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25(OH)D Status in Metabolic Syndrome, Metabolic Syndrome Components, and Healthy Adult

Status 25(OH)D pada Penderita Sindrom Metabolik, Komponen Sindrom Metabolik, dan Orang Dewasa Sehat

Efriwati Efriwati[1]*, Fitrah Ernawati[1], Nunung Nurjanah[1], Elisa Diana Julianti[1], Galih Kusuma Aji[2], Dian Sundari[1], Fifi Retiaty[1], Aya Yuriestia Arifin[1]

[1]Center for Public Health and Nutrition Research, National Research and Innovation Agency, Cibinong Science Center, Bogor, Indonesia
[2]Research Center for Agroindustry, National Research and Innovation Agency, BJ Habibie Science Center, Setu, Tangerang Selatan, Banten, Indonesia

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Correspondent:
Efriwati Efriwati
efri003@brin.go.id

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ABSTRACT

Background: Vitamin D supplementation to prevent Metabolic Syndrome (MetS) has been widely implemented in developed countries and is now being introduced in developing countries. However, recent studies have shown no association between vitamin D and MetS in adults.
Objectives: This study aims to obtain a profile of vitamin D values in adults and explore vitamin D status in MetS patients, MetS components, and healthy adults.
Methods: This research design was a cross-sectional study, utilizing both secondary and primary data from the research on the Risk Factors for Non-Communicable Diseases (RF-NCD) longitudinal study in 2021. Serum 25(OH)D levels in 956 adult respondents were determined by Enzyme-linked Immune Assay (ELISA) analysis using Stored Biological Material (SBM) serum from the 2021 RF-NCD longitudinal study. The ANOVA test was used to determine differences in vitamin D levels in various physiological conditions of respondents (MetS, MetS components, and healthy people).
Results: A total of 68.5% of respondents had inadequate (deficiency and insufficiency) serum vitamin D levels, with an average of 26.5 ng/mL. The average vitamin D levels between MetS patients, MetS components, and healthy people were not significantly different (p-value>0.05). However, the number or percentage of respondents with 25(OH)D deficiency in the MetS group (12.1%) was higher than other physiological conditions.
Conclusions: Most of the respondents in this study have inadequate vitamin D levels. The average vitamin D levels did not differ between physiological conditions. Further studies are needed on other micronutrient factors that affect the incidence of MetS and its components.

INTRODUCTION

Metabolic syndrome (MetS) is a condition characterized by the presence of three or more of the five risk factors associated with MetS (MetS component), namely Abdominal Obesity (AO), elevated Blood Pressure (BP), increased Fasting Blood Glucose (FBG), low High-Density Lipoprotein Cholesterol (HDL-C), and elevated Triglycerides (TG)[1]. MetS prevalent has become a global health problem, including in Indonesia. According to the 2018 Indonesian Basic Health Research (Riskesdas) data analysis, the national prevalence of MetS in 2018 was 24.4% and has been on the rise annually[2]. Research on the Risk Factors for Non-Communicable Diseases (RF-NCD) in densely populated areas in Bogor, Indonesia, found increased prevalence from 18.2% in the baseline (2011-2021) to 28.6% in 2018 was found[3,4]. One of the micronutrients related to the MetS is vitamin D since vitamin D plays a key role in the secretion and sensitivity of insulin, which might affect MetS development[5]. Thus, a study to relate vitamin D to MetS is crucial to be performed.

Vitamin D is a micronutrient for several body functions, such as bone mineralization and growth[6]. However, vitamin D deficiency is another significant global health issue. A meta-analysis spanning two years (January 2019 - January 2021) involving 472 research with 746,564 respondents found a high prevalence of vitamin D deficiency in the Asian population[7]. Despite being in equatorial regions that are rich in sunlight, Asian people are still susceptible to vitamin D deficiency because of lifestyle changes, including sunscreen use for skin protection, and diet[8]. This research also indicated older people had higher 25(OH)D levels than younger people. Vitamin D deficiency in Indonesia has been identified as a

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public health emergency problem[9–11]. Vitamin D deficiency occurred in almost every age group, showing a prevalence of 90% in newborns[9], 33% in children aged 6 months - 19 years old[10], and 63% in pregnant[11].

