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Increased matrix metalloproteinase-9 in male elderly with low 25-hydroxy-vitamin D

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ABSTRACT

BACKGROUND
One of the extra-skeletal roles of vitamin D for health is associated with cardiovascular disease. Poor vitamin D status has been associated with vascular endothelial dysfunction. There were controversial results about the association between vitamin D deficiency and matrix metalloproteinase 9 (MMP-9) concentration. The purpose of the present study was to determine the concentrations of 25(OH) vitamin D [25(OH)D] in an elderly population and to find any association between 25(OH)D and MMP-9 concentrations.

METHODS
This study was of cross-sectional design involving 160 male and female subjects aged 55–65 years, in South Jakarta, Indonesia. Determination of MMP-9 and 25(OH)D concentrations was done concurrently on subjects who met the inclusion and exclusion criteria after all study subjects had been selected. 25(OH)D and MMP-9 concentrations were assessed by direct competitive chemiluminescence immunoassay (CLIA) and enzyme-linked immunosorbent assay (ELISA) respectively. Statistical analysis used chi square and t tests.

RESULTS
Mean 25(OH)D concentration in the study subjects was 14.4 ± 6.4 ng/mL. A total of 68.8% of subjects had a 25(OH)D level of <20 ng/mL, and 31.2 % had a 25(OH)D level of >20 ng/mL.There was an increased MMP-9 concentration in male subjects with a 25(OH)D level of <20 ng/mL compared with subjects with 25(OH)D level of >20 ng/mL (p=0.011), but not among female subjects (p=0.809).

CONCLUSION
The MMP-9 concentration was increased among male subjects with low level of (OH)D. This study confirmed that 25(OH)D concentration may have a potential role in endothelial function.

Keywords: Endothelial function, MMP-9, 25(OH)D, vitamin D deficient, elderly

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INTRODUCTION

Vitamin D deficiency is a health problem commonly found worldwide. Vitamin D deficiency is found in around one-third to one-half of the population, from young adults to elderly. Approximately 50% and 60% of elderly in North America and the rest of the world, respectively, do not have satisfactory vitamin D levels.[1] Vitamin D concentrations are influenced by seasonal change, dietary intake, and clothing customs. Lack of vitamin D is caused by inadequate exposure to sunlight and by dietary intakes low in vitamin D. Vitamin D deficiency is most frequently associated with musculoskeletal disorders. In addition,vitamin D deficiency is also a risk factor for hypertension, various types of cancer, autoimmune diseases, type 2 diabetes mellitus, and cardiovascular diseases.[2-4] Ecological studies have shown that coronary heart disease and hypertension are frequently found in regions far from the equator, with relatively low exposure to sunlight.[5,6]

Vitamin D receptors are widely distributed in the body, e.g. in vascular smooth muscle, vascular endothelium, and in cardiomyocytes. In vitro studies show that activation of 1,25 hydroxy-vitamin D (1,25 OH D) directly inhibits renin gene expression and regulates the growth and proliferation of vascular smooth muscle cells and cardiomyocytes. Studies in knockout rats prove that lack of activation of vitamin D receptors causes disorders of the renin-angiotensin system, leading to hypertension and left ventricular hypertrophy.[1,7]

In the elderly, the prevalence of cardiovascular diseases increases exponentially with advancing age. The elderly have increased total cholesterol, low density lipoprotein cholesterol, and triglyceride concentrations, but slightly reduced high density lipoprotein cholesterol concentrations. In addition, there are changes in glucose metabolism, such that glucose concentrations tend to increase and insulin sensitivity to decrease. Other changes are in the activation of coagulation with increasing fibrinogen and plasminogen activator inhibitor (PAI-1) concentrations. In the elderly there occurs a process called endothelial dysfunction, with markers such as von Willebrand factor (vWF), endothelial leukocyte adhesion molecule-1 (ELAM-1), intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and matrix metalloproteinase-9 (MMP-9).[8,9] MMP-9 is one of the matrix metalloproteinases, a group of zinc-dependent extracellular enzymes, and plays a major role in the degradation of the extracellular matrix involving tissue remodelling. MMP-9 is also called gelatinase and has a molecular weight of 92kDa. The MMP-9 levels are increased in several cardiovascular diseases, including hypertension, atherosclerosis and myocardial infarction.[10-13]

