Expression Of Interleukin-6 Levels In Elderly Sarcopenia

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Abstract: Background/Aim: Sarcopenia is a syndrome characterized by progressive loss of muscle mass and strength with a risk of adverse clinical outcomes such as physical disability, reduced quality of life, and death. Oxidative stress and inflammatory processes are known as factors that trigger sarcopenia by releasing the catabolic stimuli of IL-6. Based on this we study the dynamics of interleukin-6 (IL-6) in elderly sarcopenia in Wahidin Sudirohusodo Hospital Makassar.Method: This study was an analytic cross-sectional design conducted at the Geriatric Polyclinic of Wahidin Sudirohusodo Hospital, Makassar in March-June 2020. The assessed variables were degree of sarcopenia (probable sarcopenia, sarcopenia, dan severe sarcopenia) based on The European Working Group on Sarcopenia in Older People 2 (EWGSOP2) criteria, age, sex, number of comorbids, and IL-6 levels examination. Statistical analysis was performed by descriptive statistical calculations and frequency distribution as well as the Independent-t statistical test, and Anova test. Results: Studied from 82 subjects (51 females, 62.2%) with the mean of 68.95 years of age. The prevalence of probable sarcopenia was 40.2%, sarcopenia 8.5%, severe sarcopenia 6.1%, and normal 45.1%. IL-6 levels were found to be significantly increased according to the severity of sarcopenia (normal, 52.81 ng/; probable sarcopenia, 67.47 ug/L; sarcopenia, 135.36 ng/L; and severe sarcopenia, 287.99 ng/L). Based on age, IL-6 levels increased significantly with age (60-74 years, 63.28 ng/L; ≥75 years, 139.35 ng/L). Based on the number of comorbids, IL-6 levels increased significantly according to the number of comorbids (1-3 comorbids, 52.86 ng/L; ≥4 comorbids (120.84 ng/L). Conclusion: In the elderly subject, IL-6 levels increases according to the severity of sarcopenia. IL-6 levels also increases with age, and the number of comorbids.

Keywords: Sarcopenia, IL-6, Elderly.

1. INTRODUCTION

Population aging has taken place rapidly, especially in developing countries in the first decades of this century. In Indonesia, in 2010 the proportion of the population over 65 years of age was 5% and by 2035 it will increase to 10.6%. According to the population census in 2010, the number of elderly people (aged) in South Sulawesi was 5.4% and in 2035 it is estimated that it will be almost double as compared to 2010, which is 9.9%. The life

expectancy of the Indonesian population (men and women) has increased from 70.1 years in the 2010-2015 period to 72.2 years in 2030-2035 period (Badan Pusat Statistik,2013).

The increase in life expectancy and well-being of the population in line with the increase in the aging population in Indonesia has led to an increasing trend in various health problems (Badan Pusat Statistik,2013). Changes that occur in aging are an irreversible and inevitable process characterized by changes in the morphology, functionality and biochemistry of the body, including the musculoskeletal system, and the most obvious changes include loss of muscle mass, and reduced muscle strength (Colón et al., 2018). Sarcopenia is a syndrome characterized by progressive loss of muscle mass and muscle strength with adverse clinical outcomes such as physical disability, decreased quality of life, and death. There are several mechanisms involved in the occurrence of sarcopenia including loss of alpha motor neuron axons, lack of physical activity, hormonal changes, impaired nutritional intake, oxidative stress, genetics, and inflammatory processes. There is evidence that pro-inflammatory mediators such as interleukin-6 (IL-6) interact with hormonal changes that occur in the aging process, thereby causing a decrease in physical activity (Aryana et al., 2018). Studies report that levels of pro-inflammatory cytokines such as IL-6 are elevated in elderly sarcopenia (Rong et al., 2018).

Based on the information above, this study aims to determine the expression of IL-6 levels in elderly sarcopenia. This research is still rarely conducted and published in Indonesia. The results of this study are expected to serve as future references for preventive and curative interventions in sarcopenia.

2. METHOD

2.1 Research Subject

This study was an analytic cross-sectional design conducted at the Geriatric Polyclinic of Wahidin Sudirohusodo Hospital, Makassar in March-June 2020. The assessed variables were degree of sarcopenia (probable sarcopenia, sarcopenia, dan severe sarcopenia) based on The European Working Group on Sarcopenia in Older People 2 (EWGSOP2) criteria, age, sex, number of comorbids, and IL-6 levels examination. The elderly patient was explained about the aims of the study and obtained consent.

