



RESEARCH ARTICLE

REVISED Relationship between *Candida albicans* and *Streptococcus mutans* in early childhood caries, evaluated by quantitative PCR [version 2; referees: 2 approved]

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Abstract

Background: The aim of this study was to analyze the synergistic relationship between *Candida albicans* and *Streptococcus mutans* in children with early childhood caries (ECC) experience.

Methods: Dental plaque and unstimulated saliva samples were taken from 30 subjects aged 3-5 years old, half with (n=15, dmft > 4) and half without (n=15) ECC. The abundance of *C. albicans* and *S. mutans* and relative to total bacteria load were quantify by real-time PCR (qPCR). This method was also employed to investigate the mRNA expression of glycosyltransferase (*gtfB*) gene in dental plaque. Student's t-test and Pearson's correlation were used to perform statistical analysis.

Results: Within the ECC group, the quantity of both microorganisms were higher in the saliva than in dental plaque. The ratio of *C. albicans* to total bacteria was higher in saliva than in plaque samples (p < 0.05). We observed the opposite for *S. mutans* (p < 0.05). The different value of *C. albicans* and *S. mutans* in saliva was positively correlated, and negatively correlated in dental plaque. Transcription level of *S. mutans gtfB* showed a positive correlation with *C. albicans* concentration in dental plaque.





Conclusion: *C. albicans* has a positive correlation with cariogenic traits of *S. mutans* in ECC-related biofilm of young children.

Keywords

Early childhood caries, C.albicans/ S. mutans, Saliva, Dental plaque, qPCR, Indonesian

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REVISED Amendments from Version 1

We have added a better suggestion at the end of the Discussion's paragraph 3, stating that more studies need to be conducted in order to obtain data regarding a carcinogenic diet. We also add a sentence at the end of paragraph 6, to state that further research needs to be conducted which explore our findings that high sucrose concentration might exist in children with oral cavities. Additionally, we include a sentence at the end of paragraph 7, mentioning that more studies regarding the involvement of *gtfB* expression, and how it affects oral health conditions, are needed.

We edit the Conclusion to mention that further studies involving preschool children may wish to include examiners more experienced in working with parents/guardians, as we have found that it can be difficult to understand information provided by the accompanying guardians regarding the children's oral hygiene and diet.

We revised Figure 2 and Figure 4 to address the reviewer's comments. Although the Figure 3 is not included in reviewer's comment, we revised the font to Times New Roman so all figures use similar font.

See referee reports

Introduction

Early childhood caries (ECC) remain the most common childhood oral health problem, globally¹, and *Streptococcus mutans* has been known for its important role in ECC development^{2,3}. However, in recent years, *Candida albicans* has frequently been linked with its synergistic relationship with *S. mutans* in dental plaque recovered from children with ECC^{4,5}. Consequently, many studies using different methods have been conducted to identify, quantify, and explored the relationship of this fungus with *S. mutans*⁶⁻⁸. However, a controversial report does exist, where *C. albicans* tends to decrease the cariogenic traits of *S. mutans* in *in vitro* dual-species biofilm⁹. Therefore, the main purpose of this study was to validate the synergistic relationships between *C. albicans* and *S. mutans*, when growing in caries-related biofilm. For this reason, we recruited preschool children with ECC experience, and we used qPCR since it is practice and reliable as a quantitative molecular tool of clinical oral samples¹⁰. The fungus and bacterium concentrations in saliva sample were used as control, and we compared the outcomes with those subjects noted as children with free caries (FC).

Methods

Subjects

Oral samples were collected from 30 required preschool children (male and female, 3–5 years old), in two different location located near (about 30 km) to Jakarta, the capital city of Indonesia. The diagnostic of ECC referred to the criteria provided by the American Academy of Pediatric Dentistry, as previously reported¹¹. Two weeks prior to collect the clinical samples, the examiners were calibrated and trained by providing with manual describing study protocol and guidance regarding examination of preschool children. Therefore, only those trained-examiners evaluated the preschool children. The preschool children were recruited to get 15 subjects for each group. Thus, in this study, children were categorized into two different groups; children without any history of caries, including white-spot lesions, (caries free; CF group) and ECC group with decay-missing-filled teeth (dmft) index >4. To be included as subjects in this study, the children were required to be free of symptomatic oral candidiasis,

have the absence of any medication therapy during the one month before this study, and have not worn any intraoral appliances. Before oral samples collection, written permission (informed consent) for children to participate was obtained from parents or guardians, according to the guidance provided by the Ethics Committee of Faculty of Dentistry, Universitas Indonesia.

Samples from supragingival plaque, obtained from the selected teeth deciduous (either molar or incisive) were isolated with sterile cotton rolls and pure cotton buds. Samples from the ECC group was obtained by gathering carious biofilm around the affected enamel¹², including dentin, as the fungus does not invade carious human dentin¹³. For the FC group, samples were obtained from enamel in clinically sound gingival areas. In each group, samples collected from molar or incisor, upper or lower teeth were not separated. Therefore, the obtained plaques were pooled to give a single sample for each subject and put in a microcentrifuge tube containing 1 ml PBS (pH 7.4). Unstimulated saliva was collected from all subjects on the same occasion and immediately after the plaque collection, by spitting the saliva into sterile Falcon plastic tubes. The minimum volume of collected saliva was 0.5 ml.

