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Oral *Candida albicans* and its correlation with caries in children *in vivo*

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ABSTRACT

Background/objectives: *Candida spp.* has been detected in the mouths with extensive carious lesions in adults as well as children with variable frequency in different geographical areas. In this study we evaluated the prevalence of *candida spp.* in the oral cavity of children of central India with and without dental caries.

Method and materials: Children (3–12 years) were examined for decayed, missing and filled teeth (deft/DMFT) scores. Children (n-25) having ≤3 score made control group while those (n-25) having score ≥4 made experimental group. Mycological examination was conducted on the collected saliva sample of selected children. Oral yeast colonization was assessed by culturing *Candida albicans* on suitable broth. Collected data was then statistically analyzed.

Results: The CFU/plate of candida in experimental group was statistically higher than in control group children. Spearman's rank correlation ($r_s = 0.643$) showed linear relationship of CFU/plate with deft/DMFT scores.

Conclusion: This study showed the active role of *C. albicans* in dental caries in children of 3–12 years of age group in Indore city of central India.

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1. Introduction

Studies of clinical and microbiological evidence in the past have suggested a correlation between high prevalence of yeast (*candida spp.*) in dental plaque/saliva and occurrence of active carious lesions. The percentage of candida species colonization ranges from 20% to 40% in healthy people and become predominant flora in more than 60% of immunocompromised subjects.[1] Nonetheless in normal individuals even, indirect evidence of association of candida species with dental caries has been suggested in past.[2,3] The authors hypothesized that candida enhanced the adherence of *Mutans streptococci* to the oral biofilm and carious tooth substance in vitro.[2]

In the recent years, there has been an increasing interest in the relationship between oral fungal microflora and dental caries. These studies focused attention on possible direct role of *Candida albicans* (*C. albicans*) in caries etiology of healthy population.[1] Moalic *et al*[3] have linked the presence of *C. albicans* in dental plaque of students to high DMFT index. Apart

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from simple correlation with caries, many studies showed that candida carriage frequency varies not only by age but also according to the geographical area.[4] Although dental caries is a multifactorial disease, it may be interesting to observe that whether its prevalence is constantly increased in children with active caries compared to those with control group children in different geographical areas.

Therefore the study aimed to evaluate the 1/- prevalence of *C. albicans* in 3–12 years old children with and without active caries and also 2/- prevalence of *C. albicans* as related to number of carious lesion in 3–12 years old children in Indore city of central India.

2. Materials and method

The study was approved by the ethical committee of the institute. Fifty patients ranging from 3 to 12 years of age amongst those reporting department of Pedodontics & Preventive Dentistry, Modern Dental College and Research Centre, Indore were enrolled for the study according to the inclusion and exclusion criteria.

Twenty five children with rampant caries, nursing bottle caries, with more than or equal to 4 active carious lesions (deft score ≥ 4 for deciduous dentition & deft + DMFT score ≥ 4 for mixed dentition) and sound periodontal conditions were included in experimental group while 25 children having deft or deft + DMFT score ≤ 3 were included in control group. Children, with prolonged antibiotic treatment in the previous 3 months atleast, fungal infection in the mouth and chronic diseases such as diabetes, allergies etc. were excluded. The parents of all enrolled children were informed about the experimental design and informed consent was obtained.

Collection of saliva: Patients were asked to rinse their mouth with 10 ml of Phosphate Buffer Saline (PBS) for 60 s. This saliva collected from each subject in a sterile universal container was stored in ice and immediately transferred to microbiological laboratory in the institute. The maximum time between sample collection and laboratory processing was 1 h.