Several studies have reported on the association between vitamin D deficiency and MMP-9 concentration. The study by Timm set al.[14] reported that vitamin D insufficiency is associated with increased circulating MMP-2, MMP-9 and C reactive protein (CRP), correctable by supplementation of cholecalciferol. The study of Wasse et al.[13] reported the finding of a significant inverse correlation between 25(OH)D concentration and MMP-9 concentration in patients with terminal renal failure. Another study by Shab-Bidar et al.[15] reported that regular consumption of vitamin D-fortified yogurt drink *(doogh)* for 12 weeks resulted in a significant improvement in endothelin-1, endothelial selectin and MMP-9 in subjects with type 2 diabetes. The study of Baker et al.[16] showed a significant inverse correlation between MMP-9 and vitamin D levels (r=-0.41; p=0.01). The study of Lucey et al.[17] which was a randomized double blind placebo controlled trial, showed no effects of vitamin D supplementation on MMP-9 concentrations.The aim of the present study was to determine the 25(OH)D concentrations in the elderly and to find any association between 25(OH)D concentrations and MMP-9 concentrations in the elderly.

METHODS

Study design

This cross-sectional study was conducted from April 2013 until June 2013 at Mampang Prapatan district, South Jakarta, Indonesia.

Study subjects

The study population used in this study comprised elderly residents of Mampang Prapatan district, South Jakarta, aged 55-65 years and selected by simple random sampling. Inclusion criteria in this study were: elderly persons aged 55-65 years, agreeing to participate in the study (by signed informed consent) after receiving explanations about the purpose of this study, ambulatory (capable of walking actively without walking aids), capable of verbal communication (capable of answering questions by themselves or aided by others). The exclusion criteria were: not being on corticosteroids, hormone replacement therapy, vitamin D supplements, not suffering from coronary heart disease, hypertension, diabetes mellitus, or stroke, ascertained from the history (anamnesis) of the subjects. To calculate the sample size, we used a previous study by Wasse et al.[13] who reported the prevalence of low 25(OH)D was 0.43. The calculated sample size was 182, which was estimated using level of significance 0.05 and the precision 0.05.

Data collection

The distributed questionnaires contained the following items: identity of respondent, health status, medical history, family history, history of habits, sports activities, sunlight exposure, clothing style, and use of sunblocking agents.

Study subjects who had been interviewed and met the inclusion and exclusion criteria by interview were asked to visit the health centre. The subjects were asked to fast overnight for 12 hours, previous to undergoing blood sample collection for routine laboratory tests. The following morning the subjects visited the health centre and underwent physical examinations, comprising blood sample collection and measurement of blood pressure using a mercury sphygmomanometer, pulse rate using a stopwatch, and body temperature. Determination of blood pressure, temperature and pulse rate was done by 2 trained nurses.

Anthropometric measurements

Height was measured in cm using a portable microtoise with a precision of 0.1 cm, weight was measured in kg to the nearest 0.1 kg using Sage portable scales. Abdominal circumference was measured in cm to the nearest 0.1 cm, using a measuring tape. Abdominal circumference was taken as the minimum circumference between the umbilicus and the xiphoid process and measured to the nearest 0.5 cm. Body mass index (BMI) was calculated as the quotient of weight in kg and the square of height in m. Determination of weight, height, and abdominal circumference was done by 2 trained nurses.

Laboratory analysis

Blood samples were collected after 10–12 hours of fasting and the laboratory tests were done on 5 mL venous blood samples. Sera were collected and stored until all subjects had finished and had been screened on fasting blood glucose to rule out diabetes mellitus. Determination of MMP-9 and 25(OH)D concentrations was done concurrently on subjects who met the inclusion and exclusion criteria after all study subjects had been selected. From the subjects who had fasted for 12 hours, 10 mL blood samples were collected for laboratory determination of 25(OH)D and MMP-9. The 25(OH)D concentrations were assessed by direct competitive chemiluminescence immunoassay (CLIA) using the Diasorin Liaison system and Diasorin reagents (integral catalog number: en 310600LIAISON 36290 8/11). Determination of MMP-9 concentrations was by enzyme-linked immunosorbent assay (ELISA) using R&D system human MMP-9 reagents (Minneapolis, MN 55413, USA, catalog number: DMP 900, lot number 307038). 25(OH)D and MMP-9 concentrations were expressed in ng/mL.

