

# Salivary *Candida*, Caries and *Candida* in Toothbrushes

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**Background:** *Candida* species are common inhabitants of the normal oral microbiota. A few studies founded a relationship between high levels of *Candida albicans* in the oral cavity and high DMF scores. Toothbrushes can also be reservoirs of microorganisms, the proliferation of these microorganism on a toothbrush could be a major factor for its distribution in the oral cavity. **Aim:** To examine the associations between salivary *Candida* and DMF, and between salivary *Candida* and *Candida* in the toothbrush. **Method:** 46 healthy school children, who attended a University pediatric dental clinic, were tested for *Candida* in their saliva and in their toothbrush. Their DMF was recorded. **Results:** 38 children were *Candida*-positive (79.2%), out of whom 5 demonstrated a positive growth of *Candida* in the toothbrushes. No correlation was found between *Candida* in the saliva and in the toothbrush. The number of *Candida*-positive girls was significantly higher than the number in boys. No significant relationship between caries experience and the presence of *Candida* was found. **Conclusions:** No correlation was found between *Candida* in the saliva and in the toothbrush. The origin of the *Candida* in the toothbrush is not totally clear.

**Keywords:** *Candida*, caries, saliva, toothbrush

## 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.<sup>1</sup> 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.<sup>2</sup> Higher frequencies of *Candida* were found in pacifier user children.<sup>2-3</sup> Among *Candida* species, the most prevalent in the oral cavity is *Candida albicans*. However, the opportunistic *C. albicans* is capable of causing a variety of infections ranging from the common denture stomatitis and thrush, to the more serious systemic infections. Other types of *Candida*, such as *C. tropicalis* and *C. krusei* where isolated from the saliva of individuals with or without oral candidiasis.<sup>4</sup>

Oral candidiasis has been associated with a deficiency of numerous dietary factors e.g. iron, zinc, vitamin K and several water-soluble vitamins. Other predisposing factors to oral candidiasis include infancy and old age, hypo-salivation, poor oral hygiene, orthodontic

appliances, mouth breathing, endocrine disturbance, antibiotics and steroids, HIV infection and immunosuppressive drugs.<sup>4</sup>

A few researches founded a relationship between high levels of *C. albicans* in the oral cavity and high DMF scores (Decayed, Missing, Filled teeth) in school- and preschool children.<sup>5-8</sup>

It has been suggested that *C. albicans* has a cariogenic potential that manifests itself by the ability to dissolve hydroxy apatite<sup>9</sup>, the ability to adhere to hydroxy apatite in the saliva, resistance to acidic medium and the ability to lower the pH of a glucose rich saliva.<sup>10</sup> Yet, the association between *Candida* in the oral cavity and dental diseases has not been fully established and it is still not clear whether *Candida* has a true contribution to the caries process or it has a commensal relationship with the bacteria.

Toothbrushes are used mainly to remove dental plaque. However toothbrushes can also be reservoirs of microorganisms that adhere to them originating from various sources such as contaminated hands, toilet aerosols and obviously from the mouth. Studies have successfully isolated microorganism from toothbrushes, among them several types of fungi, particularly *Candida*, in children and adults.<sup>11-13</sup>

Thus, the proliferation of a microorganism on a toothbrush could be a major factor for its distribution in the oral cavity and a serious source for recurrent oral infections.<sup>14-15</sup>

The aims of the present study were:

1. To examine the association between salivary *Candida* and DMF.
2. To examine the association of salivary *Candida* and *Candida* in the toothbrush.

## METHODS AND MATERIALS

The study included all school aged children, who attended the Department of Pediatric Dentistry at the Maurice and Gabriela Goldschleger School of Dental Medicine, Tel Aviv University, during a three-month-period between September and December 2009. All

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children were healthy (No illness, no medications, according to the medical records) and were not regularly using any medications. 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.

### Clinical parameters

The following information was obtained for each participant: age, gender, caries status by number of decayed-extracted/missed-filled teeth for primary and permanent dentitions (def/DMFT respectively).

