Original Article

Prevalence of candida albicans in dental plaque and caries lesion of early childhood caries (ECC) according to sampling site

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Abstract

Background: Candida albicans (C. albicans) may have cariogenic potential but its role in caries etiology has not been established. The aim of this study was to determine C. albicans in supragingival dental plaque and infected dentine of cervical and proximal in early childhood caries (ECC).

Methods: This cross-sectional study was carried out on 6o children aged 2-5 years, which were divided into 3 groups: children with at least one cervical caries; children with at least one proximal caries and caries-free. The infected dentine was collected from cervical and proximal caries lesions and plaque samples were collected from the three groups in order to compare the frequency of C. albicans in the collected sites. All samples were cultured in Sabouraud and CHROMagar medium and the cases that were positive for C. albicans were cultured in germ tube. Data were collected and analyzed.

Results: The mean age of the children was 3.9 years. From 100 samples, C. albicans samples were isolated in 55%, mold fungi were found in 29% cases and there was no fungal growth in 16% of the samples. In plaque samples, C. albicans were found in 15% of caries-free samples, 20% of the proximal and 80% of the cervical caries. In samples extracted from the caries, C. albicans were found in 60% of the proximal and 100% of the cervical caries. Mothers with university educational level had children with more cervical decays, caries free and proximal caries, respectively.

Conclusion: The results showed that prevalence of C. albicans in dental plaque and caries lesions of children with early childhood caries were relatively high and the prevalence was higher in cervical caries group.

Key words: Candida albicans, Dental caries, Children.

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Early childhood caries (ECC) is defined as the presence of one or more decayed teeth (non-cavitated or cavitated), missing (due to caries), or filled teeth surfaces in any primary tooth in a child 71 months of age or younger (1). Wetzel et al. and Moalic et al. determined the presence of candida spp. in saliva, dental plaque and infected dentine of children with early childhood caries more common than caries-free children (2, 3). de Carvalho et al. reported that according to Hodson and Craig, C. albicans in the biofilm of ECC is twice more prevalent than the caries-free children (4). Also, Thaweboon et al. showed children with rampant caries had higher levels of SIgA, mutans streptococci and candida in their oral cavities (5). Candida is a part of normal oral flora and shows large geographic variations, but an average figure of 35% has been reported in several studies. With improved detection techniques, even a prevalence up to 90% has been proposed (6). The presence of candida in oral cavity may be related to many factors such as birth infections, nurse's fingers, hospital maternity ward, baby's feeding bottles and infected pacifiers, maternal skin, air and water and carious teeth (4).

Rozkiewicz et al. revealed that C. albicans was isolated in (62.3%) of carious lesions in preschool children and in 71.4% of the school children (7). Ollila reported that colonization of candida is an important factor for caries onset in primary molars (8). Also, Klinke et al. concluded that the contribution of C. albicans to overall microbial acid formation appears to be important (9). C. albicans ferment glucose and maltose, producing both acid and gas (10). It has shown a high acidogenic potential and biofilm formation (4). However, biofilm formation of C.albicans within the oral milieu is affected to a varying extent by dietary and salivary factors (11).

Also, Hossein et al. in their study in order to identify albicans in the oral cavity and the gastrointestinal tract of pre-school children assumed that carious teeth may constitute an ecologic niche for C. albicans potentially responsible for recurrent oral and non-oral candidiasis (12). Starr et al. in a study on healthy elementary school children before and after dental caries treatment showed that children with oral C.albicans frequently maintained caries over time, even with regular dental care (13).

To sum it up, there are limited investigations about the prevalence of C. albicans in ECC children according to sampling site. The aim of this study was to determine C. albicans in dental plaque as well as caries lesion in cervical and proximal surfaces of ECC children in Babol, north of Iran.

Methods

This cross-sectional study was conducted on sixty children aged 2 to 5 years, in the kindergartens of Babol, Iran from May, 2009 until June, 2010. These children were selected on the basis of clinical characteristics of caries type. The children were generally in good health and did not take in any antibiotics for at least 1 month before the study. Also, they were not treated with local and systemic fluoride. Three groups were formed: twenty children with at least one decayed upper incisor with lesions at the cervical third of the clinical crown, twenty children with at least 1 upper incisor with strictly proximal caries in enamel and dentine (the cervical margin was sound) and twenty children without any dental caries.

Supragingival dental plaque samples were collected from the upper incisor buccal surface most affected by caries using a sterilized wooden toothpick. Samples were collected from enamel in clinically sound gingival areas for the caries-free group, and around the affected enamel for the cervical and proximal caries groups. Immediately after the sample collection, each wooden toothpick was placed in a sterilized tube containing 0.2 milliliter saline solution. The infected dentine samples were collected at the center lesion of the same upper incisor with a sterilized small excavator, careful not to allow the excavator to touch the adjacent and cervical enamel to prevent sample contamination. Dentine samples were placed in sterilized tubes containing 0.2 milliliter saline solution and then were transferred to mycological laboratory. About 100 microliter of samples cultured in Sabouraud dextrose agar contained 100 microgram chloromycetin per liter (SC medium) and then incubated at 37°C for 48 hours. All media was observed for fungal growth each day for 7 days.

