Serum and Saliva 25(OH)D Levels in Relation to Dental Caries in Young Children

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Objective: *Several studies have reported that low levels of vitamin D (25(OH)D) are associated with an increased risk of dental caries and that optimal levels may offer protection. This study aimed to assess the relationship between serum and saliva 25(OH)D levels and caries among young children.* ***Study design:*** *A total of 120 healthy children were recruited; 93 with caries and 27 caries-free. Dental caries status was evaluated using decayed, missing and filled in primary teeth (dmft) index. Blood and unstimulated whole saliva samples were collected. Laboratory analysis was performed using Enzyme-Linked Immunosorbent Assay Kit. Data were analyzed with descriptive statistics, bivariate and Spearman's rank correlation analysis.* ***Results:*** *There were no significant associations between serum and saliva 25(OH)D levels and caries status ($P > 0.05$). Levels of 25(OH)D in serum were significantly higher than levels found in saliva ($P < 0.05$), and a correlation between serum and saliva 25(OH)D levels was observed ($P < 0.05$).* ***Conclusions:*** *The association between serum and saliva 25(OH)D and dental caries in young children was inconclusive. However, a positive and significant correlation was observed between serum and saliva 25(OH)D levels. Further studies are warranted to investigate the definite relation between 25(OH)D levels and dental caries and using saliva 25(OH)D as a non-invasive alternative method over blood samples.*

Keywords: *caries, children, saliva, vitamin D, 25(OH)D*

INTRODUCTION

Dental caries in children is one of the most prevalent chronic diseases that has an adverse impact on child's general health and is still considered a serious public health issue in many parts of the world[1]. Most of the dental caries that exists in the preschool children remains untreated[1]. Untreated decay should be taken seriously as it has the possible consequences of impacting a child's overall health, well-being and quality of life of both children and their families[2,3].

Although young children may not complain of pain, they exhibit the negative effects of caries in many ways, including changes in their eating patterns[1]. Studies have shown that children with severe early childhood caries (S-ECC) may suffer from malnutrition such as anemia[4,5] or may show deficiencies in essential nutrients and vitamins such as vitamin D, which has an undesirable impact on the child's growth[6,7].

Vitamin D, commonly referred to as the sunshine vitamin, plays an important biological role in the human body, helping maintain normal growth and mineralization of bone and other calcified tissues, including teeth.[8] Recently, several studies have indicated a higher prevalence of vitamin D deficiency or inadequacy in children worldwide.[9] Furthermore, a significant association between vitamin D deficiency and insufficiency in children and dental caries has been reported.[7,10–14]

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The US Institute of Medicine (IOM) defines 25(OH)D <12 ng/mL (<30 nmol/L) as deficient; 12–20 ng/mL (30-50 nmol/L) as inadequate; and >20 ng/mL (>50 nmol/L) as adequate [9,15] The gold standard for measuring vitamin D concentrations is by assessing serum levels of 25(OH)D.[8,9] However, the collection of blood from children can be challenging considering their behavior along with the fact that the venipuncture may result in minor discomfort, bruising, and infection [16]. It is also less favored in research involving children as parents are more likely to decline to have their children participate if venipunctures are involved. Currently, saliva has been widely used as an indicator of oral health and unstimulated whole saliva can be collected from children in a less invasive method [17].

Saliva is an oral fluid that serves several purposes (e.g. buffers, lubricates, antimicrobial, remineralizers). It refers to the oral fluid that surrounds all oral hard and soft tissues. It plays a crucial role in dental caries process [18]. It is composed mostly of water (99%) and (1%) of inorganic elements for instance calcium, phosphate, sodium, potassium, and some trace elements and organic substances such as protein, lipid, free amino acids, urea, and vitamins [18].

Studies on the association between vitamin D in serum and saliva of children are scarce. Only a handful of studies have reported on the association between vitamin D levels in serum and saliva among healthy participants [19–21]. To the best of our knowledge, no studies have been conducted assessing and comparing the serum and saliva 25(OH)D levels in young children with and without dental caries. Therefore, the present study aimed to determine and compare the serum and saliva 25(OH)D levels between children with ECC and those caries-free as well as to assess the possible relationship between serum and saliva 25(OH)D levels and dental caries.

