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The correlation between the serum calcium/magnesium level ratio, body composition and sarcopenia in older adult women using Dual-energy X-ray Absorptiometry

[1,*]Sukmaningtyas, H., [2]Ngestiningsih, D., [3]Probosari, E. and [4]Hananingtyas, A.

[1]*Radiology Department, Faculty of Medicine, Universitas Diponegoro, Jalan Prof. Soedarto SH, Tembalang, Semarang, Indonesia*
[2]*Internal Medicine Department, Medical Biology and Biochemistry Division, Faculty of Medicine, Universitas Diponegoro; Jalan Prof. Soedarto SH, Tembalang, Semarang, Indonesia*
[3]*Clinical Nutrition Science Department, Faculty of Medicine, Universitas Diponegoro, Jalan Prof. Soedarto SH, Tembalang, Semarang, Indonesia*
[4]*Nutrition Science Department, Faculty of Medicine, Universitas Diponegoro, Jalan Prof. Soedarto SH, Tembalang, Semarang, Indonesia*

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Abstract

Sarcopenia is characterized by a loss of muscle mass and strength, as well as a decrease in physical performance. Furthermore, it can occur in old age and also in various pathological conditions. Ageing can lead to a deficiency of certain minerals, such as Calcium (Ca) and Magnesium (Mg), which contribute to muscle metabolism and function. The amount of these minerals consumed in the diet plays a vital role in reabsorption, inflammation and various physiological activities. Therefore, this study aimed to assess the correlation between the serum Ca/Mg ratio in elderly women with sarcopenia. A cross-sectional design was employed with an observational approach, which included twenty-eight participants aged 60 years and above. Subsequently, the body composition was determined using Dual Energy X-ray Absorptiometry (DXA), while the gait speed and handgrip tests were performed using the Asian Working Group of Sarcopenia (AWGS) algorithm, which identified ten participants with sarcopenia and eighteen participants without the condition. The results showed that the mean serum calcium levels, mean serum magnesium levels and mean serum Ca/Mg ratio in participants with and without sarcopenia were 9.42±0.50 mg/dl and 9.63±0.42 mg/dl, 2.34±0,08 mg/dl and 2.22±0,04 mg/dl and 4.20±0.10 and 4.27±0.11 mg/dl, respectively. There was no significant correlation between the serum Ca/Mg ratio and body composition with the occurrence of sarcopenia in older adult women ($p = 0.47$; $r = 0.146$).

1. Introduction

Sarcopenia is a syndrome characterized by a loss of muscle mass and strength, as well as a decreased physical performance, which occurs mostly in older adults. Furthermore, this condition is associated with a physical disability, poor quality of life and premature death (Boutin *et al*., 2015; Limpawattana *et al*., 2015). The prevalence of sarcopenia in individuals aged between 65-70 years and those above 80 years were 13-24% and more than 50%, respectively. Previous studies reported that the prevalence of sarcopenia in men and women above 60 years ranged from 10% and 8%, respectively. The reduced muscle mass in this condition is associated with functional impairment and disability, especially in older adult women. Also, the prevalence of sarcopenia in older adult men and women in Japan using the Asian criteria was 9.6% and 7.7%, respectively (Yakabe *et al*., 2015).

The cause of sarcopenia is multifactorial and can include poor nutritional intake. Several minerals including calcium and magnesium play a major role in the prevention and treatment of sarcopenia due to their importance in muscle metabolism and function (Barbagallo *et al*., 2009). Certain mineral homeostatic disorders are usually associated with the deficiency of calcium and magnesium in the elderly. Chronic Mg deficiency is associated with inflammation and oxidative stress that occurs in age-related diseases, such as sarcopenia. Meanwhile, calcium deficiency is usually

*Corresponding author.
Email: *hermina_rad@yahoo.co.id*

associated with homeostatic processes associated with muscle weakness (van Dronkelaar *et al*., 2018). Magnesium (Mg) and calcium (Ca) are antagonists during physiological activities, such as (re)absorption, inflammation and other processes. The amount of Ca and Mg absorbed depends on the Ca/Mg ratio consumed.

The serum Ca/Mg ratio is implicated as a risk factor for several diseases and studies have suggested that a high serum Ca/Mg ratio is associated with an increased risk of high-grade prostate cancer and breast cancer in postmenopausal women (Dai *et al*., 2011; Barbagallo *et al*., 2021). Calcium is responsible for the stimulation of contraction in skeletal and smooth muscles, while magnesium increases relaxation. Also, previous studies have suggested that the daily intake of calcium, magnesium and the balance between the two play a role in physiological function, which affects muscle mass. Therefore, it is assumed that the Ca/Mg ratio influences the balance of muscle homeostasis (Ismail and Ismail, 2016; Hibler *et al*., 2020), which may be due to the effect of serum Ca/Mg on the incidence of sarcopenia in older adult women. However, there has been no study to establish the relationship between the serum Ca/Mg and the incidence of sarcopenia.

