Comparison of 25-Hydroxyvitamin D Levels in Acute Coronary Syndrome

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ABSTRACT

Vitamin D deficiency is associated with cardiovascular disease, one of which is an Acute Coronary Syndrome (ACS). Some studies provide varying results, the 25 (OH)D levels, which can cause acute coronary syndrome is still controversial. This was an observational analytical study with a cross-sectional design. Samples were collected during April-September 2019 from the Dr. Soetomo Hospital, Surabaya. Patients with the ACS (70 persons) consisted of ST-Elevation Myocardial Infarction (STEMI), non-ST-Elevation Myocardial Infarction (NSTEMI), Unstable Angina (UA) were measured for 25 (OH)D and the differences in levels of 25 (OH)D between groups. Examination of 25 (OH)D used a competitive antibody method chemiluminescence immunoassay. There were different levels of 25 (OH)D patients with ACS versus healthy persons, p=0.0001. There was no difference in levels of 25 (OH)D in UA patients versus healthy persons, p=0.925. Acute coronary syndrome patients had higher 25 (OH)D levels than healthy persons, so it seemed that vitamin D did not play an essential role in the occurrence of ACS based on this study. This study showed that there were significant differences between 25 (OH)D levels in STEMI and healthy persons, NSTEMI and healthy persons, STEMI and NSTEMI, STEMI and UA, NSTEMI and UA. In the UA group and healthy persons, no statistically significant differences were found.

Keywords: 25 (OH)D, STEMI, NSTEMI, unstable angina, cholecalciferol, ergocalciferol

INTRODUCTION

Vitamin D (calciferol) is a fat-soluble prohormone that is synthesized in the skin from 7-dehydrocholesterol through the activation of ultraviolet (UV) light. Vitamin D experiences bioactivation in the liver and kidneys and is subsequently metabolized to the active form 1,25-dihydroxyvitamin D (calcitriol). Calcitriol has a variety of biological roles, including mineral regulation, bone metabolism, tissue proliferation, and several effects on the cardiovascular system and the immune system. Some studies report that vitamin D deficiency has been linked to an increased risk of various diseases, including cardiovascular disease. Vitamin D deficiency in various recent studies is a risk factor for atherosclerosis which results in the occurrence of an Acute Coronary Syndrome (ACS).[1-3]

The prevalence of vitamin D deficiency is found in almost all regions of the world, 75% is the highest in the Middle East, 6-70% in South Asia, 10% vitamin D deficiency in East Asia and Europe. Levels of 25-hydroxyvitamin D {25 (OH)D or calcidiol}

>30 ng/mL are said to be deficient. Insufficient if levels of 25 (OH)D are <30 and> 20 ng/mL, and vitamin D deficiency if levels of 25 (OH)D are <20 ng/mL.[2,4]

Vitamin D Receptors (VDR) are present in almost all tissues, including vascular smooth muscle cells, endothelial, and myocytes. Vitamin D receptors function as mediators in the action of gene transcription factors that are expressed to most cells and tissues. The interaction of calcitriol with VDR has benefits in the cardiovascular system including reducing the production of renin, vasodilation of endothelial blood vessels, and reducing the occurrence of atherosclerosis, foam cells (foam cells), thus preventing the occurrence of Acute Myocardial Infarction (AMI).[5,6]

There is a significant relationship between decreased serum vitamin D levels and cardiovascular disease. This is associated with the risk of cardiovascular disease, including hypertension, diabetes, and stroke. Vitamin D deficiency can be considered a risk factor for cardiovascular disease.[7]

Research by Mahdavi *et al.* reported that patients with ACS had levels of 25 (OH)D <20 ng/mL. Lee *et al.*

reported that patients with atherosclerosis who came to the hospital with a diagnosis of ACS turned out to be more likely to suffer from vitamin D deficiency and had a 9% increase in the risk of death due to myocardial infarction and 25% risk of ACS including STEMI. Levels of 25 (OH)D serum <15 ng/mL caused a 17% mortality rate in 384 patients with ACS including STEMI.[7,8]

