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RESEARCH ARTICLE

Analysis of Serum Vitamin D Levels in a Healthy Young Adult Population

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ABSTRACT

Vitamin D, scientifically known as calciferol, is a nutrient that is fat-soluble and crucial for controlling the metabolism of calcium and phosphorus. This vitamin can be synthesised in the human body as a result of solar exposure on the skin or obtained through food consumption. This study used 120 serum sample healthy young adult volunteer subjects consisting of 60 male subjects and 60 female subjects with an age range of 19 - 40 years who participated in the research. Vitamin D levels in the serum were measured using the Electrochemiluminescent Immunoassay (ECLIA) method with a cobas e411 device with a measurement range value of 3.00-120 ng/mL. The examination samples in this study were blood serum that met the inclusion criteria, and the lower limit measurement was <3 ng/mL, with a maximum detection limit of 120 ng/mL. The study showed found among young individuals in good health, the reference range value of serum D was 15.42 - 28.72 ng/mL, in healthy young adult male subjects 18.11 - 31.19 ng/mL and healthy young adult female subjects 15.16 - 28.14 ng/mL. Suggests that serum vitamin D reference range values in a healthy young adult population can be used for vitamin D status monitoring.

INTRODUCTION

Lack of vitamin D has been recognised as a serious worldwide health issue. Although considered healthy, young adult populations often have undetected vitamin D deficiency. Factors such as modern lifestyles that reduce sun exposure, unbalanced diets, and genetic and environmental factors can all affect vitamin D levels in the body. Therefore, establishing accurate reference range values for serum vitamin D in a healthy young adult Population is essential for a more detailed and precise health assessment. Serum vitamin D reference range analysis shows average values ranging from 20 to 50 ng/mL or 50 to 125 nmol/L. However, recent studies highlight the need to adjust these reference ranges depending on the specific Population and conditions (1).

Deficiency in vitamin D is a worldwide health issue that impacts individuals of all ages. Serum vitamin D levels differ based on a number of variables, including genetics, lifestyle, duration of sun exposure, and dietary intake (2). Prior research has demonstrated that vitamin D insufficiency may contribute to the risk of osteoporosis, autoimmune diseases, cancer, type 2 diabetes, cardiovascular diseases, infections, skin diseases, and schizophrenia (3). In Indonesia, a country in the equatorial region, the entire area is exposed to sunlight throughout the year, but this does not guarantee adequate vitamin

D levels in all individuals. Therefore, it is essential to research to determine the reference range of optimal serum levels of vitamin D in young people in good health (4).

In young people in good health, vitamin D insufficiency is an underlying problem in society. This deficiency can have profound implications for bone health and raise the chance of developing a number of chronic illnesses. However, information regarding vitamin D status in healthy young adult populations still needs to be improved. In this context, further research is essential to evaluate the extent of a lack of vitamin D and how it affects the overall health of healthy young adults (2).

The research gap on Vitamin D in the Population of Young Adults is a significant concern. Limited data and the need to understand this Population's serum vitamin D reference values limit comprehensive health monitoring. In-depth studies determining the reference range of serum vitamin D in young people in good health in Indonesia are essential to improve understanding and lead to more effective preventive efforts against various diseases associated with vitamin D deficiency (5).

Lifestyle and environmental factors also affect vitamin D levels deficiency conditions in young adults. Although Indonesia has sufficient sun exposure throughout the year, the lack of knowledge about the importance of proper sun exposure and intake of vitamin D-source foods may contribute to widespread vitamin D deficiency. This phenomenon requires an in-depth analysis of lifestyle and diet that may result in a lack of vitamin D in young adults to develop more effective prevention strategies (6).

Several previous studies have investigated how common vitamin D insufficiency is in various populations. For example, a survey conducted in Isfahan City, Iran, found that In the adult population, the prevalence of mild, moderate, and severe vitamin D insufficiency was 19.6%, 23.9%, and 26.9%, respectively (7). Another study conducted in rural communities in Indonesia found Serum 25 (OH) D levels and sun exposure ratings were positively correlated in older women (3). Then, a recent study on initial mapping of serum vitamin D levels conducted in western Jakarta with 33 subjects reported that 93.9% of healthy young adults were vitamin D deficient (2).