MATERIALS AND METHOD

Ethical approval and sample size calculation

This study was approved by Human Ethics Committee of Universiti Teknologi MARA (UiTM), Malaysia [600-RMI (5/1/6)] and Medical Research and Ethics Committee [(MREC)NMRR-15-1857-25950 (IIR)]. Additionally, written informed consent was obtained from parents.

G*Power software was used to determine the power of the study (http://www.gpower.hhu.de/) and evaluating any statistically significant difference in the means of the variables that intended to be studied between children with and without caries experience. With 27 caries-free and 93 children with caries (N=120 children participated), $\alpha = 0.05$, an effect size of 0.6 and two-tailed testing, 78% power was obtained to determine any statistically significant difference [22].

Study design and sampling

A total of 120 healthy Malaysian children under 72 months of age were recruited in this case-control, cross sectional study. They were divided into two groups: children with ECC who were going to have comprehensive dental care under general anesthesia at public and teaching hospitals and children with no dental caries experience attending paediatric dental teaching clinic for dental examinations and maintenance. All children were medically fit and healthy. Data was collected between November 2016 to August 2019.

Inclusion and exclusion criteria

Healthy children < 72 months of age were included. Cases were children with ECC (dmft ≥1), whereas controls were caries-free children with no evidence of dental caries experience clinically (dmft = 0). Children with any systemic disease, disabilities or not able to cooperate and follow instructions were excluded. Children whose parents did not consent were also excluded.

Dental examination

Before starting this study, inter-and intra-examiner reliability was assessed between the postgraduate student (MM) and the supervisor (AH) for dmft scores in a sample of 20 children and the agreement was 90% and 95% respectively. Clinical examinations were performed by (MM) after recording child's information and answering the questionnaire by the parents. The dental caries assessment was carried out using a dental mirror according to World Health Organization (WHO) criteria [23] and the caries experience was recorded using the decayed, missing and filled teeth index (dmft). The children were categorized into caries (ECC: 93 children) and caries-free (CF: 27 children) groups accordingly.

Collection of saliva and serum samples

Unstimulated whole saliva sample (2-5 ml) was collected once using the expectorating method in a sterile disposable container. The samples were obtained between 8.00 and 11.00 am to avoid the diurnal effect on salivary content. Meanwhile, fasting blood sample (3 ml) was obtained between 8.30 am and 12.00 pm. It was drawn into a 4 ml vacutainer tube with no additives (BD, Franklin Lake, NJ, USA). Saliva and blood samples were transported in a cooler box within 1–4 hours after collection to the research laboratory for processing. Serum samples were centrifuged at 1500 rpm for 5 minutes at room temperature [24] whereas saliva samples were centrifuged at 3000 rpm for 10 minutes at 4 °C, then the supernatant was collected. The obtained serum and the supernatant saliva were transferred into sterile cryovials and stored immediately at–80°C until further analysis. [25]

Measurement the levels of serum and saliva 25(OH) D

Serum and saliva samples analyzed using commercial 25(OH) D Enzyme-Linked Immunosorbent Assay (ELISA) Kit (Enzo Life Sciences, Switzerland) [26]. The cut-off was based on the US Institute of Medicine (IOM) which defines 25(OH)D levels < 12 ng/mL (i.e. < 30 nmol/L) are considered deficient, 25(OH)D levels between 12-20 ng/mL (30-50 nmol/L) are deemed inadequate, and are adequate when serum 25(OH)D levels > 20 ng/mL (> 50 nmol/L).

Statistical analysis

Data were entered and analyzed using Statistical Package for Social Science (SPSS) version 23.0. SPSS (SPSS Inc, IBM, New York, USA). Descriptive statistics including frequencies, median and interquartile range (IQR) were calculated. Nonparametric tests were used because of unmet assumption of normal distribution based on the results of histogram and Kolmogorov-Smirnov test ($P < 0.05$). Mann–Whitney U test and Kruskal-Wallis test were used to compare continuous variables between the groups, Chi-Square test was used for categorical variables whereas Spearman rank correlation coefficient test was used to determine the correlation between 25(OH)D and dmft score as well as between serum and saliva 25(OH)D levels. A scatter plot was used to visualize the correlations. The statistical significance was defined as $P \leq 0.05$.

