Original Article

Myostatin and Sarcopenia in Elderly Among Haemodyalisis Patient

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Abstract

Background and Objective: Increased serum myostatin level might be one of the causes of impaired protein synthesis and protein degradation associated with decreased muscle mass accompanying older age, which is a component of sarcopenia in CKD patients undergoing hemodialysis. There are still very limited number of studies with varying and contradictory results. This study aims to analyze the relationship between serum myostatin and sarcopenia levels in elderly chronic kidney disease patients undergoing hemodialysis at Dr. Soetomo Hospital Surabaya.

Methods: The design of this study is observational analytics using cross-sectional study design. The scope of population is all chronic kidney disease patients aged ≥60 years old who are registered and undergo hemodialysis at Dr. Soetomo Hospital Surabaya. So it gained 40. All data is analyzed with SPSS statistical program version 25.0.

Results: The study subjects experienced sarcopenia as much as 55% and severe sarcopenia 22.5%, while those who experienced presarcompenia and normal by 10% and 12.5% respectively. The median value of serum myostatin levels in this study was 23.75 ng/mL, age range with high myostatin status is obtained in the age group 60-69 years (55%). There was a significant correlation between serum myostatin status with sarcopenia (p=0.002)

Conclusion: There is a significant correlation between serum myostatin status and sarcopenia severity in elderly undergoing continuous hemodialysis. The higher the serum myostatin status, the higher the degree of sarcopenia.

Keywords: elderly, hemodialysis, serum myostatin, sarcopenia.

Introduction

The human body is constantly in need of oxygen Chronic Kidney Disease (CKD) is increasing in the elderly population with a prevalence of around 44% and is considered a significant new problem in geriatrics.[1] The metabolic imbalance associated with CKD leads to an increase in protein catabolism which results in a decrease in muscle sufferers to more easily experience a progressive loss of muscle mass and muscle strength called sarcopenia.[2] The proportion of sarcopenia itself in geriatrics in Surabaya in 2018 using the AWGS (The Asian Working Group for Sarcopenia) criteria was 41.8%.[3] In end-stage chronic kidney disease undergoing continuous hemodialysis (HD), muscle function is more decreased than pre-dialysis.[4] During HD there is an increase in muscle proteolysis, about 10 grams of amino acids are lost permanently during the HD process, which

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will cause a loss of muscle mass of 1% per year, so that elderly patients undergoing HD are more prone to suffer from sarcopenia.[5]

Myostatin was the first myokine identified in 1997 with a molecular weight of 25kD as a negative regulator of skeletal muscle growth, a member of the transforming growth factor beta (TGF-β) group. Myostatin signals through activin type IIB receptor (ActRIIB) which circulates in the blood and is expressed at various sites and circulates in the blood forming a heterodimer with activin-like kinase 4 (ALK4) or ALK5 in skeletal muscle which ultimately forms the Smad complex which will translocate to the nucleus to regulate gene transcription in the proliferation and differentiation of skeletal muscle precursor cells, as well as protein degradation in mature muscle fibers. Activation of Smad2 and 3 by myostatin also inhibits the mammalian target of rapamycin (mTOR) in response to pro-growth signals and suppresses protein synthesis.[6-8] Serum myostatin levels have also been found to be elevated in CKD elderly people who have undergone hemodialysis.[9] There are no data regarding myostatin and its relationship with sarcopenia in elderly CKD patients undergoing hemodialysis in Indonesia.

Previous study by Kim *et al.*[10] showed the prevalence of sarcopenia among community-dwelling older individuals varied depending on which components of the revised EWGSOP2 definition were used, such as the tools used to measure muscle strength and the ASM indicators for low muscle mass. While, Kang *et al.*[11] conclude that in older adults with diabetes, muscle mass index and muscle strength were lower than in those without diabetes. However, the prevalence of sarcopenia and frailty was higher and physical performance was lower only in women with diabetes. Swan *et al.*[12] showed that overall, 23.4% of the population had probable sarcopenia and was significantly higher in the subset with low compared with high SEP (28.9% vs 18.1%, p<0.001). In contrast with this study which aims to analyze the relationship between serum myostatin and sarcopenia levels in elderly chronic kidney disease patients undergoing hemodialysis

Research Methodology

The design of this study was analytic observational using an analytic cross-sectional design to analyze the relationship between myostatin and sarcopenia in elderly chronic kidney disease patients undergoing hemodialysis at Dr. Soetomo Hospital Surabaya.