The necessity to look into vitamin D levels in healthy young adults has been brought to light by the lack of knowledge and health issues related to vitamin D insufficiency. Serum vitamin D is a laboratory test that can be evaluated clinically. Determining vitamin D reference ranges for serum vitamin D status data of young, healthy populations is a global health concern due to variations in serum levels based on location, genetic composition, and Population demographics (8).

Laboratory examination methods are crucial to obtaining accurate and valid data when determining the reference range for serum vitamin D examination in healthy young adults. One commonly used method is Electrochemiluminescence Immunoassay (ECLIA). This method measures the concentration of vitamin D in serum using reagents containing compounds that bind to vitamin D. The ECLIA method allows sensitive, quantitative detection of vitamin D in serum samples, enabling precise and accurate concentration determination (8).

Selecting an appropriate examination method is very important in deermining the reference range. The ECLIA method has proven to be a reliable and tested approach for serum vitamin D analysis in clinical research. Its validity is indispensable in generating reliable data to determine reference range values, thus enabling more precis diagnosis and effective health monitoring in healthy young adults.

METHODS

Research Design: This research is Observasional Study using the Samples were collected from March to May 2024 at the Clinical Pathology Laboratory, Research Unit of Hasanuddin University Hospital (RSUH), Makassar City, Indonesia.

Population and research samples: The population of this study was a group of healthy young adult subjects with 120 healthy individuals at Hasanuddin University Makassar. The sample in this study was the blood serum of healthy young adult patients who volunteered to be research subjects.

Data analysis

Version 26 of the Statistical Package for Social Sciences (SPSS) software was used to analyse the data. The serum vitamin D reference range values were set by setting boundary values between percentiles 2.5% and 97.5%.

RESULTS AND DISCUSSION

This study was conducted from March to May 2024 at Hasanuddin University Hospital Makassar and the Labuang Baji Hospital Clinical Pathology Laboratory. The total sample size was 120 subjects, 60 males and 60 females, with an average age of 30, as listed in Table 1.

Table 1: Characteristics of research subjects

Characteristics	Unit	Mean±SD	Median	Min-Max
Age	Year	30.48 ± 5.532	31.00	19-40
BMI	Kg/m^2	23.05 ± 2.7	23.25	18.1-27.69
FBG	mg/dL	96.24 ± 10.34	96.00	77.03-118.00
Systolic Blood Pressure	mmHg	115.8 ± 8.88	120	100-130
Dystolic Blood Pressure	mmHg	75.1 ± 7.63	77.5	60-90

Table 1. shows the criteria for research subjects in healthy young adults. Based on the characteristics of the research subjects, the average *Body Mass Index* (BMI) was 23.05 kg/m^2, Fasting Blood Glucose (FBG) averaged 96.24 mg/dL, The average blood pressure was 75.1 mmHg at the diastolic and 115.8 mmHg at the systolic. Based on the research subjects collected, this fits the inclusion criteria for healthy young adult subjects.

Table 2: Normality test

	Gender	N	*p-value*	Description
Serum Vitamin D (ng/mL)	Whole lSample	120	< 0.05*	Not normally distributed
	Male	60	> 0.05	Normally distributed
	Female	60	< 0.05*	Not normally distributed

***Kolmogorov-Smirnov test (SPSS version 26)**

The normality test is carried out as a condition for parametric data testing. The Kolmogorov-Smirnov test was employed to determine if the data was normal distribution based on the test results. Based on Table 2, the results show that serum vitamin D data distribution is not normally distributed in all samples (p-value <0.05), although it is normally distributed for males (p-value >0.05) and non-normally distributed for females (p-value <0.05). The statistical tests obtained do not impact the *reference range of* serum Vitamin D. Still, the *reference range* is determined using test results from a healthy Population meeting the minimum inclusion criteria of 120 people (9).

Based on the results of the 2.5%-97.5% percentile test, the reference range of serum vitamin D in the healthy adult Population is 15.42 - 28.72, the reference range of serum vitamin D in the healthy adult, Population of the male sex is 18.11- 31,19 ng/mL and in the female sex is 15.16 - 28.14 ng/mL Table 3.

