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Vol 10 No 1 (2022) DOI 10.5195/d3000.2022.311

Is Oral Candida associated with Dental Caries in Children—A Cross Sectional Analysis.

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Abstract

BACKGROUND: Dental caries is a world-wide public health issue and is considered the most common of all non-communicable disease. There has been a growing interest in association between oral fungal flora especially *candida* and dental caries. Hence the aim of the study was to assess the correlation between active caries status and oral candida carriage in children aged twelve years.

MATERIALS AND METHODS: This is a cross-sectional study where a multiphase sampling was done and a total of thirty children were selected randomly among children screened previously. Data was collected through clinical oral examinations and structured interviews. The oral rinse technique described by Samaranayake *et al.* was used for extracting *candida* spp. The correlation between *candida spp* level and caries experience was performed by the Pearsons correlation coefficient.

RESULTS: The mean DMFS and defs scores of the children were 3.70±4.61 and 5.83±5.51 respectively. Significant association with decay scores for deciduous teeth (P<0.05) and defs (p= <0.001) was demonstrated. *Candida species* was associated highly with caries prevalence and significant positive correlation was found between *Candida* (CFU/plate) and defs scores (r=0.790) (p= <0.001)

CONCLUSION: The study results suggested that there is a significant positive correlation between candida carriage and caries, especially in children in the age group of 12 years.

KEYWORDS: Candida; Children; Dental caries; DMFT

Citation: Pai, M et al. (2022) Is Oral Candida associated with Dental Caries in Children—A Cross Sectional Analysis.
Dentistry 3000. 1:a001 doi:10.5195/d3000.2022.311
Received: February 2, 2022
Accepted: August 8, 2022
Published: August 31, 2022

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Introduction

Dental caries is a world-wide public health issue and is considered the most common of all non-communicable disease. Caries is ranked First among all diseases in global burden of disease study affecting 2.3 billion people for permanent teeth and 12th for deciduous teeth affecting 560 million children [1]. Identifying high caries risk individuals and groups is a matter of prime importance for all public health programs worldwide. It is widely known that caries prevention and management would be more effective if individuals or groups under greatest risk are identified. Hence, methods for predicting caries risk are being investigated in greater detail.

C. albicans is a common oral commensal and can be commonly isolated from the oral environment of majority of individuals. It is noted that candidal carriage is common in many individuals and as much as 80 percent health individuals have candidal

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isolates in their oral cavity, but the fact that just candidal carriage does not mean actual infection as it is considered as a normal flora of the oral ecosystem. *Candida* is a highly adaptable commensal organism and any alterations in the host micro ecosystem that favor its proliferation provide this commensal with the opportunity to essentially invade any site and proliferate causing infection. This invasion usually manifests from surface infections to invasive disseminated disease with multiple organ involvement [2].

Oral microbiota consists of not only bacteria but also numerous other microorganisms, which cause mixed infections in the oral cavity. Hence the establishment and maintenance of oral microbiota is related not only to interbacterial co aggregations but also interactions of these bacteria with yeasts such as *Candida albicans*. Fungi are isolated on several sites in the oral cavity, including tongue, buccal mucosa, palate, oral biofilm, carious lesions, and prosthetic appliances. It has been suggested that a possible relation may occur between *C. albicans* and periodontal disease, dentin and/or root caries. *C. albicans* also has capacity similar to that of *S. mutans* for colonizing hydroxyapatite and invade the tooth structure. The mode of action for such invasion may be adherence of *Candida* to salivary proteins, acid-producing capability, penetration into the dentinal tubules, and its enzymatic activity to degrade collagen which in turn may be responsible for causation of dental caries; however, these may be entirely different to that to the mechanisms of *S. mutans*, in initiation and progression of dental caries [3,4].

Studies have also demonstrated that Candida species are more prevalent in saliva of caries-active subjects than that of subjects with no caries especially in children. Enamel and dentin demineralization produced by organic fungal acids, as well as the presence of *C. albicans* hyphae invading dentinal tubules, prove the ability of fungi to invade and destroy inorganic and organic dental tissues [5].