Saliva and plaque samples were immediately cold-transported to the laboratory. For saliva samples, after centrifugation, the sediments were washed three times with 0.5 ml sterile milli Q water between each centrifugation and kept in -80°C until use. Similarly, plaque samples from caries-free or those with ECC were cold-transported to the laboratory and processed as mentioned above, then stored at -80°C until use.

Quantification of *C. albicans*, *S. mutans*, and total bacteria by qPCR

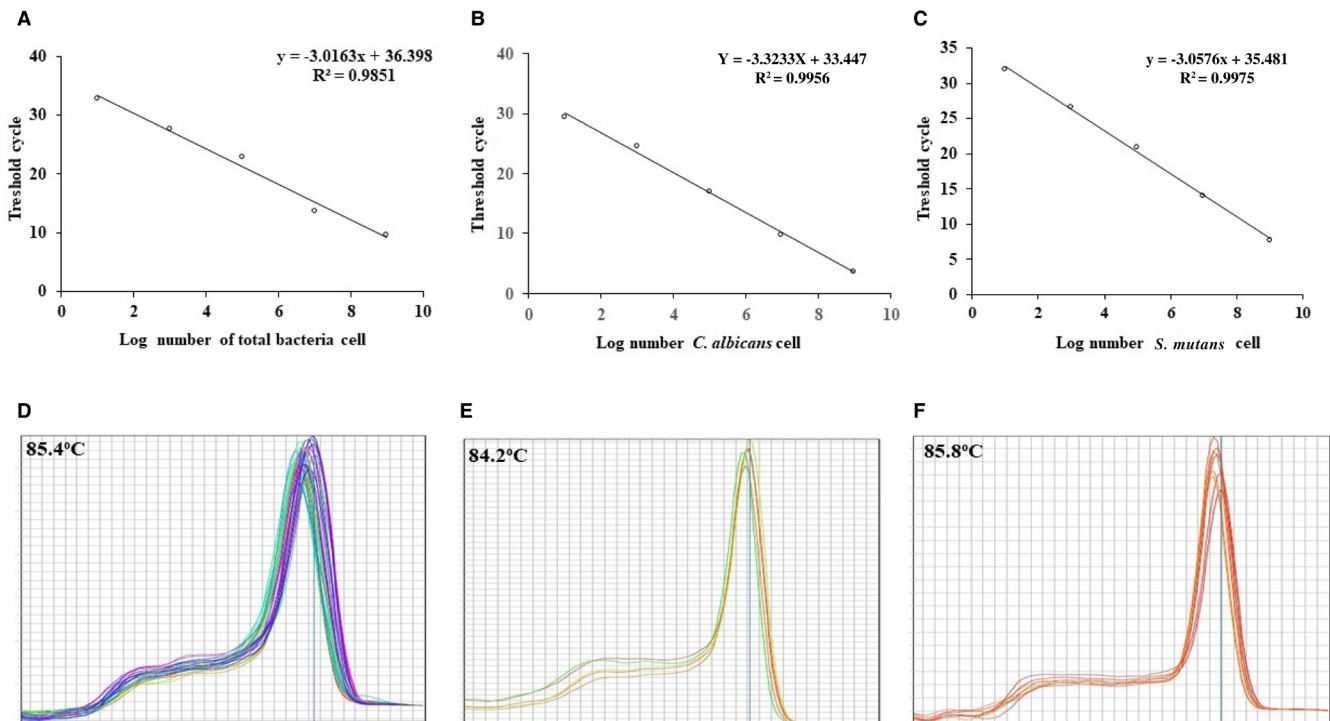
Bacterial/fungal DNA was obtained by centrifugation each sample in the microtube, using Trizol reagent (Sigma-Aldrich, Dorset, UK). Extracted DNA sample was kept at -20°C after determining the concentration and the quality by Qubit assay reagents (Invitrogen, Carlsbad, CA). The genomic DNA samples were dissolved in Tris-EDTA (TE) buffer and kept in -20°C freeze until used. Further, the DNA samples were quantified through a qPCR reaction with universal primers for the 16S rRNA genes and *C. albicans*/*S. mutans*-specific primers as shown in Table 1. For PCR-quantification, each sample was run in triplicate on an ABI StepOnePlus Real-Time PCR System with SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol.

The PCR conditions were run in a final reaction volume of 10 µl, composed of 50 ng of sample DNA and 1 µM of each primer (Table 1), with thermal cycling condition consisted of a 10 min initial denaturation at 95°C, followed by 40 cycles of 95°C for 15 s and 65°C for 60 s. The cycle threshold (Ct) were determined automatically by the instrument, and a dissociation curve of the amplified fragment set as follow; 95°C for 15 s, 60°C for 60 s, and 95°C for 15 s.

Estimating the amount of genomic DNA of both microorganisms tested was determined by constructing standard curves with r² values for both organisms tested as well as total bacteria (Figure 1A–C). To do this, we used a 10-fold serial dilution of

Table 1. Primers used in this study.

