ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4

Journal of Coastal Life Medicine

Association of Candida Albicans Carriage and Dental Caries in Children

Received: 19 February 2023, **Revised:** 21 March 2023, **Accepted:** 23 April 2023

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Keywords:
Dental caries, C. albicans, Candidal carriage, cariogenicity.

Abstract
Background: In the recent years C. albicans has been identified by researchers for having an important role in cariogenicity along with bacteria like S. mutans and lactobacilli, attributing to its acidogenic, aciduric and hetero-fermentive properties.
Objective: The present study evaluated the frequency, intensity, and species of oral Candidal carriage in children with primary and mixed dentition and to identify the association between oral Candidal carriage in children and dental caries.
Participants: 100 healthy children of age groups: group A: 4-7 years; and group B: 8-12 years, both caries active (divided into dmf/dmf+DMFT index 1-5, 6-10 and >10) and caries free were randomly selected for the study.
Study design: After a detailed intraoral examination, swab samples were taken from tongue and carious lesion for mycological examinations isolate Candida from their oral cavities in order to study the Candidal carriage in children with and without dental caries.
Results: Candidal carriage among children with high caries index was significantly higher that caries free children. 92.86% of the children from group A (4-7 years) having dmf index > 10 and 76.93% from group B (8-12 years) having dmf + DMFT index > 10 showed positive candidal carriage. Also, carriage of non albicans species of Candida increased with age.
Conclusion: The present study suggests a strong relation between Canddial carriage and dental caries in children. Further work needs to be done on larger samples recruited in community-based settings to substantiate the association of oral Candidal carriage with dental caries.

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1. Introduction:

Oral cavity is inhabitated by over 400 species of microbes [1]. The percentage of Candidal colonization in oral cavity is found in good amount in immunocompetent persons and the most dominant is the *Candida albicans*. [2]. Candida spp. is endowed with "dimorphism", in fact, a polymorphism [3-5] There has been an increasing interest in investigating the potential cariogenic properties of the oral fungal flora especially *Candida* and both presence and abundance of *Candida albicans* have been linked to high DMF indices [6,7].

In addition to streptococcus mutans and lactobacilli, *Candida albicans* has been frequently isolated from carious lesions in children. [8, 9]. The production of acid by acid producing organisms as the central etiological factor in the initiation of carious lesions has been considered in the 'Miller's acidogenic theory'. Lactobacilli along with other microbes like yeasts were mainly identified as possible candidates [10] *and* studies of oral *Candida* species prove their acidogenic and hetero-fermentative properties, especially in the presence of carbohydrates [11]. In a recent, ultrastructural study Dige and Nyvad in 2019 [12] showed co existence of cariogenic bacteria and fungi. Nikawa *et al* in 2003 [13] reported that *C. albicans* dissolved hydroxyapatite in a liquid culture at a 20-fold higher rate than *Streptococcus mutans*, despite a lower number of yeast cells. Klinke *et al in 2009* [14] Candida remains active at a more acidic pH where streptococcus becomes inactive.

Children with high caries indices have shown *Candida* species in their saliva and it has been reported that the level of oral *Candida* species could be a risk factor in caries [15,16]. Hence, the present study was carried out to examine the occurrence of oral *Candida* in children with primary and mixed dentition and to identify the association between oral Candidal carriage in children and dental caries.

The present study was carried out in 100 school children, in age groups 4-6 years and 7-12 years, by performing mycological examinations to isolate *Candida* from their oral cavities with the aim to study the Candidal carriage in children with and without dental caries.

2. Materials and Methods

PATIENT SELECTION:

In the present study, the correlation between dental caries and *candida albicans* carriage in school children was determined by selecting 100 healthy children of age groups of : group A: 4-7 years; and group B: 8-12 years. Children who were having no relavent health histories were selected.

After the child was selected, his/ her demographic details were recorded and a thorough intraoral examination was done, which involved exclusion of any oral mucosal lesions, determination of the dmf or the dmf + DMFT index.

SAMPLE COLLECTION AND MYCOLOGICAL EXAMINATION

For mycological examination, a sterile mucosal swab was taken aseptically from dorsum of tongue and transported to the laboratory within 2 hours. The swab was inserted into 0.5 ml of sterile saline in a centrifuge tube, and a vortex mixer was used to rigorously mix for thirty seconds. 0.15 ml of the wash was spread onto three Sabouraud's Dextrose medium containing chloramphenicol agar plates. The plates were incubated at 37 ^{o}C for 48— 72 h.