RESULTS

Of the 120 children, 120 blood samples were taken and available for serum 25(OH)D analysis. However, nine children were unable to provide a saliva sample and four samples were discarded because of inadequate saliva volume. Hence, only 107 saliva samples were available for saliva 25(OH)D analysis.

Children's characteristics

Table (1) reports the socio-demographic characteristics of the children. There were 27 CF children and 93 children with ECC. Sixty participants were females (50%) and most of the children were Malay (88%). The overall mean age for the children was 55.1 ± 11.0 months, ranging from 31 to 71 months. (Table 1).

Table 1: Caries experience by children's socio-demographic characteristics

Variables	n (%)	Caries status		*P*-value
		CF	ECC	
		n (%)	n (%)	
Sex				
Males	60 (50.0)	14 (23.3)	46 (76.3)	.83
Females	60 (50.0)	13 (21.7)	47 (78.3)	
Total	120 (100.0)	27 (22.5)	93 (77.5)	
Age (months)				
<60	108 (89.2)	26 (24.3)	81 (75.7)	.19
≥60	3 (2.5)	1 (33.3)	2 (66.7)	
Total	120 (100.0)	27 (22.5)	93 (77.5)	
Mean±SD	55.1 ± 11.0	53.4 ± 11.2	55.6 ± 11.0	.37
Race				
Malay	106 (88.3)	26 (24.5)	80 (75.5)	.39
Chinese	2 (1.7)	0 (0.0)	2 (100.0)	
Indian	12 (10.0)	1 (8.3)	11(91.7)	
Total	120 (100.0)	27 (22.5)	93 (77.5)	

P values were determined using Chi-square tests; significant at $P \leq .05$; CF=caries-free, ECC=early childhood caries

Comparison of 25(OH)D levels in children by their demographics

Table (2) reports the median values of 25(OH)D levels in serum and saliva of the children by their demographic characteristics. Overall, there were no significant differences in 25(OH)D levels with regards sex, age and race groups in both serum and saliva ($P > 0.05$).

Table 2: The level of 25(OH)D by children's socio-demographic characteristics

Variables	25(OH)D nmol/L			
	n= 120	Serum	n= 107	Saliva
		Median (IQR)		Median (IQR)
Sex				
Males	60	41.69 (44.26)	51	27.98 (51.82)
Females	60	44.39 (44.32)	56	27.41 (27.29)
Z statistic[a] (*P*-value)		-0.39 (0.70)		-0.57 (0.58)
Age (month)				
<60	71	37.12 (44.20)	59	27.41(48.50)
≥60	49	47.69 (41.47)	48	29.02 (30.62)
Z statistic[a] (*P*-value)		-0.79 (0.43)		-0.81 (0.42)
Race				
Malay	106	41.59 (43.31)	95	27.41 (41.61)
Chinese	2	63.72 (N/A)	2	56.16 (N/A)
Indian	12	51.84 (29.31)	10	24.71 (40.32)
X statistic[b] (*P*-value)		3.61 (0.17)		0.81 (0.67)

[a]Mann-Whitney test, [b]Kruskal-Wallis test, significant at $P \leq 0.05$

Comparison of 25(OH)D levels in children by caries experience

While the median serum 25(OH)D level appeared to be higher among CF children when compared to ECC group, this difference was not statistically significant ($P = .33$). Similarly, no significant difference in salivary 25(OH)D levels were observed between CF and ECC children ($P = .74$) as shown in Figure 1.

