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RESEARCH ARTICLE

A STUDY TO ISOLATE AND SPECIATE CANDIDA IN COMPLETE DENTURE WEARERS AND ITS RELATION TO DENTURE HYGIENE

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ABSTRACT

Objective: The purpose of this study was to determine the prevalence of denture induced stomatitis (DIS) in denture wearers in this part of India and to analyse the shift in epidemiology from Candida albicans to non albicans Candida (NAC) species and their antifungal resistance.

Materials and Methods: Our study included 30 patients who were subjected to multiple choice questionnaire followed by clinical evaluation. The denture hygiene was assessed using a disclosing agent which was applied on fitting surface of maxillary dentures to assess the quantity of plaque. Based on Budtz- Jorgensen's index, dentures were rated as: Excellent, Fair, Poor. The observations were tabulated and subjected to statistical analysis (chi- squared test). Oral candida colonization was assessed with concentrated oral rinse technique. The mouth rinse was collected and sent for further processing and sensitivity tests to widely used antifungal agents like voriconazole and fluconazole.

Results: A significant correlation between DIS and poor denture hygiene (85%) and emerging resistance to common antifungal drugs was established.

Conclusion: Our study indicated that the predisposing factors to DIS are associated with the method of denture hygiene and use of dentures while sleeping. Also, a shift in epidemiology towards antifungal resistant NAC is a worrying trend in context of their tendency to cause fungaemia.

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INTRODUCTION

Candidiasis is a curse of the prosthesis and the only spell against it is denture hygiene. Edentulous condition makes it necessary that people wear dentures which results in changes in the oral micro- environment. Studies have shown that majority of denture wearing population are failing to maintain their denture hygiene (Sadig, 2010) which is an important etiologic factor in a condition known as denture induced stomatitis (DIS) which manifests as erythematous, edematous mucosa confined to denture bearing area. Studies have shown that 70% of individuals with clinical signs of denture stomatitis exhibit fungal growth, (Sadig *et al.,* 2010) the most common oral yeast infection caused by members of the genus Candida. The genus candida is predominated by the species Candida albicans but there are various other species which are together known as the non albicans Candida (NAC). A lot of attention is being paid to NAC these days owing to their presence in systemic infections in vulnerable elders (Meurman *et al.,* 2007; Larone, 1987).

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Therefore, the need for this study was to determine the prevalence of DIS in denture wearers in this part of India and to analyse the shift in epidemiology of candida species through isolation and speciation and also to determine their antifungal resistance. This was done by assessing the denture hygiene levels and denture induced stomatitis objectively and subjectively via a questionnaire; isolation and speciation of candida; to correlate denture hygiene and to assess sensitivity of candida species to antifungal drugs.

MATERIALS AND METHODS

The study included 30 old complete denture wearers visiting the Department of Prosthodontics in SDM College of Dental Sciences and Hospital, Dharwad. The study was initiated after obtaining ethical clearance from the Institutional Review Board. The purpose of the study was explained to all participants and the informed consent was obtained. To standardize the effect of diurnal variations, the investigations were carried out at midmorning at least 2 h after drinking, eating or any oral hygiene procedure. A multiple choice questionnaire (Annexure-1) was presented to the participants.

The questionnaire had 3 parts for recording the Participants demographic data

- Objective and subjective problems in relation to the denture
- Denture hygiene habit practiced by the participants

This was followed by clinical evaluation for presence of denture induced stomatitis and papillary hyperplasia and graded as mild or moderate. The cleanliness of the dentures was assessed using Budtz- Jorgensen's index of denture cleanliness. Examination was made using a disclosing agent which was applied on fitting surface of maxillary dentures to disclose the plaque and its quantity. Based on staining the dentures (Fig-1) were divided into 3 groups:

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

The observations were tabulated and subjected to statistical analysis (chi- squared test). Oral Candida colonization was assessed with concentrated oral rinse technique. Each participant was given 10ml of sterile phosphate buffer saline solution (0.1M, 7.2 pH) and was asked to rinse mouth for 60s with this solution. The mouth rinse was collected in sterile container and was sent to Department of Microbiology in SDM College of Medical Sciences, Dharwad, for further processing (Boparai *et al.,* 2014; Arirachakaran *et al.,* 2013). Oral rinse samples were then inoculated onto chromagar plates. The oral rinse was centrifuged prior to plating. A drop of oral rinse sample was taken using an inoculating loop and spread across the plate. The plates were then inoculated for 48 h at 37°C. The number of colonies on each plate was counted after 48 h incubation and number of colony forming units were counted and tabulated (Fig-2). The suspected growth of candida species was confirmed by Gram's staining. Speciation of each isolate was performed by Germ Tube Test and depending on the morphology of growth on corn meal agar. Confirmation of species was done by axonometric plate method of sugar assimilation test. The sensitivity of these candida isolates to widely used clinical antifungal agents like voriconazole and fluconazole was performed.