There has been a growing interest in association between oral fungal flora especially *candida* and dental caries, this has resulted in research focusing on the possible direct role of *C. albicans* in etiology of dental caries. The presence of *C. albicans* in dental plaque of children have been associated with high DMF (decayed, missing, filled) Index. Reduced growth has been associated with increased filled component of the individuals and abundant growth is associated with a high decay component [6].

Hence in this study we assessed the correlation between active caries status and oral candida carriage in children aged twelve years in the coastal city of Karnataka as we had no previous studies conducted in the present geographical area. The objectives were, to assess the frequency of oral *Candida* species in children aged 12 years, to evaluate the caries experience of children aged 12 years and to correlate the frequency of oral candidal species with the caries status of the children.

Material and Methods

The study subjects included children from a primary school in the city of Mangalore, Karnataka, India. A prior permission from the respective authorities were obtained before the start of the study process. This is a cross-sectional study conducted with the aim of detecting *Candida* carriage in the oral cavity of healthy school children and to correlate that with candidal occurrence and dental caries in this population. The students in the school were screened previously and caries status of the children was obtained. A multiphase sampling was done, where a total of thirty children were selected randomly among children screened previously. This was a pilot study to know the prevalence of candida in dental caries.

The sample size was calculated by using G*Power version 3.1 with a statistical power of 0.8 and a significant level of 0.05 and estimating effect size of 0.5 a sample size of 27 was calculated, hence a sample of 30 was included in the study.

Ethical committee clearance was obtained prior to the study from the Institutional Ethics Committee and

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voluntary informed consent was obtained from the parents or guardians of the subjects prior to the study process. All infection control protocols were strictly followed, and participants were referred for further treatment if necessary to the parent institute after the examination.

The study group included children randomly selected from the sample frame who fulfilled the inclusion criteria. The inclusion criteria were children with caries who were selected through random multiphase sampling technique. Children in the age group of 12 years with no clinical signs of oral candidiasis and free from any known systemic disease.

Exclusion criteria included children with special needs, antimicrobial medications one month prior to the study and children with orthodontic appliances.

Data was collected through clinical oral examinations and structured interviews. A Single blinded examiner examined all the children and two supports who helped with the recording of data participated in the study. Intra examiner reliability was assessed and was found to be good (Cohen's Kappa $\kappa = 0.72$) during calibration. The structured interview included questions on age, gender, dental hygiene (frequency of brushing, flossing, and the use of toothpaste or mouthwash use), and dietary habits. Decayed Missing Filled Surfaces (DMFS) index for permanent teeth and decayed, missing due to caries, or indicated for extraction, filled surfaces (defs) index for primary teeth given by Grubbel AO was used to assess the caries experience in primary dentition. Each child was examined in a chair using natural light. White spot decalcifications were not considered to be caries, and loss of mineral substance with cavitation was recorded as caries [6]. Evaluations was done according to the criteria recommended by the World Health Organization.

Mycological Examination

The oral rinse technique described by Samaranayake *et al.* [7] was used for extracting *candida* spp from the subjects. The procedure involved the participant holding and rinsing 10 ml of sterile phosphate buffered saline (PBS) in the mouth for one minute. The expectorate was then transported at room temperature to the laboratory where it was centrifuged for ten minutes as described by Samaranayake *et al* [7]. Then 30µl of concentrate was inoculated onto Sabouraud's agar medium. The plates were incubated at 37°C for 72 hours, and semi-quantitative assessment of *Candida* growth was made by counting the total number of yeast colonies developed on the plates at the end of 72 hours and were quantified by the visual methods.

Data were analysed using Statistical Package for Social Sciences (SPSS), version 16.0 (SPSS Inc, Chicago IL). Mean (X), median, inter quartile distribution and Standard Deviation (SD) was calculated for describing the data. The difference was considered to be significant when $P < 0.05$. The presence or absence of *Candida spp* in each subject was compared with gender and caries prevalence. The correlation between *Candida spp* level (CFU/plate) and caries experience was performed by the Pearsons correlation coefficient. The Mann-Whitney U and Kruskal-Wallis H test was used to assess the significance between gender, caries, dental hygiene methods and candidal count.

