ORIGINAL RESEARCH

Prevalence of *Streptococcus mutans* and *Candida dubliniensis* in Plaque of Caries-free and Caries-active 3–6-year-old Children by Using Polymerase Chain Reaction: A Clinical Study

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Abstract

Introduction: Oral cavity harbors numerous types of microbial flora, which change frequently with changes in the environment and which in turn leads to the process of caries.

Aims: To evaluate the prevalence of *Streptococus mutans (S. mutans)* and *Candida dubliniensis (C. dubliniensis)* in dental plaque of caries-free (CF) and carries-active (CA) children aged 3–6 years using polymerase chain reaction (PCR).

Material and methods: A total of 18 CA and CF children in the age group of 3–6 years were randomly selected. Plaque samples were collected using sterile micro brushes from teeth. Specific primers were used to carry out PCR in the plaque samples. Statistical analysis was done using Fisher's exact test.

Results: In CA group, *S. mutans* were seen in 61.1% of children which is statistically significant with a *p*-value of 0.04 and *C. dubliniensis* in 27.8% of children whereas, in CF group, *S. mutans* were present in 27.8% of participants and *C. dubliniensis* in 5.6%. These results show that both organisms were more predominant in the CA group.

Conclusion: Though both *S. mutans* (61.1%) and *C. dubliniensis* (27.8%) were present in CA group, *S. mutans* was strongly associated with dental caries. In the individuals with high scores of def and international caries detection and assessment system II, both species were more prevalent.

Keywords: *Candida dubliniensis*, Dental caries, Polymerase chain reaction, *Streptococcus mutans*.

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Introduction

Caries is a dynamic process involving interactions between the tooth structure and the microbial biofilm, along with salivary and genetic influences. The rapid alternating periods of tooth demineralization and remineralization result in the initiation of carious lesions.[1] Caries is considered a chronic childhood disease and its global prevalence rapidly increasing in children of 2–5 years of age, making this age group a global priority action area.[2]

Streptococcus mutans (S. Mutans) is one of the most important bacteria involved in the etiology and progression of the carious lesion as they can metabolize carbohydrates and produce acids, tolerate extreme acidic environments, and also can synthesize extracellular polysaccharides which improve their adherence to other microorganisms and tooth surface.[3]

Candida species causes superficial and systemic infections in immunocompromised patients whereas, in healthy children, the predominant oral habitats of *Candida* cause carious lesions. There are several characteristics of *Candida* that are related to cariogenicity. The fungal H^+-ATPase, which actively pumps protons out of the cell causes an extraordinarily high acid tolerance and enables rapid extracellular acidification. *Candida* adheres to saliva-coated hydroxyapatite and binds to native or denatured collagen leading to caries.[4]

Many techniques are employed for the detection of microbes involved in the carious process which include: cultivation, cultures, direct microscopy, enzyme tests, enzyme-linked immunosorbent assays, and species-specific deoxyribonucleic acid (DNA) probes. PCR is well known for its sensitivity as a diagnostic tool for the detection of microbes as compared to other techniques.[5]

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To come up with proper treatment options, it is necessary to have adequate knowledge of the disease and the pathogens involved in its process. Dental caries occurs due to the involvement of various microbes and studies for the isolation of new species associated with dental caries is always a topic of research.

Hence, this study will determine the presence of *S. mutans* and *C. dubliniensis* in the plaque of CF and CA children in the age group between 3–6 years using PCR.

Methods

This research project was approved by the Institutional Ethics Research Committee (CODS/181/2018-2019), and written informed consent was obtained from the parents or guardians.

Sample Size Determination

Using the following formula:

- $n = Z2\partial 2/e2$
- $n = (1.96)2(1.56)2/(0.72)2$
- $n = 18.01 \approx 18$

Samples were collected from 36 patients (19 males and 17 females) aged 3–6 years who visited the outpatient department for dental treatment. Children who were using antimicrobial mouthwashes, presented with any systemic disease, or had used antibiotics within the previous 3 months were excluded from the study.

Sample Collection

A total of 18 samples were collected from CF individuals and 18 samples from CA individuals aged 3–6 years. Before collecting samples, the children were asked to rinse their mouth with water to remove the debris.