Each participant was asked to spit into a test tube, one Milliliter of unstimulated saliva was collected from every participant. Immediately afterwards, 0.1 mL of the saliva was plated on a selective agar medium for *Candida* – CHROMagar *Candida* (Hy Laboratories Ltd, Rehovot, Israel).<sup>16</sup> This medium is selective for the isolation and presumptive identification of yeast and filamentous fungi and differentiation of *Candida albicans*, *C. tropicalis* and *C. krusei*. The plates were incubated at 30°C for 48 hours. Subject's samples were grouped into salivary *Candida*-positive and negative groups.

Every child received an identical toothbrush and was instructed to use it twice a day for a week and to return it at the following appointment. The head of the toothbrush was immersed in 5 mL of saline and vortexed for 30 seconds. One sample (0.1 mL) was plated on the same medium as the saliva sample and incubated at 30°C for 48 hours. Subject's samples were grouped into toothbrush *Candida*-positive and negative groups.

Data was analyzed utilizing an SPSS (Statistical package for the social sciences) 15.0 software (SPSS Inc., Chicago, IL, USA). Levene's test for equality of variance was performed to assess the association between *Candida* presence in the saliva and in the toothbrush. Chi square was used to assess the association between *Candida* presence and gender. Student t-test was used to assess the association between *Candida* presence and age and def/DMF.

### RESULTS

The distribution of *Candida* colonies in the saliva and in the toothbrush is summarized in table 1. Out of 48 saliva samples, 38 were *Candida*-positive (79.2%) and 10 were *Candida*-negative (20.8%).

*C. albicans* was identified in 24 samples (50%), while *C. tropicalis* was found in 8 samples (16.7%), and *C. krusei* was found in 4 samples (8.3%).

The 38 children whose saliva contained *Candida* received a toothbrush. Five toothbrushes were *Candida*-positive (13.2%) and 33 were *Candida*-negative (86.8%). *C. krusei* was identified in 5 samples (13.2%) and one sample (2.6%) was identified as *C.*

*albicans*. No *C. tropicalis* colonies were identified. Some samples demonstrated more than one *Candida* species.

The number of *Candida*-positive girls was significantly higher than the number in boys ( $p < 0.05$ ). No gender difference was observed in regard to *Candida* in the toothbrush.

No correlation was found between *Candida* in the saliva and in the toothbrush.

Table 2 shows the number of extracted primary teeth in relation to salivary *Candida*. *Candida*-positive children experienced extractions of primary teeth while *Candida*-negative children had no extractions at all ( $p = 0.032$ ).

The scores of DMF and the total DMF (TDMF = def + DMF) were significantly higher among salivary *C. tropicalis*-positive children than among salivary *C. tropicalis*-negative children ( $p = 0.035$ ,  $p = 0.049$  respectively).

Comparing the caries status of salivary *C. albicans*-positive children to salivary *C. albicans*-negative children, revealed higher levels of total DMF, DMF and def among salivary *C. albicans*-positive children, however this trend was not statistically significant.

No correlation was found between salivary *C. krusei* to caries experience.

*C. krusei*-negative children demonstrated significant large number of restored teeth while *C. krusei*-positive children had no restorations at all ( $p < 0.001$ ).

No correlation was found between *C. albicans* or *Candida* in general in the toothbrush to caries experience.

### DISCUSSION

Caries is a multifactorial disease, its formation requires biofilm, fermentable carbohydrates, tooth surface and time. Studies have found high levels of *Candida* in subjects with high caries experience, and a correlation between the salivary *Candida* and the development of caries lesions.<sup>6-17</sup> It may be that *Candida* may be one of the many factors that are involved in the caries process.