Figure 1: Median values of serum and saliva 25(OH)D in ECC and CF children

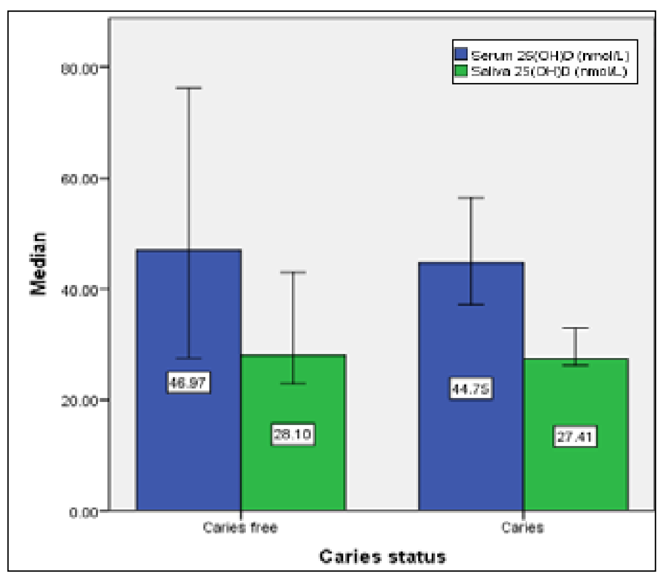


Table (3) shows the distribution of cases according to the children's caries status and their 25(OH)D levels in both serum and saliva. While more children with ECC were found to have insufficient and deficient levels of serum 25(OH)D compared to CF children, no significant differences were observed for both serum and saliva 25(OH)D levels in cases and controls ($P > .05$).

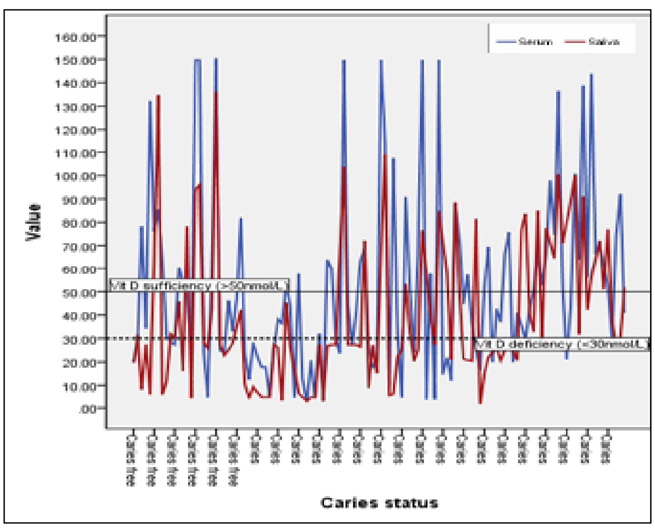
Table 3: The status of 25(OH)D by children's caries experience.

25(OH)D thresholds	Serum			Saliva		
	CF n (%)	ECC n (%)	p-value	CF n (%)	ECC n (%)	p-value
Sufficient (>50 nmol/L)	12 (22.2)	42 (77.8)	.95	6 (19.4)	25 (80.6)	.45
Insufficient (30-50 nmol/L)	6 (25.0)	18 (75.0)	.74	6 (33.3)	12 (66.7)	.32
Deficient (<30 nmol/L)	9 (21.4)	33 (78.6)	.84	14 (24.1)	44 (75.9)	.97
Total	27	93		27	93	

P values were determined using Chi-square tests, significant at $P \leq 0.05$; CF=caries-free, ECC=early childhood caries

The Figure (2) illustrates the levels of serum and saliva 25(OH) D of children with ECC and those CF. Undoubtedly, there is fluctuation in its levels in serum and saliva for both CF and ECC groups. There were wide variations between lowest and highest levels in serum and saliva in each group. Generally, the level of saliva 25(OH)D was lower than its in serum, although some children had higher levels in their saliva compared to serum. Since the IOM's threshold of 50 nmol/L of 25(OH)D in serum is the necessary level for good health in all individuals, this cut-off value was set for this figure. It illustrates that less than half number of the children had adequate 25(OH)D level in their serum whereas less than one third of the children who their level above 50 nmol/L in saliva.

Figure 2: Serum and saliva 25(OH)D levels in ECC and CF children



Comparison of 25(OH)D levels in serum and saliva

Overall, the median values (IQR) of serum and saliva 25(OH) D levels of the children regardless of their caries experience were [44.90 nmol/L (43.66); 27.41 nmol/L (37.39) respectively]. It was clearly that the level of 25(OH)D in serum was higher than in saliva with significant differences between them (Z statistic = -4.99; $P <$ 0.001).

The distribution of cases for serum and saliva 25(OH)D status among the young children is shown in Table (4). Less than half of children had sufficient serum levels of 25(OH)D and less than one third in the saliva [54 (45.0%); 31 (29.0%), respectively]. Whereas 42 (35%) and 58 (54.2%) of them had deficient 25(OH)D levels in their serum and saliva respectively. Sensitivity and specificity tests were performed. The sensitivity test is the capability to correctly determine those cases with the disease (true positive) while the specificity test is the capability to correctly determine those with no disease (true negative). The results of the sensitivity and specificity of saliva were 81.8% and 47.1%, respectively.