Vitamin D deficiency associated with cases of metabolic syndrome (MetS) has been reported by many researchers, where higher serum 25(OH)D concentrations decrease the risk of MetS. Proposed supplementation of Vitamin D to increase serum vitamin D concentrations in the population has been suggested as a strategy for preventing MetS as optimal vitamin D concentrations indicate a better metabolic profile[12,13]. Vitamin D supplementation to reduce the incidence of MetS and overcome the increase in CVD and type 2 diabetes, has been implemented in developed countries and has also begun to be adopted in developing countries. However, several current studies found no association between serum vitamin D levels and the incidence of MetS in adults[14–16]. They stated that lower serum 25(OH)D levels were significantly related to one of MetS components, such as abdominal obesity, hypertension, and glucose homeostasis abnormalities, but were not to metabolic syndrome[15]. Research in Lebanon's adult population found inconclusive evidence regarding the relationship between vitamin D and MetS[16]. In addition, the benefits of vitamin D supplementation in adults with MetS were not convincing[17]. However, solutions are needed to reduce MetS and vitamin D deficiency in Indonesian society. Implementation of vitamin D supplementation to reduce MetS cases must be based on strong supporting data.

The Indonesian government, through the Ministry of Health, between 2011 and 2021, conducted a RF-NCD longitudinal study, involving a large population with respondents over 25 years old (adults). This research highlights a high case of MetS, which was 28.6% in 2018[4]. However, the research to explore the relationship between MetS cases and vitamin D levels in Indonesian adults is still limited, especially for large populations. Therefore, this research was conducted to determine the Vitamin D value in adults and investigate the profile and status of serum vitamin D levels on various physiological conditions (MetS, MetS components, and health in the studied population of RF-NCD)[4].

METHODS

Research Design

This research design is a cross-sectional study utilizing both secondary and primary data (N=956) from the RF-NCD longitudinal study in 2021. The Primary data consists of vitamin D levels, obtained from the laboratory analysis of 25(OH)D serum SBM. On the other hand, the secondary data includes information on the health condition of respondents with or without MetS, specifically focusing on all MetS components as per the NCEP-ATP III for Asians in 2005[1] (Table 1).

Table 1. Cut-off component risk factors for metabolic syndrome (MetS)*

Risk Factor MetS	Measuring Results	Male	Female
AO	Abdominal Circumference	≥90 cm	≥80 cm
Low HDL-C	Results of HDL Cholesterol Levels	<40 mg/dL	<50 mg/dL
Elevated TG	Triglyceride Levels	≥150 mg/dL	≥150 mg/dL
Elevated BP	Blood Pressure	≥130/85 mmH	≥130/85 mm Hg
Increased FBG	Fasting Blood Glucose	≥100 mg/dL	≥100 mg/dL

*) National Cholesterol Education Program Expert Panel and Adult Treatment Panel III (NCEP-ATP III) for Asians in 2005[1].

Licensing and Ethical Considerations

Permission to utilize secondary data, specifically letter SK NO IR.03.01/H.I/5559/2023, was obtained after the submission of a formal request letter to the National Research data to the Health Development Policy Agency (BKPK) under the Indonesian Ministry of Health for the use of national data from the RF-PTM longitudinal study in 2021. Authorization to use serum SBM was acquired through a permit for SBM usage and laboratory facilities with the number HK.01.03/H.III/12397/2023. This SBM serum was collected in 2021 and stored properly in an ultra-low temperature freezer at -80^{0}C in the Laboratory Management Informatics System (LIMS) unit and Biorepository of the Indonesian Ministry of Health. Ethical approval for research using SBM as a material was obtained from the BRIN Ethics committee under permit number: 091/KE.03/SK/08/2023.

Sample Selection and Primary Data Analysis

The analysis of all SBMs that meet the inclusion criteria (1543 samples) was impossible due to limited research funding. The selection of samples for primary data analysis was based on secondary data processing to find information on the physiological conditions of respondents. The inclusion criteria for Mets and Met component samples were 1) having secondary diagnostic data that meets the Mets criteria (Table 1), 2) having serum with sufficient volume and good quality (not lysed and/or lipemic). The sample exclusion criteria were that the serum was lysed, lipemic, or if there was insufficient for a complete analysis of all parameters. From the secondary data, the samples were prioritized on individuals with MetS, who had three or more risk factors for metabolic disorders (n=263). The healthy normal individuals without MetS component (n=207) were also chosen as controls. In addition, respondents (n=486) who did not have MetS but had one or two risk factors for metabolic syndrome were also analyzed as MetS component samples with disturbed metabolism. In total, 956 respondents were obtained as primary data. The sample consisted of 278 males and 678 females, with a minimum of age 30 years and a maximum of 76 years.