The samples were collected using sterile micro brushes from the buccal surface starting from the most posterior teeth toward the anterior teeth in a sweeping motion. The collected samples were placed in reduced transport fluid (RTF) by cutting the tips with sterile scissors. The samples were stored in RTF at −80°C.

Microbiological Assessment

The samples of dental plaque were mechanically dispersed (vortexed for 30 seconds) and serially diluted with phosphate-buffered saline. From appropriate dilutions, aliquots of 0.05 mL were inoculated onto the agar media, that is, Mitis Salivarius Agar for *S. mutans* and Sabouraud dextrose agar for *Candida*. For DNA extraction, culture colonies from agar plates were isolated.

Deoxyribonucleic acid (DNA) extraction was done by modified proteinase K method using lysis buffers (Chromous Biotech, Bengaluru, India) and proteinase K enzyme (Chromous Biotech, Bengaluru, India). The primers used were for:

Streptococcus mutans (S. mutans) primers

- GTFB- F 5′-ACTACACTTTCGGGTGGCTTGG-3′
- GTFB- R 5′-CAGTATAAGCGCCAGTTTCATC-3′

Carries dubliniensis (C. dubliniensis) primers

- GTFB- F 5′-AGTTACTCTTTCGGGGGTGGCCT-3′
- GTFB- R 5′-AAGATCATTATGCCAACATCCTAGGTAAA-3′
- Deoxyribonucleic acid (DNA) extraction was followed by DNA amplification using PCR master mix (Ampliqon red) (Ampliqon, Odense M, Denmark) followed by gel electrophoresis, in which the amplified product of size 517 base pair and 175 base pair was identified with the help of DNA ladder. Amplified product of size 517 base pair and 175 base pair was identified as *S. mutans* and *C. dubliniensis,* respectively.

Statistical Analysis

The data were subjected to statistical analysis. The statistical test which was used is Fisher's exact test.

Results

Table 1 shows *S. mutans* and *C. dubliniensis* in CA and CF groups. In CA group, *S. mutans* were present in 61.1% of children which is statistically significant and *C. dubliniensis* in 27.8% of children whereas, in the CF group, *S. mutans* were present in 27.8% of participants and *C. dubliniensis* in 5.6%. These results demonstrate that both organisms were more predominant in the CA group.

When different age groups were compared, *S. mutans* and *C. dubliniensis* both were more prevalent in the 5-year age group, in both CA and CF groups (Table 2).

Discussion

Research on microbial involved in dental caries began in the 1980s and the predominant species that were found are: *mutans Streptococci* (MS), others include *Actinomyces, Lactobacillus, Candida, and Veillonella*.[6] *Candida* species display relevance from childhood until old age, that is, thrush, caries, periodontitis, infection of dental implants, and denture stomatitis.[7] The fungus *Candida* is recognized for its involvement in biofilm formation and it inhabits around 30–40% of oral microflora in healthy individuals.[8] Henriques et al.[9] stated that *C. dubliniensis* could form a mature biofilm which represents one of the pathogenic features of *Candida* species.

Table 1: Distribution of *S. mutans* and *C. dubliniensis* in CA and CF group

	S. mutans		*C. dubliniensis*	
	CA	*CF*	*CA*	*CF*
Absent	7	13	13	17
	38.9%	72.2%	72.2%	94.4%
Present	11	5	5	1
	61.1%	27.8%	27.8%	5.6%
Fisher's exact test	*p*-value = 0.04*		*p*-value = 0.18 (NS)	

* significant; NS, not significant

Table 2: Distribution of *S. mutans* and *C. dubliniensis* according to different age groups

	S. mutans		*C. dubliniensis*	
Age	*Absent*	*Present*	*Absent*	*Present*
3 years	6	3	7	0
	85.7%	14.3%	100.0%	0.0%
4 years	7	4	8	2
	70.0%	30.0%	80.0%	20.0%
5 years	3	5	8	4
	25.0%	75.0%	66.7%	33.3%
6 years	4	6	7	0
	57.1%	42.9%	100.0%	0.0%
Fisher's exact test	*p*-value = 0.06 (NS)		*p*-value = 0.19 (NS)	

