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Research Article

Scardovia Wiggsiae in Children

Identification of *Scardovia Wiggsiae* in Children Between Severe Early Childhood Caries and Caries-Free Children – A Polymerised Chain Reaction -Based Study

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Abstract: Dental caries is the most common microbial disease of oral cavity, affecting 60 – 90% of children worldwide. Early Childhood Caries (ECC) is a major health problem in developing countries. These lesions are destructive and can affect the development of permanent successors. Although *Streptococcus mutans* is the primary cause of ECC, some studies support the view that caries can develop in the absence of mutans streptococci. *Scardovia wiggsiae*, a gram-positive anaerobe was recently found to be associated with Early Childhood Caries in the presence and absence of *Streptococcus mutans*. The aims were to investigate the cariogenic potential of *S. wiggsiae*, in ECC. The study was intended to compare the presence of *S. wiggsiae*, in the saliva samples of caries-free children and children with severe ECC, thereby determine this new acidogenic species noxiousness as a pathogen in Early Childhood Caries.Saliva samples were collected from 40 children, age ranging between three to six years. The sample thus collected at room temperature was then subjected to DNA extraction and Polymerase Chain Reaction (PCR) analysis to evaluate the presence of *S. wiggsiae*. Numerical parameters were tested for significance using Fisher's Exact Test and the statistical analysis using Epi Info software.The tested samples revealed almost equal proportion of *Scardovia wiggsiae* in caries-free group and severe ECC group. *S. wiggsiae* was isolated and detected in both the study and the control groups. Statistical significance was not pronounced claiming the falsehood of the association between Early childhood caries and *Scardovia wiggsiae*.

Keywords: Early Childhood Caries, *Scardovia Wiggsiae* and Polymerase Chain Reaction.

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1. INTRODUCTION

Early childhood caries (ECC) is on the rise in many countries and has emerged as a major health concern, particularly among socially underprivileged youth. The occurrence of one or extra decayed, missing or filled dentition in any primary tooth in a person aged 71 months or younger is categorised as ECC. It has several distinct clinical attributes, such as quick caries development, that affects a large number of teeth shortly after they spring up in the oral cavity. These lesions affect tooth surfaces that are less susceptible to caries formation. Nursing bottle caries, nursing caries, widespread caries, baby bottle caries, baby bottle tooth decay, milk bottle syndrome, and sustained nursing habit caries are some of the phrases used to refer to the condition. . ECC is a complex disorder triggered by the interaction of several factors, including cariogenic microorganisms, improper feeding practises, and a wide range of social variables. [1]". The interaction of acidogenic bacteria, a carbohydrate substrate that is frequently sucrose, and host vulnerability causes dental caries. Streptococcus mutans, an acidogenic and acid-tolerant bacterial species, is recognised as the primary bacterium in early childhood caries. S. mutans is found in caries-free communities but not in each case of childhood caries, implying that other organisms may be cariogenic microbes. Though, *S. mutans* is the primary cause of ECC, recent studies have isolated *Scardovia wiggsiae*, a gram-positive bacterium from children with severe early childhood caries which makes it necessary to clarify the cariogenic potential of the bacteria. The majority of the microbiology in ECC clinical studies tends to focus on mutans streptococci (MS) and lactobacilli (LB), both of which have been routinely identified utilising selective-culture-based methodologies. The microbiota of biofilms collected from ECC patients, on the other hand, has been shown to contain a diverse range of bacteria. Some research suggests that caries can develop in the absence of MS. Newly developed molecular techniques have revealed that the traditional MS and LB species look to be less important or absent, implying that other species besides MS and LB may be mainly accountable for ECC. Thus, in addition to MS, several bacterial species, either alone or in groups, may play important roles in progression of caries Presently, approaches for detecting periodontal and cariogenic bacteria are mostly semi-quantitative and centred on cultivation techniques on various selective media, immunochromatic identification of bacterial antigens, or recognition of specific DNA sequences using various methods such as checkerboard hybridization, polymerase chain reaction (PCR), or real-time PCR. Research findings of the microbiome related to dental caries have relied primarily on 16S rRNA sequence analysis, which has been linked to PCR biases, low taxonomic resolution, and a loss of the ability to investigate functions precisely [2] Thus, the study is intended to isolate *S. wiggsiae* from saliva of caries-free children and severe ECC affected children using PCR thereby find its association with severe Early childhood caries.