The affordable population is all chronic kidney disease patients aged 60 years who are registered and undergoing hemodialysis at Dr. Soetomo Hospital Surabaya. The sample of this study were all patients with chronic kidney disease aged 60 years who were registered and undergoing hemodialysis at Dr. Soetomo Hospital Surabaya who met the inclusion criteria and did not meet the exclusion criteria from the study. So that obtained 40 people.

Inclusion Criteria

a. All male and female chronic kidney disease patients aged 60 years who were enrolled and undergoing hemodialysis at RSUD Dr. Soetomo Surabaya
b. Able to communicate actively
c. Willing to participate in research and sign an informed consent.

Exclusion Criteria

a. Unable to understand examination instructions (severe cognitive decline with MMSE score < 18 and severe functional impairment with ADL≤ 8 score).
b. History of recent fractures and/or history of surgery <6 months.
c. History or currently suffering from stroke or history of severe motor disorders (paresis, paralysis).

Measurement of Biomarkers

Serum myostatin is a hormone that plays an important role in growth, measured using a Rayto device with the Enzyme-linked Immunosorbent Assay (ELISA) method in ng/ml units.

Muscle Mass Measurement

Muscle mass is appendicular skeletal mass (ASM) divided by height squared. Appendicular skeletal mass is obtained from the percentage of muscle mass in the upper and lower extremities as measured by using bioimpedance analysis brand Omron Karada Scan model HBF 362. The results of muscle mass in men are said to be low if <7 kg/m2, while in women <5.7 kg / m2.[13]

Handgrip Strength Measurement

Muscle strength using hand grip strength was measured 3 times with the highest hand grip strength taken using the Dynamometer TKK 5001 Grip A. Data collection was carried out at a distance of 30 minutes. Subjects were prepared to stand and grip the dynamometer with the dominant hand and the arm should not be flexed. Muscle grip strength is declared low if <26kg for men and <18kg for women.[13]

Physical Performance Measurement

Physical Performance by measuring walking speed by walking 6 meters divided by the time it takes. The method of measurement is that the inspection distance is calculated with a meter and marked with a measuring tape. Running time is calculated with a stopwatch. Physical performance is declared low if 0.8 m/s.[13]

All data will be analyzed by statistical program SPSS version 25.0. Data on the basic characteristics of

research subjects will be presented descriptively in the form of tables and diagrams covering age, education level, occupation, economic status, nutritional status, comorbidities, geriatric profile of research subjects. The presentation is in the form of frequency and percentage for categorical data types (ordinal) while the median, average and standard deviation are for numeric data types (ratio). The type of data is ordinal for sarcopenia and ratio data for serum myostatin levels so that the Anova test was carried out (if the data is normally distributed) or Kruskal Wallis (if the data is not normally distributed) to compare serum myostatin levels between no sarcopenia, pre sarcopenia, sarcopenia and severe sarcopenia. (2) The data of this research can be useful for determining the correlation between serum myostatin status and sarcopenia severity in elderly undergoing continuous hemodialysis.

Finding of Research

General Characteristics of Research Subjects

Overall demographic data, geriatric assessment and biochemical data for a total sample of 40 subjects are summarized in Table 1.

