Molecular Screening of *Scardoviawiggsiae* - An Emerging Cariogenic Pathogen in Children below 5 Years with and Without Dental Caries

C. Prathima Anusha¹, Dr. Karthika Jayakumar², Dr. Priya Ramachandran³, Dr. Manikandan Gunasekaran⁴, Dr. Priyadarshini Shanmugam⁵, Dr. Gopal Muthu⁶

¹PhD Scholar, Department of Microbiology, Shri Sathya Sai Medical College & Research Institute, Ammapettai, Chennai, India Orcid ID: 0000-0001-8648-2730

Corresponding Author Mail id: prathimasureshkumar[at]gmail.com

Department of Microbiology, Shri Sathya Sai Medical College& Research Institute, Ammapettai, Chennai, India

Department of Dentistry, Shri Sathya Sai Medical College& Hospital, Ammapettai, Chennai, India

Abstract: Early childhood caries (ECC) is a major health concern in the population of India. Cariogenic bacteria like Streptococcus mutans are the major factor in the disease progression. The Discovery of Scardoviawiggsiae relating to early childhood dental caries supported the evidence of multispecies infection in carious lesions. The study aims to qualitatively assess the presence of an emerging cariogenic pathogen Scardoviawiggsiae in children below 72 months with and without dental Caries using real-time polymerase chain reaction (RT-PCR). In our study 100 children below 5 years are randomly recruited from the Dental Department and pediatric department at Shri Sathya Sai Medical College and Hospital, Ammapetai, Chengalpattu District, Tamilnadu, India. Fifty children suffering from ECC and 50 children without caries were enrolled in the study. About 1-2 mL of unstimulated saliva was collected and subjected to microbial analysis using Real-time PCR.ECC group (n = 50) was found to have a significantly higher expression of S. wiggsiae (19) compared to the control group (n = 50). S. wiggsiae was detected at 38% in the ECC group and was detected negligibly (2%) in the control (caries-free) group. In our study the presence of S. wiggsiae was significantly associated with ECC in children. S. wiggsiae represents a new perimeter in the microbial etiology of ECC.

Keywords: Early childhood caries, real-time polymerase chain reaction, Scardoviawiggsiae

Abbreviations: ECC- Early childhood caries, RT-PCR - Real-time polymerase chain reaction

1. Introduction

Early childhood caries (ECC) is a specific form of severe dental caries in infants and young children. This type of caries initially presents with an early lesion on the smooth surface of primary maxillary incisors and progresses rapidly to other teeth in the oral cavity upon the sequence of tooth eruption, except the mandibular incisors that are protected in the self-cleansing area by the movement of the tongue and salivary flow [1]. Early childhood caries (ECC) is a virulent form of the disease that is a public health problem that continues to affect infants and preschool children worldwide [2]. Historically, severe tooth decay in young children was recognized as nursing caries, nursing bottle syndrome, night bottle mouth, or baby bottle tooth decay [3]. Inappropriate bottle-feeding resulted in the above terms. However, current evidence suggested that bottle nursing was not the only cause of disease, but several factors contributed to the etiology of ECC. ECC has a diverse etiology involving microbiologic, dietary, behavioral, and socioeconomic factors [4]. The American Academy of Pediatric Dentistry in 2011 defined ECC as the presence of 1 or more decayed,(non-cavitated or cavitated lesions), 1 or more missing teeth (due to caries), or 1 or more filled surfaces (dmfts)in any primary tooth in a child under the age of 6 or younger. . The term "Severe Early Childhood Caries" refers to "atypical", "progressive", "acute", or "rampant" patterns of dental caries [5]. ECC is considered a significant public health problem both in developing as well as in industrialized countries [6]. The prevalence of ECC is reported as 44% in 8- to 48-month old children [7].