The analysis of 25(OH)D levels was performed with Enzyme-linked Immune Assay (ELISA) from the Cortez Diagnostic kit, namely AccuDiagTM 25-OH Vitamin D. Lypochek assayed chemistry control Level 1 C-310-5 by Bio-Rad, used as CRM for quality control in addition to the kit. The cut-off status for 25(OH)D refers to the Endocrine

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Society Practice Guideline (ESPG)[18], as presented in Table 3.

Data Analysis

Primary and secondary data on respondents integrating resulted in a new data set. Statistical analysis was performed on this new combined dataset, using SPSS Software Version 25. Summary statistics, namely number, percentage, mean, and standard deviation of variables calculation were used to summarize the test results. ANOVA analysis was done to determine the difference among test variables. Statistical significance is determined by a p-value<0.05.

RESULTS AND DISCUSSIONS

Secondary Data Analysis

The analysis process of processing secondary data to identify respondents' physiological conditions data refers to NCEP ATP Panel III (2005)[1] (Table 1). A respondent is classified into MetS respondents if they exhibit three or more MetS risk factor components. Since there are 5 components of MetS risk factors, there are 16 different variations of MetS components in the MetS profile and 15 in MetS components with compromised physiological conditions (with one or two MetS components disorder). These variations depend on the MetS risk factors of the components present in each respondent. Table 2 displays the respondents' physiological conditions divided into 3 groups: the MetS group, the metabolic disorders group (with 1 or 2 MetS components), and the healthy group. A total of 16 combinations of MetS components in respondents in the MetS group (≥3 MetS components) are then grouped into 3 sub groups, namely consisting of 5 MetS components, 4 MetS components, and 3 MetS components. On the other hand, the MetS components of respondents with metabolic disorders consist of 15 combinations and are then divided into 2 subgroups, namely 2 MetS components and 1 MetS component.

Nationally, the prevalence of MetS in Indonesia among individuals over 40 years old was 21.66%[19]. The prevalence of MetS in a longitudinal study population in Bogor during 2011-2012 (baseline) was 18.2%[3] and increased to 28.6% in 2018[4]. The results of this study, using the same longitudinal study population in 2021, showed that 27.5% of subjects in the same population experienced MetS. This is a slightly lower percentage than that found by Rahmawati et al., (2024)[4] does not mean decreasing in MetS cases in this population. However, this occurred due to differences in the number of samples analyzed. Rahmawati et al., (2024)[4] found 28.6% in 2018 of all RF-NCD study longitudinal samples (n=5690) followed for 6 years. It is known that the implementation of the RF-NCD study was divided into two years (even years and odd years)[3,4]. This research only uses sample data from 2021 (odd years) with a total number of respondents namely 1554 with vitamin D analysis only 956. As a result, this data cannot be used to compare MetS cases in the population.

Table 2. Profile of respondent's physiological conditions from secondary data processing

Physiological Condition of Respondent					
MetS (≥3 MetS components)			MetS Component (2 and 1 MetS components)		Healthy
5 Components	4 Components	3 Components	2 Components	1 Component	
41 (4.3%)	119 (12.4%)	103 (10.8%)	240 (25.1%)	246 (25.7%)	207 (21.7%)
263 (27.5%)			486 (50.8%)		

Primary Data Analysis

ELISA analysis on the SBM of respondents produces primary data on vitamin D levels as 25(OH)D levels in serum. The data were converted into vitamin D status with a cutoff referring to the Endocrine Society Practice Guideline (ESPG)[18], presented in Table 3. Most of the respondents in the study were classified as vitamin D hypovitaminosis (Table 3). Only 31.5% of respondents had adequate 25(OH)D levels. The prevalence of 25(OH)D inadequate with cut-off <30 ng/mL in this study population was notably high, at 68.5% (38.4% deficiency, and 30.1% insufficiency) (Table 3). This finding supports research conducted in 2000-2020 which found 47.9% that serum 25(OH)D levels in the global population were classified as vitamin D hypovitaminosis[20]. The case of vitamin D hypovitaminosis is higher in lower-middle-income countries than in high-income countries. This study is also relevant to the research in Asian countries, which found that vitamin D deficiency in South Asia was around 70%, and in Southeast Asia, it varied between 6–70%[8].

