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, 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

Saliva examination is more focused on hormonal, immunological, and infection tests, but it is rarely used

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 78.6% 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 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 weak correlation for levels of 1.25(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.

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

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Introduction

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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].

Methods

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

Table 1: Characteristic of the subjects

| Parameters of sociodemographic | Mean ± SD | n (%) |
|--------------------------------|-----------|-------|
| Age (years)                    | 41.32 ± 10.68 | 56 (100) |
| Minimum                        | 18         |       |
| Maximum                        | 58         |       |
| Median                         | 42         |       |

Table 2: Anthropometry parameters of the subjects

| Variable                     | Mean ± SD     | n (%) |
|------------------------------|---------------|-------|
| Body mass index (BMI) (kg/m²)| 26.71 ± 11.76 |       |
| Minimum                      | 16.69         |       |
| Maximum                      | 88.95         |       |
| Median                       | 24.62         |       |
| BMI classification           |               |       |
| <18 kg/m²                    | 3.54          |       |
| 18–22.9 kg/m²                | 17.304        |       |
| 23–24.9 kg/m²                | 11.963        |       |
| >25 kg/m²                    | 25.446        |       |
| Waist circumference measurement and classification | | |
| Men (cm)                     | 83.57 ± 11.07 | 16 (96.6) |
| Minimum                      | 79.06         |       |
| Maximum                      | 88.95         |       |
| Median                       | 83.57         |       |
| <90 cm                       | 16 (96.6)     |       |
| ≥90 cm                       | 1 (6.3)       |       |
| Women (cm)                   | 82.36 ± 12.14 | 13 (29.4) |
| Minimum                      | 70.08         |       |
| Maximum                      | 96.95         |       |
| Median                       | 82.36         |       |
| <80 cm                       | 13 (29.4)     |       |
| ≥80 cm                       | 20 (46.6)     |       |

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 = 0.393) 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             | 2.06       | 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: Means±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].

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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References

1. Sari DK, Mega JY, Harahap J. Nutrition status related to clinical improvement in AFB-positive pulmonary tuberculosis patients in primary health centres in Medan, Indonesia. Open Access Maced J Med Sci. 2019;7(10):1621-7. https://doi.org/10.3889/oamjms.2019.338

2. Chaiprasongsuk A, Janjetovic Z, Kim TK, Jarrett SG, D’Orazio JA, Holick MF, et al. Protective effects of novel derivatives of Vitamin D3 and lumisterol against UVB-induced damage in human keratinocytes involve activation of Nrf2 and p53 defense mechanisms. Redox Biol. 2019;24:101206. https://doi.org/10.1016/j.redox.2019.101206

PMid:31039479
3. Alshahrani FM, Almaliki MH, Aljohani N, Alzahrani A, Alsaleh Y, Holick MF. Vitamin D: Light side and best time of sunshine in Riyadh, Saudi Arabia. Dermatoendocrinol. 2013;5(1):177-80. https://doi.org/10.4161/derm.23351
PMid:24494051

4. Gannmaa D, Munkhzul B, Fawzi W, Spiegelman D, Willett WC, Bayasgalan P, et al. High-dose Vitamin D3 during tuberculosis treatment in Mongolia. A randomized controlled trial. Am J Respir Crit Care Med. 2017;196(5):628-37. https://doi.org/10.1164/rccm.201705-0936oc
PMid:28692301

5. McCullough PJ, Lehrer DS, Vitamin D, cod liver oil, sunlight, and phototherapy: Safe, effective and forgotten tools for treating and curing tuberculosis infections a comprehensive review. J Steroid Biochem Mol Biol. 2018;177:21-9. https://doi.org/10.1016/j.jsbmb.2017.07.027
PMid:28756294

6. Wahyunitisari MR, Mertaniasih NM, Amin M, Artama WT, McCullough PJ, Lehrer DS. Vitamin D, cod liver oil, sunshine, Vitamin C, and probiotics to prevent the incidence of severe respiratory infections among children in Indonesia: A randomized, placebo-controlled, double-blind, parallel group trial. J Pediatr. 2019;209:72-8. https://doi.org/10.1016/j.jpeds.2019.03.003
PMid:30820594

7. Bochen F, Balensiefer B, Görner S, Höfler F, Heitmann BL, Nadanovsky P, da Veiga AB, Balcells ME, García P, Tiznado C, Villarroel L, Scioscia N, Fanidi A, Muller DC, Midttun O, Ueland PM, Vollset SE, Relton C, Koch A, et al. Vitamin D deficiency in head and neck cancer patients - prevalence, prognostic value and impact on immune function. Oncoimmunology. 2018;7(9):e1476817. https://doi.org/10.1080/2162402x.2018.1476817
PMid:32082845

