidiasis. The highest MCCS was noted in subjects with medically compromised conditions and dentures.

Mucins present in saliva have a role in tissue protection and modulation of oral micro flora and are the primary carriers of ABH antigen. It has been suggested in subjects with blood group O, the inability to secrete ABH antigen in saliva is a risk factor in the development of candidiasis^{2,18}. It has also been suggested that individuals with blood group O present with H antigen and Le^a antigen on their epithelial cell surfaces which act as binding sites for candidal colonization. However, the correlation of blood group secretor status and candidal carriage is inconclusive as contradictory results have been reported.¹⁸ In the present study, blood group O was associated with a higher MCCS in comparison with subjects of other blood groups, although it was not statistically significant.

CONCLUSION:

In the present study, subjects with multiple predisposing factors were prone to increased candidal carriage than subjects with a single predisposing factor. Amongst subjects with a single predisposing factor, denture wearing was found to be the most important predisposing factor affecting the candidal carriage followed by medically compromised conditions. Bronchial asthma was found to have the highest association with candidal carriage amongst the medically compromised group. Subjects with hypertension and hypothyroidism had a low candidal carriage. Subjects with tobacco habits and potentially malignant conditions had a low MCCS suggesting their negligible role in candidal carriage. Individuals with Blood group O had higher

candidal carriage although the difference was not statistically significant. It may be inferred from the present study that hypertension, hypothyroidism, tobacco habits and blood group secretor status do not influence the candidal carriage status to a large extent. However, larger samples need to be included to arrive at definite conclusions.

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