Early childhood dental caries is a complex and the second most common chronic disease caused by many factors including of many cariogenic bacteria, majorly Streptococcus mutans. Streptococcus mutanscan even infect pre-dentate infantsas it can spread from mother to baby during infancy. These bacteria break down sugars for energy, causing an acidic environment in the mouth and resulting in demineralization of the enamel of the teeth and dental caries [8]. Streptococcus mutans and Lactobacillus are known as the two most common responsible microorganisms to cause caries [9, 10].

Tanner et al. in 2011 discovered a novel cariogenic bacteria Scardoviawiggsiae, associated with Early childhood dental caries. Scardoviawiggsiaewas named after Vittorio Scardovi, an Italian microbiologist, and Lois Wiggs, an American microbiologist for her contributions to anaerobic microbiology. Scardoviawiggsiae is a gram-positive, anaerobic, non-spore-forming and non-motile bacilli measuring 0.6-0.7 μ m x 1.6-4 μ m [11, 12, 13, 14]. It was found that S. wiggsiae, Veillonellaparvula, Streptococcus mutens, and Actinomycesgerensceriae are the major species that were seen in children with carious lesions. More interestingly, S. wiggsiaewas observed in ECC even in the absence of S. mutens [15]. Based upon these new findings, the goal of this study is to understand the presence of this

Volume 11 Issue 6, June 2022 www.ijsr.net

Licensed Under Creative Commons Attribution CC BY

new cariogenic pathogen S. wiggsiae from saliva samples in Tamilnadu children below 5 years with and without dental Caries using real-time polymerase chain reaction.

2. Materials and Methods

Study population:

The Paediatric population in this study are below 5 years and 100 in number. The severe ECC children (50) were recruitedfrom the Dental Department (50) and caries-free childrenwere recruited from Pediatric department at Shri Sathya Sai Medical College and Hospital, Ammapetai, Chengalpattu District, Tamil Nadu.

Informed consent was obtained from each parent or guardian; and the study procedures were approved by Institutional Ethics Committee, Shri Sathya Sai Medical College & Research Institute (2019/519).

DNA isolation:

The isolation of DNA from saliva samples was performed using the QIAGEN DNA Purification from Blood or Body Fluids (Spin Protocol) using the manufacturer's recommended protocol.

Real-time PCR Screening

Molecular screening for the presence of S.wiggsiae was accomplished using Bio-Rad Real-time PCR machine (BIORAD CFX96) with the following reaction parameters: Initial Denaturation at 95°C for 30sec followed by denaturation at 95°C 5 sec and 40 cycles at the annealing (melting) temperatures (Tm) 60°C 30 sec (TB GreenTM Premix Ex Taq TM (TliRNaseH Plus) For the CFX96 Real-Time PCR Detection System). Data attainment was done at the annealing step.

Scardoviawiggsiae primer set - 172bp[16]. Forward Primer - 5'GTG GAC TTT ATG AAT AAG C 3' Reverse Primer - 5'CTA CCG TTA AGC AGT AAG 3'

Positive control

16s rRNA bacterial primer set Forward Primer - 5' ACG CGT CGA CAG ACT TTG ATC CTG GCT 3' Reverse Primer- 5' GGG ACT ACC AGG GTA TCT AAT 3'

3. Results

Demographic analysis:

Demographic analysis of the samples revealed that the percentage of females and males within the study sample (46% and 54%, respectively) was not significantly different. The reported ethnic background of the study sample isolates was also similar to the overall clinic population with approximately 48% of the urban samples and 52% of the rural sample. [Table 1]

Real-time PCR analysis

The Real-Time PCR screening results for S. wiggsiae revealed that approximately one-third (n=19/50 or 38%) of these isolates harbored this organism. A more detailed analysis of the S. wiggsiae (SW)-positive and SW–negative

samples was performed to determine if sex or ethnicity were associated with a positive screening result. [Table 2]

The percentage of SW-positive samples in the male and female pediatric population with caries is 8 and 11 respectively, with no significant difference in the Male and female population at P<.05. (Table 2). In addition, the percentage of SW-positive samples that were derived from an urban and rural pediatric population (47.3% and 52.6%) where the rural children showed a higher prevalence of S.wiggisiae when compared to urban children [Table 3]

4. Discussion

In the present study presence of S.wiggsiae in the pediatric population with and without ECC has been analyzed. The results of this study suggest a one-third percentage of the pediatric population with ECC harbor oral S. wiggsiae, which may be a significant concern due to the cariogenic potential of this organism.