Ethical clearance

The study was performed after approval of the procedure by the Ethical Committee, Faculty of Medicine, Trisakti University, Jakarta, under registration number 51/KER/FK/05/2013. At recruitment specially trained field workers explained the study protocol to the study subjects before requesting their written informed consent.

Statistical analysis

Tests on the normality of the data distribution using the Kolmogorov-Smirnov test showed that the data were normally distributed. Subject characteristics were presented as mean and standard deviation. To evaluate factors affecting 25(OH)D concentrations in male and female subjects, the t and chi square tests were used.

RESULTS

A total of 225 respondents aged 55-65 years who were willing to participate in this study after receiving explanations about the purpose of this study were enrolled. Among the 225 subjects who had filled in the study questionnaire, there were 52 who did not meet the criteria, among whom 17 subjects aged above 65 years, 7 subjects aged below 55 years, 2 subjects consuming antiplatelet agents such as aspirin, 2 subjects had suffered from stroke, 2 subjects were suffering from asthma, 6 subjects were suffering from heart disease, 2 subjects were suffering from goiter, 1 subject was suffering from liver disease and 13 subjects did not appear on the appointed days during the study, although they had expressed their willingness to participate in the study. There were 173 study respondents who came for physical and laboratory examinations and met the inclusion and exclusion criteria. Among them were found 13 respondents with diabetes mellitus according to fasting glucose measurements, yielding 160 subjects meeting the inclusion and exclusion criteria. The flowchart of subject participation is shown in Figure 1.

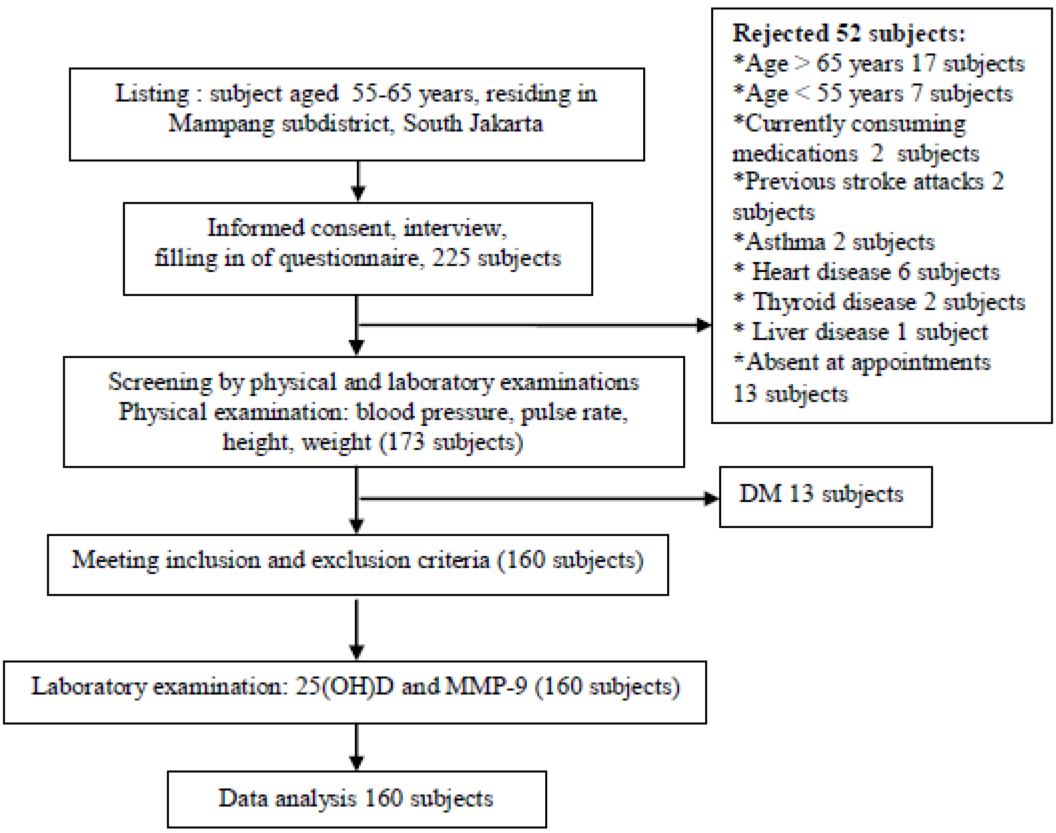

Figure 1. Flow of subject participation during study

Table 1. Distribution of characteristics of study subjects (n=160)

