SHORT COMMUNICATION

Quantity of the antigens of *Streptococcus mutans* serotype e and *Candida albicans* and its correlation with the salivary flow rate in early childhood caries

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Background: *Streptococcus mutans* involved in caries pathogenesis is classified into four serotypes, namely serotypes c, e, f, and k. *Candida albicans* can be found in the plaque of children with early childhood caries (ECC). Aims: The aim of this study was to analyze the quantity of the antigens of *S. mutans* serotype e and *C. albicans* and its correlation with the salivary flow rate in ECC. **Materials and Methods:** The antigen quantities of caries plaque samples and caries-free were determined using an enzyme-linked immunoassay with 450-nm optical density. **Results:** There was a significant difference between the quantity of *S. mutans* serotype e and *C. albicans* antigens in each salivary flow rate category ($P < 0.05$). The relationship between the antigen quantity of *S. mutans* serotype e and *C. albicans* was $r = 0.624$ ($P > 0.05$) for caries plaque samples and $r = 0.628$ ($P > 0.05$) for caries-free samples. **Conclusion:** the antigen quantities of *S. mutans* serotype e and *C. albicans* and the salivary flow rate might correlate to the pathogenesis of ECC.

Key words: *Candida albicans,* dental plaque, early childhood caries, flow rate, saliva, serotype, *Streptococcus mutans*

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INTRODUCTION

According to the American Academy of Pediatric Dentistry, early childhood caries (ECC) is a terminology used when there is a presence of more cavities or missing or filled teeth due to caries in children up to 71 months of age.[1,2] The etiology of ECC is multifactorial, resulting from interactions between microorganisms, including *S. mutans*.[3] and sugar on the surface of the teeth.[4] Host factors contributing to caries development are low salivary flow rates, immunological factors, damaged or imperfect enamel, tooth morphology itself, and poor tooth structure.[5] Saliva contains minerals that play an important role in the process of tooth remineralization.[6] Immunoglobulin A contained in saliva and gingival crevicular fluid acts as a immune defense against *Streptococcus mutans*.[7]

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S. mutans is a Gram-positive bacterium and one of seven species of the mutans streptococci.[8] Based on its serotype-specific polysaccharide composition, namely, rhamnose-glucose polymers (RPG), *S. mutans* is classified into four types: serotypes c, e, f, and k.[9] Polysaccharides in serotypes c, e, and f consist of RPG, whereas, for serotype k, the identified glucose side chains are much reduced with only rhamnose chains present.[10,11]

In addition to *S. mutans*, opportunistic fungal *C. albicans* also plays an active role in the pathogenesis of caries and is often found in children's oral cavity with ECC.[12,13] A higher number of *C. albicans* can be found in the saliva, plaque, and caries tissue of kids with ECC than that in kids without caries.[14]

The prevalence of *S. mutans* serotype e was found to be 5%.[15] Moreover, still rare study analyzing this serotype

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e that might has a correlation to ECC.[16] Future research with is required to determine the role of *S. mutans* serotype e to assess the preventive role against caries development. Analysis of the antigen quantities of *S. mutans* serotype e and *C. albicans* in related to the salivary flow rate has not been reported previously.

In this study, we aimed to analyze the quantity of the antigens of *S. mutans* serotype e and *C. albicans* and its correlation with the salivary flow rate in ECC.

MATERIALS AND METHODS

Subjects

This study was conducted as an observational study. The samples were obtained through a purposive sampling technique. Sampling was carried out at the TPA Harapan Ibu Ministry of Social Affairs Ministry of Social RI on 50 children: 36 children with ECC and 14 caries-free children. Plaque samples were taken from the first deciduous molars using cotton buds and stored in Eppendorf tubes containing 1 mL of phosphate-buffered saline (PBS).