RESULTS

A total of 30 participants, 70% males and 30% females were examined. DIS occurred in 36.6%participants. However, no significant difference in DIS incidence as related to age or gender of the participants was found. A significant association was seen between presence of denture stomatitis and denture hygiene habits and sleeping with dentures (Table-1). On objective assessment of hygiene levels majority of participants with poor denture hygiene showed greater incidence of DIS (85%)**. (**Table-2**)** The methods of cleaning dentures included brushing, soaking in water/solution or brushing and soaking. Participants who practiced denture hygiene by brushing as well as soaking showed least incidence of DIS (47%). (Table-3) A majority of participants used to keep their dentures in water when not in use (93%). Those who left in open or kept it moist with wet cotton or wet cloth showed definite presence of DIS.

Figure 1.

Figure 1.

Table 1. Association between presence of denture stomatitis and sleeping with dentures

Sleeping with denture	Stomatitis		Total
	Absent	Present	
Yes	3	3	6
no	16	8	24
total	19	11	30

Table 2. Association between denture hygiene and DIS

Method of cleaning	Stomatitis		Total
	Mild	Moderate	
Brushing only	1	2	3
Soaking in Water	1	1	2
Brushing and Soaking	17	8	25
Total	19	11	30

Table 3. Association between method of cleaning denture and DIS

Budtz- Jorgensen's index	Stomatitis		Total
	Mild	Moderate	
Excellent	9	1	10
Fair	10	2	12
Poor	0	8	8
Total	19	11	30

Table 4. Budtz –Jorgensen's index and DIS

GRADE	Mean	N	Std. Deviation
Mild	77.37	19	228.778
Moderate	229.18	11	383.071
Total	133.03	30	297.698

Table 6. Antifungal sensitivity of Candida albicans and NAC to Fluconazole and Voriconazole

CANDIDA TYPES	Fluconazole		Voriconazole	
	Sensitive	Resistant	Sensitive	Resistant
Candida albicans	93%	7%	93%	7%
Non albicans Candida (NAC)	63%	37%	72%	27%

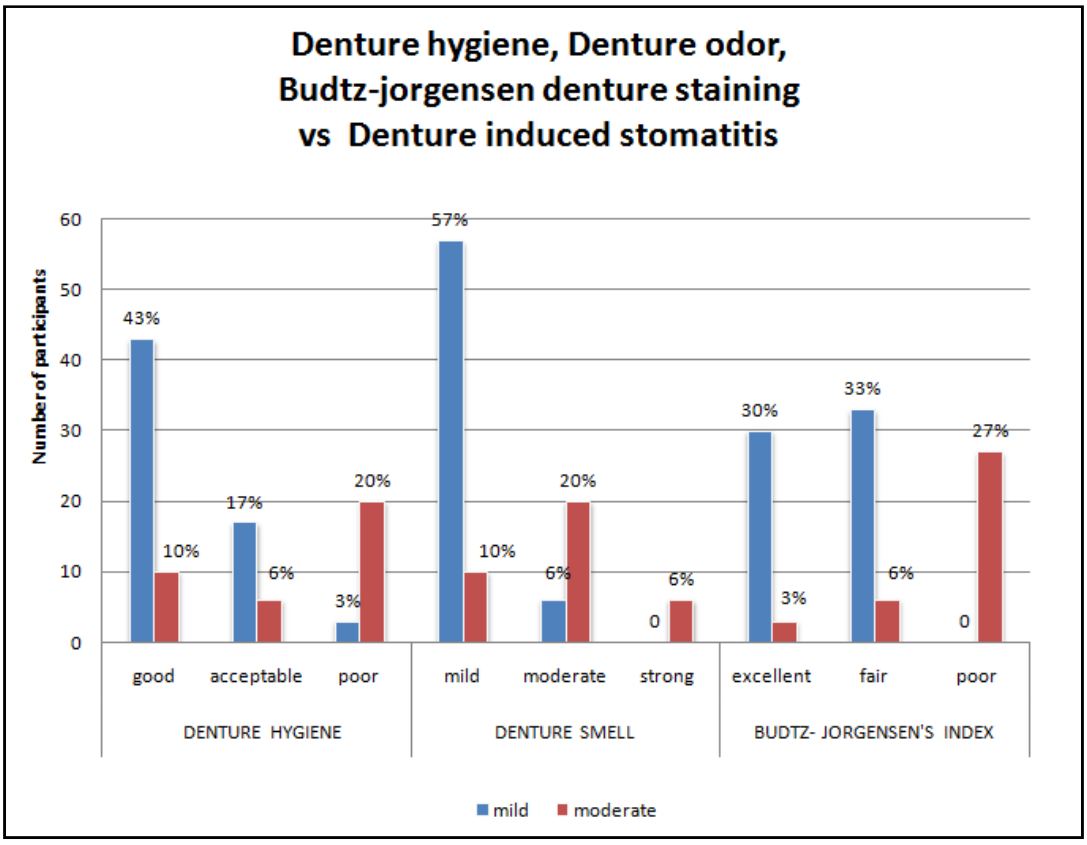

Figure 3.