Primer name	Sequences	References
<i>C. albicans</i>	Forward: 5'-CACGACGGAGTTTCACAAGA -3'	14
	Reverse: 5'-CGATGGAAGTTTGAGGCAAT-3'	
<i>S. mutans</i>	Forward: 5'-CCTACGGGAGGCAGCAGTAG-3'	14
	Reverse: 5'-CAACAGAGCTTTACGATCCGAAA-3'	
Universal bacterial 16S rDNA	Forward: 5'-GTGSTGCAYGGYTGTCGTCA-3'	15
	Reverse: 5'-ACGTCRTCCMCACCTTCCTC-3'	
<i>gtfB</i>	Forward: 5'-AGCAATGCAGCCAATCTACAAAT-3'	14
	Reverse: 5'-ACGAACCTTGCCGTTATTGTCA-3'	

**Figure 1.** Standard curve construction and melting temperature of qPCR. Standard curves of total bacteria (A), *C. albicans* (B) and *S. mutans* (C). Also shown are melt curve profiles and melting temperatures for total bacteria (D), *C. albicans* (E), and *S. mutans* (F).

extracted fungal and bacterial genomic DNA from overnight cultured of *C. albicans* ATCC 10231, *S. mutans* Xc, and *Escherichia coli* JM 107, respectively. The number (CFU/ml) assessed by plating culture dilutions on sabouraud agar, tryptone-yeast extract cysteine with sucrose and bacitracin (TYCSB) agar and Luria Bertani (LB) broth for *C. albicans*¹⁶, *S. mutans*¹⁷, and *E. coli*¹⁸, respectively. The same strains were used as positive control for qPCR. Therefore, quantification of *C. albicans* and *S. mutans* from plaques and saliva achieved by plotting the Ct values against the log of the respective standard curve. In this study, the ratio of *C. albicans* or *S. mutans* in the microbial community, in each sample, was determined as each microorganism proportion to total bacteria.

For both *C. albicans* and *S. mutans*, the detection limit by qPCR method was determined according to the limit of quantification (LOQ), obtained by the highest dilution of the template of the standard curve. When the Ct value of samples was higher than the LOQ, they would be considered positive, but their melting curve profile should be the same as those of the standards included when running the qPCR.

qPCR analysis of *S. mutans gtfB* in dental plaque samples

RNA isolation, purification, and reverse transcription of cDNA were performed similarly those in the previous study¹⁹. Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen Life Technologies, Carlsbad, CA, USA), passive reference (ROX, Invitrogen),

and *S. mutans* *gtfB* primers (Table 1), as well as 1 µg of cDNA, were used to quantify the cDNA, and non-transcribed RNA samples were used as control for genomic DNA contamination. The qPCR reaction was run on a similar machine as stated above with cycling conditions consisted of a 10 min initial denaturation at 95°C followed by 40 PCR cycles of 15 s at 95°C, and 60°C for 1 min. The formula of fold change $2^{-\Delta\Delta Ct}$ was used to calculate *S. mutans* *gtfB* gene expression that was normalized to the 16S rRNA, a well-established housekeeping gene²⁰, and *gtfB* expressed in dental plaque of FC group was set to be the control.

Statistical analysis

The variables for quantification, proportion, and the mean quantitative gene expression were assessed using Student's t-test, while Pearson's correlation two-tailed test was used to depict a linear association. Microsoft Excel software was used to perform statistical analysis, and a p-value < 0.05 was considered significant.

Results

Quantitative levels of *C. albicans* and *S. mutans* and their proportion in saliva and dental plaque samples

Standard curves were used to determine the corresponding number of microorganism tested while melting peaks were used to assess the specificity of the amplicon using saliva and plaque samples (Figure 1D–F).

In general, this study showed that in all saliva and plaque samples, from either FC or ECC children, both *C. albicans* and *S. mutans* were present. The quantification (log DNA copies) and proportion (% to total bacteria) of *C. albicans* and *S. mutans* in saliva, as well as plaque samples, are presented in Figure 2. Comparatively, it was observed, in either sample tested, a

significantly higher number of both microorganisms in ECC children was found more than in those of the FC children ($p < 0.05$). However, in either group, the quantitative level of *C. albicans*, in the saliva sample was found to be significantly lower than those of *S. mutans* ($p < 0.05$). When comparing plaque and saliva samples within ECC children, we observed that the load (log DNA copies) of either microorganism in plaque was significantly lower than that in saliva ($p < 0.05$, Figure 2A).

Furthermore, we compared the proportion of *C. albicans* and *S. mutans* DNA relative to total bacterial DNA in saliva and dental plaque samples, in FC and ECC children. Within ECC children, there was a significantly higher proportion ratio (Ca/Tb) of *C. albicans* in saliva samples (35.5%), than that in plaque samples (13.5%) ($p < 0.05$). For FC children the ratio was not statistically different (saliva, 8% and plaque, 5.3%) (Figure 2B). Similar analysis was carried out for the *S. mutans* proportion ratio (Sm/Tb). The result showed a different trend, with a significantly higher proportion of *S. mutans* in plaque (99%) than that in the saliva (62%) of ECC children (Figure 2B). The result also showed the proportion of this bacterium to total bacteria in saliva and plaque samples showed a significant difference between ECC and FC children ($p < 0.05$). Interestingly, there was a trend within dental plaque sample in ECC children, *S. mutans* DNA increased most with increased of *C. albicans*' DNA (Figure 2B).