Salivary flow rate

Salivary flow rate test was performed on each subject. The examination was performed by placing one ply of tissue on the subject with the dried lower lip and observing how much time it took for the tissue to get wet using a stopwatch.[16] The results were categorized into two groups: one with a salivary flow rate <30 s and the other with 30–60 s.[17]

Enzyme-linked immunoassay

Indirect enzyme-linked immunoassay (ELISA) was carried out in this study.[17,18] Briefly, the samples that were diluted 1/10 in PBS (Sigma-Aldrich Dorset UK. Cat. P4417) were added to the wells and incubated at 4°C overnight. After incubation and washing, blocking buffer (5% skim milk in PBS-0.1% Tween^{R}20 [Sigma-Aldrich, Cat. P9416]) was added to each well, followed by incubation for 1 h at 37°C. Rabbit sera anti-*S. mutans* serotype e or anti-*C. albicans* diluted in PBS at a 1:1,000 ratio were added to each well and bound to the coated antigen of *S. mutans* and *C. albicans*. After incubation and washing, tetramethylbenzidine (TMB substrate) (Sigma-Aldrich, Cat. T0440) was added to the wells, followed by the addition of the stop solution. The optical density (450 nm) of the complex antigen antibody was determined using an ELISA reader (AccuReader. M965/M965+ Nangang, Taipei, Taiwan).[19]

Statistical analyses

The Kolmogorov–Smirnov normality test was used, and the Mann–Whitney U-test comparative test was done to compare the quantity of *S. mutans* serotype e and *C. albicans* antigens between the two groups. The correlation of both microorganisms in both ECC and caries-free samples was tested using the Spearman test.

RESULTS

Data distribution

We have totally 50 subjects included for this study, with 36 caries active and 14 of caries-free aged between 2.4 and 4.5 years. Children with salivary flow rate <30 s in caries active were 20 subjects and 16 children have salivary flow rate of 30–60 s. Whereas 10 children with caries-free have salivary flow rate <30 ($P > 0.05$).

Quantities of *Streptococcus mutans* serotype and *Candida albicans* antigens in children with and without caries

The Kolmogorov–Smirnov test shows that the data were not normally distributed; hence, the Mann–Whitney U comparative test was done. Figure 1a shows that the average ± standard deviation of *S. mutans* serotype e antigens was 2.361 and the average optical density of *C. albicans* antigens was 1.646 ± 0.23. For caries-free samples and *S. mutans*, serotype e antigens was 2.028 ± 0.41, and the average of *C. albicans* antigens was 1.429 ± 0.37 ($P < 0.05$). In children with active caries, the average of the quantity of *S. mutans* serotypes e and *C. albicans* antigens was higher than in the caries-free group.

The average of quantities of *Streptococcus mutans* serotype e and *Candida albicans* antigens associated with the salivary flow rate

Figure 1b shows the average *S. mutans* serotype e and *C. albicans* based on the salivary flow rate in caries and caries-free samples. *S. mutans* serotype e and *C. albicans* antigens in caries were higher than those in caries-free in both categories of salivary flow rate ($P < 0.05$). In caries active and caries-free, the OD 450 of *S. mutans* in <30 s salivary flow rate 2.331 versus 1.985 and in 30–60 s were 2.463 versus 2.135, whereas *C albicans* 1.632 versus 1.428 and in 30–60 s were 1.728 versus 1.433, respectively ($P > 0.05$). Further analysis showed that the average of quantity of *S. mutans* serotypes e and *C. albicans* antigens, higher than in the free caries group both in children with a salivary flow rate <30 s and in the group with salivary flow rate of 30–60 s ($P > 0.05$).

Spearman test yielded a value of $r = 0.624$, with $P < 0.05$, indicating that there was a positive correlation between the antigen quantities of *S. mutans* and *C. albicans* in the plaque of subjects with ECC [Figure 2a]. A value of $r = 0.628$, with $P > 0.05$, was obtained from the Spearman correlation test, suggesting a positive correlation between the antigen quantities of *S. mutans* serotype e and *C. albicans* in the plaque of caries-free subjects [Figure 2b].