Identification of *S. mutans* can be done in various ways, these include the methods which morphologically differentiate the bacteria using culture, biochemical tests, and PCR.[10] In this study, PCR was used (Figs 1 and 2), as it is more sensitive and accurate; hence it is significant in eliminating any false positives or false negative results; also, it is faster and less technique sensitive than traditional methods.[11]

The results from the present study demonstrated the high number of *S. mutans* in CA children as compared to the CF population. Out of 36 children, *S. mutans* strain was found in plaque samples of 16 children (11 CA children and five CF children) (Table 1). Fragkou et al.[12] found similar results that CA children harbored more frequently and significantly higher numbers of *S. mutans* that is, in 15 out of 39 children, mostly with high deft. Similar results were seen in a study by Fujiwara et al.[13] where 39.9% of the total population harbored *S. mutans* with the majority of the population with high deft. Results showed a significant global/overall relationship between MS acquisition and dental caries. A study by Hata et al.[14] and Valdez et al.[3] found counts of MS in biofilms of children having early childhood and severe childhood caries higher than those found in CF children. Vacharaksa et al.[15] also found a high count of *S. mutans* in his study when compared to children of the CA group than to CF. In contrast, Loyola-Rodriguez et al.[16] found the percentage of *S. mutans* isolation similar in CA and CF children.

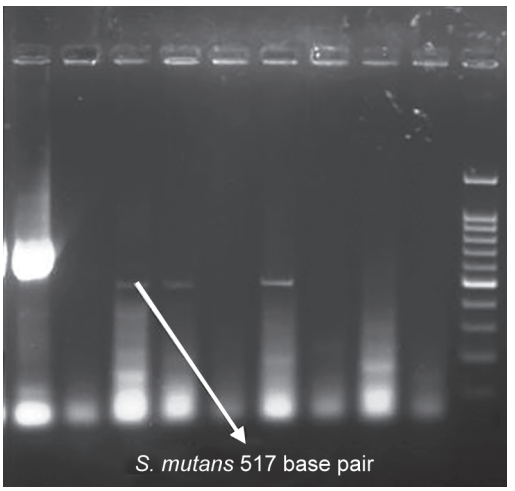

Fig. 1: *Streptococcus mutans* identified under ultraviolet light

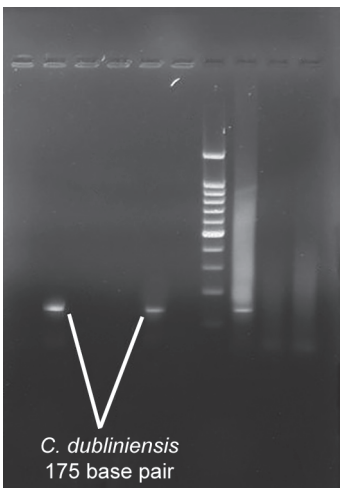

Fig. 2: *Candida dubliniensis* identified under ultraviolet light

Matee et al.[17] reported high levels of *S. mutans* in some CF children. All these results are suggestive that *S. mutans* is one of the most prevalent bacterial species in CA children.

Kneist et al.[18] found the presence of *C. dubliniensis* more in plaque and carious dentin samples. In a study by Al-Ahmad et al.[7] *C. dubliniensis* species were found in more than one quarter (27%) of plaque samples of the CA children but were never detected in the control specimen, that is, CF. Lozano Moraga et al.[19] found that *C. dubliniensis* were present only in the most caries-affected group. In a study by de Jesus et al.[20] authors concluded that in the pooled plaque mycobiome of 40 children with early childhood caries with the same number of CF children, *C. dubliniensis* dominated the mycobiome of children with caries. All these results are similar to the results of the present study, that is the prevalence of *C. dubliniensis* was however nonsignificant, but the number of candidates having the species was more in the CA group than that of CF. A total of six children displayed the presence of *C. dubliniensis* in plaque samples out of which five were from the CA group and one CF (Table 1). Hence, *C. dubliniensis* is positively correlated with caries and it increases steadily as caries severity increases.[21]

Milgrom et al.[22] in their study found that the proportion of children colonized by *S. mutans* increased with age. Karn et al.[23] also found similar results and quoted that there is a trend toward an increasing percentage of children colonized with *S. mutans* with an increase in age. Okada et al.[5] suggested that *S. mutans* are generally established in the oral cavity of children before 3 years of age. The findings of a study conducted by Ghazal et al.[24] was that the median time without MS acquisition (50% of the children not having positive MS test) was 2 years. Approximately 79% of the children had positive salivary MS tests by the age of 4 years. In the present study, we found that the subjects in the higher age group had more caries as compared to lower age groups and proportionately higher counts of *S. mutans* and *C. dubliniensis* were seen with an increase in age (Table 2).