Characteristic	n (%)
Age (years)	59.4 ± 3.1
Gender	
Female	106 (66.3)
Male	54 (33.7)
Education	
No formal education	8 (5)
Not finishing primary school	23 (14.4)
Primary school	55 (34.3)
Junior high school	31 (19.4)
Senior high schoo	40 (25)
Academy	3 (1.9)
Employment status	
Employed	38 (23.8)
Not employed	122 (76.2)
Ethic group	
Sundanese	16(10)
Javanese	82 (51.3)
Betawi/Jakarta	57 (35.6)
Other	5 (3.1)
Abdominal circumference (cm)	88.8 ± 11.5
Hip circumference cm)	95.1 ± 8.6
Weight (kg)	59.9 ± 11.2
Height (cm)	153.1 ±7.5
Body mass index (kg/m^2)	25.5 ± 4.3
< 25	77 (48.1)
25-29.9	61 (38.1)
≥30	22 (13.8)
Systolic pressure (mmHg)	137.8 ± 25.8
Diastolic pressure (mmHg)	83.9 ± 12.9
Pulse rate (per minute)	78.2 ± 9.5
Lipid profile	
Total cholesterol (mg/dL)	201.6 ± 36.5
Triglycerides (mg/dL)	139.9 ± 72.6
HDL cholesterol (mg/dL)	48.9 ± 8.7
LDL cholesterol (mg/dL)	134.9 + 33.6
Fasting blood glucose (mg/dL)	101.6 ± 37.6
MMP-9 (ng/mL)	789.8 ± 414.7
25(OH)D (ng/mL)	14.4 ± 6.4
≤20 ng/mL)	110 (68.8)
>20 ng/mL	50 (31.2
Nutrient intake per day	
Energy (kcal)	1757.5 ± 705.2
Carbohydrate (g)	276.6 ± 103.8
Protein (g)	39.9 ± 18.3
Fat (g)	54.9 ± 31.5
Vitamin D (mg)	6.6 ± 2.4

*Mean ± S.D.

Characteristics of the study subjects are presented in Table 1. The respondents participating in this study totalled 160 subjects, with a mean age of 59.4 ± 3.1 years. Most study subjects were females (66.3%). With regard to education, the majority of the subjects, i.e. 53 respondents (33.8%), had finished primary school. Most of the respondents, totalling 122 subjects (76.2%), were unemployed. The largest ethnic group in this study were the Javanese with 82 subjects (51.3%). Mean 25(OH)D concentration of the subjects was 14.4 ± 6.4 ng/mL, and 110 (68.8%) of subjects had a concentration of <20 ng/mL. Mean MMP-9 concentration was 789.8 ± 414.7 ng/mL (Table 1).

Table 2. Distribution of characteristics of subject activities associated with sunlight exposure

Characteristic of activities	n (%)
Exposed to sunlight	
Yes	141 (88.1)
No	19 (11.9)
Sunbathing period/day	
<15 minutes	69 (48.9)
≥15 minutes	72 (51.1)
Sunbathing frequency/week	
<3 times	72 (51.1)
≥3 times	69 (48.9)
Sunbathing hours	
<9.00 AM	128 (90.7)
≥9.00 AM	13 (9.2)
Use of full-body clothing	
Yes	93 (58.1)
No	67 (41.9)
Use of protection against the sun	
Yes	66 (41.3)
No	94 (58.7)

In Table 2 may be seen the subject activities associated with exposure to sunlight. The majority of subjects (141 subjects = 89.1%) stated that they were exposed to sunlight in the morning. The most common sunbathing period was >15 minutes in 72 subjects (51.1%). The most common sunbathing frequency per week was <3 times/week in 72 subjects (51.1%). The most frequent sunbathing hour was before 9:00 AM (90.7%). There were 93 subjects (58.1%) who used full-body clothing and 94 subjects (58.7%) who did not use proctective measures against the sun.