Table 1: General Profile of Research Subjects

Variable		Total N (%)
Age (years)	median (range)	64 (60-93)
Age range	60-69 years old	35 (87)
	70-79 years old	3 (7.5)
	80 years	2 (5)
Gender	Men	25 (62.5)
	Woman	15 (37.5)
Basic disease	Diabetes	22 (55)
	Hypertension	15 (37.5)
	Kidney stones	3 (7.5)
BMI	Underweight	0 (0)
	Normal	33 (82.5)
	Overweight	7 (17.5)
	Obesity	0 (0)
MNA Score	Normal	8 (20)
	At risk of malnutrition	32 (80)
	Malnutrition	0 (0)
MMSE Score	Normal	31 (77.5)
	Mild cognitive impairment	9 (22.5)
	Severe cognitive impairment	0 (0)
GDS Score	Normal	29 (72.5)
	Possible depression	11 (27.5)
	Depression	0 (0)
ADL Score	Independent	26 (65)
	Mild dependency	11 (27.5)
	Moderate dependency	3 (7.5)
	Severe dependency	0 (0)
	Total dependency	0 (0)
PASE Score	Low	31 (77.5)
	Mild	9 (22.5)
	Moderate-intense	0 (0)
HD duration (years)	median (range)	4 (0.5-21)
HD Frequency	2x a week	40 (100)
	1x a week	0 (0)
1x HD duration (hours)	median (range)	4.5 (4-5)
HD duration a week (hours)	median (range)	9 (8-10)
Transfusion history in the last 3 months	Yes	21 (52.5)
	Not	19 (47.5)
Received erythropoietin in the last 3 months	Yes	30 (75)
	Not	10 (25)
Hemoglobin (mg/dl)	median (range)	9.15 (5.9-13.4)
BUN (mg/dl)	median (range)	71 (26-105)
Serum creatinine (mg/dl)	median (range)	12.2 (4.4-17.4)
Transferrin saturation (%)	median (range)	30.9 (12.66-86.96)
Albumin (mg/dl)	median (range)	3.39 (2.76-3.8)

Subjects with an age range of 60-69 years were the largest age group, namely 35 people (87%). The proportion of men is greater than women by 25 people (62.5%) and 15 people (37.5%). The basic disease of diabetes is the

most basic disease of research subjects by 55%, followed by hypertension by 37.5%. Risk of malnutrition in 80% of subjects based on MNA scores. The MMSE score of the research subjects was still found to be 22.5% having mild cognitive impairment and the possibility of depression was 27.5%. There were still subjects with 7.5% moderate dependence and 27.5% mild dependence (ADL score). Based on the PASE variable, most of the subjects still had a low score of 77.5%. The research subjects had a median of 4 years with all research subjects carrying out HD with a frequency of twice a week. The duration of one HD with a median of 4.5 hours, while the duration of HD in 1 week with a median of 9 hours. In the last 3 months, 52% of the study subjects had received transfusions and 75% had received erythropoietin, but on average the subjects still had a HB 9.15 mg/dL.

Profile of the Degree of Sarcopenia in CKD in the Elderly Undergoing Continuous Hemodialysis

Table 2: Degrees of Sarcopenia of Research Subjects based on the 2014 AWGS

Component of Sarcopenia		Sarcopenia Degrees			
		Normal	Presarcopenia	Sarcopenia	Severe Sarcopenia
		N = 5 (12,5%)	N = 4 (10%)	N = 22 (55%)	N = 9 (22,5%)
Muscle Mass	Normal, N (%)	5 (12,5)	0	0	0
	Low, N (%)	0	4 (10)	22(55)	9 (22,5)
Handgrip Strength	Normal, N (%)	5 (12,5)	4 (10)	9(22,5)	0
	Low, N (%)	0	0	13(32,5)	9 (22,5)
Physical Performance	Normal, N (%)	3 (7,5)	4 (10)	14(35)	0
	Low, N (%)	2 (5)	0	8(20)	9 (22,5)

Table 2 shows that most of the subjects aged patients with CKD who underwent continuous HD had 55% sarcopenia, 22.5% severe sarcopenia, 10% presarcopenia, and only 12.5% had no sarcopenia. In the muscle mass component, only 12.5% of subjects did not experience a decrease in muscle mass. In the hand grip strength component, 55% of the subjects had low hand grip strength. On the physical performance component, it can be seen that 5% of subjects who did not suffer from sarcopenia had decreased physical performance.

Myostatin Serum Profile in CKD Elderly Undergoing Continuous Hemodialysis

In this study, the minimum serum myostatin level was 13.08 ng/mL and the maximum level was 51.10 ng/mL. The median for myostatin in this study was 23.75 ng/mL. The results of this study showed that the median myostatin was found to be higher in women than in men (33.79 ng/mL vs 23 ng/mL). In the age group profile, the median myostatin age 70 years was higher than the age < 70 years, but it was not significant.