Results

Out of 30 children selected for this study, the sample contained equal number of children from both genders. The subjects selected for the study had a mean age of 11.12±0.33 years. A total 73% (n= 22) of individuals reported to brush their teeth at least once a day using tooth paste and toothbrush, and only 11% brushed their teeth twice a day. All the individuals reported to brush their teeth with fluoridated toothpaste. The individuals who brushed once daily using finger had higher frequency of Candidal occurrence which was statistically significant (Table I (a)).

Pearson's Correlation between the colony forming units and demographic and dental caries experience in the sample is summarized in Table I (b).

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Table I (a): Demographic variables, oral hygiene habits and colony forming units for Candida.

		N	MEAN CFU(SD)	RANGE	MEDIAN	Mann whitney u / kruskal wallis	P value
Gender	Male	15	202(455)	0-1335	0(0,0)	110	0.935
	Female	15	123(276)	0-847	0(0,0)		
Type of cleaning	Brush	21	96(248)	0-847	0(0,0)	6.773*	**0.036**
	Finger	5	573(660)	0-1335	299(0,1229)		
	Both	4	0(0)	0-0	0(0,0)		
Material	Paste	22	105(246)	0-847	0(0,0)	0.996*	0.608
	Other	7	366(626)	0-1335	0(0,1229)		
	Both	1	0(.)	0-0	0(0,0)		
Frequency	Once	22	35(115)	0-462	0(0,0)	48	0.063
	Twice	8	514(584)	0-1335	350(0,1038)		
Time of Brushing	Morning	23	33(112)	0-462	0(0,0)	11.642*	**0.003**
	Night	5	513(703)	0-1335	0(0,1229)		
	both	2	773(105)	699-847	773(699,847)		
Diet	Vegetarian	16	29(116)	0-462	0(0,0)	77.5	0.154
	Mixed	14	315(496)	0-1335	0(0,699)		

*-Kruskal -Wallis test
*($p < 0.05$) Significant difference between/among subgroups
CFUS= Colony forming Units

Table I (b): Demographic Correlation between dfs score and DMFT scores and CFU of Candida.

Pearson Correlation								
		Gender	Type of cleaning	Material	Diet	Df SCORE	DMFS	CFUS
GENDER	Pearson Correlation	1	-.326	.000	.267	-.076	-.265	-.108
	P		.079	1.000	.153	.691	.157	.571
TYPE OF CLEANING	Pearson Correlation	-.326	1	.056	-.193	.073	.115	.096
	P	.079	-	.768	.307	.702	.544	.614
MATERIAL	Pearson Correlation	.000	.056	1	.040	.199	.281	.261
	P	1.000	.768	-	.833	.292	.133	.164
DIET	Pearson Correlation	.267	-.193	.040	1	.393*	-.133	.390*
	P	.153	.307	.833	-	.032	.482	.033
DFSCORE	Pearson Correlation	-.076	.073	.199	.393*	1	.497**	.790**
	P	.691	.702	.292	.032	-	.005	.000
DMFS	Pearson Correlation	-.265	.115	.281	-.133	.497**	1	.404*
	P	.157	.544	.133	.482	.005	-	.027
CFUS	Pearson Correlation	-.108	.096	.261	.390*	.790**	.404*	1
	P	.571	.614	.164	.033	.000	.027	-

P =*. Correlation is significant at the 0.05 level (2-tailed).
P= **. Correlation is significant at the 0.01 level (2-tailed).
DMFS: Decayed, Missing, and Filled Surfaces
CFUS= Colony forming Units
dfs= decayed filled for Deciduous tooth

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Of the 30 oral samples collected, 24 (80 %) did not present any fungal growth and 6 (20 %) presented fungal microbiota consisting of Candida with an average of 162.3 CFU/plate. The mean DMFS and defs scores of the children with caries was 3.70±4.61 and 5.83±5.51, respectively. The individual characteristics of index is presented in Table II, other than the deft index, the results of the questionnaire and oral examination revealed no significant differences between the children and there was no association between candida carriage within gender for DMFS (p=0.436) and defs (p=0.389).