2.2 Inclusion Criteria and Exclusion Criteria

The inclusion criteria include subjects >60 years old, willing to take part in the study, can walk alone or stand for 5 minutes without other assistive devices. The exclusion criteria include subjects with malignant disease, acute arthritis or acute infection, experience cognitive impairment.

2.3 Data Collection

Data collection was carried out by interview method, anthropometric measurement, handgrip (HG) strength dynamometer examination, muscle mass examination using Bioelectrical Impedance Analysis (BIA), physical performance examination with Time Up And Go test (TUG), and serum IL-6 examination.

2.4 Anthropometry

Anthropometric examination is carried out by measuring body weight (BW) and height (H) based on knee height. Measurement of height (cm) in the elderly using knee height, then converted through the calculation of the formula for Chumlea's knee height.

2.5 Sarcopenia Parameters

Based on the recommendations of EWGSOP2, the parameters of sarcopenia measured in this study were muscle mass, muscle strength, and physical performance. Measurement of muscle mass using the BIA tool by measuring the total skeletal muscle mass (muscle mass of the

upper leg, lower leg) divided by the square of height in meters and expressed in units of kg/m^2. Examination of muscle strength was performed with an HG strength dynamometer, expressed in kilograms (kg), and physical performance checks were measured using the TUG instrument in seconds.

2.6 Diagnostic Criteria

The diagnostic criteria are based on the diagnostic criteria for sarcopenia established by the EWGSOP2. Muscle mass is said to be low if the value of BIA <7.0 kg/m^2 for male subjects and the value of BIA <5.5 kg/m^2 for female subjects. Muscle strength is said to be low if the value of HG strength dynamometer <27 kg for male subjects and the value of HG strength dynamometer <16 kg for female subjects. Physical performance was assessed by the TUG instrument, and said to be low if it was 20 seconds.

2.7 IL-6 Levels

Measurement of levels or concentrations of IL-6 with the ELISA test. IL-6 levels are checked from blood serum. About 3 mL of the subject's venous blood was drawn by the research assistant. Blood in serum was centrifuged at 2000-3000 RPM for 20 minutes and stored at - 80 ° C. IL-6 examination using quantikine HS human IL-6 immunoassay (catalog list no E0090Hu) was measured by the ELISA method in ng/L units. This measurement is carried out once without repetition. The sensitivity of the detected concentration was 1.03 ng/L, and the cut-off value in normal people ranged up to 7 ng/L.

2.8 Statistic Analysis

Statistical analysis was carried out electronically using the SPSS version 22 device. The statistical analysis carried out was descriptive statistical calculations and frequency distribution as well as the Independent-t statistical test, and Anova test. The result of the test is statistically significant if the p value <0.05.

2. 9 Ethical License

Ethical permission has been obtained from the Health Research Ethics Committee of the Hasanuddin University Medical Faculty / Wahidin Sudirohusodo Hospital with letter number: LB.02.01/2.2/4071/2020.

3. RESULTS

3.1 Characteristics Research Subjects

Data analysis was carried out on 82 research subjects, obtained 31 male (37.8%) and 51 female (62.2%). Mean age was 68.95 (6.34) years. The number of subjects in the age range of 60-74 years was 76.8%, and aged ≥75 years was 23.2%. Based on the degree of sarcopenia, 33 people (40.2%) had probable sarcopenia, 7 (8.5%) of sarcopenia, 5 (6.1%) of severe sarcopenia, and 37 (45.1%) normal). Based on the parameters of sarcopenia, 12 people (14.6%) had low muscle mass, 44 people (53.7%) low muscle strength, and 30 (36.6%) low physical performance. The number of comorbids obtained in subjects with a number of 1-3 comorbids was 52 people (63.4%), and with a total of ≥4 comorbids as many as 30 people (36.6%). The mean value of IL-6 obtained in this study was 80.9 (62.7) as seen in Table 1.

