ORIGINAL RESEARCH

Evaluation of Changes in Oral Microflora in Children with Early Childhood Caries after Full Mouth Rehabilitation

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Abstract

Aim: To evaluate the change in microflora in children suffering from severe early childhood caries (ECC) after full mouth rehabilitation.

Materials and methods: A total of 60 children, aged 3–5 years suffering from severe ECC who fulfilled the inclusion and exclusion criteria were included in the study. Pooled plaque samples were taken and subjected to quantitative reverse transcriptase polymerase chain reaction (PCR) to obtain baseline mean values of *Streptococcus mutans* (*S. mutans*), *Streptococcus sobrinus* (*S. sobrinus*), *Candida albicans* (*C. albicans*), and *Candida dubliniensis* (*C. dubliniensis*) before full mouth rehabilitation was done under general anesthesia. Posttreatment samples were collected at 6, 12, and 18 months. Wilcoxon signed-rank test was used to compare the mean values of *S. mutans*, *S. sobrinus*, *C. albicans,* and *C. dubliniensis* before and after full mouth rehabilitation.

Results: A total of 60 patients recruited for the study were present at the follow-up at 6 and 12 months. At 18 months, 55 patients returned, and five were lost due to follow-up. A statistically significant reduction was seen in all microorganisms at 6, 12, and 18 months compared to baseline values. At 18 months a slight increase in *S. mutans*, *S. sobrinus,* and *C. albicans* was seen. *C. dubliniensis* was not detected in any cases after full mouth rehabilitation. Caries recurrence was seen in four patients at 18 months.

Conclusion: Significant reduction of *S. mutans*, *S. sobrinus*, *C. albicans,* and *C. dubliniensis* was seen at 6, 12, and 18 months. A complete reduction of only *C. dubliniensis* was seen. A significant but not permanent reduction of *S. mutans*, *S. sobrinus*, and *C. albicans*. Caries recurrence was seen in 7.27% of patients at 18 months.

Keywords: Early childhood caries, Full mouth rehabilitation, General anesthesia, Oral microflora.

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Introduction

Early childhood caries (ECC) is a multifactorial dental issue, seen in children who are <6 years of age. ECC is a global issue, affecting over half a billion children.[1] An array of factors such as a diet rich in fermentable carbohydrates, the existence of oral biofilm, and *Streptococcus mutans* (*S. mutans*) are mainly responsible for the occurrence of ECC. The high prevalence of ECC in children along with delay in seeking treatment often result in a negative impact on the quality of life.[2]

The vertical and horizontal transmission of microorganisms to the oral cavity coupled with a high-sugar diet creates an environment that allows the demineralization of enamel, resulting in ECC. *S. mutans* are considered to be one of the primary etiological agents responsible for ECC.[3] *S. mutans* have the ability to form cariogenic biofilms on the tooth surface in the presence of a cariogenic diet. *Streptococcus sobrinus* (*S. sobrinus*) is associated with more severe cases of caries by enhancing its initiation progression and development.[2,4]

In recent years, it has been found that *Candida albicans* (*C. albicans*) have also been isolated from children suffering from ECC. It is an intriguing finding since *C. albicans* cannot colonize a tooth on its own. *C. albicans* can adhere to mucosal and tooth surfaces and interact with *S. mutans* to result in ECC. The interaction between *S. mutans* and *C albicans* during caries development results in a new biofilm.[5,6] *C. albicans* has demonstrated the ability to highly cariogenic biofilms which allows ECC to spread rapidly. *Candida dubliniensis* (*C. dubliniensis*) is a candidal species that is capable of building biofilms in a similar manner to *C. albicans*. *C. dubliniensis* is active only in certain environments and could be responsible for the quick progress of ECC.[7]

Children suffering from ECC require multiple treatments. Due to their young age, they are usually uncooperative and often require invasive treatment. In order to provide comprehensive treatment with optimal results, treatment is usually done under general anesthesia.[8] The elimination of carious lesions under general anesthesia results in a significant reduction of cariogenic microorganisms.