Microbiological Procedures: Saliva samples, prior to microbiological lab procedures, were thoroughly shaken for 30 s in a vortex mixer and exposed for further 30 s to an ultrasonic bath. This treatment helped in obtaining the highest disaggregation and dispersion of micro-organisms in the sample without interference with their culturability.

a) **Culturing procedure**: 10 ml of this above treated oral rinse was centrifuged at 4000 rpm for 10 min. The collected sediment was added to 1000 μl of normal saline solution to build up the quantity to 1 ml 50 μl of concentrated oral rinse was then inoculated on Hichrome Agar Medium plate (Himedia) and CFU/plate (Colony forming unit per plate for the colony growth of *C. albicans*) was assessed.
b) **Culturing and colony counting**: The culture plates were labelled and enclosed in an aluminium foil. These plates were then further incubated aerobically in a Biological oxygen demand chamber (BOD) at (35 ± 0.5 °C) for 48 h. Colony counting and its characteristics that appeared on the culture plates were carried in an inoculation chamber with naked eye by the help of magnifying lenses.
c) **Colony identification and characteristics**: *C. albicans* typically grew as spherical to oval budding yeast cells 3–5 × 5–10 μm in size on Hichrome Candida Differential Agar (HCDA) media as light green coloured smooth colonies.

Percentages, means, standard deviation (SD), median, minimum and maximum values of variables in experimental group and control group were calculated. Categorical variables were analyzed using Pearson's chi-square test. Shapiro–Wilks test showed that age, CFU per plate of *C. albicans* and deft/DMFT scores did not follow normal distribution. Hence for comparison between experimental group and control group non-parametric test, Mann–Whitney U test was applied. Correlation between CFU/plate of *C. albicans* and deft/DMFT scores was evaluated by Spearman's rank correlation test. P values <0.05 were considered statistically significant and data analysis was done using Statistical Package for Social Sciences (SPSS v.21.0, IBM, Armonk, New York) for windows.

3. Results

Among the experimental group, 22 (88%) subjects had *C. albicans* colonies whereas in control group 16 (64%) had *C. albicans* colonies. Pearson's chi-square test showed significant difference ($\chi^2 = 3.947$, $P = 0.047$) between the two groups for presence or absence of *C. albicans* colonies (Table 1). Mann Whitney comparison between two groups for values of CFU/plate of *C. albicans* showed significantly higher number of CFU/plate in experimental group than control group (MW = 89.500, $P = 0.000$) (Table 2 and Fig. 1).

Experimental group was further categorised according to deft/DMFT scores i.e (1) deft/DMFT range 4–8 and (2) deft/DMFT range 9–12. Control group was categorised in deft/DMFT range 0–3. Pearson's chi-square test showed significant difference ($\chi^2 = 21.413$, $P = 0.000$) for CFU/plate of *C. albicans* among different deft/DMFT scores (Table 3, Fig. 2).

Spearman's rank correlation showed significant relationship of CFU/plate with deft/DMFT scores ($P = 0.000$). Spearman's rho ($r_s = 0.643$) revealed a strong positive relationship between these two variables. There was a linear relationship of CFU/plate with deft/DMFT scores (Table 4, Fig. 3).

4. Discussion

Candidal counts can be misleading if proper sampling and identification methods not followed. Among various methods like swab samples, oral rinse & imprint culture etc., oral rinse with phosphate buffer was used as probability of false negative culture is less in this technique.[5]

The study showed that *C. albicans* carriage in experimental group (88%) was much more than control group (64%) i.e. children without active caries (Table 1). The results were similar to the study by Hodson and Craig[6] who found that the *C. albicans* in the biofilm of ECC (56%) were twice more prevalent than that in children without active caries (33%).

Table 1 – Comparison of presence or absence of *C. albicans* colonies between control group and experimental group.

Candida albican's colonies	Groups		Total {n (%)}	Chi-square test value	P value
	Control group {n (%)}	Experimental group {n (%)}			
Yes	16 (64.0)	22 (88.0)	38 (76.0)	3.947	0.047 (<0.05), Sig diff.
No	09 (36.0)	03 (12.0)	12 (24.0)		
Total	25 (100.0)	25 (100.0)	50 (100.0)		

Table 2 – Comparison of Colony forming unit (CFU) per plate of *C. albicans* between control group and experimental group.

