Candida, Mutans Streptococci, Oral Hygiene and Caries in Children

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Objective: *to test the association between* Candida *and* mutans streptococci (ms)*, oral hygiene and caries levels and in children.* ***Methods:*** *22 boys and 12 girls (age 6 to 14.5 years) participated in the study. Each participant received a toothbrush, and was asked to brush his/her teeth after proper instructions. Dental caries and oral hygiene were recorded.* Candida *and* ms *levels were determined in saliva samples.* ***Results:*** *Candida colonies were observed in 70.5% of the children. No association was found between* Candida *and caries or plaque and gingival indices.* C. albicans*-positive children demonstrated significantly higher brushing scores.* ***Conclusions:*** *Our findings may suggest that there is no clear association between* Candida *in saliva, and levels of cariogenic bacteria and caries risk in children.*

Keywords: Candida, mutans streptococci*, oral hygiene, caries*
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INTRODUCTION

Candida species are common inhabitants of the normal oral microbiota, thus do not cause any symptoms in healthy individuals. However, in immune-compromised patients, *Candida* is an opportunistic pathogen.[1] Among *Candida* specie, the most prevalent in the oral cavity is *Candida albicans*. The frequency of the *Candida* varies with age: 24% in premature newborns, 4% in 4-5 days old babies, and 30% in children aged 3-12 years.[2] Higher frequencies of *Candida* were found in pacifier user children.[2,3]

Another factor with high levels of *Candida* albicans in the oral cavity is high caries rate. In children with low caries rates, *C. albicans* was the only *Candida* species found, while in children with high caries rates, other species of *Candida*, in addition to *C. albicans*, could be established.[4, 5]

Several studies have demonstrated an association between high counts of *C. albicans* and DMF scores (Decayed, Missing, Filled teeth) among students and among school children.[5-7] It has been shown that the presence of *Candida* was in positive correlation with the number of new carious lesions (whether active or treated) also in adolescents who had been caries free at the beginning of the study.[4]

High counts of *Candida* in the oral cavity of adolescents, both males and females, were associated with ΔDFS (the increment of the score of Decayed, Filled, Surfaces, which represents the development of new carious lesions). The presence of *Candida* at baseline could predict the development of caries among adolescents in 70% accuracy, thus, *Candida* could be a caries-prediction tool in adolescents.[8] The odds of caries development increase as the number of *Candida*-positive samples increases.

Immediately after dental treatment, the rate of *Candida* presence in the oral cavity usually decreases; however, it stays in the oral cavity of 44% of the children even after 3 years.[8]

As mentioned above, many studies address *Candida* as a predictor for caries risk. Yet, there are a few reports which claim that the *Candida* itself has a cariogenic potential which can be even higher than that of the *mutans streptococci* (ms) when it is in contact with a hydroxyapatite substrate.[4] It is possible that this potential is attributed to the ability of *Candida* to produce increased levels of pyruvate and acetate which, in turn, contribute to an acidic environment, and to consequent caries process.

In contradiction to the above studies, several clinical studies have failed to prove the association between the *Candida* levels in the oral cavity and caries, and it is still unclear whether high *Candida* levels could predict the development of caries.[4]

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Regarding the effect of oral hygiene on *Candida* persistence in the oral cavity, a large reduction in *Candida* colonization rates was observed after caries treatment, yearly dental hygienic care, and dental hygiene education for routine oral care.[6]

Another research has concluded that poor oral hygiene may contribute to the high prevalence and diversity of fungal colonization in children.[9]

The aim of the present study was to test the association between *Candida*, *mutans* streptococci, oral hygiene and caries and in children's saliva.

MATERIALS AND METHOD

The study population included 34 healthy children (22 boys and 12 girls), aged 6 to 14.5 years (mean age 9.0 ± 2.4), who were treated in the Department of Pediatric Dentistry at the Tel Aviv University School of Dental Medicine during a 3-months period (April 2008 – July 2008), and whose parents agreed to participate in the study. All children were healthy and were not using any medications permanently. The research was approved by the Tel Aviv University Helsinki Ethics Committee. Parental informed consent form was signed for all the children included in the study.

