INTRODUCTION

Early Childhood Caries (ECC) also known as early childhood tooth decay is a destructive form of dental caries that afflicts the young children. The older terminologies like “nursing caries” and “baby bottle tooth decay” have been replaced with broader term ECC. It is one of the most common chronic childhood diseases in this age group; and though not life-threatening, it affects normal health and well-being of the child. The prevalence of dental caries has reduced worldwide, yet that of ECC remains high and so it is currently a WHO concern.1,2 The difference between ECC and the dental caries is that, here the progression of caries is very rapid and widespread; and because of this rapid progression, its prevention and management is a challenge. American Academy of Pediatric Dentistry (AAPD) defines early childhood caries (ECC) as the presence of one or more decayed (non cavitated or Cavitated), missing (due to caries), or filled tooth surface in any primary tooth in a child 71 months of age or younger. Presence of any smooth surface caries in children younger than three years of age; one or more cavitated, missing due to caries or filled smooth surfaces of the primary maxillary anterior teeth in children from ages three to five; or a decayed, missing or filled score of ≥ (age 3), ≥ (age 4), ≥ (age 5) surfaces are termed as Severe-Early Childhood Caries (S-ECC).3 Oral bacteria like, Streptococcus mutans and Lactobacillus spp, are the main microorganism implicated for the initiation and progression of caries respectively. Mutans streptococci colonize the host only after the first teeth erupt, and their preferential colonization site is the teeth.4,5 They are highly localized on the surfaces of the teeth, abundant in the plaque of carious lesions;6,7 and are isolated from both initial and established carious lesion sites.8,9,10 They can rapidly produce acids from simple carbohydrates, including sucrose, and are aciduric;11,12 And also can synthesize certain macro-molecules from sucrose that foster their attachment to the teeth surfaces.13,14,15 Their virulence expression is strongly associated with consumption of sucrose.16,17,18 Even though mutans streptococci are strongly implicated with caries, their association is not unique; mutans streptococci could also persist without any evidence of detectable demineralisation.19,20 Lactobacilli do not avidly colonize the teeth; but are found in the oral cavity before the teeth erupt. Lactobacilli preferentially colonize the dorsum of the tongue and are carried into saliva by the sloughing off the tongue epithelium;21 are highly acidogenic, acidicuric and are often cultured from established caries lesion.22 Its presence was found strongly associated with the presence of caries especially in children aged 2-5 years.23 Studies have found direct correlation between its numbers in saliva and the consumption of simple carbohydrates by the host.24,25 And Lactobacillus count have been used to predict the increment of new carious lesions. Streptococcus sanguinis are non-pathogenic and non-cariogenic normal commensals of the oral cavity; that are isolated from both initial and non-cariogenic and are often cultured from established caries lesion.26 They are shown to play an important role in the causation of bacterial endocarditis in persons with pre-existing cardiac lesion. Streptococcus sanguinis are acidogenic to carbohydrates and are acid tolerant.27-28 Studies have shown that

ABSTRACT

Aim:
To determine the correlation between Candida albicans, Streptococcus mutans, sanguinis and Lactobacillus in ECC, SECC and Caries free.

Materials and Methods:
30 healthy children below 4 years of age were divided into Caries free, ECC and SECC based on AAPPD definition. Non-stimulated whole saliva samples were microbiologically evaluated.

Results:
An Inversely proportional relation was found between Candida colonization and Streptococcus sanguinis colonization in whole sample and, ECC group. (p=0.04 sig) . A directly proportional relation was found between Candida colonization and Lactobacillus colonization in whole sample and, caries free groups. (p = 0.02 sig). No significant relation was found between Candida and Streptococcus mutans colonization. (p=0.571 ns).

Conclusion:
Candida albicans may be contributing for initiation; and also for the rapid and widespread progression of ECC in the very young children with immature immune system.

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colonization of Streptococcus sanguinis may influence the colonization of Mutans streptococci by competing with their nutrients and by production of mutacin and hydrogen peroxide; and this suggests an ecological approaches towards controlling dental caries. Candida species are fungi which are common inhabitants of the normal oral microbiota found in infants. Candida is an opportunistic pathogen and in immunocompromised individuals has the ability to cause a variety of infections. For instance till date, oral thrush in infants and chronic atrophic candidiasis (denture induced stomatitis) in adult are the known most common clinical manifestations of oral candidiasis. Presently researchers are implicating the propable role of candida, a fungi in caries etiopathogenesis. Hence this study aims to correlate the role of C.albicans, Lactobacillus, S.mutans and S.sangunis in ECC, S-ECC and caries free children below 4 yrs of age.

MATERIALS AND METHODS
A cross-sectional study was conducted by randomly selecting healthy children below 4 years of age.