Correlation of serum and saliva 25(OH)D levels and dmft score

A good positive correlation between serum and saliva 25(OH) D levels was observed using Spearman's rho correlation coefficient test [ρ (rho) = 0.61; $P < 0.001$)]. A linear regression analysis was used to predict serum 25(OH)D based on saliva 25(OH)D. The equation was: Serum 25(OH)D = 9.06+0.81 [Saliva 25(OH)D] (Figure 3). On the other hand, there was no significant correlation between serum and saliva 25(OH)D concentrations and dmft score [ρ (rho) = 0.02, 0.06; *P* = .09, .52 respectively].

Figure 3: The correlation between serum and saliva 25(OH)D

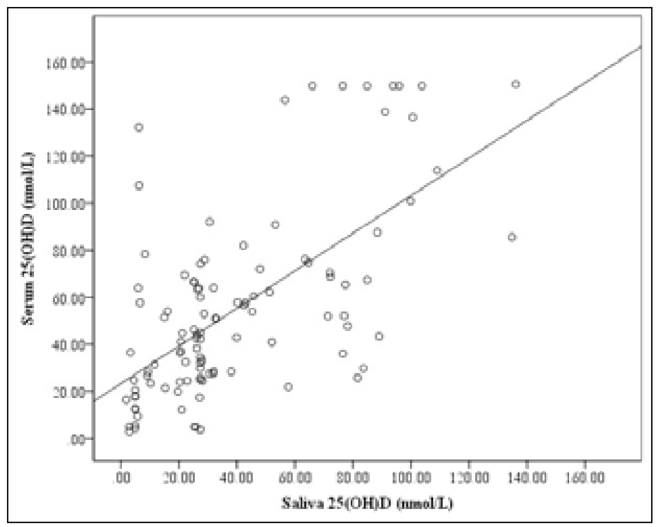


Table 4: The Distribution of cases of serum and saliva 25(OH) D status

Total 25(OH)D thresholds	25(OH)D	
	Serum	Saliva
	n (%)	n (%)
Sufficient (>50 nmol/L)	54 (45.0)	31 (29.0)
Insufficient (30-50 nmol/L)	24 (20.0)	18 (16.8)
Deficient (<30 nmol/L)	42 (35.0)	58 (54.2)
Total	120	107

DISCUSSION

This study aimed to investigate whether serum and saliva 25(OH)D concentrations in Malaysian children are correlated and associated with increased risk for dental caries. Overall, 120 children participated, the findings of this study identified differences in serum and saliva 25(OH)D levels between children with and without caries experience. Although these differences with caries experience were not significant, a moderate positive correlation was observed between serum and saliva 25(OH)D. To date, no previous studies comparing vitamin D in serum and saliva in relation to dental caries experience have been reported.

Vitamin D inadequacy is considered a major public health concern all over the world, especially in young children.[27,28] Dental caries is a complex multifactorial disease and is recognized as the most common children's oral health problem especially in early childhood.[9] Despite the widespread prevalence of vitamin D inadequacy and dental caries as well as growing evidence linking the two conditions, a limited number of studies have been published on the association between these two global problems in young children.

Even though the evidence supports an association between lower level of vitamin D and the incidence of dental caries, the mechanism is still uncertain.[9,29,30] While several studies have compared the levels of vitamin D between caries and caries-free children, some reports that lower vitamin D levels are associated with caries experience while others have observed no significant association.[8,9] As expected, all these studies have assessed 25(OH) D in blood samples as serum is considered the gold standard for assessing one's vitamin D status. Saliva can be considered a good alternative as it is less invasive and a potential alternative to young children and those afraid of needles [17]. In addition, no special equipment is needed for saliva samples collection and it is less costly compared to blood samples. Hence, using such non-invasive sample and easy to be collected from young children is highly recommended in child's health. Unstimulated whole saliva has many advantages and more convenient compared to stimulated saliva exclusively in young children [31]. Therefore, assessing vitamin D status in relation to dental caries among young children using unstimulated saliva and correlating its level in their serum is indispensable.