The following information was collected for each participant: age, gender, caries status by number of decayed-missed-filled teeth for primary and permanent dentitions (dmft/DMFT respectively), and number of decayed-missed-filled surfaces in the primary and permanent dentitions (dmfs/DMFS respectively), plaque Index (PI)[10] and gingival Index (GI).[10, 11]

Each participant received a toothbrush in the clinic area, and was asked to brush his/her teeth after proper instructions. Brushing efficiency was evaluated by one of the authors (ND), by recording the percent of the segments which were brushed in the maxilla and in the mandible (there were 24 segments: left and right posterior areas, left and right canine areas, anterior regions in the buccal and palatal areas, and posterior occlusal surfaces). The brushing duration of each segment was recorded.

Information about the frequency of morning and evening brushing as well as whether tooth brushing was performed by the children themselves or helped by the parents was obtained from the patients.

One Milliliter of unstimulated saliva was collected from every participant. Saliva samples were immediately processed as follows.

***Candida* levels**: One sample (0.1 ml) of the saliva was plated on a selective agar medium for *Candida* – CHROMagar *Candida* (Hy Laboratories Ltd, Rehovot, Israel).[4] In addition, another sample (0.1 ml) of the saliva was serially diluted 2 ten-fold dilutions in saline, and a sample of 0.1ml from both dilutions was plated as described above. Plates were incubated at 30ºC for 48 hours. *Candida* presence was analyzed as positive and negative (including indication of the various species according to colony color).

***Mutans* streptococci levels**: were assessed by RY kit (in use at the School of Dental Medicine, Tel Aviv University, Israel) – a kit for caries risk evaluation, which was adjusted for use at the School of Dental Medicine at Tel Aviv University, based on a previous caries risk test.[12] The kit is based on the ability of cariogenic bacteria to adhere to the bottle glass bottom when grown in a liquid medium in the presence of sucrose. Each saliva sample (0.2 ml) was transferred into a bottle containing an RY Kit selective liquid medium (20% sucrose, 0.5 unit/ml bacitracin, 0.001% potassium tellurite, Gentian violet and Trypan blue). The RY bottles were incubated at 37ºC for 24 hours. Following incubation, blue ms colonies were observed on the glass bottom of the RY bottle and the colony density was compared to the kit chart. Results represent caries risk and ms levels as follows. The RY scores were divided into three categories: CFU/ml $\leq 10^3$ (representing low caries risk, CFU/ml = 10^4-10^5 (representing medium caries risk), and CFU/ml > 10^5 (representing high caries risk).

Statistical analysis

Fisher Exact test was performed to assess the association between positive or negative *Candida* colonization and gender, active caries sites and morning and evening brushing scores. T-test was used to evaluate the association between *Candida* levels and age, dmf/DMF (s/S and t/T), PI, GI, and brushing efficiency scores. Pearson chi-square was used to test the association between positive or negative *Candida* colonization and RY results (mutans streptococci levels). Spearman Rho correlation was used to evaluate the association between RY results and presence of active caries (D) and between RY results and brushing indices. Mann-Whitney test was applied to evaluate the difference in active caries among *Candida*-positive vs. *Candida*-negative patients.

RESULTS

Among the 34 children who participated in the study, 24 participants (70.5%) had mixed dentition, 4 had permanent dentition and 6 had primary dentition.

Four children were caries-free. The mean DMFT was 6.7 ± 4.5 while mean DMFS was 10.5 ± 6.9. The mean plaque index (PI) was 0.91 ± 0.56, and the mean gingival index (GI) was 0.1 ± 0.2.

Mutans streptococci were found in all saliva samples tested. Eighteen children (53%) had high ms counts (>10^5) therefore were scored as high caries risk group, 9 children (26%) had medium ms counts (10^4-10^5) therefore scored as medium risk, and 7 children (20%) had low mS counts (≥10^3), therefore scored as low risk. Table 1 shows the mean DMFT, DMFS and D, and the caries risk score by RY kit.

Table 1. Mean DMFT, DMFS and D, and caries risk score (by mutans streptococci levels).

Caries risk	Mean DMFT	Mean DMFS	Mean D
Low (N=7)	6.28	8.57	2.42
Medium (N=9)	9	14.33	1.22
High (N=18)	6	9.5	2.5

Candida colonies were observed in 24 children (70.5%), while no growth of *Candida* was observed in the remaining 10 children. *Candida* colonies were identified as *Candida* albicans among 21 children (61.7%), and as Candida tropicalis among 4 children (11.7%). No Candida krusei colonies were observed.

No association was found between positive *Candida* colonization and DMFT, DMFS, PI and GI (Table 2), and between *Candida* and caries risk or active carious lesions (Table 3). In addition, no association was found between *Candida* and the type of dentition (primary or permanent), and between *Candida* and gender.