Association between the value of *C. albicans* and *S. mutans* in dental plaque and saliva of ECC children

We further evaluated the possible linear relationship of *C. albicans* and *S. mutans* load or their proportion and ECC experience in the subjects. Pearson correlations coefficient analysis revealed that the association between the numbers of these two microorganisms in saliva was moderate positively significant

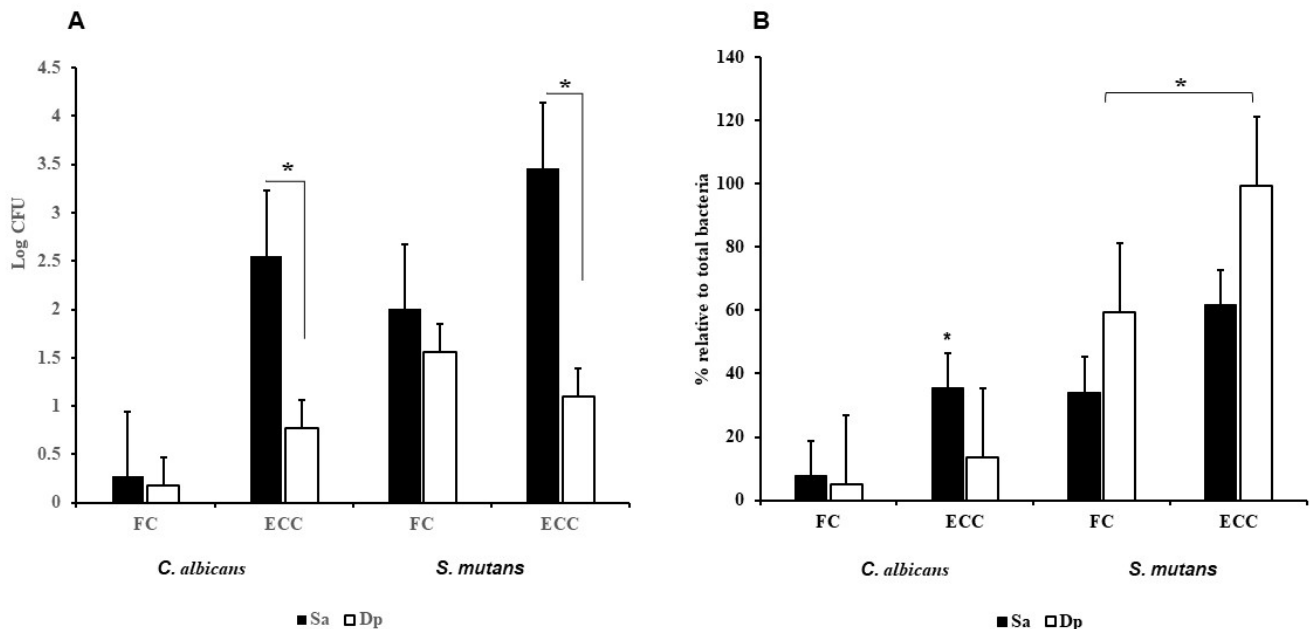


Figure 2. Mean and standard error of absolute numbers (A) and ratios (B) of *C. albicans* and *S. mutans* detected within the same saliva (Sa) and dental plaque (Dp) samples. CF, caries-free; ECC, early childhood caries. * $p < 0.05$.

($r = 0.1$, $p < 0.05$). On the contrary, a weak negative not substantial ($r = 0.03$, $p > 0.05$) between the decreasing number of *C. albicans* and the quantity of *S. mutans* in plaque samples observed in the ECC group (Figure 3A and B).

Quantification of *gtfB* gene transcription and its correlation with *C. albicans* and *S. mutans* amount in dental plaque

To confirm all the above results, we selected the *gtfB* gene, which has been reported to be mostly involved in the synergistic

relationship between *C. albicans* and *S. mutans* in biofilm development⁷, and compared its expression in each dental plaque of children tested. The qPCR result showed that level of mRNA *gtfB* was induced approximately 4.5-fold in ECC-derived dental plaque samples, and it was a significant difference compared to transcription level of *gtfB* mRNA in dental plaque sampled from children with FC ($p < 0.05$) (Figure 4A). Also, *gtfB* transcription levels and the amount of *C. albicans* and *S. mutans* (CFU/ml) in dental plaque of children with ECC