Groups	CFU/Plate			Mann Whitney U test value	P value
	Mean ± SD	Median	Min-max		
Control group (n = 25)	1.80 ± 2.27	1.00	0–9	89.500	0.000 (<0.001), Sig diff.
Experimental group (n = 25)	11.08 ± 8.58	10.00	0–30		
Total (n = 50)	6.44 ± 7.78	3.00	0–30		

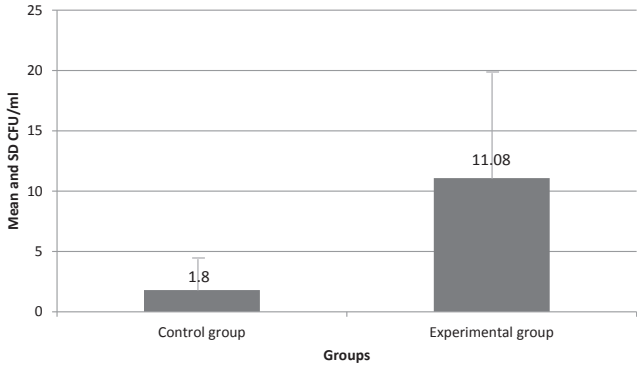


Fig. 1 – Comparison of Colony forming unit (CFU) per plate of *C. albicans* between control group and experimental group.

In the study by Sziegoleit *et al*[7] candida species were isolated from saliva of 66.7% of subjects with active caries, but only 2% from saliva of caries free subjects. Akdeniz *et al*[8] reported that 69% of childrens were found to be carriers of candida and the difference in candida prevalence between caries active and caries free groups was significant. Similar to their finding we also found high association of candida species in caries active (experimental group) children. The high occurence of *C. albicans* in experimental group may be because of formation of biofilm which is a major virulence factor in the pathogenicity of candida. Also candida biofilms are difficult to eradicate with routine oral hygiene methods.[9]

There may be some more reasons associated with increased prevalence of *C. albicans* in experimental group. It has been found that the children with ECC have a frequent habit of night bottle feeding too. Milk itself contains lactose, which, following sucrose addition, is degraded to glucose and galactose. Carvalho *et al*[10] performed a study on 2–5 years old children in Brazil and found that the outbreak of *C. albicans* in ECC group (60.4%) was significantly much more than control group (14.3%). They suggested that in an infant with ECC there is higher adhesion of *C. albicans* due to presence of above sugars. Kukletova *et al*[11] also reported that *C. albicans* and other fungi are constantly found in the dental plaque of children suffering from ECC suggestive of their contribution in fermenting carbohydrate, leading to the destructive course of the disease. Therefore *C. albicans* probably is indirectly related to deft/DMFT count i.e. sugars need to get fermented for its adherence on the tooth surface. *C. albicans* ferments the sugar component like glucose and maltose, producing acid and gas.[12] At pH 7, *C. albicans* produces 5-fold more acid per colony forming unit than lactobacilli. This contribution of *C. albicans* to total microbial acid production appears to be relevant for progression of caries.[13]

To know the CFU/plate counts of *C. albicans* for different deft/DMFT scores, experimental group and control groups were further split into three categories i.e. deft/DMFT range 0–3, 4–8 and 9–12 and CFU/plate counts (0–10, 11–20 and 20–30). Our study may be the first suggesting that *C. albicans* may be associated with caries initiation owing to strong positive co-relation of deft/DMFT scores and CFU/plate of *C. albicans* (Table 3, Fig. 2). Also Spearman's rho (r_s = 0.643)

Table 3 – Comparison of Colony forming unit (CFU) per plate of *C. albicans* according to deft/DMFT scores.