All of the isolated fungi (yeast or mold) were diagnosed by routine laboratory methods (macroscopy, microscopy and biological methods). The isolated yeast was tested by germ tube production, candida CHROMagar biochemically using method, and cornmeal agar.

The data were collected and analyzed by SPSS version 12 and Chi-square test. All statistical tests were considered at the level of significance of 0.05%.

Results

The mean age of children in cervical caries, proximal caries and caries-free group was 48.4, 47.4 and 46.9 months, respectively (p=0.124).

There were no significant differences between the length and the kind of feeding between the three groups (p>0.05) but there was significant relationship between the parents' level of education in the three groups. Parents with high school level of education had children with more proximal decay. Mothers with the same university level of education same had children with more cervical decay, caries free and proximal caries respectively and fathers with this education had children with caries free, cervical and proximal caries, respectively.

The most distribution of C.A. in dental plaque and caries lesion was in cervical decay group (table 1). Prevalence of C. albicans in dental plaque and caries lesion in three study groups were compared through the three methods of Germ tube, CHORMagar and Sabouraud. There were no significant differences between the three methods.

Table 1. Distribution of Candida albicans in Dental plaque and caries lesion in three groups

Group / Kind of fungi	Caries free	Plaque of proximal caries N (%)	Lesion of proximal Decay N (%)	Plaque of cervical caries N (%)	Lesion of cervical Decay N (%)
Candida albicans	3 (15)	4 (20)	12 (60)	16 (80)	20 (100)
moulds fungi	4 (20)	16 (80)	5 (25)	4 (20)	0 (0)
No growth	13 (65)	0 (0)	3 (15)	0 (0)	0 (0)

Discussion

Candida is the major fungal pathogen of human causing a variety of afflictions ranging from superficial mucosal diseases to deep seated mycoses. Biofilm formation is a major virulence factor in the pathogenicity of candida, and candida biofilms are difficult to eradicate especially because of their very high antifungal resistance (14).

In this study, C. albicans was discovered in the samples taken from the plaque of 15% of the cases without caries, 20% of the dental plaque of ECC children with proximal caries and 80% of them with cervical caries. The samples which were taken from caries lesion of ECC children had shown 60% of lesions with proximal caries and 100% of the cervical caries samples were C. albicans positive. There are a few reports about these issues in the medical literature. Carvalho et al. performed a study on 2-5 years old children in Brazil in 2006 and showed that the outbreak of C. albicans in cervical decay of ECC group (60.4%) was significantly more than the caries free group (14.3%) and proximal caries (12.5%) and these findings were similar to our results (15).

In the study of Sziegoleit et al., candida species was isolated from the saliva of 66.7% subjects with active caries, but only 2% from the saliva of caries free subjects (16). Ugan-Can et al. reported that the frequency of oral candida of the 4-6 years old children with moderate and high df-t (dental caries index) was statistically higher than in caries-free children (17). Akdeniz et al. reported that 69% of the children with caries and 5% of caries-free children were found to be candida carriers and the difference in candida prevalence between these two groups was significant (18). As pointed out in many studies, candida's outbreak especially C. albicans in the ECC affected children is more common in caries free children. And also, this study has the same direction. Kukletova et al. reported that C. albicans and other fungi are the constant components of the dental plaque in children suffering from ECC and can contribute in their ability to ferment carbohydrate to the destructive course of the disease (19). C. albicans ferments glucose and maltose, producing both acid and gas (10).

At pH 7.0, C. albicans produced 5-fold more acid per colony forming a unit than lactobacilli. The contribution of C. albicans to total microbial acid formation appears to be relevant for caries progression (9). Also, Signoretto and Thaweboon support the role of candida spp in children with rampant caries (20, 5). Ten Cate et al., suggested that C. albicans adhere to HAP (Hydroxy Apatite) specifically through electrostatic interaction, in a much smaller number (1.0/7.4 x 10(5)), C. albicans possesses the ability to dissolve HAP to a greater extent (approximately 20-fold) when compared with S. mutants (21). Also, C. albicans has a high collagenolytic activity and can adhere to the intact and denaturated collagen exposed from dentine. This process may contribute to the persistence of C. albicans on the surface of dissolved hydroxyl apatite because of high adherence capacity of this yeast to collagen (4). It appears that these data suggest C. albicans can develop caries. Maijala s' study did not support the previous suggestion that C. albicans is important in the dentine caries pathology (22).

Carious teeth may constitute an ecologic niche for the progression and dissemination of C. albicans in oral cavity (15). Also, Mondin et al. investigated the decrease in colonization of candida after dental treatment in ECC children (4). Sziegoleit et al. concluded that dental treatment alone eliminated candida from oral cavity in 90% cases, whereas, the local application of amphotericin B alone had a

minimal effect and the combination of dental treatment plus amphotericin B eliminated candida from oral cavity completely (16). Despite the extensive investigations, the role of C. albicans in dental caries has not been established clearly (15). In conclusion, the results show that the prevalence of C. albicans in dental plaque and caries lesions of children with early childhood caries are relatively high. This prevalence was higher in cervical caries group. Consequently, this result should be considered by dentists in the therapy and prevention program.

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