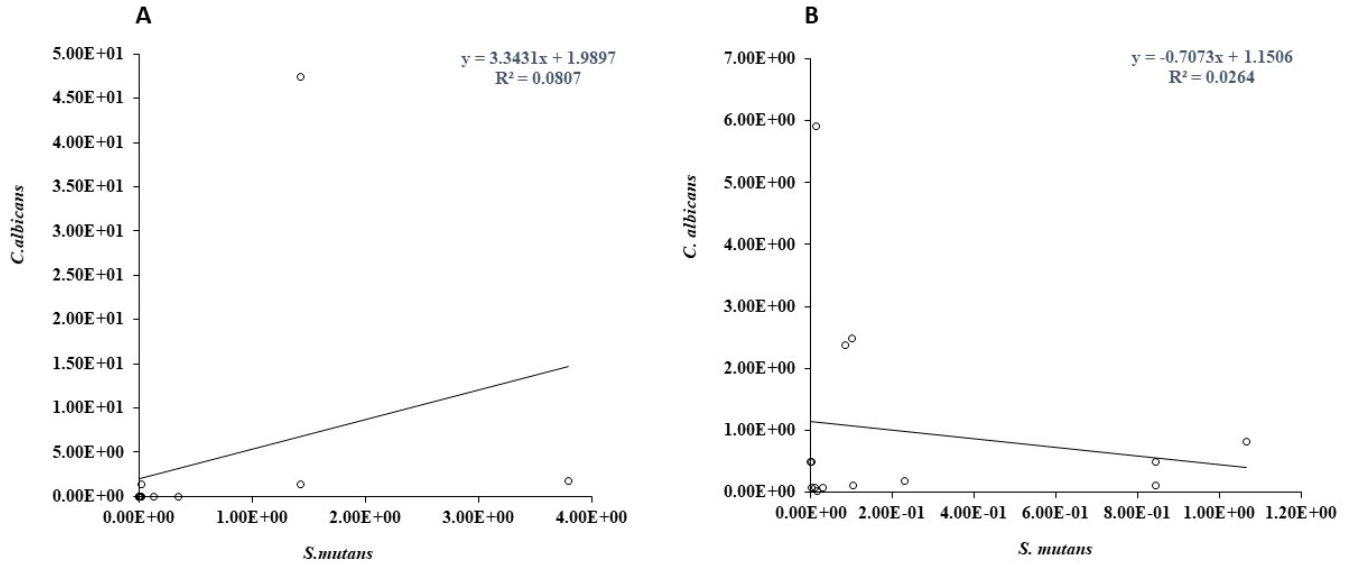


Figure 3. Correlation between *C. albicans* and *S. mutans* loads in saliva (A) and in dental plaque (B), in the same subjects. Each circle depicts the value of *C. albicans* (Y-axis) and *S. mutans* (X-axis) in log CFU/ml for each subject.

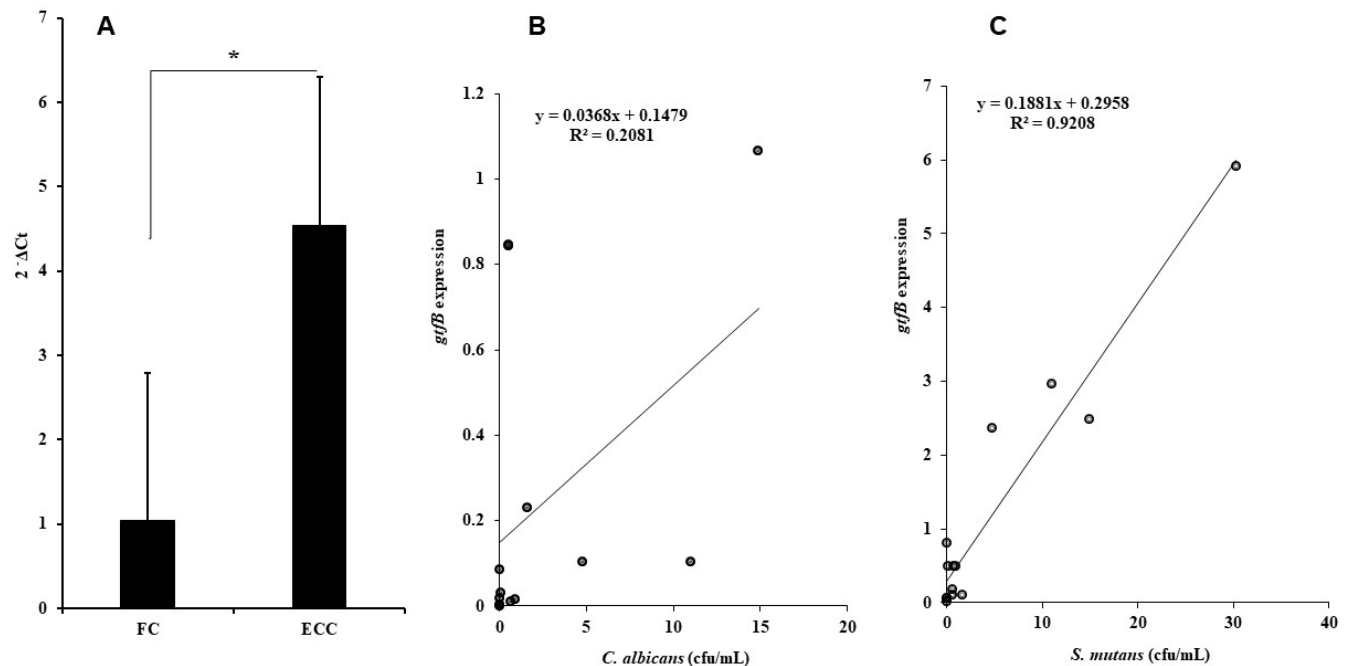


Figure 4. *S. mutans gtfB* gene expression. (A) The fold regulation of *gtfB* cDNA that was normalized to the amount of cDNA of 16S rRNA. (B, C) the correlation between *gtfB* transcription rate and the amount of *C. albicans* and *S. mutans*, respectively.

showed moderate ($r = 0.2$) and strong ($r = 0.9$) positive correlations, respectively, which was statistically significant ($p < 0.05$, Figure 3B and C).