CFU/Plate	Experimental group (n = 25)		Control group (n = 25)	Total (n = 50)
	deft/DMFT range 4–8 {n (%)}	deft/DMFT range 9–12 {n (%)}	deft/DMFT range 0-3{n (%)}	deft/DMFT range 0–12
0–10	12 (57.1)	01 (25.0)	25 (100.0)	38 (76.0)
11–20	06 (28.6)	03 (75.0)	00 (0.0)	09 (18.0)
21–30	03 (14.3)	00 (0.0)	00 (0.0)	03 (6.0)
Total	21 (100.0)	04 (100.0)	25 (100.0)	50 (100.0)

Chi-square test value = 21.413, P value = 0.000 (<0.001), Significant difference.

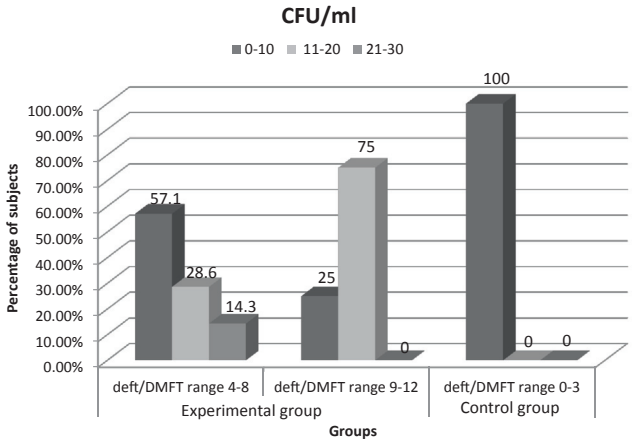


Fig. 2 – Comparison of Colony forming unit (CFU) per plate of *C. albicans* according to deft/DMFT scores.

Table 4 – Correlation between Colony forming unit (CFU) per plate of *C. albicans* and deft/DMFT scores.

Correlation between	Correlation coefficient (Spearman's rho)	P Value
CFU/plate and deft/DMFT scores	0.643 Strong positive relationship	0.000 (<0.001), Significant correlation

reveals that as number of CFU/plate increases deft/DMFT scores also increases and vice versa (Table 4, Fig. 3). Our results support the explanations by Ten Cate *et al*[14] who suggested that *C. albicans* adhere to hydroxyapatite (HAP) of enamel specifically through electrostatic interaction and probably does not need sugar fermentation for its adherence on enamel. Although this adherence is in a much smaller number, they possess the ability to dissolve HAP to a greater extent (approximately 20-fold) when compared with *S. mutans*. Also *C. albicans* has got high collegenolytic activity and can adhere to the intact and denatured collagen from dentin.[15] Therefore, on the basis of results, the role of *C. albicans* can be said to be of initiator of dental caries, but future studies are needed to validate the statement.

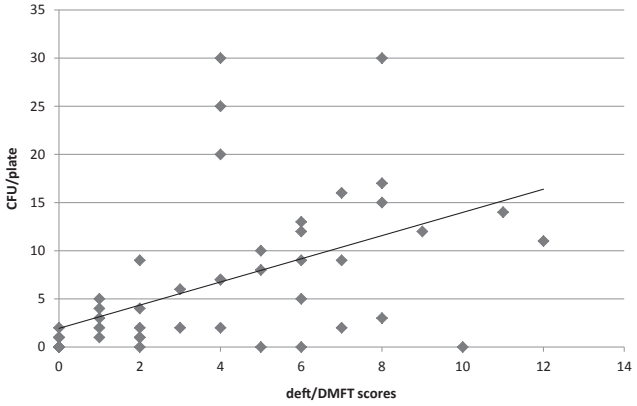


Fig. 3 – Correlation between Colony forming unit (CFU) per plate of *C. albicans* and deft/DMFT scores.

5. Conclusion

Within the limitation of the study, following conclusion could be deduced-

1) Prevalence of oral *C. albicans* was significantly higher in experimental group children indicating its active role in extensive carious lesions, in Indore city of central India.
2) Strong positive co-relation between CFU/plate and deft/DMFT count is suggestive of its role in dental caries initiation.

Conflicts of interest

All authors have none to declare.

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