8. Fanidi A, Muller DC, Middutn O, Ueland PM, Vollset SE, Selton C, Vineis P, et al. Circulating Vitamin D in relation to cancer incidence and survival of the head and neck and oesophagus in the EPIC cohort. Sci Rep. 2016;6:36017. https://doi.org/10.1038/srep36017
PMid:27812016

9. Fairney A, Saphier PW. Studies on the measurement of 25-hydroxy Vitamin D in human saliva. Br J Nutr. 1987;57(1):13-25. https://doi.org/10.1079/bjn19870005
PMid:3001379

10. Gottlieb CW, Relief FP, Herbert V. Blockade of Vitamin B12-binding sites in gastric juice, serum and saliva by analogues and derivatives of Vitamin B12 and by antibody to intrinsic factor. Biochim Biophys Acta. 1967;141(3):560-72. https://doi.org/10.1016/0304-4165(67)90185-7
PMid:3233998

11. Flusser J, Chvojikova V, Kozička V. [Relationship between saliva and Vitamin B12. (Contribution to the problem of the intrinsic factor)]. Cas Lek Cesk. 1961;100:199-205. PMid:13700414

12. Heaney RP, Holick MF. Why the IOM recommendations for Vitamin D are deficient. J Bone Miner Res. 2011;26(3):455-7. https://doi.org/10.1002/jbmr.328
PMid:21337617

13. Grant WB, Holick MF. Benefits and requirements of Vitamin D for optimal health: A review. Altern Med Rev. 2005;10(2):94-111. PMid:15989379

14. Baggerly CA, Cuomo RE, French GB, Garland CF, Gorham ED, Grant WB, et al. Sunlight and Vitamin D: Necessary for public health. J Am Coll Nutr. 2015;34(4):359-65. PMid:26098394

15. Cannell JJ, Grant WB, Holick MF. Vitamin D and inflammation. Dermatoendocrinol. 2014;6(1):e983401. PMid:26413186

16. Cannell JJ, Vieth R, Umhau JC, Holick MF, Grant WB, Madronich S, et al. Epidemic influenza and Vitamin D. Epidemiol Infect. 2006;134(6):129-40. https://doi.org/10.1017/s0950268806007175
PMid:16959053

17. Evans LW, Omaye ST. Use of saliva biomarkers to monitor efficacy of Vitamin C in exercise-induced oxidative stress. Antioxidants (Basel). 2017;6(1):5. https://doi.org/10.3390/antiox6010005
PMid:28085082

18. Rakhashbhuvankar AA, Clarke MW, Simmer K, Pateo SK, Pillow JJ. Saliva for assessing Vitamin A status in extremely preterm infants: A diagnostic study. Neonatology. 2020;117(3):365-8. https://doi.org/10.1159/000506132
PMid:32114572

19. Annweiler C, Kabeshova A, Callens A, Paty ML, Duval GT, Holick MF. Self-administered Vitamin D status predictor: Older adults are able to use a self-questionnaire for evaluating their Vitamin D status. PLoS One. 2017;12(11):e0186578. https://doi.org/10.1371/journal.pone.0186578
PMid:29091930

20. Biancuzzo RM, Clarke N, Reitz RE, Travison TG, Holick MF. Serum concentrations of 1,25-dihydroxyvitamin D2 and 1,25-dihydroxyvitamin D3 in response to Vitamin D2 and Vitamin D3 supplementation. J Clin Endocrinol Metab. 2013;98(3):973-9. https://doi.org/10.1210/jc.2012-2114
PMid:23386645

21. Cocate PG, Kac G, Heitmann BL, Nadanovsky P, da Veiga Soares Carvalho MC, Benaim C, et al. Calcium and Vitamin D supplementation and/or periodontal therapy in the treatment of periodontitis among Brazilian pregnant women: Protocol of a feasibility randomised controlled trial (the IMPROVE trial). Pilot Feasibility Stud. 2019;5:38. https://doi.org/10.1186/s40814-019-0417-6
PMid:30873290

22. Figueiredo AC, Trujillo J, Freitas-Vilela AA, Franco-Sena AB, Rebelo F, Cunha GM, et al. Association between plasma concentrations of Vitamin D metabolites and depressive symptoms throughout pregnancy in a prospective cohort of Brazilian women. J Psychiatria. 2017;95:1-8. https://doi.org/10.1016/j.jspychi.2017.07.009
PMid:28755554

23. Isola G, Polizzi A, Muraglie S, Leonardi R, Giudice AL. Assessment of Vitamin C and antioxidant profiles in saliva and serum in patients with periodontitis and ischemic heart disease. Nutrients. 2019;11(12):3193. https://doi.org/10.3390/nu11123193
PMid:32114572

24. Balcels ME, García P, Tiznado C, Villarroel L, Scioscia N, Carvajal C, et al. Association of Vitamin D deficiency, season of the year, and latent tuberculosis infection among household contacts. PLoS One. 2017;12(4):e0175400. https://doi.org/10.1371/journal.pone.0175400
PMid:28403225