UNIVERSITI TEKNOLOGI MARA

THE ASSOCIATION OF VITAMIN D AND SEVERE EARLY CHILDHOOD CARIES: A CASE-CONTROL STUDY

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ABSTRACT

Objectives: This research consists of in vivo and in vitro studies. The in vivo study aims to study the association between serum and saliva vitamin D and severe early childhood caries (S-ECC) in a group of young Malaysian children. The other objective was to determine if salivary LL37 and mutans streptococci levels are related to S-ECC. The in vitro study aims to evaluate the antimicrobial effects of the cholecalciferol vitamin D_3 against *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*). **Methods:** The in vivo study included the recruitment of 120 healthy children; 93 with S-ECC and 27 were caries-free (CF). Clinical examinations were performed, blood and saliva samples were collected, and parents completed a questionnaire about family demographic characteristics, child's oral health and nutritional habits. The ELISA Kit was used to determine vitamin D and salivary LL37 levels. Mutans streptococci bacteria were isolated and identified using a modified SB-20 culture medium. In the in vitro study, the antimicrobial effects of vitamin D_3 were evaluated against *S. mutans* and *S. sobrinus* using the agar disc diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of vitamin D_3 were determined using a microdilution method. Scanning electron microscope (SEM) was used to evaluate the morphological changes of bacterial cells following exposure to vitamin D_3. Data were analysed with descriptive statistics, bivariate, Spearman's rank correlation and multiple regression analysis. A p-value $\leq$ 0.05 was considered statistically significant. **Results**: S-ECC children had lower median serum and saliva vitamin D levels compared to CF children; however, these differences were not significant. Vitamin D levels in serum were significantly higher than in saliva, with a positive moderate correlation between them. S-ECC children had significantly lower median salivary LL37 levels and exhibited a higher count of *S. mutans* and *S. sobrinus* compared to CF children, with an inverse weak correlation between salivary LL37 levels and caries experience. Regression analysis disclosed that mothers with higher education levels were 92.9 % less likely to have children with dental caries and children that had both *S. mutans* and *S. sobrinus* were almost 12 times more likely to have S-ECC compared to children with no bacteria. In vitro results demonstrated that *S. sobrinus* was more sensitive to vitamin D_3 compared to *S. mutans* bacteria. The MIC values of vitamin D_3 against *S. sobrinus* and *S. mutans* were 60 µg/mL and 250 µg/mL respectively whereas the MBC values were 120 µg/mL and 500 µg/mL, respectively. Moreover, significant changes in the bacterial morphology were observed in treated bacterial cells with vitamin D_3 as compared to the untreated control bacteria using SEM. **Conclusion:** The association between serum and saliva vitamin D and dental caries in young children was inconclusive. S-ECC children exhibited lower serum and saliva vitamin D levels, lower salivary LL37 levels and higher *S. mutans* and *S. sobrinus* counts compared to CF children. Mother's education level and the presence of both *S. mutans* and *S. sobrinus* were the main factors that predicted dental caries experience in this age group. Also, this study supported the protective role of salivary LL37 against dental caries. Vitamin D_3 showed excellent antimicrobial effects against *S. mutans* and *S. sobrinus* in vitro. Further studies are warranted to investigate the definite relation between vitamin D levels and dental caries, and the use of saliva vitamin D as a non-invasive alternative method over blood samples, and to elucidate the mechanism of vitamin D_3 on *S. mutans* and *S. sobrinus*.

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