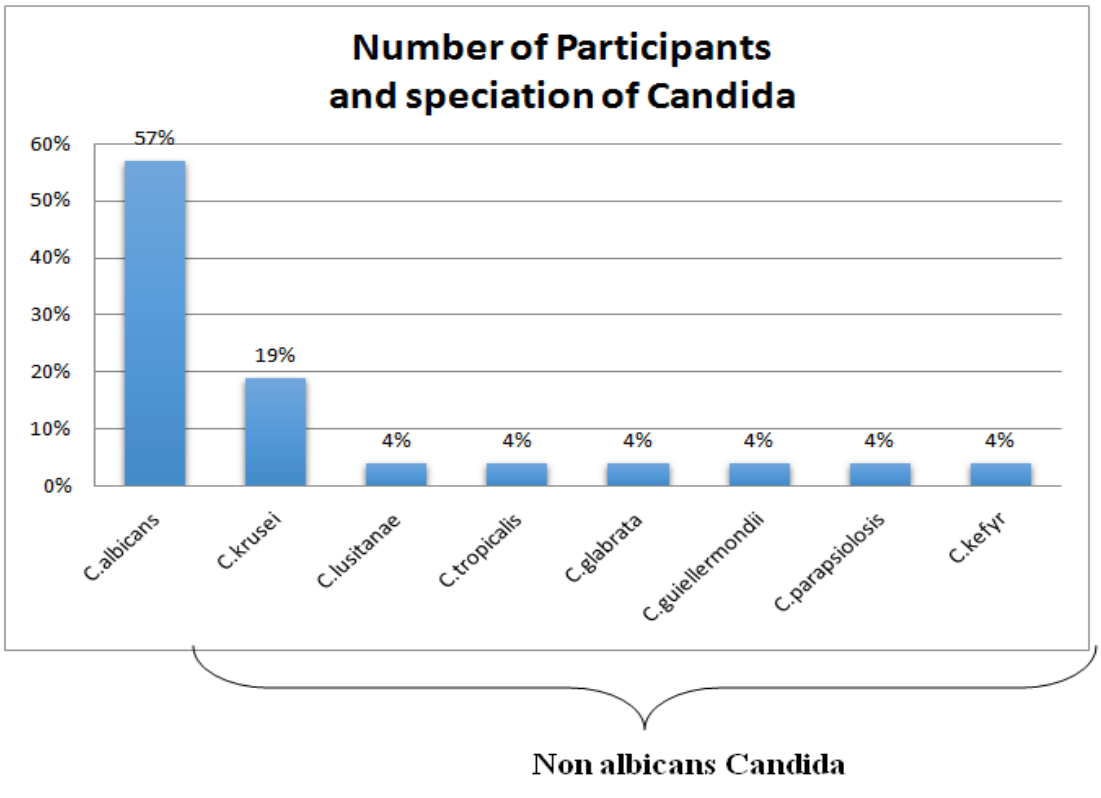

Figure 4.

According to Budtz-Jorgensen's index of staining a definite presence of DIS was seen in dentures which were rated poor. (Table-4) A positive correlation was established between denture hygiene, denture odor, Budtz- Jorgensen staining with denture stomatitis (Fig-3). On isolation the predominant species was found to be C. albicans (57%). The rest was formed by group of various other species of candida together known as the Non Albicans candida among which Candida krusei was the predominant species (19%). (fig-4) The results of our study showed a higher value of mean colony forming unit in participants who slept with their dentures (340.33); dentures where hygiene levels were rated poor (310); also in those dentures which were rated poor according to Budtz-Jorgensen's index (307.50). The results of our study showed no significant co-relation between the type of cleansing agent and mean CFU number. A significant relation between mean CFU number and denture stomatitis was also established. (Table-5) The results of the sensitivity tests showed a majority of C. albicans being sensitive to commonly used antifungal agents (Table-6). However, NAC species have shown higher resistance to fluconazole and voriconazole.