Eren *et al.* suggested that there were differences of opinion regarding the relationship between vitamin D and ACS, showing 81 patients with ACS, 41 of them with STEMI. However, there was no relationship between vitamin D deficiency and acute coronary syndrome. This was supported by several studies by Luis *et al.* who stated that many factors caused vitamin D deficiency including race, different individual activities, seasons, and nutritional differences.[9,10]

Based on this background, the researchers wanted to compare vitamin D levels in patients with acute coronary syndromes and healthy persons at the Dr. Soetomo Hospital, Surabaya, and to know the vitamin D levels in healthy persons in Indonesia.

METHODS

This was an observational analytical study with a cross-sectional design. The study sample consisted of 4 groups, namely 70 patients with ACS (STEMI, NSTEMI, UA) and 30 healthy persons.

Inclusion criteria for patients with ACS were adults with complaints of chest pain and diagnosed with STEMI, NSTEMI, UA by cardiologists under the supervision of the doctor in charge supported by electrocardiography examinations read by cardiologists under the supervision of the doctor in charge and troponin examinations with a cut-off value 0.04 ng/mL, and who were willingness to follow the research by signing informed consent.

In STEMI, typical ST-segment elevation persists for hours and is followed by an inversion of ST-segment depression followed by a T-wave reversal without the evolution of Q-waves, which may result from NSTEMI. Unstable angina was considered to be present in patients with ischemic symptoms suggestive of an ACS and no elevation in troponin, with or without ECG changes. Researchers grouped vitamin D levels into vitamin D deficiency state (<20 ng/mL), vitamin D insufficiency (20-29 ng/mL).[9]

Inclusion criteria for healthy persons in healthy adults (adjusted for the age of patients with ACS) who had no complaints and did not suffer from a disease based on history taking, physical examination, and willing to participate in research by signing an informed consent.

Exclusion criteria were patients taking vitamin D supplements, patients with kidney failure, and patients with liver disease.

The sample of ACS group were adults suffering from complaints of chest pain and diagnosed by STEMI, NSTEMI, UA by a cardiologist under the supervision of the doctor in charge who came to the Emergency Ward of the Dr. Soetomo Hospital Surabaya supported by electrocardiography examination which was read by a cardiologist under the supervision of the doctor in charge and troponin examination.

Healthy persons samples were all healthy adults of age (adjusted for the age of the sick population) without complaints and illness based on history taking and physical examination. All groups of patients with ACS and healthy persons who met the inclusion and exclusion criteria were examined for vitamin D levels using the competitive antibody method chemiluminescence immunoassay Advia Centaur XPT made in Germany.

The study was conducted in the Clinical Pathology Laboratory Dr. Soetomo Hospital. Research samples using venous blood samples were taken as much as 5 mL from the mediana cubiti vein, inserted into a yellow closed vacutainer tube. The serum is stored at -80ºC, and before running the sample had to be thawed first.

Research permission was obtained from the Health Research Ethics Committee of the Dr.Soetomo Hospital, Surabaya, with number 1241/KEPK/VI/2019.

RESULTS AND DISCUSSION

The collection of research subjects was carried out during June-August 2019, who came to the Emergency Room, and Inpatient Room of the Integrated Cardiac Care Center, Dr. Soetomo Hospital Surabaya, who was approved and the study was declared to be ethical.