Table 1. Characteristics of Research Subjects (n=82)

Variables		n	%
Sex	Male	31	37.8
	Female	51	62.2
Age	60-74 years	63	76.8
	≥ 75 years	19	23.2

Degree of Sarcopenia	Normal	37	45.1	
	Probable Sarcopenia	33	40.2	
	Sarcopenia	7	8.5	
	Severe Sarcopenia	5	6.1	
Muscle Mass	Normal	70	85.4	
	Low	12	14.6	
Muscle Strength	Normal	38	46.3	
	Low	44	53.7	
Physical Performance	Normal	52	63.4	
	Low	30	36.6	
Number of Comorbids	1-3	52	63.4	
	≥ 4	30	36.6	
Interleukin-6 Levels		80.9 (62,7)		

3.2 Description of Interleukin-6 Levels based on Sex, Age, Degree of Sarcopenia, and Number of Comorbids

Interleukin-6 levels were found to be higher in women (84.04) than in men (75.74), but the statistical test results showed that the difference was not significant (p>0.05) as shown in Table 2.

Interleukin-6 levels were found to be higher at age ≥75 years (139,35) than at age 60-74 years (63.28) and there was a visible pattern of increasing IL-6 levels with increasing age. The results of statistical tests showed that this difference was significant (p<0.01) as shown in Table 2.

Levels of IL-6 were found to be the highest in severe sarcopenia (287.99) and lowest in normal (52.81) and this result was statistically significant (p<0.001) as shown in Table 2.

Table 2. Levels of Interleukin-6 based on Sex, Age, Degree of Sarcopenia, and Number of Comorbids

Variables	n	Mean	Standard Deviation	p
Sex				
Male	31	75.74	52.16	0.565
Female	51	84.04	68.70	
Age				
60-74 years	63	63.28	29.39	0.004
≥ 75 years	19	139.35	100.24	
Degree of Sarcopenia				
Normal	37	52.81	9.81	
Probable sarcopenia	33	67.47	29.11	<0.001
Sarcopenia	7	135.36	42.15	
Severe Sarcopenia	5	287.99	47.37	
Number of Comorbids				
1-3	52	57.86	15.85	0.001

≥4	30	120.84	89.18	

Interleukin-6 levels were found to be significantly higher in the comorbid count ≥4 (120.84) compared to the comorbid counts 1-3 (57.86) and this result was statistically significant (p<0.01) as shown in Table 2.

4. DISCUSSION

This study included 82 research subjects with female (62.2%) and male (37.8%) subjects. The study of Riviati et al. found 60.2% women and 39.8% men (Riviati et al., 2017).

The age of the research subjects is 60 years and over. The mean age was 68.95 (6.34) years. The number of subjects in the 60-74 years age range was 6.8%, and at ≥75 years old was 23.2%. Research from Aryana et al. obtained a mean age of 69.52 ± 8.71 years. (Aryana et al., 2018). Meanwhile, the mean age retrieved from the research subjects by Patel et al. was 67 ± 2.6 years (Patel et al., 2013).

The degree of sarcopenia obtained in this study was found to have probable sarcopenia by 40.2%, sarcopenia 8.5%, severe sarcopenia 6.1%, and the remaining 45.1% were normal. In the study of Sobentiansky et al. of the 287 research subjects, it was found that probable sarcopenia was 73% and sarcopenia was 20% (Sobestiansky et al., 2019). Meanwhile, in the study of Pang et al. from 536 study subjects, it was found that probable sarcopenia was 15.3%, sarcopenia was 15.5%, and severe sarcopenia was 10.1% (Pang et al., 2020). From the results of this study, there was quite a lot of probable sarcopenia (40.2%), and this suggests that it turns out that quite a lot of subjects are at risk of sarcopenia, therefore, it is necessary to immediately conduct an assessment to find the causative factors and initiate effective early interventions, so that the incidence of sarcopenia can be prevented. This is consistent with the algorithm from EWGSOP2 for the earliest possible diagnosis and intervention (Cruz-Jentoft et al., 2019).

Of the 82 research subjects, 70 people (85.4%), subjects with normal muscle mass and 12 (14.6%), subjects with low muscle mass. Research by Bian et al. of the 441 study subjects, 82% of subjects with normal muscle mass and 18% of subjects with low muscle mass (Bian et al., 2017). As many as 38 people (46.3%), subjects with normal muscle strength and 44 people (53.7%), subjects with low muscle strength. The research of Riviati et al. at the age of more than 75 years, there were 56.5% subjects with low muscle strength and 43.5% subjects with normal muscle strength (Riviati et al., 2017). A total of 52 people (63.4%), subjects with normal physical performance and 30 people (36.6%), subjects with low physical performance. Research by Guedes et al. was found that subjects with low physical performance were 34.3%, while subjects with normal physical performance were 65.7% (Guedes et al., 2019).