Literature on the long-term effects of dental treatment on the oral microflora in ECC is very scarce.[4] Hence, this prospective cohort study was undertaken to understand the long-term effects of full mouth rehabilitation on the oral microflora before and after treatment for a period of 24 months.

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Materials and Methods

This study was conducted after receiving approval from the ethical committee (IHEC/SDC/FACULTY/21/PEDO/132a).

The sample size was calculated using the G-Power sample power calculator (Universität Kiel, Kiel, Germany). A *post hoc* analysis of the previously published study showed that the studies had based their conclusion on a sample size that had a power of 0.95 when calculated with an effect size of 0.8 was 50 patients. Around 10 more patients were added to account for the loss of follow-up.

A total of 60 children, aged 3–5 years diagnosed with ECC requiring full mouth rehabilitation under general anesthesia were included in the study. Children with long-term chronic illnesses and those on medications for chronic illness were excluded from the study.

Oral examinations were performed using a dental mirror and explorer in a dental chair with optimal artificial light by a qualified pediatric dentist. Dental examinations were completed in standard dental operatories under ideal conditions. If the child was uncooperative for a dental examination, parents were informed that a treatment plan would be decided in the operating room. The procedure, risks, possible discomforts, and benefits were explained to parents/guardians of the children who gave written consent for participation.

Sample Collection

Pooled plaque samples were taken from each participant at four points—(1) before the initiation of full-mouth rehabilitation, (2) at the 6-month posttreatment visit, (3) at the 12-month posttreatment visit, and (4) at the 18-month posttreatment visit. Patients were instructed not to brush or eat 2 hours prior to collecting samples. All teeth were isolated with cotton rolls and gently dried with compressed air. One clinician collected supragingival plaque samples from all proximal surfaces as well as the gingivobuccal surfaces of all teeth present using a sterile universal scaler. Plaque samples were placed into marked sterile Eppendorf tubes containing 200 µL of 10 × TE buffer (Tris-hydrochloride ethylenediaminetetraacetic acid) as transport media. Samples were then transferred immediately to the laboratory and stored at –70° C until they could be further processed.

Treatment

Full mouth rehabilitation for all patients was done under general anesthesia by a trained pediatric dental team. The team followed the standardized treatment protocol used in previous studies.[9,10]

All children received instructions on oral hygiene maintenance and dietary counseling regarding caries-promoting food. Toothbrushing was demonstrated on a supersized model followed by hands-on practice by the children. Parents were also instructed on how to brush their children's teeth. At 6, 12, and 18 months from the baseline, clinical measurements and microbial samples were repeated. Reminders were used to optimize subjects returning for posttreatment checkups, including two telephone calls, and each child was given a small gift as a reward. Children who developed new lesions after initial therapy were later retreated after monitoring and microbial sampling.

Sample Processing

Analysis of *S. mutans*, *S. sobrinus*, *C. albicans*, and *C. dubliniensis* was carried out using quantitative reverse transcription polymerase chain reaction (qRT-PCR) at the Blue Lab in Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences (SIMATS) (Deemed to be University), Chennai, Tamil Nadu, India. DNA extraction of the samples was done using a highly purified Invitrogen DNA isolation kit (Purelink DNA extraction kit, Applied BioSystems, Thermo Fisher Scientific, Waltham, Mass., United States of America). The standard "proteinase K" method was followed for DNA isolation. The extracted DNA was purified using a procedure designed for purifying genomic DNA using a spin column-based centrifugation procedure for 10–15 minutes. For *Streptococcal* species, custom Synergy Brands Green assay reagents (applied biosystems) were added. The sequence of the forward primer for *S. mutans* was 5′-GCCTACAGCTCAGAGATGCTATTCT-3′, and the sequence of the reverse primer was 5′-GCCATACACCACTCATGAATTGA-3′. The sequence of the forward primer for *S. sobrinus* was 5′-TGC TAT CTT TCC CTA GCA TG-3′, and the sequence of the reverse primer was 5′-GGT ATT CGG TTT GAC TGC-3′. For *C. albicans* the forward primer sequence was 5′-GGATTTACTGAAGACTAACTACTG-3′ and the reverse sequence primer was 5′-GAACAACAACCGATCCCTAGT-3′. For *C. dubliniensis*, the sequence of the forward primer was 5′-AGT TAC TCT TTC GGG GGT GGC CT -3′, and the sequence of the reverse primer was 5′-AAG ATC ATT ATG CCA ACA TCC TAG GTA AA-3′.