Dual-energy X-ray absorptiometry (DXA) is selected in this study for the routine assessment of bone mineral density because it is a well-defined method for analyzing body composition. According to studies, the amount of appendicular skeletal muscle mass (ASM) can be estimated using the bone-free and fat-free mass of the arms and legs as measured by DXA. The diagnosis of sarcopenia requires the measurement of muscle mass and strength, as well as physical performance. Currently, operational definitions and diagnostic strategies of sarcopenia in Asia are based on the consensus of the Asian Working Group of Sarcopenia (AWGS), which was modified from the European Union Geriatric Medicine Society (EUGMS) and the European Working Group on Sarcopenia in Older People (EWGSOP) (Pahor *et al*., 2009; Cooper *et al*., 2013; Rubbieri *et al*., 2014). Therefore, this study aimed to assess the correlation between the serum Ca/Mg ratio in elderly women with sarcopenia.

2. Materials and methods

A cross-sectional study was conducted to evaluate older adult women who visited several geriatric health centres in Semarang, Indonesia. The AWGS algorithm was used to identify subjects who met the inclusion criteria. The current study included a total of 28 older adult women aged 60 years and older consisting of 10 sarcopenia participants and 18 non-sarcopenia participants. Physical performance and muscle strength were assessed on-site, while the muscle mass was measured using a DXA machine. The serum Ca and Mg levels were measured at the SMC Telogorejo Hospital in Semarang, Indonesia.

The physical performances of the subjects were assessed using the standard gait speed test without declaration, where they were asked to walk in normal and comfortable steps. Walking paths are 4 or 6 m long, with two marking signs at 8 m apart and a stopwatch starting at 2 m between the first and second sign. Furthermore, this was to create a 2 m acceleration zone, a 4 m walk time section and a 2 m deceleration zone. The time taken to walk 4 m was recorded using a stopwatch and a Gait speed <0.8 m/s was defined as poor physical performance (Kon *et al*., 2013)

The handgrip strength was used in this study to assess muscle strength using a JAMAR mechanical hand dynamometer. The subjects sat in chairs and were strongly prompted to exert the greatest amount of force possible during the examination. The best three attempts with each hand were averaged in this analytical procedure. According to the AWGS, the criteria for low grip strength was defined as <18 kilograms (kg) for women (Chen *et al*., 2020).

A fan-beam dual-energy x-ray absorptiometry was used to assess the total body fat mass and total bone-free lean mass (kg) using standard image analysis protocols. Appendicular lean mass (ALM) is defined as the amount of shear mass from both arms and legs. Meanwhile, the ASM is the muscle mass in the four limbs determined by a DXA scan. The skeletal muscle mass index (SMI) was calculated as ASM/height2 (kg/m^2), where a low muscle mass was characterized as SMI <5.4 kg/m^2 (Miyake *et al*., 2019).

The participant's blood was collected with an Auto Hematology Analyzer in order to measure the serum calcium levels using the photometric endpoint determination method with phosphonazo III. The working principle of this method was the formation of a purple-blue complex from the reaction of the calcium with phosphonazo III in an acidic medium. Subsequently, the calcium was bounded to a cheating agent, which removed the specific signal. The difference in absorbance is directly proportional to the concentration of calcium in the sample and can be used to ascertain the specific calcium serum levels. Subsequently, the serum magnesium levels were determined using a photometric test method and xylidyl blue. The working principle was the formation of a purple complex from the reaction between magnesium ions and xylidyl blue in an alkaline solution. The presence of calcium ions in GEDTA complexes caused

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the reaction to be specific, while the intensity of the purple colour indicated the serum magnesium levels. The results were measured in mg/dl and the normal levels of serum calcium and magnesium were 8.8-10.2 mg/dl and 1.8-2.6 mg/ dl, respectively (DiaSys (Diagnostic System), 2015, 2019). The ratio of Ca/Mg was obtained by dividing the serum calcium levels by the serum magnesium levels.

The data normality test was conducted using Shapiro -Wilk, while the parametric test between the sarcopenic group and non-sarcopenic group was performed using an independent t-test for normal data distribution and Mann -Whitney for skewed distribution. Additionally, the Spearman test was used for the non-parametric test. The data were analyzed statistically using SPSS at a p-value <0.05.

