Comparison of Saliva and Serum Total 25(OH)D Levels in Young Children: A Pilot Study

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Abstract

Vitamin D deficiency is associated with different health conditions including dental caries in children. This pilot study aimed to assess 25-hydroxyvitamin D [25(OH)D] levels in saliva of young children and investigate the possible association between saliva and serum total 25(OH)D levels.

A total of 25 healthy children were enrolled in this study. Paired serum and unstimulated whole saliva samples were collected from each child. Serum and saliva values of total 25(OH)D levels were measured using Enzyme-Linked Immunosorbent Assay (ELISA) kit. Descriptive statistics, bivariate and Pearson's correlation analysis were performed. The significance level for alpha was set at 0.05.

Two children had deficient serum 25(OH)D while seven children had deficient levels of 25(OH)D when measured from saliva. The mean 25(OH)D level were higher in serum compared to saliva (25.66±9.52 ng/mL vs. 23.38±13.92 ng/mL). However, the difference was not significant (p=0.306). There was a significant association between serum and saliva 25(OH)D levels (r2 = 0.626.; p < 0.001).

Saliva analysis of 25(OH)D yielded lower levels than serum samples. The findings revealed a positive correlation between serum and saliva values of total 25(OH)D suggesting saliva 25(OH)D may potentially be used as screening test for vitamin D status in young children.

Clinical article (J Int Dent Med Res 2020; 13(4): 1405-1410)

Keywords: Children, Saliva, Serum, Total 25-hydroxyvitamin D, Vitamin D.

Received date: 10 February 2020 **Accept date:** 13 March 2020

Introduction

Vitamin D is a sunshine vitamin. It consists of two compounds, vitamin D_3 and vitamin D_2.[1] Vitamin D is metabolized in the liver into the 25-hydroxyvitamin D [25(OH)D] which is the predominant circulating form and it is used to assess the status of vitamin D in the body.[2] The 25(OH)D is further metabolized in the kidney into the 1α,25-dihydroxyvitamin D [1,25$(OH)_2$D] which is considered to be the active form of vitamin D.[1,2]

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Recently, several studies have reported a higher prevalence of vitamin D deficiency in children worldwide.[3] Levels of 25(OH)D ≥ 50 nmol/L are considered sufficient, those between 30-50 nmol/L are deemed insufficient, while those <30 nmol/L are considered deficient.[4] Vitamin D has an important role in the human body, such as maintaining normal growth, and mineralization of the bone and other calcified tissues, including teeth.[5,6]

Of late, some investigators have observed a significant association between vitamin D deficiency and occurrence of dental caries in children.[7-11] Two mechanisms were suggested explaining the role of vitamin D status in modulating the dental caries incidence.[6,12] First, vitamin D regulates serum calcium, phosphate and parathyroid hormone, which are very imperative in the formation and preservation of the teeth and increasing the resistance of enamel

to the demineralization process. Second, it regulates and modulates the immunity system. It binds with Vitamin D Receptor (VDR) and regulates certain antimicrobial peptides such as defensins and cathelicidin, which may provide protection against cariogenic bacteria.[6] However, evidence from this area is inconclusive.

The gold standard for measuring 25(OH)D levels in the body is by using blood sample.[6] However, the collection of blood from children can be challenging considering the children's behavior, and the venipuncture can be associated with discomfort, bruising, and infection.[13] It is also less favored in research involving children as parents are less likely to agree to have their children participate if venipunctures are involved. Currently, saliva samples are used as a diagnostic medium for identification of some biological markers such as cortisol and enzymes.[14,15] Several studies have reported similar values of these markers in serum and saliva, suggesting that these markers can be used as diagnostic laboratory tests.[16] Saliva has also been widely used as an indicator of oral health.[17] Unstimulated whole saliva can be collected in a less invasive method. It often correlates to systemic clinical conditions more accurately than stimulated saliva because some materials that use to stimulate flow may change salivary composition.[18] Therefore, unstimulated whole saliva may be considered as another method for measuring vitamin D levels in the body.[2]