Estimation of Streptococcus Mutans and Sanguinus
By means of sterile disposable syringe 1ml aliquot of saliva was transferred from the universal container to the previously labelled sterile tube containing 4ml of broth (thioglycolatebroth). The saliva sample was vortexed, to get a uniform mix, containing saliva and the broth using a cyclomixer. Using an inoculation loop (4mm inner diameter) 10 ul of the vortexed 1:5 dilution sample was streaked in duplicate on Mitissalivarius agar (MS) selective for S.Mutans. The MS agar plates (Figure 4) were incubated in an anaerobic jar for 24 to 48 hrs at 37°C. Based on colony characteristics S. Mutans and S. Sanguinis were identified. Streptococcus mutans appeared as rough, heaped irregular yellow color colonies resembling frosty glass and could be picked off from the agar (Figure 5). Whereas Streptococcus sanguinis were having smooth, hard rubbery grayish white color colonies, and they adhered firmly to the agar media. Identification for S. Mutans and S. Sanguinis were further confirmed by biochemical tests; mannitol fermentation and gram staining catalyst test respectively. Colony counting was done on the digital colony counter, and the count was expressed as the number of colony forming units per millimeter (CFU/ml) of saliva. Semiquantification of the number of colonies was done by multiplying the actual colony count with 1x 103to adjust for the dilution factor.

Estimation of Lactobacilli
20 l of saliva samples were spread on plates of Rogosa agar (Figure 4) (Unipath, Basingstoke, UK) for the count of total lactobacilli. And the plates were incubated anaerobically in Mac Intosh Field Jar (85% N2, 5% CO2 and 10% H2) at 37°C for 48-72 hrs. Lactobacilli were identified with colony characters and by the gram staining method (Figure 5). Lactobacilli appeared straight rod shaped, and in pairs of varying length. Number of colonies was counted using digital counter and its concentration in saliva was expressed in colony forming unit per ml (CFU/ml).

Table 1
Correlation between species colony count and dfs (Caries experience)

<table>
<thead>
<tr>
<th>Species</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus mutans</td>
<td>-0.279</td>
<td>0.135</td>
</tr>
<tr>
<td>Streptococcus sanguinis</td>
<td>-0.30</td>
<td>0.875</td>
</tr>
<tr>
<td>Candida</td>
<td>0.503</td>
<td>0.005*</td>
</tr>
</tbody>
</table>

Table 2
Correlation between Candida, Streptococcus mutans, Streptococcus sanguinis and Lactobacilli in control, ECC, S-ECC groups and total sample

<table>
<thead>
<tr>
<th>Group</th>
<th>Species</th>
<th>Streptococcus mutans</th>
<th>Streptococcus sanguinis</th>
<th>Lactobacilli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Control</td>
<td>Candida</td>
<td>-0.394</td>
<td>0.259</td>
<td>0.414</td>
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<td></td>
<td>Lactobacilli</td>
<td>-0.234</td>
<td>0.516</td>
<td>0.495</td>
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<tr>
<td></td>
<td>Streptococcus sanguinis</td>
<td>-0.222</td>
<td>0.538</td>
<td></td>
</tr>
<tr>
<td>ECC</td>
<td>Candida</td>
<td>0.093</td>
<td>0.797</td>
<td>-0.609</td>
</tr>
<tr>
<td></td>
<td>Lactobacilli</td>
<td>-0.533</td>
<td>0.113</td>
<td>0.386</td>
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<tr>
<td></td>
<td>Streptococcus sanguinis</td>
<td>-0.569</td>
<td>0.086</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Candida</td>
<td>0.28</td>
<td>0.431</td>
<td>-0.035</td>
</tr>
<tr>
<td>SECC</td>
<td>Lactobacilli</td>
<td>0.097</td>
<td>0.790</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td>Streptococcus sanguinis</td>
<td>-0.186</td>
<td>0.608</td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>Candida</td>
<td>-0.108</td>
<td>0.571</td>
<td>-0.463</td>
</tr>
<tr>
<td>Total</td>
<td>Lactobacilli</td>
<td>-0.363</td>
<td>0.049*</td>
<td>0.156</td>
</tr>
<tr>
<td></td>
<td>Streptococcus sanguinis</td>
<td>-0.422</td>
<td>0.02*</td>
<td></td>
</tr>
</tbody>
</table>

*** vhs <0.001, **hs <0.01, *sig <0.05
Estimation of Candida

0.1ml of saliva was plated on Sabourand's dextrose agar medium (Figure 4) with 0.1mg/dl of chloramphenicol. Plates were incubated at 370 C for 48 hrs. Candida presence was analyzed as positive or negative. Agar plates were evaluated daily for growth of yeast colonies. Candida albicans colony characters appeared white creamy, medium sized, circular convex, colonies, and were buttery in texture. The counting of colony-forming units per millimeter was carried out after the growth of characteristic yeast colonies (Figure 5,6). The Candida colonies were counted on each plate, and then multiplied by the appropriate dilution factor to yield the colony-forming units per milliliter of the original saliva. A germ tube test was carried out for all Candida positive samples; by inoculating 5ml of serum, containing 0.5% glucose and incubated at 350C for 2-3 hours (Reynold – Braude phenomenon). Samples which showed germ tube formation of yeasts, were confirmed the presence of Candida albicans (Figure 7,8). Gram staining was done to see the presence of hyphal and pseudohyphal form under light microscope.