The MMP-9 level was significantly higher in male subjects with a 25(OH)D concentration of <20 ng/mL (p=0.011). In female subjects, BMI was significantly higher in study subjects with

Table 3. Factor affecting vitamin D concentration in male and female

Factors	25 (OH) D in male			25(OH) D in female		
	<20 ng/mL (n= 30)	>20 ng/mL (n= 24)	p	<20 ng/mL (n= 80)	>20 ng/mL (n= 26)	p
Age (years)	59.5 ± 3.5	60.2 ± 2.7	0.447	59.1 ± 3.0	60.0 ± 2.7	0.343
BMI (kg/m²)	23.9 ± 3.6	22.8 ± 4.2	0.274	26.7 ± 4.3	24.3 ± 2.8	0.034*
Waist circumference	86.7 ± 11.0	80.9 ± 12.7	0.087	91.5 ± 10.9	85.4 ± 7.5	0.045*
MMP-9 (ng/mL)	909.0 ± 455.6	621.9 ± 338.7	0.011*	780.3 ± 414.4	724.6 ± 312.6	0.809
Vitamin D intake (mg/day)	6.5 ± 2.3	6.7 ± 2.0	0.916	6.6 ± 2.5	7.1 ± 2.7	0.855
Physical acitivities **						
Regular	10	11	0.491	26	12	0.284
Not regular	20	13		54	14	
Sunbathing time (minutes)**						
<15 (n=69)	18	11	0.199	28	12	0.518
≥15 (n=72)	10	7		41	14	
Sunbathing frequency/week **						
<3 times (n=72)	12	8	0.478	46	6	0.161
≥3 times (n=69)	18	15		29	7	
Sunbathing hours **						
<9.00 (n=128)	28	18	0.528	68	14	0.373
≥9.00 (n=13)	2	3		5	3	
Use of full body clothing **						
Yes (n=93)	20	12	0.553	45	16	0.440
No (n=67)	10	12		35	10	
Use of protection against the sun**						
Yes (n=66)	16	12	0.156	26	12	0.534
No (n=94)	14	12		54	14	

*p value with significance at p< 0.05 (independent t test) **(Chi Square) Mean ± S.D.

25(OH)D <20 ng/mL (p=0.034). Waist circumference was higher in females with 25(OH)D <20 ng/mL (p=0.045) (Table 3).

DISCUSSION

The results of this study showed that the mean concentration of 25 (OH)D was 14.4± 6.4 ng/mL.This value represents the concentration of vitamin D below 20 ng/mL, indicating vitamin D deficiency. The majority of study subjects (110 or 68.8%) had 25(OH)D concentrations of ≤20 ng/mL or vitamin D deficiency, whereas 50 subjects (31.2%) had 25(OH)D concentrations of >20 ng/mL.To date there is no consensus about the optimal 25(OH)D concentration, but many experts define 25(OH)D deficiency as a 25(OH)D concentration of < 20 ng/mL. A 25(OH)D concentration in the range of 21-29 ng/mL is considered to indicate 25(OH)D insufficiency, while a 25(OH)D concentration of >30 ng/mL is termed a sufficient or normal concentration.[5,18,19]

The results of this study differ considerably from those of the study by Setiati [20] conducted in Jakarta and Bekasi on 74 elderly females. The latter study showed a lower prevalence of vitamin D deficiency of only 35.1%, wereas in our study vitamin D deficiency was found in 82.8% subjects. The difference between these results may have been due to the different 25(OH)D assessment methods used. Our study used direct competitive chemiluminescence immunoassay (CLIA) whereas Setiati's study used ELISA. In addition, it has been only since the year 2009 that a standard calibrator for 25(OH)D measurement became available, such that the variability of measurements has decreased, from >30% in 1995 to 15% in 2011.[13,14] The 25 (OH)D is a difficult analyte to measure, since it is a hydrophobic molecule with several molecular forms and is strongly bound to vitamin D-binding protein (VDBP) and since there are no standardized reference materials and measurement procedures for 25(OH)D assessment.[18,21,22]