Overall, the results of statistical analysis showed that there was a significant difference in gender and age with a p-value of ≤ 0.0001 and medical history of patients with hypertension in STEMI, NSTEMI, UA, and in healthy persons, there was a significant difference with a value of p ≤ 0.0001 (Table 1). Diabetes mellitus showed a significant difference with a value of p=0.003, a history of heart disease showed a statistically significant difference with p=0.002. Smoking habits showed statistically significant differences with

Table 1. Characteristics of the research subjects

	STEMI n=30	NSTEMI n=30	UA n=10	Healthy Persons n=30	P-value
Gender					< 0.0001
Male	26 (86.7%)	21 (70%)	7 (70%)	10 (33.3%)	
Female	4 (13.3%)	9 (30%)	3 (30%)	20 (66.7%)	
Age (years old)					< 0.0001
Median	53	51.5	50.5	40.5	
Minimum	39	39	39	37	
Maximum	64	67	61	65	
Medical history and risk factors					
Hypertension	12 (40%)	20 (66.7%)	4 (40%)	0	< 0.0001
Smoking	16 (53.3%)	9 (30%)	5 (50%)	3 (10%)	0.003
Diabetes mellitus	4 (16.7%)	19 (63.3%)	2 (20%)	0	0.003
Heart disease	8 (26.7%)	9 (30%)	5 (50%)	0	0.002

Kruskal Wallis test ; p = 0.0001
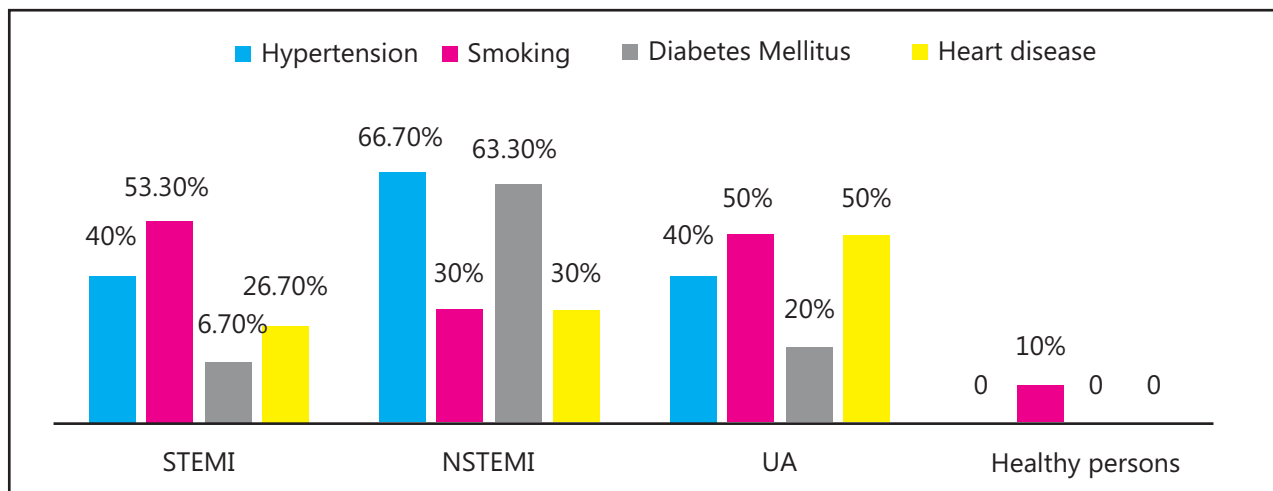
Figure 1. Bar charts of medical history percentages and patient risk factors from each group of subjects

p=0.003. Statistic analysis using the Kruskal Wallis test with p ≤ 0.0001 was statistically significant (Figure 1).

Overall differences in serum 25 (OH)D levels between groups showed significant differences with p-values ≤ 0.0001 (Table 2).

