The Use of the 25 (OH) D Saliva Test as a Substitute for the 25 (OH) D Serum Test in Healthy People

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ABSTRACT

Background: Routine examination of vitamin D levels is carried out by checking serum 25 (OH)D levels, which indicate circulating vitamin D levels. While serum 1.25 (OH) D levels are less frequently utilized, they represent the active form of vitamin D and could be a substitute for checking vitamin D levels.

Objective: This study aims to find the correlation between vitamin D levels, namely 25 (OH) D and 1.25 (OH) D saliva, which correlate with serum 25 (OH) D and 1.25 (OH) D levels in the examination of salivary vitamin D, and which could be a substitute for checking serum vitamin D levels.

Design: This study is a cross-sectional study involving healthy men and women, aged 20–50 years, from Lima Puluh Village, Batubara District, North Sumatra Province, Indonesia. The parameters studied were 25 (OH) D and 1.25 (OH) D levels of saliva and serum.

Results: This study involved 56 study subjects, male and female, with a percentage of deficiency of 78.6% found by examining 25 (OH) D saliva, and 76.8% found by examining 25 (OH) D serum. For the 1.25 (OH) D examination of saliva and serum, all were within normal limits. The analysis showed that a moderate correlation was obtained for levels of 25 (OH) D saliva with serum 25 (OH) D ($p = 0.424$), and a weak correlation for levels of 1.25 (OH) D saliva with serum 25 (OH) D ($p = 0.339$).

Conclusions: Salivary 25 (OH) D assay can be used to replace serum 25 (OH) D assay in healthy people, as a non-invasive alternative.

Keywords: vitamin D; cross-sectional; non-invasive; correlation; deficiency; insufficiency

Introduction

Vitamin D deficiency occurs in various parts of the world, including in the tropics, and occurs in both healthy and sick people.[1, 2] Examination of serum 25 (OH) D levels is needed to determine the level of vitamin D in circulation and in the active form, namely 1.25 (OH) D serum.[3] However, this examination often causes discomfort in the patient, so an easier examination is needed during which the patient does not feel pain.

This examination of vitamin D is necessary due to the role of vitamin D, which can increase body immunity through its role as an endocrine. [4-7] It also acts as an anti-inflammatory and has a regulatory effect on the immune system. The effects of vitamin D therapy can be felt in various metabolic diseases and cancer. [7, 8]

Examination of vitamin D levels in the form of 25 (OH) D and 1.25 (OH) D in saliva has not been used often for diagnostic purposes of vitamin D levels, because these levels may not show the actual levels in the body.[9] Saliva examination is more focused on hormonal, immunological, and infection tests, but it is rarely used.[10, 11] In addition, saliva examination may reveal many other contamination factors, so saliva examination in diagnostic tests is often neglected.[11] However, if done according to the correct procedure, then the risk of contaminants can be removed.

Based on the results of the above research, we conducted a study that looks at the correlation between levels of 25 (OH) D and 1.25 (OH) D of saliva and serum. The goal was to find an alternative for serum testing, which is invasive and causes

discomfort to the patient. It is hoped that with the findings of this research, saliva examination could replace serum testing.

Methods

This research was conducted following the ethics committee protocol, and was approved by the ethics committee of the Universitas Sumatera Utara (number 63 / KEP / USU / 2020). The subjects of this study have their signed informed consent prior to being included. During the study, no therapy or intervention was carried out and the research subjects were not charged any fees for the laboratory examination.

This research was conducted in Lima Puluh Village, Batubara District, Simalungun Regency, North Sumatra, Indonesia. This area is about 153 kilometers from the city center of Medan City, near rubber and oil palm plantations, and the hope was that the research subjects would be healthier because of high activity levels. This study included 56 study subjects: the inclusion criteria were men and women aged 18–60 years, not currently experiencing chronic pain, kidney problems, liver problems, or other hormone disorders. Exclusion criteria were study subjects who consumed vitamin D supplements regularly, were pregnant, or were breastfeeding.