For collection of sample from the carious lesion, food debris and plaque was first removed thoroughly from the surface of carious lesion and carious dentine was excavated from the centre of lesion using an excavator. Care was taken to prevent contact of the excavator with the adjacent non carious enamel. The carious dentine was placed immediately into 0.5 ml of normal saline and was triturated using a sterile mortar and pestle. The triturated material was immediately inoculated on the Sabouraud's Dextrose medium and incubated at 37 ^{o}C for 48-72 h and the formed colonies were identified as shown in the figure 1.

The colony forming units formed on each Sabouraud's agar plate was counted using a semi automatic colony counter. Depending upon the number of colony forming units, they were divided into three groups: C.F.U. <10, 10-100 and > 100.

After that, germ tube test and chlamydospore formation on cornmeal agar were performed on the Candida isolates and an isolate with positive germ tube test and

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positive chlamydospore test was considered as *Candida Albicans* whereas an isolate with a negative germ tube and chlamydospore test were considered other candidal species. Sugar fermentation and assimilation tests were performed to detect the presence of species like *C. Tropicalis, C. Kefyr, C. Guillermondii ,C. Parapsilosis, C. Krusei, C. Glabrata*. Sugar assimilation test result of *C.Albicans* is shown in figure 2.

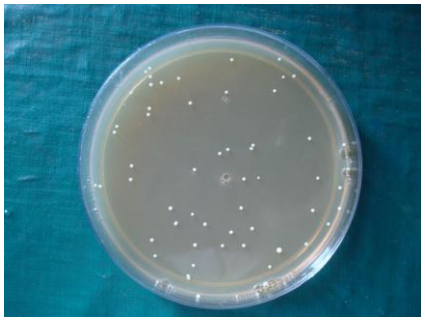

Figure 1 - Figure showing candidal colonies formed on Sabouraud's Dextrose medium and incubated at 37ºC for 48-72 h.

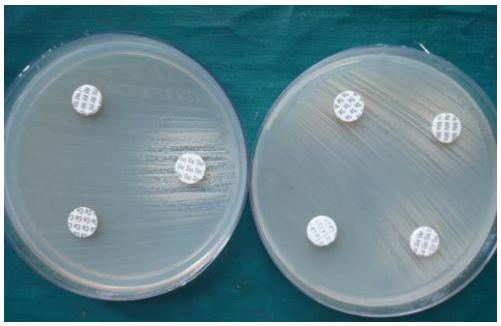

Figure 2 - Figure showing results of sugar assimilation test for C. Albicans species.

STATISTICAL ANALYSIS

The data was statistically analyzed using the SPSS statistical software. *p*- values, p< 0.001 was considered highly significant and *p*< 0.05 was considered as significant.

3. Results:

In the present study, 100 children were selected and divided into two equal groups, 50 children each in Group A [4-6 years] Group B [7-12 years], out of which, 40 [80 %] children were caries active and 10 [20%] children were controls in each group as shown in the table 1.

As shown in the table 2, 54% of children in the group A and 42% children in group B showed Candidal carriage. In the group A, out of the 14 children with df-t index greater than 10, only 1 [7.14%] child was non-carrier and 13 [92.86%] children were carriers and from them, 11 [84.62%] children showed *Candida albicans*, 2 [15.38%] children showed *Candida tropicalis*. Similarly, among group B children, out of children with df-t +DMFT index greater than 10, 3[23.07%] children were non-carriers and 10 [76.93%] children were carriers and from them, 6[60%] children showed *Candida albicans*, 2 [20%] children showed *Candida tropicalis*, 1 [10%] child showed *Candida guillormondi* and 1 [10%] child showed *Candida parapsillosis*.

These findings suggested that the Candidal carriage was very strongly associated with dental caries activity showing a high statistical significance [p< 0.001]. Thus, suggesting an important role of *Candida* in causation of dental caries. Moreover, the age of the child may have an influence on the isolation of *Candida* species as the carriage of *Candida albicans* species decreased with age and there was a simultaneous increase in the carriage of non- albicans species in our study population.

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ISSN: 2309-6152 (Online)
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Table 3 shows correlation between df-t +DMFT index and colony forming units [C.F.U.] isolated from tongue and carious lesion in group A and group B. The findings suggested that high caries indices are significantly associated with heavy Candidal colonization in both the carious lesions and tongue are strongly associated with causation of dental caries which are statistically significant [$p< 0.05$].

Table 1: Table showing demographic details and caries activity status of the children in group A and group B.