Statistical analysis showed that there were significant differences between 25 (OH)D levels in STEMI and healthy persons (p ≤ 0.0001), in NSTEMI, and healthy persons, there were significant differences (p ≤ 0.0001). There were no significant differences between levels of 25 (OH) D in UA and healthy persons with a p-value = 0.925. There was a significant difference between 25 (OH)D levels in STEMI and NSTEMI with p=0.008. The STEMI and UA

Table 2. Differences in levels of 25 (OH)D between groups

| Groups	n (amount)	Median (ng/mL)	Minimum (ng/mL)	Maximum (ng/mL)
STEMI	30	22.67	14.20	27.12
NSTEMI	30	17.59	10.42	26.85
UA	10	8.50	5.93	23.49
Healthy persons	30	9.31	5.21	21.00

Kruskal Wallis test ; p = 0.0001

groups showed a significant difference with a value of p ≤ 0.0001. Levels of 25 (OH)D in NSTEMI and UA showed significant differences with p=0.003. Box plot curves of 25 (OH)D levels in each study subject showed a higher median value in the STEMI group than in the other groups (Figure 2).

Percentage bar charts of vitamin D status for each group of study subjects, referring to vitamin D deficiency and vitamin D insufficiency (Figure 3) and some research results at 25 (OH)D levels in patients with ACS and healthy people (Table 3).
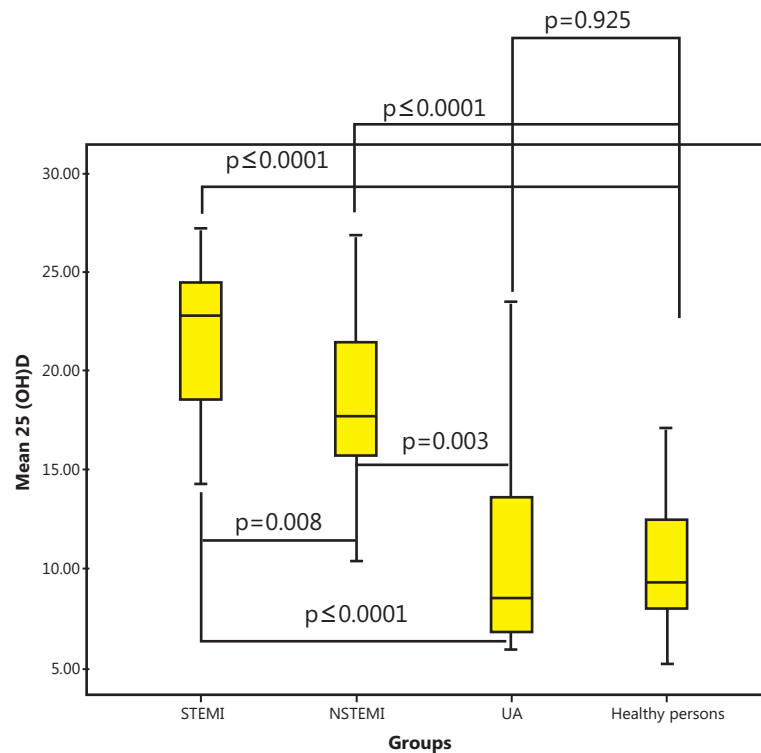
Figure 2. Box plot curves of 25 (OH)D levels in each study subject showed that the median value in the STEMI group was higher than the other groups
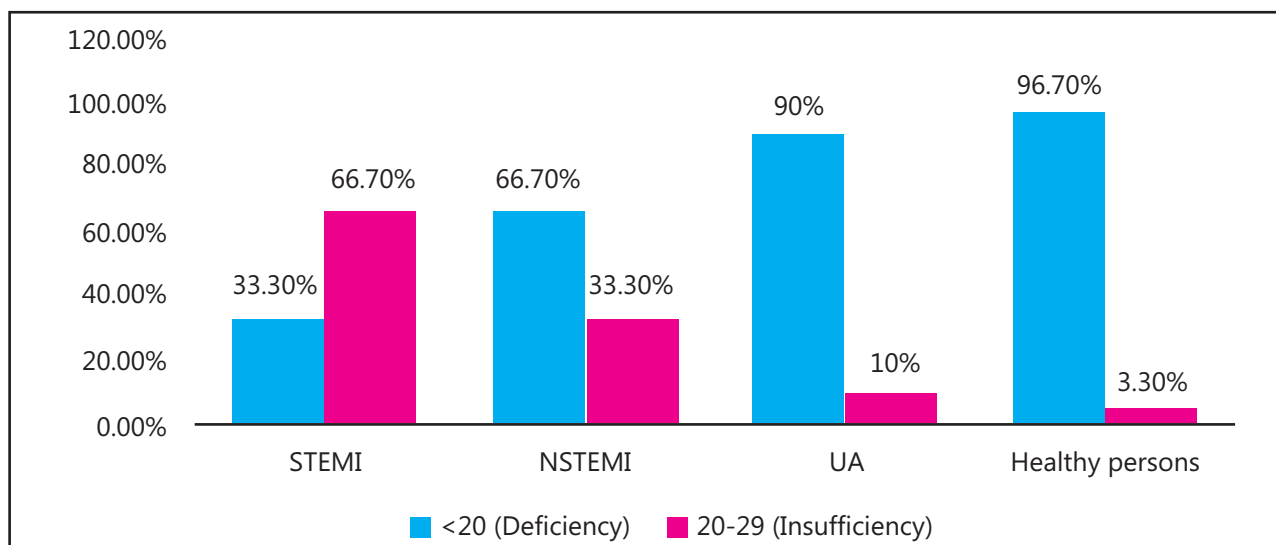
Figure 3. Bar graph of the percentage of vitamin D status for each group of study subjects