The tests carried out were examination of 25 (OH) D and 1.25 (OH) D levels of serum and saliva, examination of demographic data, and other anthropometries. The examination was carried out by taking 5 mL of blood and 2 mL of saliva, and then

checking the serum and saliva levels of 25 (OH) D and 1.25 (OH) D. Before the examination, the research subjects were asked not to consume food or drink for at least 90 minutes before being examined. Centrifugation was carried out (2500 g, 10 minutes) and the product immediately stored at –20 ^{o}C. In the serum examination, after the blood was drawn, centrifugation was carried out and the product stored at –20 ^{o}C for further determination of the levels of 25 (OH) D and 1.25 (OH) saliva.

The serum and salivary 25 (OH) D level is defined as a deficiency if below 10 ng/mL, insufficiency if between 11–20 ng/mL, and optimal if ≥20 ng/mL. The 1.25 (OH) D serum and saliva are deemed deficient if ≤48 pmol/L and normal if >48 pmol/L.[12, 13] Examination of 25 (OH) D and 1.25 (OH) D serum and saliva was carried out using the Bio-Rad ELISA technology tool, California, United States of America, using the Enzyme-Linked Immunosorbent Assay (ELISA) kit, Brand Bioassay, Bioassay Technology Laboratory, Shanghai, China.

Statistical analysis was performed by presenting the data in the form of standard deviations if the data was normally distributed, but if the data was not normally distributed, then it was presented in the form of a minimum, maximum, and median. For correlation analysis with a normal distribution, the Pearson correlation test was used, whereas if the data was not normally distributed, the Spearman correlation test was used. A weak correlation is 0.2 to <0.4, a moderate correlation is 0.4 to <0.6, and a strong correlation is 0.6 to <0.8.

Results

Based on the demographic data, it can be seen that in the research location there are more people in their mid-30s, and women are more likely to work as housewives, while men are most likely to be self-employed (**Table 1**). Based on the anthropometric examination, most of the study subjects were categorized as obese, however, based on the criteria for abdominal circumference, a greater percentage of women experienced central obesity (**Table 2**).

Table 3 shows that the percentage of deficiency was 78.6% when testing with 25 (OH) D saliva and 76.8% when testing with serum 25 (OH) D. As for the 1.25 (OH) D examination of saliva and serum, it was shown that 100% of the study subjects were within normal limits.

The results of the study shown in **Table 4** suggest that there is a moderate correlation for levels of 25 (OH) D saliva with 25 (OH) D serum (p = 0.424), and a weak correlation for levels of 1.25 (OH) D saliva with 25 (OH) D serum (p = 0.339) using the Spearman test.

Discussion

This study shows that vitamin D deficiencies occur in groups of healthy research subjects, although they do not tend to be accompanied by diseases caused by vitamin D deficiency. [1, 14] Examination of vitamin D has become routine, and most often uses serum. [3, 15, 16] This examination is often uncomfortable and invasive. Various studies have also been conducted to compare this examination with examination of other body fluids. [17, 18]

In addition to serum examinations, some studies have discussed the diagnosis of vitamin D status using a questionnaire. [19] This suggests that an invasive examination is not required to establish vitamin status, but the study focused only on the elderly. [19] Other studies have shown that serum levels give more precise results compared to other body fluids. [20-22]

The saliva examination in this study showed a higher level than the serum level, which was probably due to the different sensitivities in detecting 25 (OH) D in serum and saliva. Saliva examination is also considered to be heavily influenced by contaminants, so these results cannot be adjusted to the levels in serum. However, this study showed that there was a moderate correlation between saliva and serum levels, especially in 25 (OH) D levels.

The examination of 1.25 (OH) D serum showed a higher limit compared to serum, so with a cutoff point of 48 pmol/L, it showed that no study subject had a vitamin D deficiency. All study subjects belonged to the normal group. This result is certainly different from other studies, which showed a deficiency both through serum and saliva. [2, 5, 17, 22-24]

This study showed a moderate strength of correlation between saliva and serum for 25 (OH) D levels, and this moderate correlation shows that saliva assessment can be used as the same test as that for serum. Examination of 1.25 (OH) D showed a weak correlation; this needs further analysis, and indicates that salivary examination cannot reveal the correlation between saliva and serum.

This study also has limitations, particularly around the abnormal data for vitamin D levels that were very high and very low. This study also does not assess the levels of calcium and parathyroid hormone, which would have allowed discussion of the relationship between the three nutrients, and this study did not compare vitamin D levels in people experiencing disease, which would have shown a more pronounced difference.