GROUP	TOTAL NO.OF CHILDREN	SUBGROUP	NO. OF CHILDREN	SEX	
				MALES	FEMALES
	NO. [%]		NO. [%]	NO. [%]	NO. [%]
GROUP A [4-6 YRS]	50 [100]	CARIES ACTIVE	40 [80.00]	22 [55.00]	18 [45.00]
		CONTROLS	10 [20.00]	3 [30.00]	7 [70.00]
GROUP B [7-12 YRS]	50 [100]	CARIES ACTIVE	40 [80.00]	23 [57.50]	17 [42.50]
		CONTROLS	10 [20.00]	4 [40.00]	6 [60.00]
TOTAL			100 [100]	52 [52.00]	48 [48.00]

Table 2: Table showing correlation between df-t + DMFT index and Candidal carriage in group A and group B.

GROUP	df-t +DMFT INDEX	TOTAL NO. OF CHILDREN	CANDIDA CARRIAGE		CANDIDA SPECIES			
			NON-CARRIERS	CARRIERS	C.ALBICANS	C. TROPICALIS	C.GUILLORMONDI	C.PARAPSILLOSIS
		NO. %	NO. %	NO. %	NO. %	NO. %	NO. %	NO. %
GROUP A [50]	0	10 [20.00]	9 [90.00]	1 [10.00]	1 [100]	0 [0.00]	0 [0.00]	0 [0.00]
	1− 5	12 [24.00]	8 [66.67]	4 [33.33]	4 [100]	0 [0.00]	0 [0.00]	0 [0.00]
	6 − 10	14 [28.00]	5 [35.67]	9 [64.33]	5 +1* [66.67]	3 +1* [44.44]	0 [0.00]	0 [0.00]

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	> 10	14 [28.00]	1 [7.14]	13 [92.86]	11 [84.62]	2 [15.38]	0 [0.00]	0 [0.00]
	TOTAL	50 [100]	23 [46.00]	27 [54.00]	22 [81.48]	6 [22.22]	0 [0.00]	0 [0.00]
GROUP B [50]	0	10 [20.00]	8 [80.00]	2 [20.00]	2 [100]	0 [0.00]	0 [0.00]	0 [0.00]
	1− 5	14 [28.00]	11 [78.60]	3 [21.40]	2 [66.67]	1 [33.33]	0 [0.00]	0 [0.00]
	6 − 10	13 [26.00]	7 [53.90]	6 [46.10]	6 [100]	0 [0.00]	0 [0.00]	0 [0.00]
	> 10	13 [26.00]	3 [23.07]	10 [76.93]	6 [60.00]	2 [20.00]	1 [10.00]	1 [10.00]
	TOTAL	50 [100]	29 [58.00]	21 [42.00]	16 [76.19]	3 [14.29]	1 [4.76]	1 [4.76]
* Shows one child with mixed carriage of C. albicans & C. tropicalis								

Table 3: Table showing correlation between df-t +DMFT index and colony forming units [C.F.U.] isolated from tongue and carious lesion in group A and group B.

GROUP	DMFT INDEX	TOTAL NO. OF CHILDREN	C.F.U. ISOLATED FROM TONGUE				C.F.U. ISOLATED FROM CARIOUS LESION			
			0	< 10	10-100	>100	0	< 10	10-100	>100
		NO. %	NO. %	NO. %	NO. %	NO. %	NO. %	NO. %	NO. %	NO. %
GROUP A [50]	0	10 [20.00]	9 [90.00]	0 [0.00]	1 [10.00]	0 [0.00]	10 [100]	0 [0.00]	0 [0.00]	0 [0.00]
	1− 5	12 [24.00]	8 [66.67]	1 [8.33]	1 [8.33]	2 [16.67]	9 [75.00]	0 [0.00]	2 [16.67]	1 [8.33]
	6 − 10	14 [28.00]	5 [35.71]	0 [0.00]	8 [57.14]	1 [7.14]	8 [57.14]	0 [0.00]	3 [21.43]	3 [21.43]

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	> 10	14 [28.00]	1 [7.14]	2 [14.29]	6 [42.86]	5 [35.71]	5 [35.71]	0 [0.00]	6 [42.85]	3 [21.42]
	TOTAL	50 [100]	23 [46.00]	3 [6.00]	17 [34.00]	7 [14.00]	32 [64.00]	0 [0.00]	11 [22.00]	7 [14.00]
GROUP B [50]	0	10 [20.00]	8 [80.00]	1 [10.00]	1 [10.00]	0 [0.00]	10 [100]	0 [0.00]	0 [0.00]	0 [0.00]
	1− 5	14 [28.00]	11 [78.57]	1 [7.14]	2 [14.29]	0 [0.00]	12 [85.71]	1 [7.14]	1 [7.14]	0 [0.00]
	6 − 10	13 [26.00]	7 [53.84]	0 [0.00]	6 [46.15]	0 [0.00]	9 [69.23]	0 [0.00]	2 [15.38]	2 [15.38]
	> 10	13 [26.00]	3 [23.07]	0 [0.00]	7 [53.84]	3 [23.07]	4 [30.77]	0 [0.00]	5 [38.46]	4 [30.77]
	TOTAL	50 [100]	29 [58.00]	3 [6.00]	15 [30.00]	3 [6.00]	35 [70.00]	1 [20.00]	8 [16.00]	6 [12.00]