Table 3: Data AnalysisProfile of Study Subjects based on Serum Myostatin Status

Variable		Myostatin status (ng/mL)		P value
		median	Range	
Profile based on sociodemographic variables				
Gender	Male (25)	23	(13.08 – 51.1)	0.158
	Female (15)	33.79	(15.68 - 47.76)	
Age range	60-69 years (35)	23	(13.08 – 51.1)	0.063
	70-79 years (3)	45.91	(39.25-47.74)	
	80 years (2)	27.5	(22.81 – 32.22)	
Basic disease	Diabetes (22)	31.11	(15.23 – 51.1)	0.021
	Hypertension (15)	20.87	(13.08 – 47.74)	0.035
	Kidney stones (3)	23.27	(17.64 – 32.09)	0.626
Profile based on geriatric variables				
MNA Score	Normal (8)	19.26	(13.08 – 51.1)	0.156
	At risk of malnutrition (32)	28.67	(13.56 – 47.89)	
MMSE Score (9)	Normal (31)	23	(13.08 – 51.1)	0.225
	Mild cognitive impairment 32.74	(15.68 - 47.76)		
ADL	Independent (26)	22. 37	(13.08 – 51.1)	0.232
	Dependency			
	Light (11) 23	(15.16 – 47.76)		
PASE	Moderate Addiction (3)	37.87	(32.2 - 45.91)	
	Low (31)	32.2	(13.56 – 51.10)	0.001
	Light (9)	18.85	(13.08 – 24.22)	
Profile based on hemodialysis variables				
HD duration (years)	4	23.75	(13.08 – 51.1)	0.148
HD duration 1 week (hours)	9	23.75	(13.08 – 51.1)	

Serum Myostatin Status with Sarcopenia in CKD Elderly Undergoing Continuous Hemodialysis

In this study, the results of the myostatin normality test were abnormal, so the test used was Kruskal Wallis to compare four groups of sarcopenia (normal, presarcopenia, sarcopenia, severe sarcopenia). The results of the test showed that there was a significant difference in serum myostatin levels between groups of sarcopenia degrees (p=0.001). It can be concluded that the higher the degree of sarcopenia, the higher the serum myostatin level. The median and range values for each group can be seen in table 4.

Table 4: Analysis of Serum Myostatin Levels with Sarcopenia Degree

Degree of Sarcopenia	Subject (N)	Rate Myostatin Serum		p Value
		median (ng/mL)	Range (ng/mL)	
Normal	5	18.85	(13.08-19.67)	0.001
Presarcopenia	4	19.07	(15.16 – 24.22)	
Sarcopenia	22	24.79	(13.56-51.1)	
Severe Sarcopenia	9	39.25	(22.81-47.89)	
Total	40	23.75	(13.08-51.1)	

In this study, the Mann Whitney test was used to determine the difference because the data distribution was not normal. The results of the Mann Whitney analysis showed that there were statistically significant differences in the normal group with sarcopenia (p=0.013), the normal group with severe sarcopenia (p=0.03), the presarcopenia group and the severe sarcopenia group (p=0.009), and the sarcopenia group. with severe sarcopenia (p=0.007). There was no significant relationship between the normal group with presarcopenia and the presarcopenia group with sarcopenia, each having p = 0.327 and p = 0.102 (p>0.05).

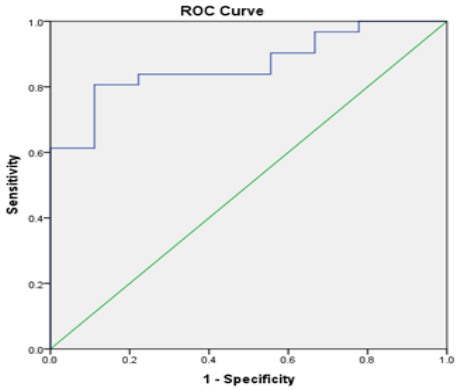

Figure 1: Myostatin ROC curve

The analysis is continued by using the ROC curve. Based on the ROC curve graph, the cut-off value of serum myostatin levels was 20.28 ng/mL. From the results of the cut off, it was concluded that serum myostatin levels 20.28 ng/mL were declared as high myostatin status. High myostatin status was present in 62.5% of the subjects in this study.