This study contributes to determining the prevalence of *S. mutans* and *C. dubliniensis* in CA and CF children however more such studies are required to determine new microbial species with different age groups and among the different populations which will benefit in understanding the pathogenesis and etiology of the disease and will also contribute in considering new treatment modalities. Very few studies have been reported on identifying *Candida* species as a cariogenic organism and establishing the pathogenesis of *C. dubliniensis* in caries progression; hence more literature is needed in this context.[25] The limitation encountered with PCR is that it cannot differentiate dead from live bacteria. Also, different methods for microbial isolation should be determined as no suggested method acts as a gold standard for the isolation and identification of microbes.

Conclusion

The present study provided the corroboration of oral carriage of *C. dubliniensis* and *S. mutans* in 3–6-year-old CA children. The association of *S. mutans* as an active cariogenic organism has been proved several times by various authors in the past, but very fewer shreds of evidence have demonstrated the role of *Candida* species in caries. Considering results from previous studies and the present study, *C. dubliniensis* can be contemplated as one of the associated pathogens in dental caries. However, future research on these species has to be carried out to unearth the etiology of dental caries.

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References

1. Pitts NB, Zero DT, Marsh PD, et al. Dental caries. Nat Rev Dis Primers 2017;3:17030. DOI: 10.1038/nrdp.2017.30
2. Dye BA, Arevalo O, Vargas CM. Trends in paediatric dental caries by poverty status in the United States, 1988-1994 and 1999-2004. Int J Paediatr Dent 2010;20(2):132–143. DOI: 10.1111/j.1365-263X.2009.01029.x
3. Valdez RMA, Duque C, Caiaffa KS, et al. Genotypic diversity and phenotypic traits of *Streptococcus mutans* isolates and their relation to severity of early childhood caries. BMC Oral Health 2017;17(1):115. DOI: 10.1186/s12903-017-0406-1
4. Klinke T, Guggenheim B, Klimm W, et al. Dental caries in rats associated with Candida albicans. Caries Res 2011;45(2):100–106. DOI: 10.1159/000324809
5. Okada M, Kawamura M, Oda Y, et al. Caries prevalence associated with *Streptococcus mutans* and *Streptococcus sobrinus* in Japanese schoolchildren. Int J Paediatr Dent 2012;22(5):342–348. DOI: 10.1111/j.1365-263X.2011.01203.x
6. de Carvalho FG, Silva DS, Hebling J, et al. Presence of mutans streptococci and *Candida* spp. in dental plaque/dentine of carious teeth and early childhood caries. Arch Oral Biol 2006;51(11):1024–1028. DOI: 10.1016/j.archoralbio.2006.06.001
7. Al-Ahmad A, Auschill TM, Dakhel R, et al. Prevalence of Candida albicans and Candida dubliniensis in caries-free and caries-active children in relation to the oral microbiota-a clinical study. Clin Oral Investig 2016;20(8):1963–1971. DOI: 10.1007/s00784-015-1696-9
8. Alnuaimi AD, O'Brien-Simpson NM, Reynolds EC, et al. Clinical isolates and laboratory reference Candida species and strains have varying abilities to form biofilms. FEMS Yeast Res 2013;13(7):689–699. DOI: 10.1111/1567-1364.12068
9. Henriques M, Azeredo J, Oliveira R. Candida albicans and Candida dubliniensis: comparison of biofilm formation in terms of biomass and activity. Br J Biomed Sci 2006;63(1):5–11. DOI: 10.1080/09674845.2006.11732712