2. MATERIALS AND METHODS

2.1 Study Population

Forty children between the age of three to six years, visiting the Department of Pediatric Dentistry in our institute were enrolled in this study. The present study was approved by the Institutional Review Board and Institutional Ethical Committee. (IGIDSIEC2018NRP22PGJVOPM)

2.2 Selection Criteria

The children between 3 to six years who fit into the criteria of dental caries with consenting parents were included. Children who were medically healthy, not used antibiotics within the last three months, and did not undergo orthodontic treatment were included in this study. The study was explained to the parent or the guardian and assent was obtained.
The parents who did not give any consent or children with any other systemic illness were excluded

2.3 Grouping and Design

The children were grouped as caries-free children and children with severe early childhood caries (S-ECC) by clinical examination.

2.4 Design

Unstimulated saliva of one-two ml (1-2ml) was collected using Naglers' method and was subjected to qualitative PCR analysis. (Fig1)

Fig 1: Saliva samples collected in 5ml vials. A total of 40 samples were submitted for PCR analysis.

2.5 *Bacterial Collection*

The samples were centrifuged at 10,000 rpm for ten minutes at room temperature. The supernatant was discarded and the pellet containing the bacterial cells was lysed with SDS/Triton bacterial lysis buffer containing 2% SDS (SIGMA-ALDRICH, Cat# 71736) and 10% Triton-X100 (SRL Fine Chemicals, Cat#64518). Bacterial cells were lysed by heating the samples at 95°C for ten minutes followed by centrifugation at 12,000rpm at room temperature for five minutes to pellet undigested cells and debris.

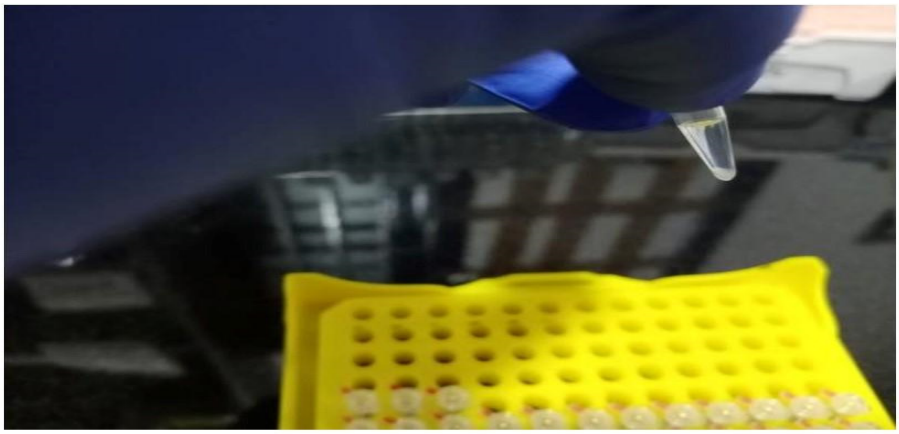

Fig 2: Sample DNA in supernatant. White precipitate is cellular protein, lipids and debris from saliva.

2.6 *Polymerase Chain Reaction*

The supernatant containing bacterial genomic (Fig2) was transferred to a fresh 0.5ml tube and five µl (5µl) of total DNA was subjected to polymerase chain reaction (PCR) amplification of the 16S rRNA gene hypervariable regions V1 to V6 with the total bacterial (universal) primers (Eurofins, Bangalore). (Table-1)

After an initial denaturation at 94°C for four minutes, the samples were subjected to 30 cycles of 94°C for 45 s, 60°C for 45 s, 72°C for one minute, with a final extension at 72°C for five minutes. All PCR amplicons were then diluted fivefold and were then used as template in the next round of PCR with *Scardovia wiggisiae* specific primers (species-specific) that are present within the 16S rRNA gene were used (Eurofins, Bangalore). (Table-1)

Table 1: Total bacterial primers and species-specific primers.

Primers	Sequence (5' –3')
Total bacteria	Forward: AGTTTGATCCTGGCTCAG
	Reverse: TAGATACCCTGGTAGTCC
Scardovia wiggsiae	Forward: GTGGACTTTATGAATAAGC
	Reverse: CTACCGTTAAGCAGTAAG

PCR conditions as developed by ACR Tanner et al was used. After an initial denaturation at 94°C for two minutes, the samples were subjected to 40 cycles of denaturation at 94°C/20 seconds, annealing at 51°C/20 seconds, and elongation at 72°C/20 seconds, with a final extension at 72°C for five minutes. The amplified region of 16s rRNA of *S. wiggisiae* were verified by electrophoresis on 1.5% agarose gels (Sigma-Aldrich, USA). (Fig3)