After determining high and low myostatin status, a 2x2 fisher test was performed, which can be seen in table 6 to determine the relationship between myostatin status and the degree of sarcopenia. This table shows a significant relationship between serum myostatin status and the degree of sarcopenia, p = 0.002 (<0.05), with a relative risk value (RR) = 2.

Table 5: Fisher's test table 2x2 between Myostatin Serum status and Sarcopenia

	Status of Sarcopenia		P Value	RR Value
	Sarcopenia	No Sarcopenia		
Serum Myostatin Status	High (N) 25 (62,5%)	2 (5%)	0,002	2
	Low (N) 6 (15%)	7 (17,5%)		

Note: No sarcopenia = combination of no sarcopenia and presarcopenia, sarcopenia = combination of sarcopenia and severe sarcopenia.

Discussion

Profile of The Degree of Sarcopenia in CKD in The Elderly Undergoing Continuous Hemodialysis

In this study, most of the elderly subjects with CKD who underwent continuous HD had 55% sarcopenia, 22.5% severe sarcopenia, 10% presarcopenia, and only 12.5% had no sarcopenia. The study conducted by Slee et al.[14] showed similar results, namely the prevalence of sarcopenia in HD patients was 55% with elderly subjects as many as 87 patients. Another study by Lamarca et al.[15] on 102 elderly subjects (>60 years) showed that the prevalence of sarcopenia varied from 3.9% to 63% in CKD according to the method used. It has been previously described that the metabolic imbalance associated with CKD causes an increase in protein catabolism which will result in a decrease in muscle function and mass, so that CKD patients are more prone to suffer from sarcopenia.[16] During HD there is an increase in muscle proteolysis, about 10 grams of amino acids are lost permanently during the HD process, which will cause a loss of muscle mass of 1% per year.[5] This

was also seen in this study, where only 12.5% of the subjects did not experience a decrease in muscle mass.

Myostatin Serum Profile in CKD Elderly Undergoing Continuous Hemodialysis

In this study, the myostatin levels ranged from 13.8 to 51.1 ng/mL, similar results were also shown by a study in continuous HD patients in Taiwan, which was 12.9 – 38.5 ng/mL.[17] In addition, the study conducted by Koyun *et al.*[18] also showed myostatin results from 31.82 to 48.54 ng/mL. The study showed that serum myostatin levels in elderly patients undergoing continuous HD were higher than normal subjects (2.5±2.4 ng/mL, p = 0.001).[18] Patients with CKD had higher myostatin levels than patients who did not suffer from CKD.

The median myostatin in diabetes baseline was 31.11 ng/mL which was significantly significant. According to a research study conducted by Allen *et al.*[19] this is possible because myostatin can increase the glycolysis process, which can cause hyperglycemia resulting in insulin resistance. Myostatin may also affect glucose uptake indirectly through the expression of TNF-a, which has an insulin antagonistic effect.[19] Subjects with underlying hypertension also had a significant difference in serum myostatin levels with those without hypertension, with a median value of 20.87 ng/mL (p = 0.035). According to Verzola *et al.*[20] this may be related to inflammation of the blood vessels caused by myostatin.

Based on the PASE variable, the group of subjects with low PASE had higher myostatin levels compared to the normal PASE group (p = 0.001). A study conducted by Hjorth *et al.*[21] showed that physical exercise can cause changes in myokine secretion so that myostatin expression decreases so that the less physical activity, the higher the serum myostatin level. Other studies have also shown that plasma myostatin levels in healthy young men have shown a significant decrease in the 24 hours after exercise when compared to before exercise.[21-23]

Serum Myostatin Status with Sarcopenia in CKD Elderly Undergoing Continuous Hemodialysis

Myostatin is the first myokine that plays a role in muscle synthesis and degradation.[24] Moorthi *et al.*[25] mention that increased myostatin also contributes to muscle degradation in CKD patients. This effect of myostatin occurs not only on muscle size, but also on muscle mass.[25] In the normal group, all subjects had low serum myostatin status with a median of 18.85 ng/mL. The presarcopenia group had a median of 19.07 ng/mL, this result was higher than the normal group. In the presarcopenia group, there were still subjects who had high myostatin levels, this could be due to some of the presarcopenia subjects having low muscle mass.