Vitamin D plays a crucial role in bone and tooth mineralization. Rachitic tooth may occur as a result of insufficient level of serum vitamin D [32]. This tooth is a defective and hypomineralized tooth which is highly susceptible to decay.[8] Vitamin D levels could influence the tooth susceptibility to dental caries by regulating the levels of calcium and phosphate in the body. These ions are vital during tooth formation period. Low calcium and phosphate levels in the body found to be linked to defect in the enamel and dentin.[8,9] Furthermore, vitamin D is able to control of tooth germ formation and maturation as well as the stages of primary tooth crown development based on research on the molecular basis of vitamin D and specific vitamin D Receptor.[8,9] Hence, in this study the levels of serum 25(OH)D were compared between ECC and CF groups. The results revealed serum 25(OH) D level in ECC group was lower than in CF group; however, no significant differences were observed. Similarly, no significant correlation between serum 25(OH)D level and dmft score was found. Our findings from this study are similar to those of Kim *et al.* who have found that no significant differences between the lower level of 25(OH)D below 50 nmol/L and dental caries incidence in permanent molars among Korean children (10-12 years) [33]. Another study was carried out by Herzog et al. to explore the association of 25(OH)D deficiency with dental caries in US children (5-12 years) [34] In fact, it was unable to determine an actual association between vitamin D and caries experience. While several studies from Canada, Qatar, Pakistan and India have reported a significant association between lower level of 25(OH) D and dental caries incidence in children.[11–13,22] These dissimilarities with our findings may be due to different age group, small sample size or different assays for analysis.

In the present study, whole unstimulated saliva was also collected to assess 25(OH)D level. Whole unstimulated saliva is a mixture of salivary gland secretions and reveals the status of the oral cavity environment. Our findings indicated saliva 25(OH)D level is lower in ECC group rather than CF group, but the differences were not statistically significant. These results could not be compared with the others owing to lack of studies that assessed saliva 25(OH)D in relation to dental caries experience whether in children or adults. Moreover, there was no significant correlation between saliva 25(OH)D and dmft scores which suggesting that the occurrence of dental caries is influenced by other factors such as plaque control, dietary habits and acid resistance of teeth [35] rather than 25(OH)D status in saliva. More studies about salivary vitamin D and dental caries are strongly recommended.

Overall, nearly half number of children (45%) in the present study had satisfactory level of 25(OH)D in their serum compared to only one-third of children (29%) in their saliva. Moreover, the most interesting finding in the present study that children with ECC had adequate 25(OH)D levels in both serum and saliva when compared to the controls (77.8%, 80.6%; 22.2%; 19.4% respectively). These findings indicated that dental caries is a multifactorial disease and can be occurred even with adequate level of vitamin in both serum and saliva. Although currently vitamin D is considered a promising caries-preventive agent as vitamin D supplementation was associated with a 47% caries reduction in children [36], the incidence of dental caries in primary teeth of the participated children with sufficient level might be due to the presence and the effect of other risk factors for instance inappropriate feeding practice, oral hygiene practice, dietary habits, oral bacterial flora, fluoride exposure, enamel defects, salivary flow, demographic characteristics and parental oral health since dental caries is a complex multifactorial disease.

Saliva 25(OH)D level found to be lower compared to serum with significant differences between them and it agrees with the findings of study by Fairney and Saphier reported significantly lower vitamin D level in saliva compared to serum in schoolchildren and adults [19]. Further, in the present study, remarkably there seems to be a moderate positive correlation between serum and saliva total 25(OH)D levels in young children. Yet, the levels vary between them and serum readings appear to be better at grouping children into 25(OH)D threshold based on their level which is in line with previous findings who reported a significant and positive correlation between serum and saliva levels of 25(OH)D [19–21,37]. Therefore, saliva test of 25(OH)D levels may not possess enough specificity to rule out cases of true 25(OH)D deficiency. However, it could be helpful as screening tool that used for preliminary evaluation of vitamin D level and assistance in the selection of cases for further serum assessment. It could also help in assessing child's caries risk.

Our study is not without limitations. First, it was case-control study of a cross-sectional nature with comparatively small number of children, especially the smaller number of caries-free controls, which may limit the conclusion that can be drawn from the present study. Furthermore, the correlation between vitamin D and dental caries maybe inconclusive due to half number of controls had 25(OH)D insufficiency and deficiency. Lastly, children's oral hygiene practices and dietary habits and their association with the level of vitamin D were not studied as the focus was comparing serum and saliva 25(OH)D levels. Notwithstanding these limitations, these investigative data about serum and saliva 25(OH)D levels in relation to dental caries are considered novel and open the door for further research in this area to find out the role of saliva vitamin D that may play in dental caries process.