This research revealed that 7 out of 10 adults in the population studied have 25(OH)D deficiency, an average of 26.51 ng/mL (Table 3). This research strengthens the conclusion of Octavius et al. (2023) who stated that 25(OH)D deficiency in Indonesia is a public health emergency[9–11]. In the Indonesian population, vitamin D deficiency occurs in almost every age group, not only in Indonesian children, but also among teenagers, adults, and other groups. A systematic and meta-analysis study found that the prevalence of hypovitaminosis D in children aged 6 months to adolescents 19 years in Indonesia was 33%, with an average serum vitamin D level of 22.74 ng/mL[10] and in pregnant women 63% with an average serum vitamin D level of 16.24 ng/mL[11]. Another study found that the prevalence of vitamin D deficiency in newborns in Indonesia was 90%[9].

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Table 3. 25(OH)D status of each physiological condition on the respondent in the population

Vitamin D Status	Cut-off (ng/mL)	Total Frequency (%)	Physiological Conditions			
			MetS Frequency (%)	MetS Components Frequency (%)		Healthy Frequency (%)
				2 MetS components	1 MetS component	
Deficiency	<20	367 (38.4)	116 (12.1)	76 (7.9)	92 (9.6)	83 (8.7)
Insufficiency	>20 - <30	288 (30.1)	67 (7.0)	84 (8.8)	75 (7.8)	62 (6.5)
Sufficient	>30 - <100	301 (31.5)	80 (8.4)	80 (8.4)	79 (8.3)	62 (6.5)
Toxic	>100	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total		956 (100)	263 (27.5)	240 (17.2)	246 (25.7)	207 (21.7)

Indonesia is a tropical country, with abundant sunlight throughout the year, and faces a significant problem of vitamin D deficiency. This deficiency may be attributed to a low intake of vitamin D from foods. In addition to being synthesized in the skin in the presence of UV rays from the sun, vitamin D can also be obtained from foods of animal-based foods (in the form of cholecalciferol) or plant-based foods (in the form of ergocalciferol). A study in Indonesia found that the average vitamin D among adolescents was only 25.8% of the recommended dietary intake[21]. In elderly people (>60 years old) who lived in institutionalized care units, the vitamin D intake was far less consumed than the adolescents, showing 0.6 IU of vitamin D consumption[22]. This low or inadequate nutritional intake occurs due to an unbalanced diet, and several other factors such as eating habits, nutritional knowledge, infections, and socioeconomic status[23]. On the other, for people living in South Asian and Southeast Asian countries like Indonesia, the vitamin D status in the community is also influenced by various factors, namely skin pigmentation, aging, sunscreen use, religious beliefs, and lifestyle changes[8]. Higher levels of skin pigmentation result in increased melanin, which protects against UV radiation and decreases the ability to synthesize vitamin D[24]. Sunscreen protection with improper usage also reduces vitamin D production due to its inhibiting effect on UV rays[25]. Aging also contributes to lower vitamin D levels since it diminishes vitamin D synthesis, reduces nutrient intake and absorption (including vitamin D), and low outdoor activity time[8,26]. Vitamin D status is affected by religious practices, such as fasting at a specific time, which causes reduced nutrient intake, and wearing modest clothes for women, which reduces sun exposure[8]. Lastly, lifestyle changes often encourage people to be actively indoors, causing lower sun exposure and reduced vitamin D levels[27].

In addition, the differences in cut-off used as vitamin D status are still a problem in several countries. The cut-off for vitamin D status for Indonesian society that has been used may be unsuitable. This cut-off may need to be reviewed, as done by the Indian government for its people. The Indian National Consultation Committee, consisting of doctors, epidemiologists, endocrinologists, and nutritionists in India has confirmed that the serum 25(OH)D limit is used explicitly for the Indian community. The serum 25(OH)D limit used for the Indian community is lower than the cut-off used in Western countries, for deficiency, insufficiency, and sufficient to be <12 ng/mL, 12-20 ng/mL, and >20 ng/mL, respectively[28]. The new cut-off deficiency of Indian people might be suitable for Indonesia since both countries live in tropical countries, which are different from Western countries.