Table 3. Results of researches on 25 (OH)D levels in patients with ACS and healthy persons

The Researcher	Subjects	Methods	Results
Malhotra and Mithal, 1970. India.[11]	Healthy persons (91%)	-	Vitamin D status in Asia : Indonesia = 19.2 ng/mL Malaysia = 17.6 ng/mL China = 27.6 ng/mL Hong Kong = 28.4 ng/mL Japan = 30 ng/mL
Lund *et al*. 1978. Denmark.[12]	53 STEMI and NSTEMI 75 UA	DEAE Sephadex ion exchange chromatography	Levels of 25 (OH)D patients with AMI (STEMI, NSTEMI) and UA respectively 24±10 ng/mL and 23.5±9.6 ng/mL
Setiati, 2008. Indonesia.[13]	74 healthy persons (average age 18-70 years old)	ELISA method was performed in the clinical laboratory which was internationally standardized (ISO 9001)	Most Indonesians were vitamin D deficient with cut-off 25 (OH)D 30 ng/mL
Han *et al*. 2011. Korea.[14]	Healthy people 3.047 ♂ and 3.878 ♀ (average age 20-80 years old)	RIA (Diasorin)	Mean 25 (OH)D (ng/mL) : ♂ = 30±1 ♀ = 29±1 Mean 25 (OH)D < 20 ng/mL : 47% ? and 65% ? Mean 25 (OH)D < 30 ng/mL : 87% ? and 94% ?
Nimithpong and Holick, 2013. Thailand.[15]	Healthy people 1.321 ♂ and 1.320 ♀	LC-MS/MS (Liquid chromatography/tandem mass spectrometry)	Mean ± 25 (OH)D (ng/mL) : ♂ = 53±19 ♀ = 46±18 Mean 25 (OH)D < 20 ng/mL : 2% ♂ and 9 % ♀ Mean 25 (OH)D < 30 ng/mL : 33% ♂ and 57 % ♀
Kirchberger *et al*. 2015. Jerman.[16]	314 STEMI and 473 NSTEMI	-	155 (19%) insufficiency vitamin D and 566 (70%) deficiency vitamin D. There were no significant differences between STEMI and NSTEMI patients.
Mitchell *et al*. 2017.[17] Boston.	170 healthy persons (average age 18-65 years old)	Chemiluminescent immunoassay (Abbott ARCHITECT i2000, Illinois, U.S.A)	Mean 25 (OH)D = 30 ng/mL : 34% ♂ and 65% ♀ Mean 25 (OH)D = 30 ng/mL : 63% ♂ and 37% ♀
Luis *et al*. 2013. Brazilia.[10]	204 patients with ACS	Competitive CLIA ADVIA Centaur (Siemens Healthcare Diagnostics).	Mean 25 (OH)D = 20 ng/mL : 19.5±8.2 ng/mL
Eren et al. 2014. Turki.[9]	47 STEMI and 41 UA (average age 18-70 years old)	Competitive CLIA (LIAISON)	Patients STEMI Mean 25 (OH)D 20-60 ng/mL : 8.7±3.3 Patients UA Mean 25 (OH)D 20-60 ng/mL : 10.7±4.5
Safaie *et al*. 2018.[18]	88 STEMI and NSTEMI (average age 18-67 years old)	Microplate Absorbance Reader (2020 SW-version 2.0 up) and factory kit (Euroimmun, Germany)	Patients STEMI Mean 25 (OH)D = 20 ng/mL : ♂ = 13.3±7.5 ♀ = 14 ± 8.2 Patients NSTEMI Mean 25 (OH)D = 20 ng/mL : ♂ = 26.5±15.5 ♀ = 17.7±10.8
Bijaya *et al*. 2018 India.[19]	100 patients with ACS	-	The level 25(OH)D of healthy persons= 32.97 ng/mL was higher than STEMI, NSTEMI patients, whereas at UA = 28.30 ng/mL which showed statistically significant differences