DISCUSSION

Previous studies of different populations have shown a variation in the prevalence of denture stomatitis from 11 to 67% (Kulak-Ozkan *et al.,* 2002; Sadig, 2010; Budtz-Jörgensen *et al.,* 1972). Different diagnostic criteria or differences in the groups could explain the variation. The present study showed a prevalence of 36.6% which was similar to Ozkan et al,[2] and Sahebjamee *et al.,* (2010) and differ greatly from the 65% found by Budtz-Jorgensen *et al.,* 1975 and the 50% found by Sadig *et al.,* 2010 The differences may be explained by different diagnostic criteria or differences in the groups such as age, sample size or even the influences of other factors such as drugs or diseases. Symptom-free oral carriage of candida organism has been recognized for many years and the reported prevalence in clinically normal mouths of healthy adults range from 3 to 48%. In our study significant candidal colonization was identified in 73 % of participants irrespective of presence or absence of DIS. This disparity may reflect differences in sampling technique, the sites sampled and the selection of participants. The correlation between poor denture cleanliness and denture stomatitis was statistically significant. This finding supports those of Budtz-Jorgensen and Bertram, 1970; Kulak Ozkan, 2002 and Sadig *et al.,* 2010 Because denture plaque is an important factor in stomatitis in participants who wear dentures, cleaning of dentures and the removal of plaque are obviously important steps in the maintenance of good oral health. The relationship between frequency of denture cleaning, denture stomatitis and yeast colonization showed that more stomatitis and yeast colonization was seen in participants who wore dentures throughout the day as found in our study and also by Sadig (Sadig *et al.,* 2010). Effective plaque removal requires a degree of manual dexterity that is often lacking, especially among older adults. The finding of our survey is similar to those of earlier workers that many older adults apparently do not know how to keep dentures clean (Budtz-Jorgensen and Bertram *et al.,* 1970; Kulak Ozkan, 2002; Sadig, 2010). It is clearly an important duty of the dental profession, at a time when the older population is rapidly rising, to ensure that the denture wearing public know how to clean their dentures properly. The predominant candida species among denture wearers were C. albicans, C. krusei, C. tropicalis, C. lucitone, C. guellermondi, C. kefyr, C. parapsiolosisis. This was different from the report by Prakash *et al.,* 2015 and Arirchakaran *et al.,* 2013 in which C. tropicalis was predominant species among NAC and other reports wherein C. glabrata was the dominant species (Vanden Abbeele *et al.,* 2008). In our study C. krusei and C. guiellermondii were found to be less susceptible to common antifungal drugs than C. albicans. Thus identification of candida species has been found to be increasingly important for determining the appropriate course of treatment. However, it is not clear yet whether species co-existence plays an integral or antagonistic role in pathogenesis and virulence. Furthermore, the co-existence of mixed species could complicate treatment modalities. Whether the co-existence of species is limited to certain combinations of species, and whether the co-existence is mutually beneficial, have yet to be determined (Arirachakaran *et al.,* 2013). A significant epidemiological shift to higher isolation of NAC species was noticed. The high usage of fluconazole appears to have played a role in this shift, however, it may be recognised that other events like participant's specific risk factors might have also contributed for selection of few species (Oberoi *et al.,* 2012). Decreased susceptibility to fluconazole with cross resistance to voriconazole in the background of high azole consumption may limit the use of these agents as empirical therapy for candida before species identification and results of antifungal susceptibility testing are known.

Conclusion

Our study indicated that the predisposing factors to DIS are associated with the method of denture hygiene and use of dentures while sleeping. This makes it important that we as dentists provide post insertion denture hygiene instructions and motivate them to brush their dentures and soak them in water when not in use and to avoid wearing their dentures during sleeping. Proper education and motivation would improve the quality of life of the denture wearing population. Our study shows a shift in epidemiology towards NAC which is a worrying trend in context of their tendency to cause fungaemia. Also, the rise of resistant species makes it important that changes need to be made in the present regimen for treatment of fungal infection. This also makes the role of a dentist important as oral cavity is the most common route for systemic candida infections.

The following conclusions were drawn from our study:

- 36.6% of participants from our sample size showed the presence of DIS
- A positive correlation was established between denture odour, denture hygiene, and denture staining with DIS.
- The commonly isolated species in our sample group were: C. albicans (57.6%), C. krusei (19.2%), C. tropicalis, C. lucitone, C. guellermondi, C. kefyr, C. parapsiolosisis constituting the rest.
- The predominantly isolated NAC species in our study was found to be C. krusei
- The candida colonization was found to be less in the participants who use to wear their dentures throughout the day and practiced the habit of sleeping with the denture.
- C.albicans was found to be quite susceptible to the antibiotics used in our study.
- Among NAC, C. krusei, C. guiellermondii were found to be resistant to antibiotic i.e. voriconazole and fluconazole.

The limitations encountered in our study were:

- A limited sample size. The study needs to be done on a larger sample size.
- The presence of DIS must be evaluated among diabetic and nondiabetic individuals
- C.albicans and NAC percentage needs to be evaluated in diabetic and nondiabetic
- Antifungal susceptibility of C. albicans and NAC needs to be studied in diabetic and nondiabetic participants
- The next step would be analytical studies with larger subject cohorts to determine, if preferential co-existence of particular candida species can be linked to increased risk of denture stomatitis or its severity.

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