Molecular Detection of *Streptococcus sobrinus* in the Supragingival Microbiome in a Cohort of Sri Lankan Pre-School Children

J.A.C.K. Dahampath[1]*, V.D. Keerthithilaka[1], L.K.D.N. Wijewardhana[2], U.S.S. Udagedara[2], L.K.N. Premathilaka[3], E.M.U.C.K. Herath[3], B.G.T.L. Nandasena[2], C.S. Dhanapala[1], M.P. Paranagama[2]

[1]*Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, Peradeniya, 20400, Sri Lanka*
[2]*Department of Basic Sciences, Faculty of Dental Science, University of Peradeniya, Peradeniya, 20400, Sri Lanka*
[3]*Department of Community Dental Health, Faculty of Dental Science, University of Peradeniya, Peradeniya, 20400, Sri Lanka*
**s19031@sci.pdn.ac.lk*

Early childhood caries (ECC) is a global oral health concern, with a notably high prevalence in Sri Lanka. *Streptococcus sobrinus* is a key cariogenic bacterium in the dysbiotic carious microbiome and has potential for use in caries risk assessment and targeted preventive strategies. However, its prevalence or association with ECC has not yet been studied in Sri Lanka. This study, therefore, represents the first cross-sectional investigation in Sri Lanka to detect *S. sobrinus* in supragingival biofilm samples from caries-free (CF), mild ECC (M-ECC), and severe ECC (S-ECC) children using molecular methods and to assess its association with caries severity. A total of 169 children under 6 years were enrolled in the study (mean age:48.33 months;83 females,86 males). Children with recent antibiotic or mouthwash use, systemic illnesses, or orthodontic appliances were excluded. They were classified based on dmft scores into CF (n = 44), M-ECC (n = 35), and S-ECC (n = 90) groups; the mean dmft score of ECC children was 6.77. Their supragingival plaque samples were collected and cultured on Mitis salivarius agar, and bacterial DNA was extracted using a boiling method. PCR was performed using species-specific primers targeting the *gtfU* gene to detect *S. sobrinus.* The resulting 155 bp amplicon was detected using agarose gel electrophoresis and further validated by bidirectional Sanger sequencing, which showed 100 % identity with the *S.sobrinus* reference sequence in the NCBI database. The highest prevalence of *S.sobrinus* was in the M-ECC group (28.6%), followed by the S-ECC (17.7%) and CF groups (9.1%). Multinomial logistic regression using CF as reference demonstrated a clinically relevant trend toward higher odds of ECC with *S. sobrinus* positivity; however, associations did not reach statistical significance (M-ECC; OR=2.97, 95% CI:0.76-11.6, p=0.12, S-ECC; OR=1.70, 95% CI=0.75-4.55, p=0.43), likely attributable to the limited sample size and low overall prevalence of *S.sobrinus*. The higher prevalence in M-ECC than S-ECC may partly reflect the small M-ECC sample size or the higher contribution of *S.sobrinus* for caries initiation. These preliminary findings warrant a larger cohort study to clarify the true association between *S. sobrinus* and ECC severity in Sri Lankan preschool children.

Keywords: early childhood caries, dental molecular epidemiology, *gtfU* gene, *Streptococcus sobrinus*, Sri Lanka

Acknowledgement: *Financial assistance from the University Research Grant URG/2023/10/D is gratefully acknowledged.*