S. wiggsiae, which is a member of the Bifidobacteriaceae family, is recently identified and thought to be an important part of the caries-related microbial complex [17]. Studies have shown that there is a relationship between the presence of caries and Bifidobacteriaceae Bifidobacteria and Scardovia's high prevalence [18].

The Prevalence of ECC is slightly higher in females (52%) as compared to males (48%), which is in contrast with the study where Boys (57%) were affected more than girls (43%)[19]. This may be due to the difference in feeding habits in different areas of India. The prevalence of SW-positive samples in the male and female pediatric population is 8 and 11 respectively, the female pediatric populations showed a higher frequency [20].

The rural Paediatric population has a higher prevalence of ECC 52.6% compared to the urban pediatric population 47.6%. This is supported by a study where oral Health-Related Quality of Life among preschool children living in urban India was found to be better than those in rural India [21]. The association of ECC was found Significant with sociodemographic factors such as gender, parental education, parental occupation, socioeconomic status, and oral hygiene practices

Within the limitations of the study, we conclude that the presence of S. wiggsiae was significantly associated with ECC in children. S. wiggsiae represents a new frontier in the microbial etiology of ECC. This may lead to the development of new antimicrobial agents targeted at this organism and improve the treatment of ECC.

Conflict of interest

The authors have declared no conflict of interest

References

[1] Ripa LW. Nursing caries: a comprehensive review.Pediatr Dent 1988;10:268-82.

Volume 11 Issue 6, June 2022 www.ijsr.net

Licensed Under Creative Commons Attribution CC BY

- [2] Berkowitz RJ. Causes, treatment and prevention of early childhood caries: A microbiologic perspective. J Can Dent Assoc 2003;69:304-7.
- [3] Reisine S, Douglass JM. Psychosocial and behavioral issues in early childhood caries. Community Dent Oral Epidemiol 1998;26:32-44
- [4] Takahashi N, Nyvad B. Caries ecology revisited: Microbial dynamics and the caries process. Caries Res 2008;42:409-18
- [5] American Academy of Pediatric Dentistry. Policy on Early Childhood Caries (ECC): Classifications, Consequences, and Preventive Strategies. Pediatr Dent 2011;33:47-9.
- [6] Bucher K, Tautz A, Hickel R, Kuhnisch J. Longevity of composite restorations in patients with early childhood caries (ECC). Clin Oral Investig 2013;18:775-82
- [7] Jose B, King NM. Early childhood caries lesions in preschool children in Kerala, India. Pediatr Dent. 2003;25:594–600. [PubMed: 14733478]
- [8] Douglass JM, Douglass AB, Silk HJ. A practical guide to infant oral health. Am Fam Physician. 2004;70:2113–20. [PubMed: 15606059]
- [9] Balhaddad AA, Kansara AA, Hidan D, Weir MD, Xu HHK, MeloMAS.Toward dental caries: Exploring nanoparticle-based platforms and calcium phosphate compounds for dental restorative materials. BioactMater 2019;4:43-55.
- [10] Peterson SN, Snesrud E, Liu J, Ong AC, Kilian M, Schork NJ, et al.The dental plaque microbiome in health and disease. PloS One2013;8:e58487.
- [11] vanRuyven FO, Lingström P, van Houte J, Kent R. Relationship among mutans streptococci, "low-pH" bacteria, and iodophilic polysaccharide-producing bacteria in dental plaque and early enamel caries in humans. J Dent Res.2000;79:778-784.
- [12] Tanner AC, Mathney JM, Kent RL, Chalmers NI, Hughes CV, Loo CY, Pradhan N, Kanasi E, Hwang J, Dahlan M, Papadopolou E. Cultivable anaerobic microbiota of severe early childhood caries. J Clin Microbiol.2011;49:1464-1474.
- [13] Tanner AC, Kent RL, Holgerson PL, Hughes CV, Loo CY, KanasiE, Chalmers NI, Johansson I. Microbiota of severe early childhood caries before and after therapy.J Dent Res. 2011;90:1298-1305.
- [14] Tanner AC, Sonis AL, LifHolgerson P, Starr JR, Nunez Y, Kressirer CA, Paster BJ, Johansson I. Whitespot lesions and gingivitis microbiotas in orthodontic patients. J Dent Res. 2012;91:853-858.
- [15] Tanner ACR, Mathney JMJ, Kent RL, Chalmers NI, Hughes CV, Loo CY,et al. Cultivable anaerobic microbiota of severe early childhood caries.JClinMicrobiol 2011;49:1464-1474.
- [16] Tanner AC, Kent RL, Holgerson PL, Hughes CV, Loo CY, et al. (2011) Microbiota of severe early childhood caries before and after therapy. J Dent Res 90: 1298-305.
- [17] Kanasi E, Dewhirst FE, Chalmers NI, Kent R, Moore A, et al. (2010) Clonal analysis of the microbiota of severe early childhood caries. Caries Res 44: 485-97.
- [18] Kaur R, Gilbert SC, Sheehy EC, Beighton D (2013) Salivary levels of Bifidobacteria in caries-free and caries-active children. Int J Paediatr Dent 23: 32-8.