3. Results and discussion

A total of twenty-eight respondents (old adult women) were divided into two groups, which included ten individuals with sarcopenia and eighteen individuals without sarcopenia. The respondents aged between 60-84 were evaluated based on DXA assessment, gait speed test and handgrip strength test using the AWGS algorithm. The results showed that the mean weights and BMI ranged between 48.2±4.6 kg and 58.1±8.0 kg for the sarcopenia group, as well as 20.9±1.5 kg/m^2 and 26.0±3.8 kg/m^2 for the non-sarcopenia group, which was comparatively higher, as seen in Table 1.

According to the body composition, the sarcopenia group had lower mean total mass, lean mass and fat mass than the non-sarcopenia group. The mean total mass, mean lean mass and mean fat mass in the sarcopenia and non-sarcopenia groups were 48.0±4.7 kg and 57.4±8.3 kg, 27.8 g and 32.3±2.8 g and 18.2±3.6 g and 23.4±5.7 g, respectively, as seen in Table 1.

There was no significant difference in the handgrip strength and gait speed, which ranged between 15.9±3.8 kg and 15.7±4.0 kg in the sarcopenia group, as well as 0.67±0.17 m/s and 0.69±0.18 m/s in the non-sarcopenia group, as seen in Table 1.

All respondents had normal ranges of serum magnesium and calcium levels. Subsequently, there was no significant difference in the mean serum calcium and magnesium levels in the sarcopenia and non-sarcopenia groups. The mean serum calcium and magnesium levels ranged between 9.42±0.50 mg/dl and 9.63±0.42 mg/dl, respectively. Meanwhile, the mean serum Mg level in the sarcopenia and non-sarcopenia groups was 2.34±0.08 mg/dl and 2.22±0.04 mg/dl, respectively. There was no significant difference in the ratio of serum Ca/Mg serum levels in the two groups. The mean ratio of serum Ca/Mg in the sarcopenia and non-sarcopenia groups was 4.20±0.10 mg/dl and 4.27±0.11 mg/dl, respectively. According to the statistical analysis, there was no significant difference in the mean ratio of Ca/Mg between the sarcopenia and non-sarcopenia groups ($p = 0.69$). Subsequently, the results of the Spearman Rank test showed that there was no relationship between the serum Ca/Mg ratio and the incidence of sarcopenia (Table 2) at $r = -0.146$ ($p = 0.478$).

Table 2. Correlation serum Ca/Mg with sarcopenia (Rank Spearman).

Sarcopenia	r	p
Ratio Ca/Mg Serum Level	-0.146	0.4789

Ca: Calcium, Mg: Magnesium

Table 1. Body composition, muscle strength and function, serum calcium dan serum magnesium.

	Sarcopenia older adults n = 10	Non-sarcopenia older adults n = 18	p-value
Age, years, mean (SD)	70.9 (7.1)	66.7 (6.5)	0.12
Body Composition			
Weight, kg, mean (SD)[a]	48.2 (4.6)	58.1 (8.0)	0.001
Height, m, mean (SD)[b]	1.51 (0.05)	1.49 (0.05)	0.531
BMI, kg/m^2, mean (SD)[a]	20.9 (1.5)	26.0 (3.8)	0.000
Total Mass, kg, DXA mean (SD)[a]	48.0 (4.7)	57.4 (8.3)	0.003
Lean Mass, g, DXA, mean (SD)[a]	27.8 (1.9)	32.3 (2.8)	0.000
Tissue %lean, %, DXA, mean (SD)[a]	59.2 (3.9)	56.8 (4.3)	0.165
Fat Mass, g, DXA, mean (SD)[a]	18.2 (3.6)	23.4 (5.7)	0.017
Fat mass, %, DXA, mean (SD)[a]	38.9 (4.1)	41.4 (4.6)	0.169
Muscle Strength and Function			
Handgrip strength, kg, mean (SD)[a]	15.9 (3.8)	15.7 (4.0)	0.907
Gait-speed, m/s, mean (SD)[a]	0.67 (0.17)	0.69 (0.18)	0.788
Serum Level			
Ca Serum Level, mg/dl, mean (SD)[a]	9.42 (0.50)	9.63 (0.42)	0.241
Mg Serum Level, mg/dl, mean (SD)[a]	2.34 (0.08)	2.22 (0.04)	0.201
Ratio Ca/Mg Serum Level, mg/dl, mean (SD)[a]	4.20 (0.10)	4.27 (0.11)	0.69

[a]Independent t-test, [b]Mann Whitney, Ca: Calcium, Mg: Magnesium

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4. Discussion

The results showed that the respondents with sarcopenia were aged 60 to 84 years with an average age of 67.8 years, which was consistent with a previous study by Doherty TJ. Invited (2003) and Cruz-Jentoft AJ (2010), explained that sarcopenia begins at the age of 40-50 years and progresses by about 0.6% each year thereafter. However, the decline in muscle mass at this rate usually does not have a negative impact (Cruz-Jentoft *et al*., 2010; Doherty, 2003; Sgrò *et al*., 2019).