Dataset 1. The raw data associated with this study

<https://dx.doi.org/10.5256/f1000research.16275.d221304>

Data are grouped according to the figure with which they are associated.

Discussion

Numerous studies^{7,8,21} have supported the idea that ECC may be better understood by focusing on the effect of the functional relationship between species within consortia instead of individual pathogens. This study focused on the relationship between *C. albicans* and *S. mutans*, as these oral microorganisms are frequently detected in the plaque of children with ECC^{12,22}. Since ECC may indicate a vast proliferation of the cariogenic microorganism, we sought to evaluate the extent to which the amount of *C. albicans* might correlate to the ECC experience. To do this, we used qPCR. This method enabled the quantification of the targeted microorganisms' genomic DNA from oral samples²³. Thus, we examined the similarities and differences by comparing the amount and ratio to total bacteria of each microorganism in saliva and dental plaque samples, and verified their correlation with the occurrence of ECC. In general, we observed that the oral cavities of preschool children in this study, with or without ECC, are colonized by yeast (*C. albicans*) and bacteria, as represented by *S. mutans*. Overall, the fungus presence was always simultaneously detected with *S. mutans*, although children with ECC had a higher amount of *C. albicans* and *S. mutans* in their oral cavity, compared to those children with FC. As expected, in addition to saliva, these oral microbiotas commonly exist together in an ECC-related biofilm.

Our observation is in accordance with results from other studies, which found that in addition to *S. mutans* as a specific cariogenic bacterium^{24,25}, *C. albicans* can be part of the dental lesion^{26,27}. Moreover, previous studies showed strong synergism when *C. albicans* and *S. mutans* co-existed in biofilm, suggesting that this co-existence enhanced their virulence^{7,8,28}. However, our data showed, the fungus was detected at lower levels in dental plaque, compared to *S. mutans*. This support the previous *in vitro* study²⁹, which found that the presence of *C. albicans* might favor the extensive colonization of *S. mutans* in dental biofilm.

The causes to generate site specificity bacteria proportion are believed to include local sucrose concentration in the oral cavity^{30,31}. In addition to sucrose³², many factors may link to the presence of *C. albicans* in children oral cavity. These include infection at birth, baby's feeding bottles, infected pacifiers, and carious teeth¹². We speculate that a high cariogenic diet might influence the interaction between *C. albicans* and *S. mutans* in these children tested. In turns, it becomes critical for ECC, since the presence of sucrose in the children oral cavity may lead to the ability of this species to grow within structured

microbial biofilm^{33,34}. Further studies to obtain data regarding cariogenic diet are therefore necessary.

The presence of yeast and bacteria is one of the local factors that contributes to the etiology of ECC⁷. To obtain an overall insight into the impact of simultaneous participation of *C. albicans* and *S. mutans* when detecting together in each sample tested, we compared the percent proportion of *C. albicans* or *S. mutans* relative to total bacteria. As expected, the proportion of *C. albicans* was higher in saliva than in carious plaque among children with ECC. Other studies have reported this phenomenon, where *Candida* species were frequently isolated more, qualitatively and quantitatively, from saliva than from dental plaque³⁵ and subgingival samples³⁶. During in saliva, this fungus might act as a bridge for oral bacteria to adhere to a mucosal surface, a mechanism that may protect this bacterium from being removed by salivary flow and swallowing²¹. On the other side, biofilm formation is vital for *C. albicans* to survive as a pathogen, which involves attachment, colonization, and development of structural biofilm integrity composed of yeast and hypha^{37,38}. Our data illustrate that co-adhesion between *C. albicans* and *S. mutans* in cariogenic biofilm is one mechanism by which the fungus, in yeast form, survives in the oral cavity^{39,40}. Although the fungus morphology was not observed in this study, it has been reported by other studied that hypha morphology is not crucial for the *C. albicans*–*S. mutans* relationship when they grow in multispecies dental biofilms^{41–43}. Therefore, in addition to the morphology, both the number and proportion of salivary *C. albicans* influence the fungal–bacterial relationships, which further increases the risk of caries. Additionally, the flushing effect of saliva, as part of innate defense mechanism, might contribute to decrease *Candida* adherence to the oral surface⁴⁴, including tooth surface. Thus, the quality and quantity of saliva have an essential role in maintaining fungus behavior, as commensal or pathogen⁴⁵.

We observed that the proportion of *S. mutans* was higher in dental plaque than in saliva, and there was a tendency for the percentage of *C. albicans* to be lower in carious plaque, where the proportion of *S. mutans* increased. This observation indicates that *S. mutans* has an active role in orchestrating the development of cariogenic biofilms^{46,47}. This species has an essential part in attenuating the virulence of the fungus⁴⁸ by interfering with the fungus transition, from yeast to hypha form when these oral microflorae interact and grow in biofilm^{49,50}. This result further supported by the data of correlation analysis, in which the fungus-bacterium concentration in dental plaque sample showed a negative association, although a positive correlation was found in saliva sediment. This suggests that the ECC rate may not be connected to the quantity of *C. albicans* involves.