The study of Green et al.[23] showed a prevalence of 63% for vitamin D deficiency in 18-40 year old females in Jakarta. Goswami et al.[24] reported a prevalence of up to 90% for hypovitaminosis D in India. These values are somewhat similar to those of our study. Hypovitaminosis D in India is widely found in all age groups, including toddlers, children of schoolgoing age, pregnant women, and adult males and females in urban as well as in rural areas. This is caused by the skin type of Asian Indians, lack of exposure to sunlight, vegetarian habits, and absence of a vitamin D fortification program, although India belongs to countries with a hot climate and abundant sunshine.[24] In the present study, the high prevalence of 25(OH)D deficiency may have been due to the fact that on average the subjects took sunbaths for <15 minutes in the morning only, with 58.1% using full-body clothing and 41.3 % using protective measures against the sun (sun blocking agents and umbrellas). Sunlight in the morning contains more ultraviolet A (UV-A) than ultraviolet B (UV-B) such that only small amounts of vitamin D are produced by the skin. The best time for UV-B irradiation is between 10.00 to 15.00. The use of full-body clothing and headscarfs also decreases the skin vitamin D producing capacity.[5,19] In addition, on average the subjects of this study, as do the majority of Indonesians, seldom consume vitamin D containing foods, such as cow's milk, butter, shrimps, salmon and sardines, all of which are not widely consumed by Indonesian communities.[5] The price of vitamin D-containing foods tend also to be high, such that Indonesians have difficulties in obtaining dietary sources of vitamin D.

Vitamin D in the circulation is found in two major forms, i.e. 25(OH)D, which is biologically inert, and 1,25$(OH)_2$D, which is the biologically active form. In this study the concentrations of 25(OH)D were determined because 25(OH)D is the most abundant form of vitamin D in the circulation and 25(OH)D concentration is the best indicator of vitamin D status in the body.[18,25] The half life of 25(OH)D is 2-3 weeks, longer than that of 1,25$(OH)_2$D, which has a half life of 4 hours only. The active form of vitamin D, i.e. 1,25$(OH)_2$D, is not a good indicator for representing the vitamin D reserves in the body, because its concentrations are influenced by calcium concentrations and particularly decrease in cases of vitamin D deficiency, and more accurately reflect abnormalities of renal function.[25] In vitamin D deficiency the levels of parathyroid hormones are increased, subsequently inducing increased activity of 1á-hydroxylase, such that the concentration of 1,25$(OH)_2$D becomes normal or is increased. In addition, its concentration is very low in the blood, being 100-1000 fold lower than that of 25(OH)D, such that it is difficult to detect.[25,26]

The factors influencing 25(OH)D concentrations were BMI and waist circumference in females, while MMP-9 concentration was a factor affecting 25(OH)D concentrations only in male subjects (Table 3). With regard to BMI it may be seen that 83 subjects (51.9%) had BMI values of more than 25 kg/m^2, which indicates obesity. In this study it was found that in female subjects the 25(OH)D concentration was significantly lower in subjects with higher BMI, but not in male subjects. Vitamin D is a fat-soluble vitamin such that with increasing body fat more vitamin D is stored, thus reducing the circulating vitamin D levels. Deposition of excess fat in the body depends on the location of the body fat, which may have an adverse influence on vitamin D status. Obesity is more prevalent in women than in men. Adipose tissue acts as a metabolic well for vitamin D, therefore obesity may decrease the bioavailability of vitamin D.[27-29] Obesity is associated with increased risk of vitamin D deficiency. The inverse association between obesity and serum vitamin D concentration may have been caused by vitamin D deposition in the body fat compartment, by decreased release of vitamin D into the systemic circulation and by low sunlight exposure. Increases in the number of subcutaneous and visceral adipocytes is associated with decreased vitamin D concentration.[27,29] The study conducted by

Salekzamani et al.[28] suggested an inverse association of vitamin D status with insulin resistance, the metabolic syndrome, and type 2 diabetes mellitus. Serum levels of 25(OH)D are the best indicators to determine overall vitamin D status, since 25(OH)D is the most abundant form of vitamin D found in the human body and its concentrations are 1000-fold higher than those of 1,25 dihydroxy-vitamin D [1,25$(OH)_2$D]. The circulation of 25(OH)D is inversely associated with abdominal obesity, hypertriglyceridemia and hyperglycemia.[28]