According to Table 1, the mean BMI of respondents in the non-sarcopenia group was higher than the sarcopenia group at 26.0±3.8 kg/m2 and 20.9±1.5 kg/m2, respectively. Obesity is one of the risk factors for sarcopenia, but the results showed that the BMI of the sarcopenia group was normal, while that of the non-sarcopenia was overweight. Therefore, this could presume that the total mass, lean mass and fat mass were higher than in the non-sarcopenia group. Also, there was a possibility that the fat mass in the non-sarcopenia group contained superficial peripheral fat, which was protective against osteoporosis.

4.1 Characteristics of serum calcium and serum magnesium

The average serum calcium and magnesium in the sarcopenia and non-sarcopenia groups were still in the normal category at 9.42±0.50 mg/dl and 9.63±0.42 mg/dl, respectively, which was not consistent with a study by Cruz-Jentoft AJ (2010). According to the study, serum calcium levels decrease with age because calcium is used in large quantities in bone formation rather than absorption, which causes osteoporosis (Cruz-Jentoft *et al*., 2010; Doherty, 2003). This difference may be due to the fact that the respondents received adequate calcium intakes, such as milk, eggs, fish and vegetables.

The serum magnesium levels in the blood were normal, which ranged from 1.91 to 2.60 mg/dl, while the normal is 1.8 to 2.6 mg/dl. However, the sarcopenia and non-sarcopenia groups had magnesium levels below the normal limit, which was consistent with a previous report by Barbagallo *et al*., stating that the total plasma concentration of Mg (MgT) is generally constant throughout life in healthy humans and does not change with age (Barbagallo *et al*., 2009). According to Suranto *et al.* (2020), there is no relationship between serum magnesium levels and sarcopenia. The amount of magnesium in the blood accounts for less than 1% of the total magnesium content of the body. The 31P Magnetic Resonance Spectroscopy was used to determine the Intramuscular ionised magnesium, which was considered a better clinical measure of the magnesium status than the total serum magnesium since it could be measured when muscle weakness of unknown aetiology is detected (Cameron *et al*., 2019).

DXA was the gold standard used to measure muscle mass, which was one of the unique features of this study. Also, there was no previous study in Southeast Asia (particularly Indonesia) that established the correlation between the serum Ca/Mg ratio, body composition and sarcopenia. Calcium and magnesium play antagonistic roles in physiological reactions, such as in intestinal absorption, where magnesium shares the same transporter as calcium. Low calcium concentrations and high magnesium concentrations in the intestinal lumen can activate the transport of Mg in the mucosa since high Ca intake can reduce magnesium absorption (Dai *et al*., 2013).

According to Table 1, the serum Ca/Mg ratio in the blood of the respondents ranged between 3.52 to 5.34, where the average value in the sarcopenia and non-sarcopenia groups were 4.20±0.10 and 4.27±0.11, respectively. According to the statistical analysis using the Spearman's Rank test, there was no significant relationship between the Ca/Mg ratio and the incidence of sarcopenia ($p = 0.478$) with $r = -0.146$. Subsequently, this could be due to the numerous risk factors of sarcopenia, such as the deterioration of the neuromuscular junction (NMJ). The NMJ serves as a link between the nervous system and the muscles and it plays a role in the transmission of action potentials from lower motor neurons, which leads to muscle contraction (Bao *et al*., 2020). However, this study did not administer a food questionnaire to participants based on the type of food, frequency of intake and dietary minerals, unlike in the previous report by Verlaan *et al.* (2017), which could have been a confounding factor. Furthermore, this outcome was consistent with Dai *et al.* (2013) in China, which found no significant relationship between the Ca/Mg ratio and the risk of death. However, there was a well-established relationship between the Ca/Mg ratio and its modulatory effects on cancer death risk. In the Chinese population, a Ca/Mg ratio greater than 1.7 was associated with a lower risk of dying from colorectal cancer. Meanwhile, a high Mg intake was associated with a significantly increased risk of dying from colorectal cancer in women among respondents with a Ca/Mg ratio of 1.7 (Dai *et al*., 2013). The limitation of this study was the small sample size, which prompted the need for further studies involving a larger sample size to examine the calcium and magnesium intake of the subjects.

5. Conclusion

This study showed that there was no correlation between the serum Ca/Mg level, body composition and

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Sarcopenia.

Conflict of interest

The authors declare no conflict of interest.

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