Table 3: Vitamin D reference range values

	Gender	N	Reference Range Percentile (2.5-97.5%)
Serum Vitamin D (ng/mL)	Whole Sample	120	15.42 – 28.72
	Male	60	18.11 – l1.19
	Female	60	15.16 – 28.14

Table 3. shows the reference range value of serum vitamin D in a healthy adult Population of 120 people, which is 15.42 - 28.72 ng/mL. This shows that the healthy adult Population with these reference range values is in the vitamin D insufficiency status group.

Insufficiency is a state where vitamin D levels in the body are below the level considered ideal for maintaining bone health and overall health. This indicates that while vitamin D levels may be sufficient to prevent severe symptoms of vitamin D deficiency, they still do not reach optimal levels for good health (10). Griffin *et al.* (11) found that ehen the body's vitamin D levels fall below 10

ng/mL, it is considered to be insufficient; when they fall between 10 and 30 ng/mL, it is considered to be insufficient; and when they fall between 30 and 100 ng/mL, it is considered to be sufficiently high.

The insufficiency in the healthy adults in this study may be due to the influence of an insufficient diet. Absence of ingestion of vitamin D-rich foods, including egg yolks, fatty fish (such as salmon and tuna), and fortified dairy products, can also cause vitamin D insufficiency (12). This aligns with research conducted by Cashman (13) that estimated 490 million healthy adults have vitamin D insufficiency with vitamin D levels <30 ng/mL.

Table 3 presents the findings of the statistical test of 2.5%- 97.5% percentile obtained *reference range of* serum vitamin D in the healthy adult Population of the male sex is 18.11-31.19 ng/mL. Meanwhile, for the female healthy adult Population, it is 15.16 ng/mL-28.14 ng/mL. The results of this study are also similar to research conducted by Kestanbun *et al.* (14), which states that women are more prone to vitamin D deficiency. This is also in line with research conducted by Muhairi *et al.* (15) in an observational study conducted on a group of people aged 18 years and over in the United Arab Emirates, showing a higher prevalence of vitamin D deficiency tends to occur in the female Population.

This condition may be due to male respondents in this study experiencing more frequent sun exposure than female respondents, causing an increase in vitamin D levels in the body of male respondents higher than female respondents. This is in line with the theory of Giustina *et lal.* (16) that exposure to sunlight containing *ultraviolet B* (UVB) rays stimulates the conversion of *7-dehydrocholesterol* precursor compounds contained in the skin into vitamin D3 (*cholecalciferol*). Subsequently, this cholecalciferol will be transported to the liver and kidneys to undergo a conversion process into its active form, namely 25-hydroxy vitamin D and then into 1,25-dihydroxy vitamin D. Therefore, men who are frequently exposed to sunlight have a more significant potential to produce higher amounts of vitamin D in their bodies, as sufficient sun exposure triggers the endogenous production of vitamin D (17).

Several studies have been conducted to determine the reference range values for vitamin D in laboratory standards. In general, the reference values for vitamin D show significant differences. Based on the results of research in various countries, the *reference range* value of serum Vitamin D in the Indian region is 19.3-30.1 ng/mL, Germany 30-100 ng/mL, Belgium 14.4-27.2 ng/mL, South Africa 37-43 ng/mL, Korea 35-45 ng/mL, Indonesia l9.13-26.22 and 15.42 - 28.72 ng/mL Table 4.

Table 4: Reference range of vitamin D in Indonesia and other countries

Reference	Country	Age	Inspection Method	Vitamin D *Reference Range* Value
Amitha *et al*. 2022	India	18-70 Years	ECLIA Cobas e411	19.3- 30.1 ng/mL
Zeng *et al*. 2021	Germany	18-65 Years	ECLIA Cobas e411	30-100 ng/mL
Cavalier *et al*. 2020	Belgium	20-40 Years	LCMS/MS	14.4-27.2 ng/mL
Lategan *et al*. 2011	Africa South	18-64 Years	ECLIA Cobas e411	37-43 ng/mL
Shin *et al*. 2013	Korea	20-79 Years	LCMS/MS	35-45 ng/mL
Restimulia *et al*. 2018	Indonesia	21-60 Year	ECLIA Cobas e411	9.13-26.22 ng/mL
This research 2024	Indonesia	18-40 Years	ECLIA Cobas e411	15.42 - 28.72 ng/mL