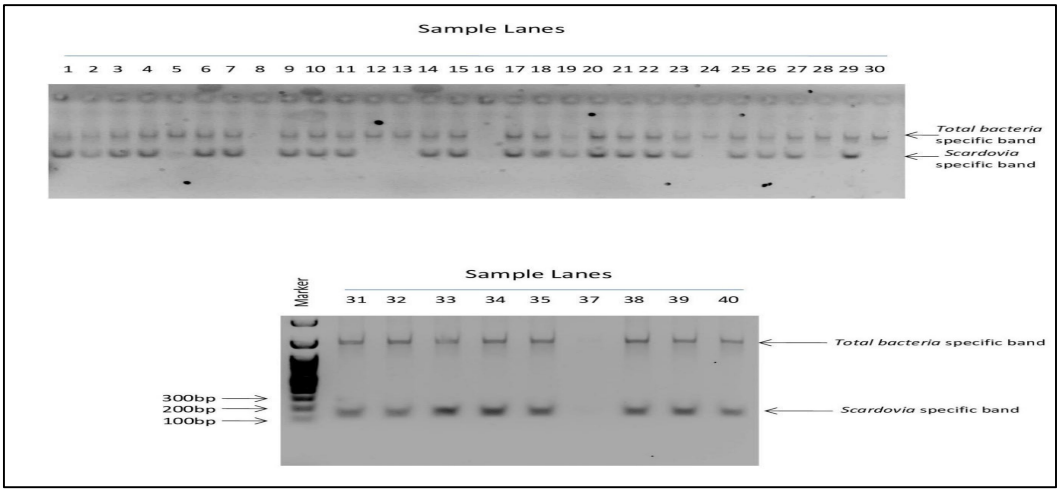

Fig 3: PCR amplification of genomic DNA with *Scardovia wiggisiae* specific primers.

3. STATISTICS

The statistical analysis was done using Epi Info software, Fisher's Exact test was used for assessment of the prevalence of *S. wiggsiae* between the groups. Using a significance level of ☐ = 0.05 and a power p = 0.80, a minimum sample size of fortyy (N = 40) was calculated. Student t test was used for analyses and comparison of parametric data. A p value of less than 0.05 was considered significant.

4. RESULTS

A total of 40 children who gave consent to participate in the study were divided into two groups based on their caries status as caries-free group and S-ECC group for the purpose of comparative analysis. Each group had 20 children of both genders. The groups were similar in terms of demographic data. Fisher's Exact test showed no statistically significant association between the caries-free and S-ECC group p>0.05(p= 0.2674). (Table-2)

Table-2: Fisher's Exact Test was used for the statistical analysis, comparing severe early childhood caries and caries-free groups.

Group	Frequency	Odds ratio	P-value
S-ECC	89%	3.6	P > 0.05 (p=0.2674)
Caries-free	70%		

The children in caries-free group showed no clinical evidence of caries while the children in S-ECC group had four or more carious teeth that were clinically evident. These children were asymptomatic and of the caries affected teeth were deciduous teeth. The alike and unlike features between the two groups has been presented in Chart-1.