Each set of PCR analyses included a negative control (water blank). The conditions for real-time PCR were—holding stage at 95°C for 10 seconds, 40 cycles of shuttle heating at 95°C for 15 seconds, and at 60°C for 1 minute. The melt curve stage was at 95°C for 15 seconds, 60°C for 1 minute, and 95°C for 15 seconds. A total of 16 small ribonucleic acid (sRNA) was used as an endogenous control (SYBR Green Assay Reagents, Applied Biosystems). The changes in steady-state messenger ribonucleic acid (mRNA) levels of a gene across multiple samples are expressed relative to the levels of an endogenous control RNA (that is, 16 sRNA), which is referred to as relative quantification (RQ). RQ for *S. mutans*, *S. sobrinus* was based on the number of PCR cycles necessary to obtain the threshold signal of fluorescence given by cycle threshold values. All calculations were performed using Applied Biosystems software.

Results

A total of 60 children participated in the study. All children attended the 6 and 12-month visits. At 18 months, only 55 children attended. This corresponded to a dropout rate of 8.33% at 18 months. Demographic details and baseline characteristics are presented in Table 1.

At baseline, the mean RQ score of *S. mutans* was found to be 1.32 ± 1.38. This further decreased to 0.69 ± 0.73 at 6 months and 0.43 ± 0.51 at 12 months. At 18 months, an increase in RQ score increased to 0.71 ± 0.47. Results were found to be statistically significant (Table 2).

Table 1: Demographic details at baseline

Variable	
Age (years)	4.3 ± 1.1
Gender	
Boys	36 (60%)
Girls	24 (40%)
dmft	10.12 ± 4.73
PI	1.11 ± 0.12
GI	0.74 ± 0.16

GI, gingival index; PI, plaque index

The *S. sobrinus* had a mean RQ of 3.12 ± 4.02 at baseline. A statistically significant reduction to 1.19 ± 1.04 at 6 months and 0.84 ± 0.93 at 12 months was seen. A slight increase to 0.97 ± 0.71 was seen at 18 months. The results were statistically significant (Table 2).

For *C. albicans*, the baseline RQ score was 1.48 ± 1.23. The RQ scores decreased to 0.68 ± 0.52 at 6 months. The score decreased to 0.52 ± 0.47 at 12 months. However, the score increased to 0.74 ± 0.61 at 18 months (Table 2).

The *C. dubliniensis* had average RQ score of 0.78 ± 0.17. At 6, 12, and 18 months, *C. dubliniensis* was found to be completely absent. The results were found to be statistically significant (Table 2).

At baseline, the average decayed, missing, and filled primary teeth (dmft) was found to be 10.12 ± 4.73. There was no recurrence of caries in any of the cases at 6 and 12 months. At 18 months 11 teeth were found to show new carious lesions from four patients (7.27%). Table 3 represents caries recurrence at 6, 12, and 18 months.

Discussion

Due to the age of children suffering from ECC, they are often difficult to manage. Due to the complexity of treatments required and the cooperative ability of the child, treatment is done in an operating room under general anesthesia. Complete treatment is often completed in a few hours under optimal conditions and patients can return home the same day. Considered an elective procedure, daycare surgery for ECC is the most common day surgery in some of the first world countries.[11,12]

It has been found that after substantial dental treatment, there is a considerable reduction in microbial load for a period of 6 months.[12] The removal of carious lesions and subsequent treatment not only restores the tooth but also removes the foci of infection. Comprehensive treatment of carious lesions under general anesthesia also results in the absolute elimination of caries as the origin of inflammation.[13]