Studies on the association between vitamin D in serum and saliva are scarce. In literature, a handful of studies have reported investigations on the association between vitamin D levels in saliva and serum of healthy participants.[2,19] In 1983, Trafford & Makin (20) reported detection of vitamin D in mass spectrometry, but none using a specific high-performance liquid chromatographic method. Fairney and Saphier in 1987[19] also detected vitamin D in saliva of adults and schoolchildren using mass spectrometry procedure. Moreover, Higashi and his team in 2008[2] reported 25(OH)D_3 can be detected in the saliva of adults participants using Liquid chromatography tandem mass spectrometry method (LC-MS/MS) and there was an association between 25(OH)D_3 levels in serum and saliva. However, to our knowledge, no studies have been conducted assessing the salivary and total serum vitamin D levels in young children. Therefore, the present study aimed to measure and compare 25(OH)D levels in saliva of healthy young children (3- 6 years old) and correlate its level with total serum levels.

Materials and methods

Study design and participants

A total of 25 healthy Malay children, in the age group of 3 to 6 years, were recruited after obtaining informed written consent from their parents. The children were medically fit and healthy and were undergoing comprehensive dental care under general anaesthesia (GA) at a public hospital, Selangor, Malaysia. Furthermore, this study was approved by Human Ethics Committee of Universiti Teknologi MARA (UiTM), Malaysia.

Collection of saliva and serum samples

On the day of operation and before sending the child to the operating theatre, a dental examination was performed and 3-5 ml of unstimulated whole saliva was collected once using the expectorating method in a sterile disposable container. All samples were obtained between 8.00 and 11.00 am to avoid the diurnal effect on salivary content. Meanwhile, serum samples were obtained between 8.30 am and 12.00 pm while the child was under GA, prior to the initiation of rehabilitative dental treatment. Fasting blood (3 ml) was drawn into a 4 ml vacutainer tube with no additives (BD, Franklin Lake, NJ, USA). Saliva and blood samples were covered with aluminum foil to avoid ultraviolet exposure and kept on dry ice then 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[21] 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 analysis.[22]

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

On the day of sample analysis, after thawed, total 25(OH)D concentrations in serum

and in saliva were determined by using commercial 25(OH)D Enzyme-Linked Immunosorbent Assay (ELISA) Kit (Enzo Life Sciences, Switzerland). This 25(OH)D ELISA kit has been validated for the evaluation of 25(OH)D in blood and other body fluid.[23] All samples were measured in duplicate and the results were obtained after plotting the standard curve. There are two classifications for serum vitamin D status. The cut-offs differ based on which organization The US Institute of Medicine (IOM) defines 25(OH)D levels <30 nmol/L are considered deficient, 25(OH)D levels between 30-50 nmol/L are deemed inadequate, and are sufficient when serum 25(OH)D levels ≥ 50 nmol/L. However the US Endocrine Society describes vitamin D deficiency when 25(OH)D levels < 50 nmol/L (20 ng/mL), insufficiency when its levels between 52.5-72.5 nmol/L (21–29 ng/mL), and the levels exceed 75 nmol/L (>30 ng/mL) are considered sufficient.

Statistical analysis

Data were entered and analyzed using Statistical Package for Social Science (SPSS) version 20.0. SPSS (SPSS Inc, Chicago, IL, USA). Descriptive statistics including frequencies mean values ± standard deviations (SD), and 95% confidence intervals were calculated. Serum and salivary 25(OH)D data distribution was checked for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests and they were normally distributed ($p > 0.05$). The correlation between serum and salivary 25(OH)D levels was determined by Pearson's correlation coefficient. Statistical significance was defined as $p < 0.05$. A scatter plot was used to visualize the distribution of 25(OH)D levels in saliva and serum.

Results

Participants' characteristics

The study was conducted from January to December 2016. Paired serum and saliva sample were obtained from 25 healthy children < 72 months. Out of the 25 children in this study, 11 (44.0%) were males and 14 (56.0%) were females. The mean age was 59.56±8.48 months.

Total 25(OH)D levels in serum and saliva

Overall the salivary levels of 25(OH)D were lower than the serum levels. The mean value of serum 25(OH)D levels was 25.7 ± 9.5 ng/mL whereas the mean value of 25(OH)D levels in saliva was 23.4 ± 13.9 ng/mL. Although, the levels of 25(OH)D in saliva were lower than in serum, but the difference was not significant (p=0.305). The descriptive statistics are shown in Table (1).