Conclusions

Salivary 25 (OH) D assay can be used to replace serum 25 (OH) D assay in healthy people as a non-invasive alternative. Examination using saliva as a substitute for serum testing is expected to facilitate the examination of 25 (OH) D.

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Availability of data and materials

All data generated or analyzed during this study are included in this article.

Ethics approval and consent to participate

All participants knowledgeably consented to participate in this study. This research was conducted following the ethics committee protocol, and was approved by the ethics committee of the Universitas Sumatera Utara (number 63 / KEP / USU / 2020).

Competing interests

The authors declare that they have no competing interests.

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Table 1. Characteristics of the subjects.

	Mean	n (%)
Age (years)	41.32 ± 10.68 Minimum: 18 Maximum: 58 Median: 42	
Age classification:		
18–25 years		7 (12.5)
26–35 years		6 (10.7)
36–45 years		19 (33.9)
46–60 years		24 (42.9)
Genders:		23 (41.1)
Male		33 (58.9)
Female		
Ethnic:		
Batak		26 (46.4)
Melayu		30 (53.6)
Occupation:		
Housewife		21 (37.5)
Entrepreneur		14 (25)
State civil apparatus		9 (16)
Farmer		8 (14.3)
Student		4 (7.2)
Education		
Strata 1		9 (16.1)
Diploma		2 (3.6)
Senior High School		33 (58.9)
Junior High School		8 (14.3)
Primary School		4 (7.1)
Vitamin D supplementation		
No		56 (100)
Yes		0 (0)

Continuous variable: mean ± SD; categorical variable: n (%); SD= standard deviation

Table 2. Anthropometry parameters of the subjects.

Variable	Mean	n (%)
Body Mass Index (BMI) (kg/m^2)	26.71±11.76	
	Minimum: 16.69	
	Maximum: 88.95	
	Median: 24.62	
BMI classification:		
<18 kg/m^2		3 (5.4)
18–22.9 kg/m^2		17 (30.4)
23–24.9 kg/m^2		11 (19.6)
>25 kg/m^2		25 (44.6)
Waist circumference measurement and classification:		
Men (cm):	83.57±11.07	
<90 cm		16 (69.6)
>90 cm		7 (30.4)
Women (cm):	82.36±12.14	
<80 cm		13 (39.4)
>80 cm		20 (60.6)

Continuous variable: mean ± SD; categorical variable: n (%); SD= standard deviation

Table 3. Vitamin D saliva and serum level.

Variable	Saliva	Serum
25 (OH) D level (ng/mL)	16.54±5.01 Minimum: 2.05 Maximum: 25.1 Median: 17.45	15.07±15.34 Minimum: 2.32 Maximum: 80.1 Median: 8.7
25 (OH) D categorized [n(%)]:		
≤10 ng/mL (Deficiency)	6 (10.7)	30 (53.6)
11–20 ng/mL (Insuficiency)	38 (67.9)	13 (23.2)
≥20 ng/mL (Optimal)	12 (21.4)	13 (23.2)
1.25(OH)D level (pmol/L)	201.15±50.58 Minimum: 52.7 Maximum: 285 Median: 221.5	268.31±219.26 Minimum: 51.7 Maximum: 884.2 Median: 182
1.25(OH)D categorized [n(%)]:		
≤48 pmol/L (Deficiency)	0 (0)	0 (0)
>48 pmol/L (Normal)	56 (100)	56 (100)

Continuous variable: mean ± SD; categorical variable: n (%); SD=standard deviation

Table 4. Correlation between vitamin D saliva and serum level.

Variable	25 (OH) D level (ng/mL) in serum	1.25 (OH) D level (pmol/L) in serum
25 (OH) D level (ng/mL) in saliva	r = 0.424 (positive-moderate) *p* = 0.001 (significant) n = 56	
1.25 (OH) D (pmol/L) in saliva		r=0.339(positive-weak) *p* = 0.01 (significant) n = 56

Analysis: Spearman test

Approval Sheet After Explanation (Informed Consent)

My Informed Consent Sheet signed below,

Name :

Age :

Gender :

Has been clearly explained by the researcher about the study "The Use of the 25 (OH) D Saliva Test as a Substite for the 25 (OH) D Serum Test in Healthy People", so I hereby voluntarily and without coercion stated :

☐ Willing to be included in the study.

Such is this statement to be used as necessary.

Sincerely,

_____