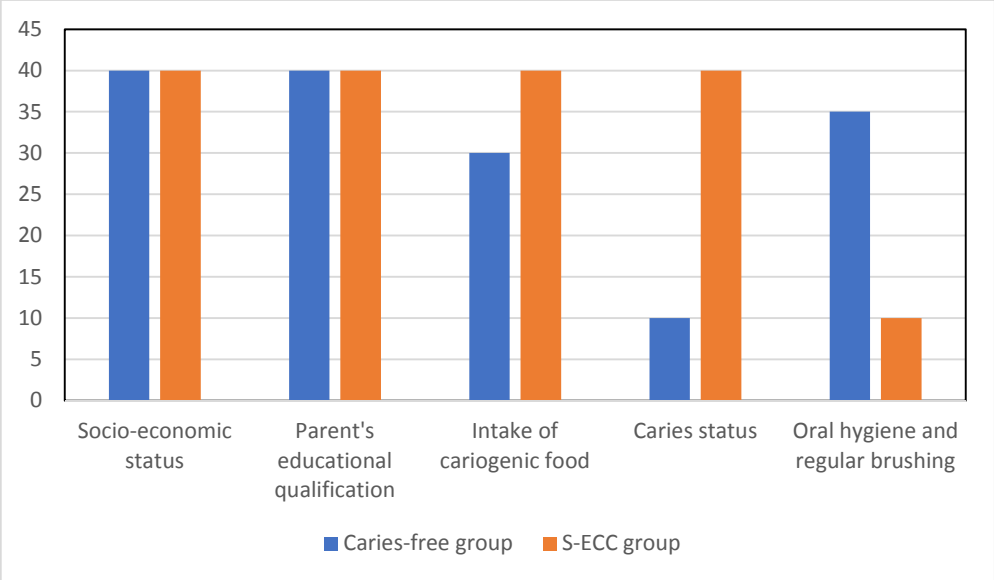

Chart – 1: Bar diagram depicting the differences in various factors between the two groups.

During processing, insufficient DNA was obtained from one sample in S-ECC group; hence DNA was recovered from 39 samples out of the 40 samples. The total 39 samples (20 caries-free and 19 S-ECC) after DNA extraction were screened using PCR to assess the presence and absence of *S. wiggsiae*. After PCR analysis, nearly three-fold of the tested samples revealed the presence of *Scardovia wiggsiae*, which included both caries-free and S-ECC groups (Table-3).

Table – 3: Tabulation showing the number of samples positive and negative for Scardovia wiggsiae.

ORGANISM	RESULT	CARIES-FREE GROUP	S-ECC GROUP	TOTAL
Scardovia wiggsiae	Positive +	14 (70%)	17 (89%)	31 (80%)
	Negative -	6 (30%)	2 (11%)	8 (20%)

Though the S-ECC group showed notable difference between presence and absence of S. wiggsiae, the caries-free group did not exhibit such significant difference. (Chart-2)

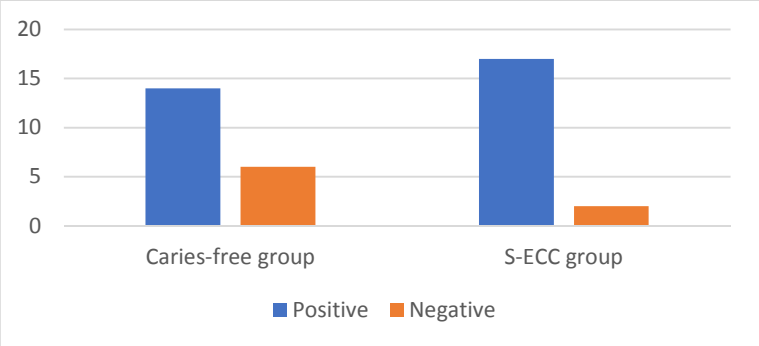

Chart – 2: Difference between the two groups that were positive and negative for S. wiggsiae