10. Villhauer AL, Lynch DJ, Drake DR. Improved method for rapid and accurate isolation and identification of *Streptococcus mutans* and *Streptococcus sobrinus* from human plaque samples. J Microbiol Methods 2017;139:205–209. DOI: 10.1016/j.mimet.2017.06.009
11. Damle SG, Loomba A, Dhindsa A, et al. Correlation between dental caries experience and mutans streptococci counts by microbial and molecular (polymerase chain reaction) assay using saliva as microbial risk indicator. Dent Res J (Isfahan) 2016;13(6):552–559. DOI: 10.4103/1735-3327.197035
12. Fragkou S, Balasouli C, Tsuzukibashi O, et al. Streptococcus mutans, Streptococcus sobrinus and Candida albicans in oral samples from caries-free and caries-active children. Eur Arch Paediatr Dent 2016;17(5):367-375. DOI: 10.1007/s40368-016-0239-7
13. Fujiwara T, Sasada E, Mima N, et al. Caries prevalence and salivary mutans streptococci in 0–2-year-old children of Japan. Community Dent Oral Epidemiol 1991;19(3):151–154. DOI: 10.1111/j.1600-0528.1991.tb00131.x
14. Hata S, Hata H, Miyasawa-Hori H, et al. Quantitative detection of *Streptococcus mutans* in the dental plaque of Japanese preschool children by real-time PCR. Lett Appl Microbiol 2006;42(2):127–131. DOI: 10.1111/j.1472-765X.2005.01821.x
15. Vacharaksa A, Suvansopee P, Opaswanich N, et al. PCR detection of *Scardovia wiggsiae* in combination with *Streptococcus mutans* for early childhood caries-risk prediction. Eur J Oral Sci 2015;123(5):312–318. DOI: 10.1111/eos.12208
16. Loyola-Rodriguez JP, Martinez-Martinez RE, Flores-Ferreyra BI, et al. Distribution of *Streptococcus mutans* and *Streptococcus sobrinus* in saliva of Mexican preschool caries-free and caries-active children by microbial and molecular (PCR) assays. J Clin Pediatr Dent 2008;32(2):121–126. DOI: 10.17796/jcpd.32.2.cm00062530v856r4
17. Matee MI, Mikx FH, Maselle SY, et al. Mutans streptococci and lactobacilli in breast-fed children with rampant caries. Caries Res 1992;26(3):183–187. DOI: 10.1159/000261440
18. Kneist S, Borutta A, Sigusch BW, et al. First-time isolation of Candida dubliniensis from plaque and carious dentine of primary teeth. Eur Arch Paediatr Dent 2015;16(4):365–370. DOI: 10.1007/s40368-015-0180-1
19. Lozano Moraga CP, Rodríguez Martínez GA, Lefimil Puente CA, et al. Prevalence of Candida albicans and carriage of Candida non-albicans in the saliva of preschool children, according to their caries status. Acta Odontol Scand 2017;75(1):30–35. DOI: 10.1080/00016357.2016.1244560
20. de Jesus VC, Shikder R, Oryniak D, et al. Sex-based diverse plaque microbiota in children with severe caries. J Dent Res 2020;99:703–712. DOI: 10.1177/0022034520908595
21. Garibyan L, Avashia N. Polymerase chain reaction. J Invest Dermatol 2013;133(3):1–4. DOI: 10.1038/jid.2013.1
22. Milgrom P, Riedy CA, Weinstein P, et al. Dental caries and its relationship to bacterial infection, hypoplasia, diet, and oral hygiene in 6- to 36-month-old children. Community Dent Oral Epidemiol 2000;28(4):295–306. DOI: 10.1034/j.1600-0528.2000.280408.x
23. Karn TA, O'Sullivan DM, Tinanoff N. Colonization of mutans streptococci in 8- to 15-month-old children. J Public Health Dent 1998;58(3):248–249. DOI: 10.1111/j.1752-7325.1998.tb03001.x
24. Ghazal TS, Levy SM, Childers NK, et al. Mutans streptococci and dental caries: a new statistical modeling approach. Caries Res 2018;52(3):246–252. DOI: 10.1159/000486103
25. Menon LU, Scoffield JA, Jackson JG, et al. Candida albicans and early childhood caries. Front Dent Med 2022;(3):849274. DOI: 10.3389/fdmed.2022.8492