Tooth brushing efficiency was evaluated by two parameters: reaching all tooth-segments, and the brushing duration of each segment. Out of the 34 participants, two children did not brush their teeth themselves and were helped by their parents, and hence their tooth brushing could not be scored. Another pair of participants brushed themselves once daily, while the second tooth brushing was carried out by their parents. Thus, these 4 children were also excluded regarding tooth brushing efficiency.

Children who were C. albicans positive have demonstrated significantly higher mean percent of segments brushed and longer mean brushing time than those who were negative for C. albicans (p = 0.029 and 0.016 respectively) (Table 4).

DISCUSSION

Table 2. Salivary *Candida* and DMFT, DMFS, PI and GI

p*	Mean	N	Saliva C.albicans	
0.546	6.3846	13	Negative	**DMFT**
	7.3333	21	Positive	
0.385	9.2308	13	Negative	**DMFS**
	11.4286	21	Positive	
0.466	0.8215	13	Negative	**PI**
	0.9681	21	Positive	
0.200	0.1785	13	Negative	**GI**
	0.0538	21	Positive	

* Student t-test

Table 3. Caries risk, active carious lesions (the D component of the DMF scale), and the presence of salivary *Candida*.

p	C. albicans presence			Caries related parameter
	Positive	Negative		
0.361*	13	5	N	High caries risk (CFU/ml > 10^5)
	72.2%	27.8%	%	
	4	5	N	Medium caries risk (CFU/ml = 10^4-10^5)
	44.4%	55.6%	%	
	4	3	N	Low caries risk (CFU/ml ≤ 10^3)
	57.1%	42.9%	%	
0.718**	9	4	N	Active carious lesions (D)
	69.2%	30.8%	%	
	12	9	N	No active carious lesions
	57.1%	4.9%	%	

* Pearson's Chi-Square test
** Spearman's Rho correlation

Table 4. Salivary *Candida* albicans presence and mean, mean percent of segments brushed, and mean brushing time.

Salivary *Candida*	N	Mean % of brushed segments	Mean brushing time
Negative	12	32.5	15.8
Positive	18	50.8	34.7
p *		0.016	0.029

* Student t-test

Caries is a multifactorial disease. Some researchers have shown higher presence of C. albicans in children with high caries prevalence,[4,7,8] which may have lead to the assumption that high C. albicans may be an indicator for high caries activity.[13–15] In our study, caries was assessed by DMF,scores and the caries risk by the *mutans* streptococci levels (which were measured by using the RY kit).

The results of our study show no correlation between *Candida* and caries levels (DMF scores), PI, GI, or caries risk (as measured by *mutans* streptococci levels). Several possible factors may explain this contradictory result: First, the dynamic nature of the biofilm, in which microorganisms exist and thrive in close relations with others. Although not fully conclusive, it might be possible that while some types of microorganism thrive with *Candida* in the biofilm, the growth of others may be suppressed.[16,17] In addition, in the present study, the children, knowing they participated in a study, could have improved their tooth brushing efficiency, which in turn, may have influenced the RY kit scores of *mutans* streptococci levels. Furthermore, children who were positive for C. albicans have demonstrated significantly higher mean percent of brushed segments and longer brushing time than those who were negative for C. albicans. A possible reason for this finding could be that toothbrushes which were more frequently used were constantly moist, thus, providing good environment for *Candida* to thrive, and for continuous contamination of the users. Secondly, since caries is a multifactorial disease, despite the fact that children with high *mutans* streptococci counts demonstrated lower caries scores, it is possible that *mutans* streptococci was not the only risk factor. Other factors such as saliva rate as well as its composition and its buffering capacity, may also influence the carious process.[18,19]

The prevalence of *Candida* among the children in our study was 70.5%. This finding is somewhat higher than found in previous studies, which demonstrated a prevalence of 30%-50% among 3 to 12-year-old children.[2,6] The most prevalent species of *Candida* found was *Candida* albicans, followed by *Candid*a tropicalis (11%). Since *Candid*a tropicalis counts were very low, all comparisons were carried out with C. albicans counts.

Additional investigations are needed to further establish the association between *Candida* and other types of microorganisms in the human mouth, and their influence on oral diseases, primarily on dental caries.

CONCLUSIONS

b. There was no clear association between *Candida* in saliva and caries or *mutans* streptococci levels..

a. Toothbrushes may be reservoirs of *Candida*, and as such, may cause reinfection of the toothbrush user with *Candida*.

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