25(OH)D levels	Minimum	Maximum	Median	Mean±SD	P value
	ng/mL	ng/mL	ng/mL	ng/mL	
Serum	6.60	51.29	26.63	25.66±9.52	0.305
Saliva	0.75	54.52	21.53	23.38±13.92	

Table 1. The levels of total 25(OH)D in serum and saliva.

Based on IOM classification, majority of children had sufficient levels of 25(OH)D in their serum and saliva (76% and 56%, respectively). Out of the 25 children, only 2(8%) were 25(OH)D deficient based on their serum, compared to 7(28%) based on saliva analysis. According to the Endocrine society classification, 24% and 44% of the children were deficient in serum and saliva, respectively. The distribution for number of cases for serum and saliva 25(OH)D status among the young children based on IOM and Endocrine society classification is shown in Table (2). In IOM classification the sensitivity and specificity of saliva were 63.2% and 66.7%, respectively whereas in Endocrine classification the sensitivity and specificity of saliva were only 33.3% and 73.7%, respectively.

Cut-off points	Variable	25(OH) D levels	Saliva			
			Sufficient >20ng/mL	Insufficient 12-20ng/mL	Deficient <12ng/mL	Total (N)
IOM	Serum	Sufficient (>20ng/mL)	12	4	3	19
		Insufficient (12-20ng/mL)	1	0	3	4
		Deficient (<12ng/mL)	1	0	1	2
		Total (N)	14	4	7	25
Endocrine Society	Serum		Sufficient ≥30ng/mL	Insufficient 20-29ng/mL	Deficient <20ng/mL	Total (N)
		Sufficient (≥30ng/mL)	2	3	1	6
		Insufficient (20-29ng/mL)	3	4	6	13
		Deficient (<20ng/mL)	2	0	4	6
		Total (N)	7	7	11	25

Table 2. The distribution of cases of serum and salivary 25(OH)D status based on IOM and Endocrine society classification.

Comparison and correlation of serum and salivary 25(OH)D levels

There was a strong positive correlation between serum and salivary 25(OH)D levels (r^2=0.626, Pearson correlation coefficient test; p=0.001). A linear regression analysis was used to predict serum 25(OH)D based on salivary 25(OH)D. The equation: Serum 25(OH)D = 15.67 + 0.43 (Salivary 25(OH)D) (Figure 1).

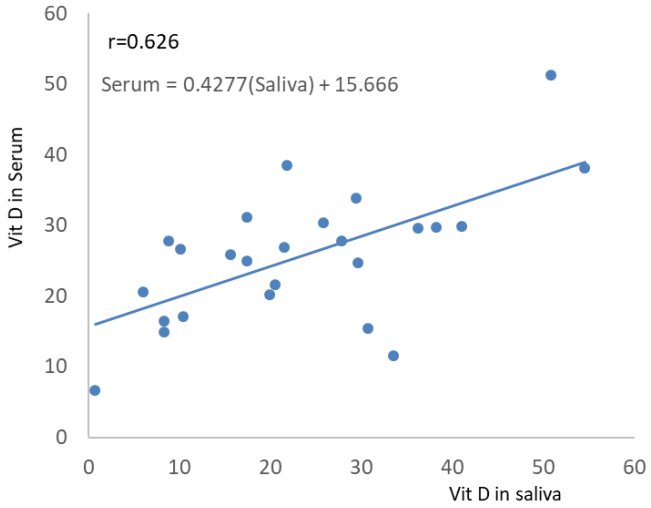

Figure 1. Association between Serum and Saliva 25(OH)D.

Discussion

In this pilot study, levels of 25(OH)D in their serum and saliva were compared among 25 healthy children aged 3-6-year-old. Even though there was a difference in saliva and serum total 25(OH)D levels, the correlation between the two was high. To the best of our knowledge, no previous studies have been conducted in young children assessing and comparing vitamin D in their serum and saliva.