One of the mediators for the synergistic relationship between *C. albicans* and *S. mutans* is the streptococcal GtfB enzyme^{7,32}. Our finding indicates that cariogenic biofilm developed in ECC children accompanied by the increased transcription level of *gtfB* mRNA and enhance of *S. mutans* growth in dental plaque derived from children with ECC. In a clinical situation, this

observation is relevant, since GtfB is the enzyme that synthesizes glucan polymers from sucrose⁶. Thus, result of this study suggests that a high sucrose concentration, which is critical for the development of dental caries, might exist in the children oral cavity. Future clinical studies regarding diet-associated ECC risk factor are thus recommended. Collectively, this study indicates that although the proportion of *C. albicans* was less in ECC-associated biofilm, it may support facilitation of a fungal-bacterial synergistic relationship. Yeast cells could be used by *S. mutans* to promote fitness and the bacterial survival, as shown by enhanced transcription level of *gtfB*, which reflects that more extracellular polysaccharides were produced to promote the fungal-bacterium relationship in caries-related biofilm⁷.

The results of the present study cannot explain the reason for such an association. However, at least this study provides information on ECC experience among preschool children. The presence of *C. albicans* in dental plaque and saliva could be merely an indicator of oral health conditions and the high carbohydrate intake among the young children selected in this study, which might confer a survival advantage for *C. albicans* and it is favorable for ECC development. More studies regarding the involvement of *gtfB* expression, and how it relates to oral health conditions as well as cariogenic intake in Indonesian preschool children, are needed.

There is some limitation in this study. First, the primary disadvantage of qPCR used in this study is its inability to separate and quantify the viable from nonviable cells. This technique may result in false positives or an overly high estimation, as all DNA extracted from live or dead *C. albicans*, or *S. mutans* cell will be amplified. Since the number of viable cells is especially significant for diagnosing and monitoring disease, adding cell viability information to qPCR-based diagnostics should be considered. Second, the number of children involved in this study was small

(15 subjects per group) because of difficulty in sample collection, primarily to obtain the plaque on the dentin surface.

Conclusions

This study shows that *C. albicans* contributes to increasing concentration of *S. mutans* by inducing the expression of *gtfB* mRNA in ECC-related biofilm. Therefore, results from this study would be useful as a starting point to consider *C. albicans*, as a potential target in prevention programs to reduce the high rates of ECC in individuals or groups of young children. Moreover, since the information regarding oral hygiene habit or diet, provided by the accompanying guardian are difficult to be understood, as found during this study, future studies involving preschool children may wish to involve examiners who are more experience in deal with such parents/guardians when planning studies.

Data availability

Dataset 1. The raw data associated with this study. Data are grouped according to the figure with which they are associated. DOI: <https://doi.org/10.5256/f1000research.16275.d221304>⁵¹.

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Shahida Mohd-Said 

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This paper reports an interesting and relevant study on ECC in children. The methods and contents of the papers are current, relevant and well-structured. However, some improvements could benefit the presentation including checking on grammar and typos, some revisions to sentence construction, highlights on the significance of this study, how the findings can be important to update of knowledge and current management of ECC, and comparison of data from Indonesia with other populations to perhaps illustrate novelty and significance to current available data.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 08 Nov 2018

Endang Bachtiar,

Dear reviewer,

I am pleased to resubmit for publication the revised version of **Relationship between *Candida albicans* and *Streptococcus mutans* in early childhood caries, evaluated by quantitative PCR.**

We appreciate all the insightful comments provided by the reviewer. Based on the reviewer's guidance, includes a number of positive changes, we endeavoured to improve the fit of the paper with the journal.

We hope that these revisions improve the paper such that the editor and reviewer now deem it worthy of publication in F1000 Research. Thank you for taking the time to help us improve the paper.

Sincerely,
Authors: Endang W Bachtiar and Boy M Bachtiar

Responses to reviewer #1:

We have fixed all grammar, typos, and some revisions to sentence construction. Furthermore all responses to the suggestions have been added in the manuscript in the yellow highlight sentences.

Competing Interests: No competing interests

Referee Report 31 October 2018

<https://doi.org/10.5256/f1000research.17775.r39491>



Zamirah Zainal-Abidin 

Centre for Craniofacial Diagnostics and Biosciences, Faculty of Dentistry, National University of Malaysia (UKM), Kuala Lumpur, Malaysia

Methods:

1. The authors did not mention how the examiners were calibrated, in order to reduce the inter- and intra-examiner variability when examining the preschool children, before grouping them into the early childhood caries and caries free groups.
2. The authors may have been able to collect more information on the oral hygiene habits or dietary intake of the subjects, before taking the oral samples.

Results:

1. In the caption for Figure 2, the symbol * $p < 0.05$ should be indicated clearly. For Figure 2A, it is to show that there is a statistically-significant difference between the *C. albicans* in saliva and dental plaque in the ECC group, and a statistically-significant difference between the *S. mutans* in saliva

and dental plaque in the ECC group. The use of top square bracket () with an asterisk would be better to indicate which parameters are statistically compared.