The *S. mutans* and *S. sobrinus* are the primary organisms associated with dental caries in the mutans streptococci group. The characteristic high caries activity of this group is due to the intrinsic attributes of adhesion, acidogenicity, and aciduricity. This allows a shift in the ecological balance to an acidic environment, thereby favoring the growth of acidogenic and aciduric organisms which result in dental caries. *S. sobrinus* is associated with more severe cases of caries by enhancing its initiation progression and development.[14,15]

In recent years, there has been growing evidence that *C. albicans* is also involved in the progression of ECC. Though the exact pathogenesis remains unclear, children who suffer from ECC are often found to have higher *C. albicans* count when compared to caries-free children. *C. albicans* is an opportunistic pathogen that can successfully adapt, proliferate, and modify host environments. *C. albicans* is often found coexisting with other microorganisms in oral biofilms due to the acidic environment.[5,6,16]

Studies in the recent past have shown that a mutual collaboration is present linking *C. albicans* and *S. mutans*. In the presence of sucrose, the adherent cooperation among both organisms is increased. *In vitro* studies have shown that the exoenzymes of *S. mutans* bind to *C. albicans* surface. With the availability of sucrose, the exoenzymes adsorb onto *C. albicans* cells and generate copious amounts of glucans. This in turn amplifies binding sites for *S. mutans*. Thus *S. mutans* and *C. albicans* symbiotically have the ability to modulate and enhance the ecosystem to an acidic environment due to the production of exopolysaccharides, thus enhancing the virulence of the plaque biofilm.[5,6,16–19]

Most studies in the past have focused on cultural methods. The process of differentiating between microorganisms using culture media is tedious, involves multiple steps, and does not provide an accurate assessment. Recent innovations in the field of molecular biology have given rise to highly accurate techniques that can detect and quantify microorganisms accurately. A fluorescence-based technique that combines these qualities and is able to differentiate between both *S. mutans* and *S. sobrinus*, as well as *C. albicans*, and *C. dubliniensis*, is available which is known as qRT-PCR. Since sample viability does not play a role, qRT-PCR has been found to be highly technique-sensitive and is considered to be effective and reliable for microbial quantification. During a PCR, double-stranded amplicons accumulate, increasing fluorescence. Since qRT-PCR can locate fluorescence over a vast range, the sensitivity is intensified in locating targets.[4,20–23]

The reduction of microorganisms after 6 months was found to be significant. This could be due to the complete removal of caries due to treatments performed such as restoration, extraction, and pulp therapy which would result in removal the of the source

Table 2: Change in oral microflora at baseline, 6, 12, and 18 months using Wilcoxon signed-rank test

Microorganism	*Pretreatment*	*6 months*	*12 months*	*18 months*	*p-value*
S. mutans	1.32 ± 1.38	0.69 ± 0.73	0.43 ± 0.51	0.71 ± 0.47	<0.001
S. sobrinus	3.12 ± 4.02	1.19 ± 1.04	0.84 ± 0.93	0.97 ± 0.71	<0.001
C. albicans	1.48 ± 1.23	0.68 ± 0.52	0.52 ± 0.47	0.74 ± 0.61	<0.001
C. dubliniensis	0.78 ± 0.17	0	0	0	<0.001

Table 3: Caries recurrence after treatment

Examination	*Pretreatment N = 60*	*6 weeks N = 60*	*12 weeks N = 60*	*18 weeks N = 55*
Plaque on front teeth (%)	67	25	20	57
Patient with new lesions (*n*)	–	0	0	4
Number of new carious lesions (*n*)	–	0	0	11
New lesions with pulpal involvement (*n*)	–	0	0	4

of infection.[12] Parents and children were taught oral hygiene instructions, with parents reinforced and given demonstrations to teach brushing techniques. Another factor could be the weekly reminders sent through WhatsApp, which was done to make sure parents were maintaining oral hygiene. Our results are similar to previously published studies.[4,24] The complete absence of *C. dubliniensis* is a unique finding in the current study.