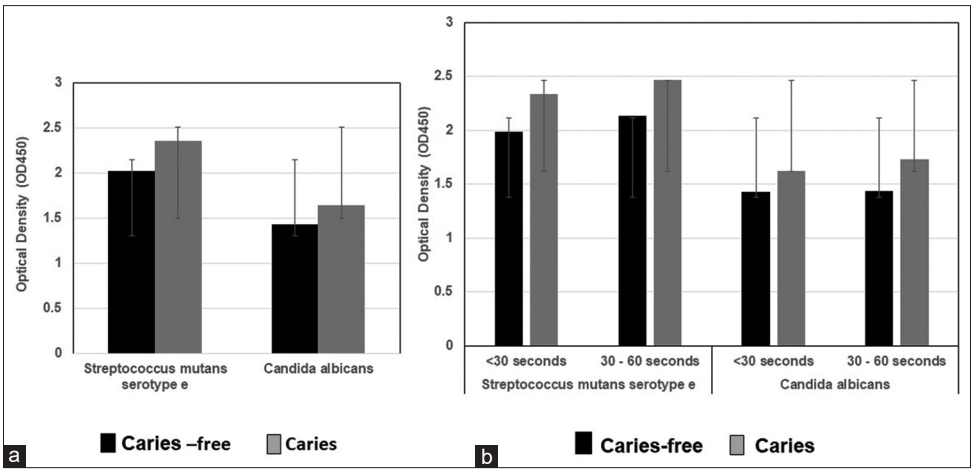

Figure 1: (a) Average of the quantities of *Streptococcus mutans* serotype e and *Candida albicans* antigens in the plaque of children with and without caries, (b) quantities of *Streptococcus mutans* serotype e and *Candida albicans* antigens in the plaque associated with salivary flow rate

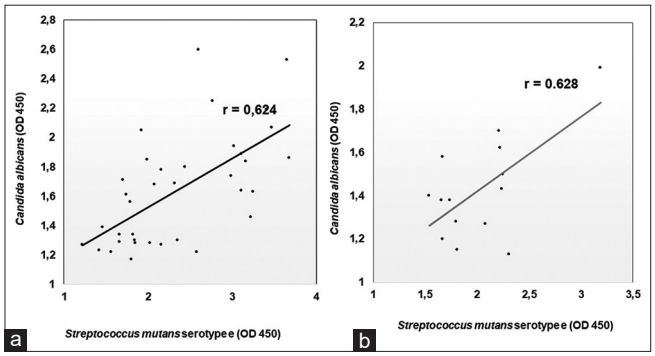

Figure 2: (a) Correlation between antigen quantities of *Streptococcus mutans* serotype e and *Candida albicans* in the plaque of subjects with caries, (b) correlation between antigen quantities of *Streptococcus mutans* and *Candida albicans* in the plaque of caries-free subjects

DISCUSSION

The key finding of our study is the quantity of *S. mutans* serotype e, and *C. albicans* antigens on the plaque of children with caries are higher than those caries-free children regardless of the salivary flow rate category.[18-22]

Our finding is supported by previous studies that stated that caries was a multifactorial condition where the number of cariogenic microorganisms did not guarantee whether a carious lesion formed. However, caries formation is also determined by a balance of protective and pathologic factors called "caries balance."[3]

Furthermore, this study showed a strong positive correlation between antigens of *S. mutans* serotype e and *C. albicans*. This result is consistent with that of a previous study by Bachtiar and Bachtiar, reporting that there is an association between antigen quantities of both microorganisms.[20] The data on the association between *C. albicans* and *S. mutans* serotype e in this study may support the clinical importance of uncovering the pathogenesis of ECC. The results of this study may contribute to find strategies to improve children's oral health. Further *in vitro* experiment study might need to investigate the molecular interaction between *S. mutans* and *C. albicans* under sugar enriched condition.

CONCLUSION

The salivary flow involved in *S. mutans* serotype e and *C. albicans* existence in ECC patients.

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Conflicts of interest

There are no conflicts of interest.

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