CONCLUSIONS

Based on the findings of the study, it is concluded that serum and saliva 25(OH)D levels are higher in controls than the ECC group but did not disclose any significant differences. Moreover, there are no associations were found between serum and saliva 25(OH)D levels and ECC. However, a significant and positive correlation was observed between serum and saliva 25(OH)D levels in young children. Further studies are warranted to understand the real role of serum and saliva 25(OH)D levels on dental caries process in young children.

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Conflict of interest:

The authors declare no conflict of interest.

REFERENCES

1. Correâ-Faria P, Viana KA, Raggio DP, Hosey MT, Costa LR. Recommended procedures for the management of early childhood caries lesions-A scoping review by the Children Experiencing Dental Anxiety: Collaboration on Research and Education (CEDACORE). BMC Oral Health. 2020;20(1):1-1.
2. Kassebaum NJ, Smith AGC, Bernabé E, et al. Global , Regional , and National Prevalence , Incidence , and Disability- Adjusted Life Years for Oral Conditions for 195 Countries , 1990 – 2015 : A Systematic Analysis for the Global Burden of Diseases , Injuries , and Risk Factors. J Dent Res. 2017; 96(4):380-387.
3. Pesaressi Eraldo; Villena Rita; Frencken Jo. Dental caries and oral health - related quality of life of 3 - year - olds living in Lima , Peru. Int J Paediatr Dent. 2020;30:57-65.
4. Schroth RJ, Levi J, Kliewer E, Friel J, Moffatt ME. Association between iron status, iron deficiency anaemia, and severe early childhood caries: a case-control study. BMC Pediatr. 2013;13(1):22.
5. Babu NSV, Bhanushali PV. Evaluation and association of serum iron and ferritin levels in children with dental caries. 2017;35(2):106-109.
6. Schroth RJ, Jeal NS, Kliewer E, Sellers EAC. The relationship between vitamin D and severe early childhood caries: a pilot study. Int J Vitam Nutr Res , 2012; 82(1):53-62.
7. Schroth RJ, Levi JA, Sellers EA, Friel J, Kliewer E, Moffatt ME. Vitamin D status of children with severe early childhood caries: a case-control study. BMC Pediatr. 2013; 13(1):174.
8. Botelho J, Machado V, Proença L, Delgado AS, Mendes JJ. Vitamin D deficiency and oral health: A comprehensive review. Nutrients. 12(5),2020. doi:10.3390/nu12051471
9. Almoudi MM, Hussein AS, Abu Hassan MI, Schroth RJ. Dental Caries and Vitamin D Status among Children in Asia: A literature review. Pediatr Int. 2019;61(4):327-338.
10. Bener A, Al-Darwish MS, Hoffmann GF. Vitamin D deficiency and risk of dental caries among young children: a public health problem. Indian J Oral Sci. 2013; 4(2):75-82.
11. Schroth RJ, Rabbani R, Loewen G, Moffatt ME. Vitamin D and Dental Caries in Children. J Dent Res. 2016; 95(2):173-179.
12. Ali N, Rahim A, Ali S, Iqbal MH. Impact of Vitamin D on Development of Early Childhood Caries. Pakistan Armed Forces Med J. 2017;67(3):429-433.
13. Gupta A, Chhonkar A, Arya V. Comparison of Vitamin D Level of Children with Severe Early Childhood Caries and Children with No Caries. Int J Clin Pediatr Dent. 2018;11(3):199-204.
14. Wójcik D, Krzewska A, Szalewski L, et al. Dental caries and Vitamin D 3 in children with growth hormone deficiency. Med (United States). 97:8(e9811),2018.
15. Institute of Medicine 2011 Dietary reference intakes for calcium and vitamin D. Washington, DC: The National Academies Press.
16. Williamson S, Munro C, Pickler R, Grap MJ, Elswick RK. Comparison of Biomarkers in Blood and Saliva in Healthy Adults. Nurs Res Pract. 2012; 2012:1-4.