Saliva offers unique advantages compared to serum because it can be collected without venipuncture, does not require special equipment for collection and is inexpensive to assay. It is a good alternative when blood taking is challenging such as among young children, obese and those who are fearful of needle.[17] Saliva is used for the diagnosis of systemic diseases such as in hypertension, cardiovascular disease or renal disease because it has serum constituents.[24,25] These constituents are derived from the salivary glands and gingival crevicular fluid. On the other hand, there is some disagreement of using saliva as a diagnostic fluid because the salivary elements and hormones concentrations are very low to be detected.[26] However, due to the development of advanced and highly sensitive detection techniques, currently saliva can be used as a diagnostic or screening or test for many diseases with no limitations.[26,27]

Serum vitamin D deficiency is associated with several chronic illness in children such as rickets, asthma, allergy, and diabetes mellitus[28-31] and recently its relevance to dental caries is being recognized.[6,12] Moreover, there is growing evidence regarding vitamin D status and severe early childhood caries in children.[11,32] Vitamin D levels could influence the tooth susceptibility to dental caries by controlling the levels of calcium and phosphate which are important elements for tooth formation. Defects in enamel and dentin layers of the tooth found to be linked to low calcium and phosphate levels in the body.[6] In fact all the above reported studies about vitamin D status and dental caries have been conducted using serum vitamin D. The ability of having initial assessment of vitamin D level using non-invasive test such as saliva sample is a highly needed in child's health. Therefore, evaluating vitamin D levels in saliva of young children is very important in assessing its level in serum and consequently in dental caries risk especially for children below 6 years.

Season, diurnal rhythm, age and ethnicity are considered to be affecting the levels of vitamin D in saliva [19]. Therefore, in this pilot study the saliva was collected at the same time between 8.00 a.m. to 10.00 a.m., no food or drinks were given to the children for at least 8 hours, and all 3-6 years old children were from same ethnic group, minimizing the impact of variabilities on 25(OH)D levels in saliva sample. Fairney and Saphier (1987)[19] reported significantly lower vitamin D in saliva compared to total serum levels in adults and schoolchildren using mass spectrometry. The salivary values were between 105 and 1000 pg/ml and there was a significant relation between saliva and serum vitamin D (r^2=0.45, P < 0.001). In this study, we also have found the total 25(OH)D measured in saliva of young children using commercial 25(OH)D ELISA Kit has the same relationship with its level in serum and to be lower in saliva compared to serum with no significant differences between them. These variations in vitamin D levels in saliva and serum between the

current study and the previous studies could be related to the method that used to measure 25(OH)D, different age group or due to small sample size in our study as a larger sample size might have found that saliva levels were significantly lower than serum.

Interestingly in the current study, there seems to be a correlation between total 25(OH)D levels in serum and saliva of young children. However, the levels differ enough and serum appears to be better at grouping children into 25(OH)D status based on their concentrations. Hence, salivary testing of total 25(OH)D levels may not have enough specificity to rule out cases of true 25(OH)D deficiency.

This pilot study is not without limitations. Sample size was small; however, it still provided useful information. Comparison of the levels of vitamin D in young children with dental caries and caries-free could not be carried out because all the participants were with dental caries. Since the incidence of vitamin D deficiency in children is on the rise worldwide and this deficiency may be considered one of the risk factors for dental caries occurrence, there is a need for early detection. Based on our finding from this pilot study, saliva can be used as screening test to assess vitamin D level in young children. Children with vitamin D deficiency in their saliva need for further evaluation of vitamin D levels in their serum to confirm that.

Conclusions

No significant differences were observed in the levels of total the 25(OH)D in the saliva and serum of young children. There is a strong positive correlation between salivary and serum total 25(OH)D levels. Assessing 25(OH)D levels in saliva can be considered as a quick screening test that may reflect its levels in the serum of young children. Further studies are recommended to investigate the association of saliva and serum total 25(OH)D to the occurrence and the severity of dental caries in young children.

Acknowledgement

The authors would like to thank all the parents for allowing their children to participate in this study as well as the Tengku Ampuan Rahimah Hospital for their ethical approval and support. This work was generously supported and funded by the Research Management Institute of Universiti Teknologi MARA (UiTM), Malaysia (600-RMI/DANA 5/3/BESTARI (P) (022/2018).

Declaration of Interest

The authors report no conflict of interest.

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