4. Discussion:

Candida albicans is the most common fungus normally seen in human oral cavity. Several factors, such as persistence, dimorphism, acidogenicity, adherence and interference with host defense system work together to enhance the virulence of the fungi[13,17]. In the recent years there has been an increasing interest in the relationship between oral fungal flora, especially the *Candida* and dental caries. *Candida* is responsible for demineralization of tooth enamel and dissolution of hydroxyapatite, thereby causing caries[18, 19].

Therefore, study was performed to find the correlation between *Candida albicans* carriage and dental caries activity in children and to investigate the contribution of *Candida* in causation of caries. As it has been reported that a highest Candidal carriage is seen during the primary dentition stage [20], the study population was divided into two age groups : group A [4-6 years] consisted of children with primary dentition and the group B [7-12 years] consisted of children with mixed dentition.

The overall Candidal carriage rate of both the groups was of 48% which is quite similar to the findings of both Raja *et al.* [2010] and Moalic *et al.* [2001] who found an overall Candidal carriage rate of about 60% [21,22]. In the present study, the Candidal carriage rate among the children in group A [primary dentition stage] was 54% and that in the children of group B [mixed dentition stage] was 42% which showed that Candidal carriage in the primary dentition stage was comparatively geater than mixed dentition stage but was statistically non significant and these findings were similar to the findings of RoñCkiewicz*et al*. [2006] and Qi *et al.* [2005] [15, 20]. According to Kadir *et al.* [2005]and Starr *et al*. [2002]a lower Candidal carriage in elder children with mixed dentition and that may be due to the alterations in the oral hygiene habits and dietary habits may explain the reduction of Candidal carriage in this age group. [6, 23].

In the present study, 92.86% of children with high caries activity [>10] in group A and 76.93% children with high caries activity [>10] in age group B showed Candidal carriage with a significant difference between the Candidal carriage in the caries active children and caries free children in both groups which suggested a strong association between Candidal carriage and caries activity with a high statistical significance. These

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findings correlated with the findings of Signorettoet *al*. [2009] [24].

In the present study, *Candida albicans* was the principal Candida species isolated and represented 81.48% of all Candida isolated in group A and 76.19% in group B. The second species identified were *Candida tropicalis* [22.22% in group A and 14.29% in group B], with very low percentage of *Candida parapsillosis* and *Candida guillormondi*. These findings were in accordance with those of Cortelli*et al.* [2006] [25].

In the present study, high DMFT indexes were strongly associated with high Candidal carriage. From the children in both group A and group B, with df-t +DMFT indexes greater than 10, 78% and 64.29% children respectively showed Candidal carriage from tongue and 76.92% and 69.23% respectively showed Candidal carriage from carious lesion which were statistically significant. These findings were in accordance with those of Hossain *et al.* [2003] who suggested that the fungi residing and cultivating in the carious tooth may spread systemically to cause extraoral disease conditions.

The present study showed that *Candida* has a significant role in establishing a high caries activity. At present, the exact mechanism of caries production by *Candida* has not been elucidated, but it has been suggested by Klinke *et al.* [2009] that Candida facilitates the enamel and dentin demineralization by a complex process of acidification of the oral environment by different mechanisms. Firstly, by production of pyruvic acid, that has a low pKa of 2.36 and has greater acidification potential than lactic acid [pKa of 3.86] secreted by lactobacilli. The amount of pyruvic acid is greatest during the initial colonization periods, but gradually decreases later apparently because of its reutilization. Secondly, the cell membrane of yeast cells consistes of H + -ATPase which expels protons out of the cells, causing acidification. Thirdly, an increased acidification occurs because of the carbon dioxide released during the metabolism in yeast bodies. [14] Also, abundance of C. albicans carriage creates an acidic environment in the oral cavity to promote dental caries. [27, 28]

5. Conclusion:

High loads of Candida may lead to occurrence of active dental decay in mixed dentition stage. Therefore, identification of high caries risk population by isolation of Candida is easier than isolation of other cariogenic microorganisms during epidemiological studies.

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