MMP-9 belongs to a family of zinc-dependent proteases and has extracellular endopeptidase activity. The substrates for MMP-9 are the extracellular matrix proteins and adhesion proteins. MMP-9 is important in the extracellular remodeling process and is the principal effector molecule of inflammatory cells. It also plays a key role in vascular diseases, including hypertension, and in the formation of aneurysms. In the present study the mean MMP-9 concentration was 789.8 ± 414.7 ng/mL (Table1). The study by Welsh et al.[30] on patients with coronary heart disease showed a mean MMP-9 concentration of 741.3 ± 274.7 ng/mL, whereas that of controls was 691.8 ± 245.0 ng/mL.The mean MMP-9 concentration in the controls of Welsh's study [30] was almost identical with the mean of 659.2 ± 262.9 ng/mL found in the present study among subjects with normal vitamin D concentrations (data not shown). On the other hand, in male subjects with 25(OH)D concentrations of <20 ng/mL, mean MMP-9 concentration was 909.0 + 455.6 ng/mL and in subjects with 25(OH)D concentration of >20 ng/ml the mean MMP-9 concentration was 621.9 + 338.7 ng/mL (Table 3). There was a significantly higher mean MMP-9 concentration in male subjects having deficient 25(OH)D concentrations in comparison with subjects having insufficient and normal vitamin D concentrations, although this difference was not found in female subjects. This statistically non-significant result in females may have been caused by the higher BMI in female subjects as compared with male subjests.

MMP-9 is frequently associated with a susceptibility to vascular plaque rupture. The study of Wasse et al.[13] reported the finding of a significant inverse correlation between 25(OH)D concentration and MMP-9 concentrations (r=-0.29; p=0.004) in patients with terminal renal failure. In the study of Wasse et al.[13] the mean MMP-9 concentration in all patients was 975.2 ± 662.9 ng/mL.The mean MMP-9 concentration in subjects with 25(OH)D concentrations of <15 ng/mL was higher, namely 1155.7 ± 666.3 ng/mL, and in subjects with 25(OH)D concentrations of ≥15 ng/mL were lower, i.e. 839.8 ± 593.9 ng/mL with p=0.01. In Wasse's study [13] the subjects involved were subjects with terminal renal failure, whereas in our study the subjects were persons in normal health. Although the results of Wasse's study showed a correlation, the correlation was low, being below 0.4. In contrast, the study of Lucey et al.[17] which was a randomized double blind placebo controlled trial, showed no effects of vitamin D supplementation on MMP-9 concentrations.

The factor with the strongest influence on 25(OH)D concentrations was time of contact with sunlight, but in our study this factor did not show any significant influence both in male and female subjects, which agrees with the pathophysiology of vitamin D synthesis. In this study most of the male and female subjects had a sunbathing frequency of <3 times/week and their sunbathing time was before 9:00 AM. The main source of vitamin D is its synthesis in the skin, which is affected by exposure to UV-B radiation, initiating vitamin D synthesis with the formation of double bonds in the B ring, leading to opening of the B ring and the formation of previtamin D that is no longer rigid.[19,31] Ultraviolet irradiation of the skin is measured as the minimal erythema dose (MED) or the total amount of UV irradiation causing minimal erythema of the skin. Exposure to 1 MED is estimated to release 10.000 to 20.000 IU vitamin D into the circulation for 24 hours.[32] Exposure of 40% of the body surface to ¼ MED results in the formation of 100 IU vitamin D per day. These values are minimum values of vitamin D synthesis required by the body. Time of exposure to sunlight in the US to obtain 1 MED at midday in the summer is 4-10 minutes for light-skinned individuals and 60-80 minutes for dark-skinned individuals.[32] Vitamin D intake in this study was not associated with 25 (OH) concentration, both in male and female subjects. Our study subjects were in the low vitamin D intake category, as compared with the recommended vitamin D intake. In Indonesia vitamin D-containing foods are seldom consumed, because those foods are more expensive.

One of the limitations of this study was the cross-sectional study design. In addition, it is necessary to conduct an experimental study on subjects receiving vitamin D supplementation. The clinical implication of this study is that the community and health personnel in areas of with high sunshine levels such as Indonesia should be alert to the necessity of sunlight exposure as a source of vitamin D, since this study found 25(OH)D deficiency in the majority of respondents.

CONCLUSION

A deficiency of 25(OH)D was found in the majority (68.8%) of the subjects. The MMP-9 concentration was increased among male subjects with low 25(OH)D concentration.

CONFLICT OF INTEREST

We hereby assure that the manuscript a) is an original work, b) has not been previously published in whole or in part, and c) is not under consideration for publication elsewhere. All authors have disclosed any actual or potential competing interests regarding the submitted article and the nature of those interests. All authors a) have read the manuscript, b) agree that the manuscript is ready for submission to the journal, and c) accept responsibility for the manuscript's contents.

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