Table 4. shows the vitamin D reference range values of various populations in Indonesia and other countries that show significant differences. Research conducted by Amitha *et al.*(2022) (18) reported

that in India, with an age range of 18 lto 70 years, the *reference* range value was 19.3 to 30.1 ng/mL. The results of *Zeng let al.* (2021) (19) reported that vitamin D *reference range* values for young adults in Germany were between 30 to 100 ng/mL.

Another study conducted in South Africa showed that in the age range of 18 to 64 years, the *reference range* value of vitamin D is 37 to 43 ng/mL. These studies were conducted in urban areas with sufficient sun exposure throughout the year. Factors such as geographical location in the tropics or close to the equator allow for higher sunlight intensity, which is necessary for vitamin D synthesis in human skin. Sunny weather conditions also favour optimal UVB absorption, which is key for vitamin D production. As a result, most participants in this study showed adequate vitamin D status, essential for optimal bone health and immune function among this Population (16). Meanwhile, in Belgium, with an age range of 20 to 40 years, *reference range* vitamin D values were found to be between 14.4 to 27.2 ng/mL (20). Research by Shin *et al.* (2013) (21) also reported that the adult Population in Korea had *reference range* values between 35 to 45 ng/mL. In Indonesia, especially in Surabaya, research conducted by Restimulia *et al.* (2018) (22) reported that the vitamin D reference range was between 9.13 to 26.22 ng/mL.

In line with a study conducted from March to May 2024 on serum vitamin D in a healthy young adult Population, serum vitamin D's reference range was 15.42 - 28.72 ng/mL. The reported *reference range l*values of vitamin D differ across countries, possibly due to various scientific factors, including geographical differences, skin color, diet, and Population health conditions. In countries such as India and Korea, genetic variability also affects how the body produces and metabolizes vitamin D, resulting in differences in the *reference range* (23). The results of other studies also report that the cause of vitamin D deficiency is not only lack of sun exposure and consumption of foods containing vitamin D, but dark skin color also affects which researchers in this study did not group by skin color. This is supported by the results of research conducted by (24), who reported that individuals with dark skin tended to have higher levels of vitamin D deficiency, estimated to be 15 to 20 times higher. Vitamin D deficiency is caused by a lack of production due to melanin in the skin that blocks the sun's UVB rays, which are necessary for vitamin D synthesis. Dark skin color and low UVB radiation can exacerbate vitamin D deficiency in high-atitude areas.

Individuals with darker skin pigmentation tend to exhibit lower vitamin D levels than those with lighter skin pigmentation (25). This is due to the role of melanin, the pigment responsible for skin colouring, which can inhibit vitamin D production by sunlight. Melanin acts as the skin's natural defence against ultraviolet (UV) light exposure (26). However, its presence also inhibits the skin's ability to synthesize vitamin D when exposed to sunlight. As a result, individuals with higher melanin levels require longer sun exposure times to achieve vitamin D levels than those with lower melanin levels. This causes individuals with darker skin pigmentation more susceptible to vitamin D insufficiency (24).

Some limitations have been identified from direct observation in this research process, which can concern future researchers who wish to conduct more in-depth data analysis. This research is also limited to specific locations or areas.

CONCLUSION

Based on the study's results, the reference range of vitamin D levels in healthy young adult subjects aged 18-40 is 15.42-28.72 ng/mL. The reference value range of vitamin D levels in men is 18.11-31.19 ng/mL, and in women, 15.16-28.14 ng/mL.

Authors' contributions

The research was conceived and planned by MM and LBK, the data collecting and collection was carried out by MM and MA, the analysis and calculation of the observational data were done by MM and AAZ, the paper was written by MM, and the findings were interpreted with assistance from AA and TE. Every author contributed to the manuscript's critical revision.

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