In the present study we isolated *Candida* from the saliva and from the toothbrushes of 46 school children (18 boys, 28 girls). The prevalence of salivary *Candida* in the in the present study is 79.2%. Previous studies reported a much lower prevalence of 60 to 66%,<sup>4-5</sup> and even 70.5% in a previous research that was held in our department.<sup>18</sup> The difference can be attributed to differences in the studied populations. It may be that the population in the present study came from a lower socioeconomic status.<sup>19</sup>

Fifty percent of the children in the present study were found to be positive for *C. albicans* in their saliva. This is higher than the percentage reported by Raja *et al* (39%),<sup>5</sup> and lower than reported by Peretz *et al* (61.7%) (18). The results seem inconsistent/incon-

**Table 1:** Distribution of *Candida* presence in the saliva and in the toothbrush\*

Specimen	<i>Candida</i> - Positive				<i>Candida</i> -Negative	Total
	<i>C. albicans</i>	<i>C. tropicalis</i>	<i>C. krusei</i>	Total <i>Candida</i>		
Saliva	24 (50%)	8 (16.7%)	4 (8.3%)	38 (79.2%)	10 (20.8%)	48 (100%)
Toothbrush	1 (2.6%)	0 (0%)	5 (13.2%)	5 (13.2%)	33 (86.8%)	38 (100%)

\* Patients may harbor more than one species of *Candida*.

**Table 2:** The number of extracted primary teeth (e) by salivary *Candida*

Negative	Positive	<i>Candida</i> in the saliva
0	5	Number of children with an extracted primary teeth
0	6	Number of extracted primary teeth
0	0.17*	Mean extracted primary teeth

\*  $p=0.032$  Levene's test for equality of variance

clusive. We could not produce a comprehensive explanation for the variations in regard to the prevalence of *C. albicans* in children's saliva. Another possibility is related to differences in the number of subjects that were examined in all studies.

The prevalence of *Candida* in the toothbrushes in the present study was 13.16%. It is lower than the prevalence that was reported by Rizk *et al*, who reported isolating *C. albicans* from the toothbrushes of 52% of the subjects.<sup>20</sup> The difference in the frequency of brushing, the period of time that the toothbrushes were used and their storage could account for these discrepancies.

When judging the relationship between the caries status in the primary and permanent dentitions to the presence of *Candida* in the saliva; only among *Candida*-positive children did extracted teeth are found (read this sentence aloud, it sounds like one master Yoda would say). It is important to note that the total amount of extracted teeth was 6 in only 5 children out of 48.

In the present study, the total DMF and the DMF were significantly higher among salivary *C. tropicalis*-positive children. The literature however refers to *C. albicans* with regard to caries. Again, the various findings may point toward inconsistency regarding the association between *Candida* and dental caries. These findings support an association between *Candida* and caries development.

Moreover, a negative trend was found between *Candida*, total DMF and DMF. This is in accordance with a previous report<sup>18</sup> in which higher levels of total DMF, DMFT and def in *C. albicans*-negative children, was noted.

When examining the correlation between caries experience and the presence of *Candida* in the toothbrushes, we found that children that were negative to *C. krusei* experienced restorations in the primary teeth as opposed to those who were positive and did not have any restorations. Despite the statistical significance ( $p<0.001$ ), the small number of positive children (5 out of 38 children) does not allow for definite conclusions.

There was no correlation between the types of salivary *Candida* and those found in the toothbrushes. This finding suggests that the origin of the *Candida* in the toothbrush may be from an external source such as dirty hands, sink or bathroom aerosol.<sup>14-15</sup>

One can conclude that correct storage and sanitization of the toothbrushes is preferable in order to minimize the potential of oral contamination, whether its origin is from the oral cavity or from exogenous source. Some researchers recommended different methods of disinfection: chlorhexidine spray or mouthwash, ozone and even microwave (14) (20-21).

When comparing the *Candida* presence between boys and girls, we found that significantly more girls had a positive growth in the saliva. One explanation can be that the majority of the subjects were girls (60.7%).

The present study has some limitations. The relatively small number of children who participated may have skewed the results. Larger cohorts are needed, as well as examining the toothbrush after dental treatment is advisable to further understand the relations role *Candida* plays in the caries process.

## CONCLUSIONS

1. No correlation was found between *Candida* in the saliva and in the toothbrush.
2. The origin of the *Candida* in the toothbrush is not totally clear.

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