The process of the mechanism of vitamin D in the heart and vitamin D deficiency status is mostly found in Asians. Black adults and Asians have a higher risk of vitamin D deficiency. Vitamin D sufficiency is more common in East Asia and Europe. This is due to more consumption of fish, fish oil, and skin and season pigmentation.[11,12]

Vitamin D deficiency found in Indonesia according to research by Setiati, was because 38.5% of Indonesian females only come out once a week and many wear a hijab as the most sun protection so that the subject had a long exposure to 30-60 minutes of sunshine for once a week, and average daily vitamin D intake was 0.6 IU.[13]

Vitamin D deficiency may be said only as a risk marker, not a major risk factor for cardiovascular disease. There may be other factors. The researchers found a variety of literature stating that many factors cause vitamin D deficiency including race, different individual activities, seasons, and nutritional differences.[14,15]

The mechanism of the role of vitamin D in the heart, according to Kassi *et al.* who stated that vitamin D was an anti-inflammatory by inhibiting pro-inflammatory cytokines that will inhibit plaque formation and can regulate acute vascular tonicity through the decreased molecular proliferation of smooth muscle cells in subendothelial cells.[1] Vitamin D could also cause the increased synthesis of antioxidant molecules in endothelial cells and decreased ROS production so that endothelial cells were protected and reduced plaque formation. The role of vitamin D could reduce the occurrence of atherosclerosis, which caused ACS. In accordance with this study which obtained low 25 (OH)D levels in patients with the syndrome, but 25 (OH)D levels in healthy persons were lower.[1]

CONCLUSION AND SUGGESTION

There were differences in the levels of 25 (OH)D in patients with the ACS with STEMI and healthy persons. There were differences in the levels of 25 (OH)D in patients with the ACS with NSTEMI and healthy persons. There was no difference in levels of 25 (OH)D in patients with the ACS with UA and healthy persons. There were differences in levels of 25 (OH)D in patients with ACS STEMI, NSTEMI, and UA. However, 25 (OH)D levels in healthy persons were lower than STEMI, NSTEMI, UA.

Not taking into account the intake of foods containing vitamin D, the length of exposure to sunlight could cause bias in this study. The study population was only in one geographical area resulting in vitamin D state, which could change with various factors, such as season, geographical location, and sun exposure.

Further research should be carried out, taking into account the intake of foods containing vitamin D, the duration of exposure to sunlight, which could cause bias in this study. However, taking into account recruiting research subjects in various geographical areas.

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