The sarcopenia and severe sarcopenia groups had medians of 24.79 ng/mL and 39.35 ng/mL, respectively. If we look at the overall median of the sarcopenia group, it can be concluded that the higher the degree of sarcopenia, the higher the serum myostatin level in elderly CKD patients undergoing continuous HD. This result is also statistically significant, namely p = 0.001 (<0.05). Researchers conducted the Mann Whitney test to determine differences in serum myostatin levels between degrees of sarcopenia. In these data, there were no significant differences in the normal group with presarcopenia. This could be because the median myostatin levels in the two groups were almost the same, as well as the number of study subjects. Significant differences were found in the normal group with sarcopenia and severe sarcopenia. This is in accordance with a study conducted by Aryana *et al.*[24], there was a significant difference in myostatin levels between the normal and sarcopenia groups in the elderly community. Serum myostatin levels in the sarcopenia group with severe sarcopenia showed the same results, this could be due to the large difference in the median values of the two groups.

In this study, the cut off of myostatin was 20.28 ng/mL. After obtaining the cut off value, the researcher can conclude that research subjects with myostatin levels 20.28 ng/mL are called high myostatin status, while those below this value are called low myostatin status. In table 5 it can be seen that the results of the Fisher 2x2 test showed a significant relationship between serum myostatin status and the degree of sarcopenia in elderly patients undergoing continuous HD (p<0.05). High myostatin status has a risk of 2 times to suffer from sarcopenia, this result is indicated by the relative risk in this study of 2. These results are in accordance with a study conducted by Aryana *et al.*[24] which showed that individuals with sarcopenia had serum myostatin levels. higher (OR=3.23; 95% CI=1.49–7.01). In addition, a study in 204 elderly patients by Delanaye et al. (2019) also showed a positive correlation (r=0.37) between serum myostatin levels and sarcopenia in HD patients.

Myostatin/ASM Ratio

Myostatin/ASM ratio is defined to know relative serum myostatin level compared to skeletal muscle mass; serum myostatin level (ng/mL) was divided by ASM (kg). Higher serum myostatin level and myostatin/ASM ratio to be associated with muscle mass and physical performance in elderly Koreans. Mean serum myostatin level in our cohort was similar to that of a previous Japanese study.[27] Fife *et al.*[28] reported that myostatin levels were not associated with muscle mass, but negatively with handgrip strength in 56 older women.

Kang *et al.*[29] reported a positive association between handgrip strength and ASM index in HD patients, although they did not measure serum myostatin level. A higher myostatin/ASM ratio reflects relatively higher myostatin levels in comparison with muscle mass because participants with lower muscle mass can secret less myostatin.[30]

Conclusions and Suggestions

Conclusion

The subjects of this study were mostly dominated by groups with an age range of 60-69 years, male, at risk of malnutrition, and had a median HD duration of 9 hours for 1 week. Therefore, the study subjects who had sarcopenia were 55% and severe sarcopenia was 22.5%, while those who had presarcopenia and normal were 10% and 12.5%, respectively.

Grade median value Serum myostatin in this study was 23.75 ng/mL with a cut off of 20.28 ng/mL. the age range with high myostatin status was found in the age group 60-69 years (55%). From the findings of this study, it can be cocluded that there are Relationship between serum myostatin levels and severity of sarcopenia. The higher the myostatin level, the higher the degree of sarcopenia.

Suggestion

a. Further research using a better design (ex: cohort study)
b. Further research on risk factors affecting serum myostatin levels in elderly patients.

Research Limitations and Weaknesses

The limitations and weaknesses of the study are:
a. This study is a cross sectional type which was conducted at a certain time so that it cannot describe the course of the patient's condition until the increase in serum myostatin levels.
b. This study has several confounding factors that cannot be excluded such as anemia, previous food intake, and physical activity carried out by research subjects

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