After 12 months, a further reduction was seen in all microorganisms except *C. dubliniensis*, which was not found in any of the children treated under general anesthesia. A possible reason for the decrease in microorganisms could be due to continued reminders for oral hygiene instructions and the interaction with the dentist for 6 months which would have continued to motivate both parents and children to maintain good oral hygiene.[25] Foster et al.[26] found the children who came for recall visits had lesser chances of relapse of dental caries.

At 18 months, a slight increase was seen with *S. mutans*, *S. sobrinus*, and *C. albicans*. This could be due to the fact that in spite of aggressive treatment under general anesthesia and preventive measures, children with ECC often return with new carious lesions.[4,11,14] Though a reduction in microbial load was seen, a complete reduction was seen only for *C. dubliniensis*. Since a permanent decrease in microbial load was not seen, the microorganisms could have grown on the new surfaces which would have replaced carious tooth structures. Stainless steel crowns are known to accumulate more plaque than normal teeth which could also be a reason for the occurrence of new caries.[27] Another factor could be a change in dietary habits and oral hygiene maintenance which would have changed over the last 18 months.[24]

At 6 and 12 months there were no new lesions in any of the patients in our study. At 18 months, recurrence was seen in 7.27% of the patients. This coincided with the increase in counts of *S. mutans*, *S. sobrinus,* and *C. albicans*. The frequency and willingness to dental home care could have probably decreased with time. This in turn would be able to provide new niche areas for cariogenic microorganisms to grow. The tooth is continuously bathed in saliva, encountering multiple changes in the oral environment, eventually leading to the emergence of a biofilm.[26,27] This results in the formation of microbial colonies which can facilitate the occurrence of dental caries in a previously unaffected area. Stainless steel crowns and space maintainers also accumulate plaque which in turn could be responsible for the increase in caries.[26,28]

A unique finding in the present study was the complete disappearance of *C. dubliniensis* after full mouth rehabilitation. *C. dubliniensis* is a fungus similar to *C. albicans* but has been poorly reported in relation to ECC. This can be attributed to the difficulty in the separation and detection of *C. dubliniensis* using culture media when compared to *C. albicans*. The presence of *C. dubliniensis* has often been associated with children who are either immunocompromised or malnourished.[7] Children with ECC are found to be underweight and anemic along with deep carious lesions which would provide a perfect environment for the growth of *C. dubliniensis*. The completion of treatment would result in the resolution of infection, thus resulting in a change of the plaque ecosystem and improvement in the quality of life which is not favorable for the growth of Candidal species, particularly *C. dubliniensis*.[24,29] Thus, the complete absence of *C. dubliniensis* could be probably due to the improvement in oral and general health after intervention.

Another strength is the use of qRT-PCR. The high sensitivity of qRT-PCR makes it a desirable diagnostic method in which a single assay can detect 10 copies of the target in 30 µL of the reaction mixture. Among the other desirable qualities of qRT-PCR are easy sample handling, the ability to detect low numbers of bacterial species, 20 stability of samples after being frozen for long periods, a faster and less cumbersome method of detection, accuracy, and speed at which results can be obtained with convenient kits that are commercially available.[4,22,30]

Prevention of ECC requires unique methods based on caries risk assessment. Daily toothbrushing with fluoride toothpaste, dietary control, and continuous monitoring should be done to reduce the recurrence of caries after full mouth rehabilitation.[31]

Conclusion

A significant decrease in *S. mutans*, *S. sobrinus*, *C. albicans,* and *C. dubliniensis* was seen after full mouth rehabilitation under general anesthesia. At 12 months a further decrease was seen in *S. mutans*, *S. sobrinus,* and *C.albicans* followed by a slight increase at 18 months. *C. dubliniensis* was completely absent in all cases at 6, 12, and 18 months. Recurrence of caries was seen in 7.27% of patients at 18 months. Appropriate counseling and follow-up along with good oral hygiene practices can prevent the recurrence of caries.

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