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The Use of 25-hydroxyvitamin D Saliva Test to Replace Vitamin D Serum Blood Test in Healthy People

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Abstract

BACKGROUND: Routine examination of Vitamin D levels is carried out by checking serum 25-hydroxyvitamin D (25[OH]D) levels which indicate circulating Vitamin D levels. While serum 1.25(OH)D levels are less frequently performed, although serum 1.25(OH)D levels represent the active form of Vitamin D be a substitute for checking Vitamin D levels.

AIMS: This study aims to see 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 so that the examination of salivary Vitamin D levels can be a substitute for checking serum Vitamin D levels.

MATERIAL AND METHODS: This study is a cross-sectional study involving healthy men and women, aged 20–50 years, sampling in 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 deficiencies of 78.6% by examining 25(OH)D saliva and 76.8% by examining 25(OH)D serum. As 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.

Introduction

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

This examination of Vitamin D is very necessary due to the role of Vitamin D which can increase body immunity, through its role as an endocrine [4], [5], [6], [7]. The role of Vitamin D in this is as an anti-inflammatory and 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, the saliva examination may reveal many other contamination factors, so that saliva examination in diagnostic tests is often neglected [11]. However, if done according to the procedure, then the possibility of contaminants can be removed.

Based on the results of the above research, it is desirable to conduct a study that looks at the correlation between levels of 25(OH)D and 1.25(OH)D of saliva and serum. The goal is to find an alternative replacement for serum testing that is invasive and gives discomfort to the patient. It is hoped that with this examination, saliva examination can replace serum testing.

Methods

This research was conducted after following the ethics committee protocol and was approved by

the ethics committee of the Universitas Sumatera Utara with number 63/KEP/USU/2020. The subjects of this study had also signed informed consents before being included in this study. During the study, no therapy or intervention was carried out and the research subject was 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 km from the city center, namely, Medan City, with the location of rubber and oil palm plantations in the hope that the research subjects will be healthier in their activities. This study included 56 study subjects with 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, and 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 anthropomentries. 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, research subjects were asked not to consume food and drinks for at least 90 min before being examined. Furthermore, centrifugation is carried out (2500 g/10 min) and immediately stored at –20°C, as well as in the serum examination, after blood is drawn, centrifugation is carried out and stored at –20°C for further determination of the levels of 25(OH)D and 1.25(OH)D saliva.

Serum and salivary 25(OH)D level category are defined as deficiency if 10 ng/mL, including insufficiency if 11–20 ng/mL, and including optimal if ≥20 ng/mL category 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 enzyme-linked immunosorbent assay (ELISA) technology tool, California, United States of America, using the ELISA kit, Brand Bioassay, Bioassay Technology Laboratory, Shanghai, China.

Statistical analysis is performed by presenting the data in the form of standard deviations if the data are normally distributed, but if the data are not normally distributed, then it is presented in the form of a minimum, maximum, and median. For correlation analysis with normal distribution, the Pearson correlation test will be used, whereas if the data are not normally distributed, the Spearman correlation test will be used. The strength of correlation is 0.2 ≤ 0.4, which means that the correlation is weak, 0.4 ≤ 0.6 is stated as moderate correlation, and 0.6 ≤ 0.8 is stated as strong.

Results

Based on demographic data, it can be seen that in the research location there are more productive ages, namely, in their mid-30s, and some of the female group work more as housewives, while the male group is self-employed (Table 1). Based on anthropometric examination, it showed that 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).

TablesTable 1: Characteristic of the subjects

Parameters of sociodemographic	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		
Male		23 (41.1)
Female		33 (58.9)
Ethnic		
Batak		26 (46.4)
Melayu		30 (53.6)
Occupation		
Housewife		21 (37.5)
Intrepreneur		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)

Continues variable: Mean±SD, Categorical variable: n (%), SD: Standard deviation.

Table 3 shows that the percentage of deficiencies is 78.6% by testing 25(OH)D saliva and 76.8% by testing 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.

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	16 (69.6)
<90 cm		7 (30.4)
>90 cm		
Women (cm)	82.36 ± 12.14	13 (39.4)
<80 cm		20 (60.6)
>80 cm		

Continues variable: Mean ± SD, Categorical variable: n (%), SD: Standard deviation.

The results of the study in Table 4 show 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.

Table 3: Vitamin D saliva and serum level

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

Continues variable: Mean±SD, Categorical variable: n (%), SD: Standard deviation.

Discussion

This study shows that it appears that the occurrence of Vitamin D deficiencies still occurs in a group of healthy research subjects, although not accompanied by diseases caused by Vitamin D deficiency [1], [14]. Examination of Vitamin D has become a routine examination performed, and most often uses serum [3], [15], [16]. This examination is often uncomfortable and invasive. Various studies have also been conducted to compare the examination with other body fluids [17], [18].

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.

In addition to serum examinations, there are studies that discuss the diagnosis of Vitamin D status using a questionnaire [19]. This study suggests that an invasive examination is not required to establish vitamin status, but this study focuses on the elderly [19]. Other studies have shown that serum levels show more precise results compared to other body fluids [20], [21], [22].

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

Where on the examination of 1.25(OH)D serum showed a higher limit compared to serum, so that with a cutoff point of 48 pmol/L, it showed that no study subjects had 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], [23], [24].

This study shows a moderate strength of correlation between saliva and serum for 25(OH)D levels, so this moderate correlation is expected to show that the saliva assessment can be used as the same test as the test on serum. Examination 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, namely, this study has abnormal data with Vitamin D levels that have very high and very low values, this study also does not assess the levels of calcium and parathyroid hormone which can describe the effect between the three nutrients, and this study did not compare with Vitamin D levels in people with the 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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