17. Arunkumar S, Arunkumar J, Burde K, Shakunthala G. Developments in diagnostic applications of saliva in oral and systemic diseases-A comprehensive review. J Sci Innov Res. 2014; 3(3):372-387.
18. Hegde MN. Saliva as a biomarker for dental caries: A systematic review. Conserv Dent Endod. 2019; 22:2-6.
19. Fairney a, Saphier PW. Studies on the measurement of 25-hydroxy vitamin D in human saliva. Br J Nutr.1987; 57(1):13-25.
20. Higashi T, Shibayama Y, Fuji M, Shimada K. Liquid chromatography-tandem mass spectrometric method for the determination of salivary 25-hydroxyvitamin D3: A noninvasive tool for the assessment of vitamin D status. Anal Bioanal Chem. 2008; 391(1):229-238.
21. Hussein, A.S., Almoudi M.M., Schroth, Abu Hassan, M.I., Robert J., Saripudin, B., Chinna K. Comparison of saliva and serum total 25(OH)D levels in young children: a pilot study. J Int Dent Med Res.2020; 13(4):1405-1410.
22. Gyll J, Ridell K, Öhlund I, Karlsland Åkeson P, Johansson I, Lif Holgerson P. Vitamin D status and dental caries in healthy Swedish children. Nutr J. 2018; 17(1):1-10.
23. WHO. Oral Health Surveys: Basic Methods. 4th ed. WHO: Geneva, Switzerland; 1997.
24. Bulent Kargi A, Demir M. Vitamin D Deficiency as a Risk Factor in Non-Squamous Lung Cancer Subgroups–A Preliminary Study. J Clin Respir Dis Care. 2016; 2(2):2-5.
25. Dwivedi R, Gupta YK, Singh M, et al. Correlation of saliva and serum free valproic acid concentrations in persons with epilepsy. Seizure. 2015; 25:187-190.
26. Sethu S, Shetty R, Deshpande K, Pahuja N, Chinnappaiah N, Agarwal A. Correlation between tear fluid and serum vitamin D levels. Eye Vis. 2016; 3(1)22.
27. Hussein AS, Almoudi MM, Zen SANM, Azmi NH, Schroth RJ, Abu Hassan MI. Parental awareness and knowledge of vitamin D and its health benefits for children. J Int Dent Med Res. 2018; 11(3):916-924.
28. Wimalawansa SJ, Razzaque MS, Al-Daghri NM. Calcium and vitamin D in human health: Hype or real? J Steroid Biochem Mol Biol. 2018; 180(2010):4-14.
29. Seminario AL, Velan E. Vitamin D and Dental Caries in Primary Dentition. J Dent Child (Chic). 2016; 83(3):114-119.
30. Singleton R, Day G, Thomas T, et al. Association of Maternal Vitamin D Deficiency with Early Childhood Caries. J Dent Res. 2019: 98(5):549-555.
31. Ribeiro TR, Dria KJ, de Carvalho CBM, et al. Salivary peptide profile and its association with early childhood caries. Int J Paediatr Dent. 2013;23(3):225-234.
32. D'Ortenzio L, Ribot I, Kahlon B, et al. The rachitic tooth: The use of radiographs as a screening technique. Int J Paleopathol. 2018; 23:32-42.
33. Kim IJ, Lee HS, Ju HJ, Na JY, Oh HW. A cross-sectional study on the association between vitamin D levels and caries in the permanent dentition of Korean children. BMC Oral Health. 2018;18(1):1-6.
34. Herzog K, Scott JM, Hujoel P, Seminario AL. Association of Vitamin D and dental caries in children Findings from the National Health and Nutrition Examination Survey, 2005-2006. J Am Dent Assoc. 2016;147(6):413-420.
35. Rugg-Gunn A. Dental caries: strategies to control this preventable disease. Acta Med Acad. 2013;42(2):117-130.
36. Hujoel PP. Vitamin D and dental caries in controlled clinical trials: Systematic review and meta-analysis. Nutr Rev. 2013;71(2):88-9.
37. Bahramian A, Falsafi P, Abbasi T, et al. Comparing Serum and Salivary Levels of Vitamin D in Patients with Recurrent Aphthous Stomatitis and Healthy Individuals. J Dent. 2018;19(4):295-300.