RESULTS

The mean age of the study sample was 3.42 (S.D 0.218) and the children in the control group was 3.4 (S.D 0.2108), ECC group was 3.5 (S.D 0.1581) and SECC group was 3.3 years (S.D 0.2359). (F= 3.029, p= 0.065 ~ sig). The Mean dfs of the population in ECC group was 2.5 (S.D 0.7071) and of SECC group was 4.4 (S.D 0.6992). (F=147.742, p <0.001 vhs). The mean”d” compound of dfs was same as that of dfs. Table 1 shows correlation of each species with dfs. Table 2 and Graph 1; shows correlation between Candida, Streptococcus mutans, Streptococcus sanguinis and Lactobacilli in whole sample, control, ECC and SECC group.

DISCUSSION

The oral cavity contains complex, multispecies microbial communities. This suggests that the residents in this community should display extensive interactions while forming biofilm structures, carrying out physiological functions, and inducing microbial pathogenesis. These interesting interactions, include (i) competition between bacteria for nutrients, (ii) synergistic interactions which may stimulate the growth or survival of one or more residents, (iii) production of an antagonist by one resident which inhibits the growth of another, (iv) neutralization of a virulence factor produced by one organism by another resident, and (v) interference in the growth-dependent signaling mechanisms of one organism by another. In a micro-Gaia community, these interactions could be envisaged as forms of “war and peace” among the bacterial residents of a biofilm. The implications of such effects are discussed below, especially in terms of progression of caries from the oral cavity of young children, where immunity is in beginning stage of development. On correlating Candida Albicans, Streptococcus mutans, Streptococcus Sanguinis and Lactobacilli count; Candida and Streptococcus mutans were found to be negatively correlated (p=0.571ns). But on correlation in each group; Candida and Streptococcus mutans were negatively correlated only in control group (p=0.259 ns). Whereas in ECC and SECC they were found to be positively correlated (p=0.797 ns and p= 0.431ns respectively). This result also probably indicates the existence of interspecies communication; ie co-aggregation of the two species in the severity of Early Childhood Caries. (Table 2 and Graph 1). Recent studies suggests that this co-aggregation may be probably due to; starvation of Streptococcus mutans which could specifically aid oral colonization of C. albicans, secretion of diffusible quorum-sensing molecule CSP (Competence Stimulating Peptide) by Streptococcus mutans that affects Candida albicans Hypha formation, and absorption of Glucosyltranferase B from saliva by Candida albicans that could regulate Streptococcus mutans carriage on Hydroxyapatite surface.

On correlating Candida and Streptococcus sanguinis in whole sample, they were found to be negatively correlated (p=0.04 sig); and also with the caries groups. (p=0.062 ~sig in ECC and p= 0.923 ns in SECC). This result probably shows that Candida flourishes in cariogenic micro flora (Table 2 and Graph 1). In our study, Candida and Lactobacilli were positively correlated in the whole sample. (p=0.02 sig). But on correlation in each groups;


Candida and lactobacilli were positively correlated in control and ECC group (p=0.013 sig and p=0.714 ns respectively). Their Zm et al, suggested that co-culture of Candida and Lactobacilli did not show any consistent reduction of yeast counts in Candida biofilm.7 Whereas, in SECC group, there was a negative correlation (p=0.723 ns). This probably indicates Candida dominance over acidogenic bacteria like Lactobacilli when severity of caries increases. (Table 2 and Graph 1). Microbial community stability can be achieved only when a natural balance is established among different species within the same biological niche, and this balance is often the result of the constant “war and peace” activities experienced by all the members of the biocommunity. The production of, and sensitivity to, certain bacteriocin or bacteriocin-like activities among oral bacteria could enable bacteria to select their neighbors, promote the establishment of a community with specific bacterial species, and play an important role in the ecological balance of the oral ecosystem.8

**CONCLUSION**

Our findings indicated that there was local micro-ecological disequilibrium in the oral cavities of children with ECC and S-ECC and they are as follows:

- An Inversely proportional relation was found between Candida colonization and Streptococcus sanguinis colonization in the whole sample, and ECC group.
- A directly proportional relation was found between Candida colonization and Lactobacilli colonization in whole sample and, caries free groups.
- No significant relation was found between Candida and Streptococcus mutans colonization.

These findings suggest that Candida albicans may be contributing for initiation; and also for the rapid and widespread progression of ECC in the very young children with immature immune system.

**References**


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