- [19] PrakashaShrutha S, Vinit GB, Giri KY, Alam S. Feeding practices and early childhood caries: a crosssectional study of preschool children in kanpur district, India. International Scholarly Research Notices. 2013;2013.
- [20] Quan, K., & Kingsley, K. (2018). Effect of Dental Sealants on Oral Microbial Burden of Scardoviawiggsiae within a Pediatric Population: A Pilot Study. Microbiology Research Journal International, 24(6), 1-10. https://doi.org/10.9734/MRJI/2018/42947
- [21] Subramaniam P, Surendran R. Oral health related quality of life and its association with dental caries of preschool children in urban and rural areas of India. Journal of Clinical Pediatric Dentistry. 2020;44(3):154-60.

Tables

Luoie Le D ennographice analysis of State, Sample

		Study sample	With	Without Caries
		(n=100)	Caries(n=50)	(n=50)
Sex	Male	46%(n=46)	48%(n=22)	52%(n=24)
	Female	54%(n=54)	52%(n=28)	48%(n=26)
Ethnicity	Urban	48%(n=48)	42%(n=20)	58%(n=28)
	Rural	52%(n=52)	58%(n=30)	42%(n=22)

 Table 2: Real Time PCR analysis for S. wiggsiaein male and female Paediatric Population

und feinale i deditatie i optimiton								
		With EC	C(n=50)	Without ECC(n=50)				
		S.wiggsiae Positive (n=19)	S.wiggsiae Negative (n=31)	S.wiggsiae Positive (n=2)	S.wiggsiae Negative (n=48)			
Sex	Male	8	14	0	24			
	Female	11	17	2	24			

No significant difference in the male and female population with ECC at p < .05.

Table 3: Real Time PCR analysis for S. wiggsiaein Urban and rural population

		With Caries(n=50)		Without Caries(n=50)	
		S.wiggsiae Positive (n=19) 38%	S.wiggsiae Negative (n=31) 62%	S.wiggsiae Positive (n=2) 4%	S.wiggsiae Negative (n=48) 96%
Ethnicity	Urban	9 (47.3%)	11 (35.4%)	0	28
	Rural	10 (52.6%)	20 (64.5%)	2 (4%)	20

Volume 11 Issue 6, June 2022 www.ijsr.net

Licensed Under Creative Commons Attribution CC BY