Table 3 illustrates that there was no specific pattern between 25(OH)D status and certain physiological conditions. While the prevalence of 25(OH)D deficiency is higher among individuals with MetS and compared to other physiological conditions (MetS components with two or one MetS component, and healthy), the sufficient status among MetS respondents is also higher. Healthy respondents do not exhibit higher levels of 25(OH)D sufficiency than other physiological conditions within the population.

Profile of 25(OH)D Value on MetS, Mets Components, and Healthy Cases

Table 4 depicts the relationship between 25(OH)D average and MetS cases by analysis of variance (ANOVA), combining vitamin D value from primary data and MetS cases from secondary data. Anova analysis reveals that the average 25(OH)D levels ranged from 25.1 to 27.7 ng/mL. All the Physiological Conditions groups showed that the average 25(OH)D levels were still categorized as hypovitaminosis vitamin D (insufficiency) (Tables 2 and 4). Further analysis with ANOVA showed no significant difference between MetS cases and other groups. The 25(OH)D values among MetS respondents (≥3 MetS components) and MetS components respondents with 2 and 1 MetS components (either in Ernawati total or individually) were quite similar, as were with the healthy respondents (without any MetS component at all).

Table 4. 25(OH)D value and MetS cases

Physiological Conditions	n	Mean	Min	Max	p-value
MetS (≥3 MetS components)	263	25.1±15.2	1.8	82.8	0.301
MetS Components with Two (2) MetS Components	240	27.7±15.8	2.3	86.3	
MetS Components with One (1) MetS Component	246	26.8±16.2	2.2	88.6	
Healthy	207	26.6±16.1	1.1	80.6	
Total	956	26.5±15.8	1.1	88.6	

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This study shows no significant 25(OH)D level exists between MetS, Mets component, and healthy adults. This result strengthens and aligns with other studies by Mansouri et al., (2018)[14] and Abboud et al., (2023)[16]. However, Mansouri et al., (2018)[14] noted that lower vitamin D in serum significantly increases the risk of some Mets components namely abdominal obesity, hypertension, and glucose homeostasis abnormalities. A study connecting vitamin D levels and MetS parameters found that increased serum concentrations of glucose, total cholesterol, low-density lipoproteins (LDLs), and triglycerides, had become negatively linked with vitamin D levels[21,29].

The vitamin D status profile in MetS respondent and those with MetS components has been studied for the first time in Indonesia through this research, which involved a large sample size. However, this study has certain limitations as it did not examining the relationship between each MetS component and 25(OH)D levels, both individually and in combination among individuals with MetS. As a result, this study cannot determine whether hypertension or one of the MetS components is more influenced by a lower of 25(OH)D or vice versa. This study only describes the difference in 25(OH)D levels between the MetS group, those with the physiological disorder (with 2 or 1 MetS component), and healthy adults without assessing the contribution of 25(OH)D to the condition of each MetS component either singly or in combination. This limitation arises from the wide range of frequency observed in the physiological profile of respondents grouped within the MetS category, which varied from just 2 to 57 respondents. Additionally, in this study, the physiological conditions of MetS with three components, namely hypertension, abdominal obesity, and HDL-C as well as hypertension, fasting blood glucose, and triglycerides, showed a zero value (not found). Further research should aim to investigate the contribution of 25(OHD) levels to each MetS component, among individuals with MetS, both individually and in combination.

CONCLUSIONS

The research highlights that seven out of ten adult respondents have inadequate 25(OH)D levels, while the average 25(OH)D levels ranged from 25.1 to 27.7 ng/mL. However, 25(OH)D values are not significantly different between the incidence of MetS, MetS components, and healthy adults. Future work on, the relationship between 25(OH)D value among MetS components or all combinations from the five MetS components and other micronutrient factors that influence the incidence of MetS and MetS components needs to be done.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURE

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AUTHOR CONTRIBUTIONS

EE, FE: conceptualization; EE, FE, NN, EDJ, DS, FR: sample and data curation; EE, FE, NN, EDJ, GKA, DS, FR: investigation; EE, FE, GKA: supervision; EE: writing–original draft; EE, FE, GKA, FR, AYA, NN, EDJ, DS: writing–review and editing.

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