2. For Figure 4A, the use of top square bracket () with an asterisk would be better to indicate the parameters which are statistically compared.

Discussion:

1. In paragraphs 3 and 7, the authors speculated that a high cariogenic/high sucrose concentration diet might influence the interaction between *C. albicans* and *S. mutans* in the tested subjects. The authors may have been able to draw a better suggestion if the information on the oral hygiene habits or diet is obtained during the recruitment of the subjects (See my comment in Methods (2)).
2. In paragraphs 3, 7 and 9, the authors could have suggested what further studies are necessary to address the suggested theories/findings.

General comments:

1. The paper has a few grammar and language issues, in the Abstract and Methods sections.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 08 Nov 2018

Endang Bachtiar,

Dear editor and reviewer,

I am pleased to resubmit for publication the revised version of **Relationship between *Candida albicans* and *Streptococcus mutans* in early childhood caries, evaluated by quantitative PCR.**

We appreciate all the insightful comments provided by the reviewer. Based on the reviewer's guidance, includes a number of positive changes, we endeavoured to improve the fit of the paper with the journal.

We hope that these revisions improve the paper such that the editor and reviewer now deem it worthy of publication in F1000 Research. Thank you for taking the time to help us improve the paper.

Sincerely,
Authors : 1. Endang W Bachtiar
2. Boy M Bachtiar

Author's response to reviewer comments
Comment provided by Reviewer #1

Methods:

Reviewer's comment:

The authors did not mention how the examiners were calibrated, in order to reduce the inter- and intra-examiner variability when examining the preschool children, before grouping them into the early childhood caries and caries-free groups.

Author's response:

We thank the reviewer for this correction. The problem has been fixed. As suggested by the reviewer, we have added sentence within material and method as follow: "two weeks prior to collect to clinical samples, the examiners were calibrated and trained by providing with the manual describing study protocol and guidance regarding the examination of preschool children. Therefore, only those trained-examiners evaluated the preschool children".

Reviewer's comment:

The authors may have been able to collect more information on the oral hygiene habits or dietary intake of the subjects, before taking the oral samples.

Author's response:

In this study, we used a questionnaire regarding the parent's/ guardian's perception concerning children's general and oral health, the risk factor for caries, dietary intake, and access to dental care. We noticed, in general, most of the responders believed that dental decay is a natural phenomenon. However, considering the complexity of the issue studied in this study, it is not the authors' intent to include oral hygiene habits or dietary intake of the subjects (preschool children). Therefore, the related-data were not included in the current study.

Reviewer's comment:

In the caption for Figure 2, the symbol $*p<0.05$ should be indicated clearly. For Figure 2A, it is to

show that there is a statistically significant difference between the *C. albicans* in saliva and dental plaque in the ECC group, and a statistically significant difference between the *S. mutans* in saliva and dental plaque in the ECC group. The use of top square bracket () with an asterisk would be better to indicate which parameters are statistically compared.

For Figure 4A, the use of top square bracket () with an asterisk would be better to indicate the parameters which are statistically compared.

Author's response:

According to the suggestions provided by Reviewer#1, we have corrected the old figures. The top square bracket has been added in Fig. 2A to indicate a statistically significant difference as suggested. For the Fig. 4A, we have added the top square bracket with an asterisk to indicate a significant difference between the parameters compared. Thank you.

Discussion:

Reviewer's comment:

In paragraphs 3 and 7, the authors speculated that a high cariogenic/high sucrose concentration diet might influence the interaction between *C. albicans* and *S. mutans* in the tested subjects. The authors may have been able to draw a better suggestion if the information on the oral hygiene habits or diet is obtained during the recruitment of the subjects (See my comment in Method (2)).

Author's response:

As mentioned above (response to the reviewer's comment in methods (2); it is not the authors' intent to include the oral hygiene habits or diet of the preschool children in this study. This is because the information regarding oral hygiene habit or diet, provided by the accompanying guardian are difficult to be understood. Therefore, it is hard to interpret oral hygiene habit or diet data.

Reviewer's comment:

In paragraphs 3, 7 and 9, the authors could have suggested what further studies are necessary to address the suggested theories/findings.

Author's responses:

According to reviewer suggestion, we have added a better suggestion at the end of paragraph-3, as follow; "Further studies to obtain data regarding cariogenic diet are therefore necessary". Additionally, we have added a sentence at the end of paragraph 7, as follow: "More studies regarding the involvement of gtfB expression, and how it relates to oral health conditions as well as cariogenic intake in Indonesian preschool children, are needed.

At the end of paragraph 9 (conclusion), as suggested by the reviewer, we added sentences; "Moreover, since the information regarding oral hygiene habit or diet, provided by the accompanying guardian are difficult to be understood, future studies involving preschool children may wish to involve examiners who are more experience in dealing with such parents/guardians

when planning studies". Thank you.

Dear Editor,

We are pleased to inform you that; 1/ for all the revision, as suggested by the reviewer, were showed in yellow-highlighting words, 2/ we revised the figure 2 and 4 to address the reviewer's comments. Although the Fig. 3 is not included in reviewer's comment, we revised the font, from the original (Arial) to new version (Times New Roman). Thus, all founds depicted in figures are similar. Thank you.

Competing Interests: No competing interests

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