Association of microbial counts with caries status

Associations between caries status (DMFS and defs scores, dependent variables) and microbial counts (independent variables) were evaluated. Significant association with ds decay scores for deciduous teeth (P<0.05) (Table II) and defs (p= <0.001) was demonstrated. Candida species was associated highly with caries prevalence and significant positive correlation was found between Candida (CFU/plate) and defs scores (r= 0.790) (p= <0.001), in the subjects but there was no positive correlation between DMFS and Candida carriage in children (r=0.404) (p=0.027).

Table II: Association (Pearsons chi square) between the Colony forming units of candida, brushing habits, and dental caries experience.

	Chi-Square value	df	P value
Gender	6.00	6	0.423
ds	139.16	72	**<0.001**
dfs	150.0	72	**<0.001**
DMFT	95.45	54	**<0.001**
Tooth cleaning methods	22.067	12	**0.037**
Frequency of brushing	12.95	6	**0.044**
Diet	7.40	6	0.285

P =*. Significant at the 0.05 level (2-tailed).
Df= degree of freedom
DMFT=Decayed, Missing, and Filled Teeth
Dfs= decayed filled for Deciduous tooth

Discussion

Several predictors up till now have been analysed and discussed in relation to dental caries. So far none of the indicators alone can correctly predict caries ranging from clinical, microbiologic, and an array of host factors. Combined information of several predictors showed some ability to distinguish subjects into high or low caries groups. Past caries experience, plaque index and microbiological factors are easy to determine and show a reasonably good association with caries risk but still prediction of caries has remained an enigma and a conundrum spinning the web of confusion in clinician's mind.

The association of Candida to caries in children is a topic of intense discussion in recent times. In the present study, the oral candidal carriage was assessed in children and an association was observed for the same. Of all the subjects in the study, Candida was present in 20 percent of the subjects and this finding is similar to that Beena et al in India [8], but much lower to that of studies by Moalic et al. in France [9], Cortelli in Brazil [10], Akdeniz et al [11]. Candida in Turkey, the difference in the Candida count can be attributed to the difference in number and age of the subjects, geographic locations, and methodology used by different authors. The study of Akdeniz used imprint method for culturing candida hence may have had higher Candida samples [11]. The imprint method though the most followed method may not be as accurate as that of oral rinse method used in the study as explained by Samaranayake et al [7].

In the present study, the prevalence of Candida was higher in sample group than the general population as all the subjects in the present study had dental caries. The results demonstrated a significant association between the presence of Candida species and caries in children. But there was no statistically significant relationship between age, gender, type, and method of cleaning teeth. Material used for cleaning teeth, frequency of cleaning teeth and diet as all these factors were similar among the sample population.

Studies suggest that Candida species have high cariogenic potential in

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vitro. Carious dentine has shown to have a higher colonization of Candida spp, providing a significant ecologic niche for the dissemination of this fungi [12,13]. The recent evidence also points at relationship between the presence of Candida and caries in children specifically in children with poor oral hygiene, carious dentinal lesions and high sugar diet [14,15].

The relationship between Candidal carriage, initiation or progression of caries lesions is unclear. To say the least, it is not evident that whether and how the disease process ante cedes or predates the adhesion of yeasts. The current insight suggests that higher frequency of sugar intake, with poor oral hygiene, open caries, and the extensive intake of sweet substances via the feeding-bottle in nursing bottle caries may be some of the important factors responsible for the high prevalence of this fungi in the mouths of children [11].

The biggest drawback of the study might be the absence of no caries-free children in, as this was a pilot study to test the contemporary hypothesis. In other words, a relationship between caries and frequency of oral Candida carriage was observed only in children with caries but a negative correlation in not obtained in non-carious age group hence further research should be directed in this direction. The results of this study are specific to children from a single location and a single cross-section. Any generalizations hence must be confirmed by repeating the study in additional age groups and different geographic locales.

Conclusion

The study results suggested that there is a significant positive correlation between candidal carriage and caries, especially in children in the age group of 12 years. Evaluation of oral candida level may help in planning preventive programs for children in public health settings. Further longer-term studies with an extended study group are needed to confirm our results in evaluating these indicators as risk predictors of caries in children. The relationship between colonization, secretor status and in-vitro adhesion of *Candida albicans* in oral cavity should be the focus of such research.

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