5. DISCUSSION

Early childhood caries (ECC) is a most common chronic infectious disease of childhood with a prevalence of 8.8% globally [3] and around 51.9% in India. According to the American Academy of Pediatric Dentistry (AAPD), early childhood caries (ECC) is defined as a severe form of tooth decay in the primary dentition of a child under the age of six years. Severe Early childhood caries (S-ECC) is a rapid, aggressive and destructive form of dental caries in children which can cause acute pain, sepsis, tooth loss and can be a risk factor for caries in permanent teeth.[4] S-ECC is indicative in children from ages three through five years who have one or more cavitated missing teeth (due to caries), filled smooth surfaces in primary maxillary anterior teeth, or decayed, missing, or filled score of ≥4 (age 3 years), ≥5 (age 4 years), or ≥6 (age 5 years) surfaces" [5]. ECC is generally not associated with bottle feeding, rather the etiology is multifactorial involving high sugar intake, interaction of microorganisms with sugars on the tooth surface, duration, diet and feeding practices, lack of oral hygiene, lack of fluoride exposure, salivary pH and flow and enamel defects. Amongst these, dietary sugar is a major factor in the development of ECC. [6-8] Most children of low-income families suffer from early childhood caries, which remains a health problem around the world[9]. In our study children from both the groups were from low sociodemographic background. Likewise, irregular brushing and milk consumption with sugar before sleep were found to be more in S-ECC group that could have contributed to their caries status. Scardovia belongs to the Bifidobacteriaceae family that are gram-positive, acidogenic, aciduric, pleomorphic, branched, non-motile, non-sporing and non-filamentous rods. *S. wiggsiae* possess a unique metabolic pathway called the fructose-6-phosphate pathway (F6PPK shunt) which differs from the glycolytic pathway of S. mutans. It utilizes both lactate formate pathway and acetate pathway to produce small quantities of lactic and formic acids and high amount of acetic acid which metabolically helps in the acidification of the oral biofilm. The high acid productivity and the distinct F6PPK shunt pathway makes it fluoride-resistant and acid-tolerant. Higher proportion of acetic acid at low pH decalcifies the enamel which indicates *S. wiggsiae* is capable to develop caries and can be a microbial risk marker[10,11]. In our study, the S-ECC group showed considerable expression for *S. wiggsiae* which relates its association with caries but at the same time its presence in caries-free subjects can be compared with a similar study in which S. mutans, a well-known cause of dental caries was also present in caries-free subjects as commensals.[12] Despite researchers have isolated *S. wiggsiae* from dentinal caries, root caries, occlusal caries and deciduous pulps,[13,14] they have also identified different strains of Scardovia from sound tooth-root surface and healthy buccal mucosal tissue. In addition, *S. wiggsiae*-specific PCR products had matched a specific strain of *S. wiggsiae* and other Bifidobacteria as well.[15] This shows that these species may not necessarily occur in relation to caries and demonstrates microbiota variability between sites within the oral cavity which implies strain-level analysis of the organism is also important[16]. Studies conducted so far had demonstrated *S. wiggsiae* associated carious lesions had a wide age range since the organism has been isolated from children as well as adults. Studies by Row L et al, Carr G et al and McDaniel et al have reported that *S. wiggsiae* positivity was almost similar in both adult and pediatric groups. They have found that *Scardovia wiggsiae* was present in both pediatric and adult populations with and without caries experience which suggests that age and Scardovia detection have no significant association [17,18,19]. Besides, the choice of an appropriate sample has become uncertain due its divergent views. Vacharaksha et al found that *S. wiggsiae* was higher in infected dentine than in dental plaque samples, whereas Vieira et al found saliva to be more diverse and stable than plaque samples[20]. On the other hand, Matondkar et al found dental plaque to be more sensitive than salivary samples[21]. These differences in the sites sampled for the analysis and the varied outcomes questions the reliability of the sample chosen. Moreover, sampling location also plays an important role as microbiomes in oral cavity are dependent on the local microecological conditions as well. Though saliva is considered to be a representation of the whole oral ecosystem, different atmospheric conditions culture different bacterial community. Therefore, saliva samples may not be representative of the bacterial diversity located at the disease site [22]. Furthermore, technical differences between methods like microbial culture and PCR and more even the type of PCR technique used had resulted in discrepancies in detection of microorganisms especially in pathogens like Scardovia which makes their role obscure in early childhood caries. This has been demonstrated by Vacharaksa et al who had found that caries-free group was completely negative for *S. wiggsiae* when end-point PCR was used, while some samples from the same group tested positive for *S. wiggsiae* using qPCR. Inferring from these findings, we suggest that an individual microorganism cannot be used as a microbial risk marker. Since children from caries-free group also expressed *S. wiggsiae* positivity and mere presence of this organism may not be considered as a risk factor for development of caries in children. As mentioned by Kashyap P, *S. wiggsiae* in combination with other cariogenic species like S. mutans can be considered as microbiological marker for better caries risk assessment in children because use of a single microorganism may compromise the sensitivity or specificity

of the test. But so far, risk factors for caries either alone or in combination with other factors that provide true positive and true negative predictive values have not been reported. Animal studies that have proved the caries-inducing capacity of S. mutans can also be performed using *S. wiggsiae* to understand its cariogenic potential. The species of Bifidobacterium like *Scardovia inopinata, S. wiggsiae, Parascardovia denticolens* have been detected from oral cavity usually at low levels which demands quantification of the organism. Advanced research on different strains of *S. wiggsiae* and their characteristics, use of better methods for assessment of ECC, selection of relevant age group, choice of suitable sample site and sampling location may enhance the knowledge on this species and facilitate better identification and association of *S. wiggsiae* with severe ECC.

6. CONCLUSION

We conclude that the detection of Scardovia wiggsiae using a PCR-based assay was comparable in children with and without caries. We assert that the presence of this microbe alone cannot be projected as a risk factor for caries in children. Other social and environmental factors must be taken into account. A multicentric trial with differing social influences is likely to confirm our single-center findings.

7. AUTHOR CONTRIBUTION STATEMENT

Vinodine Joice.C, Santhadevy.A, - concept design, manuscript, Sivaramakrishnan.M Sanguida.A, - data collection, Suganya.R, Vezhavendhan.N- manuscript, supervision

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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