A total of 52 people (63.4%), subjects had a comorbid number of 1-3. From the research of Bahat et al., The average number of comorbids was 2,6 (Bahat et al., 2013). Research by Aryana et al. was done by excluding comorbids or done without any comorbids. This is because this study used a clinical-based setting where the subject came to the polyclinic with various comorbid diseases, while the research of Aryana et al. was community-based (Aryana et al., 2018). The most comorbid diseases in this study were hypertension (85.4%) and Diabetes Mellitus (70.7%). The research of Riviati et al. obtained comorbid hypertension (78.7%) and diabetes mellitus (44.3%) (Riviati et al., 2017). In this study there was no analysis of the subjects with comorbid diseases. In the aging process, there is an increase in

8-hydroxy-2-deoxyguanosine (8-OH-dG) which is a free radical marker. It is also found in hypertension and diabetes mellitus (Nemtsova et al., 2018).

The mean IL-6 level in this study was 80.9 (62.7). Research by Bian et al., the mean IL-6 level in the group with decreased muscle mass was 49.77 (22.14) and in the group with no decreased muscle mass was 39.72 (29.53) (Bian et al., 2017). The increase in IL-6 levels was influenced by various factors. such as age, gender, comorbid diseases (Maggio et al., 2006).

Description of Interleukin-6 Levels by Sex

In this study, it was found that IL-6 levels were higher in female than male, but this difference was not statistically significant (p>0.05). Research by Aureli et al. found that the IL-6 levels were significantly higher in women than in men (Aureli et al., 2014). In this study, it was found that there was no significant relationship between sex and IL-6 levels. These results differ from some literature whereby sex association with IL-6 levels suggests that decreased production and levels of circulating sex hormones cause a mild pro-inflammatory state in the elderly (Maggio et al., 2006). One in-vitro study found that estrogen increases the synthesis of pro-inflammatory cytokines in cells that resemble macrophages (O'Connor et al., 2007).

Description of Interleukin-6 levels based on Age

In this study, it was found that IL-6 levels were higher at ≥75 years of age and lower at the age of 60-74 years and there was a statistically significant pattern of increasing IL-6 levels with increasing age (p<0.01). In the study by Kuznicka et al. it was also found that there are significantly higher IL-6 levels at older age, and this occurred in the entire study group (p<0.001) (Kuźnicka et al., 2016). Age-related increase in IL-6 levels were due to overproduction of oxygen-free radicals, which stimulate IL-6 production (Maggio et al., 2006).

Description of Interleukin-6 levels based on Degree of Sarcopenia

In this study, it was found statistically significant (p<0.001) that IL-6 levels increased according to the severity of sarcopenia. So the more severe the degree of sarcopenia, the higher the IL-6 levels. Based on the EWGSOP2 definition, probable sarcopenia is a decrease in muscle strength, sarcopenia is a decrease in muscle strength and a decrease in muscle mass, and severe sarcopenia is a decrease in muscle strength, a decrease in muscle mass and a decrease in physical performance. High levels of IL-6 result in decreased muscle mass, muscle strength and physical performance (Aryana et al., 2018; Visser et al., 2002; Grosicki et al., 2020).

Description of Interleukin-6 levels based on Number of Comorbids

In this study, it was found statistically significant (p<0.01) that IL-6 levels increased according with the number of comorbids. The physiological role of IL-6 has been mostly studied in the context of the acute phase response, although there is growing evidence that IL-6 also plays a central role in the pathogenesis of chronic disease. In chronic inflammation, IL-6 has a detrimental role that favours mononuclear cell accumulation at the site of injury (Maggio et al., 2006).

The inflammatory reaction is only one of the factors causing sarcopenia. Other factors include nutritional problems and decreased hormone levels in the body. Sarcopenia will be one of the main causes of disability and decreased quality of life in the elderly. In recent years, although many experts have explored this in various aspects, the pathological mechanism remains unclear and there are no standard diagnostic criteria. To determine the effective method to prevent and treat this disease, it is necessary to conduct a more large-scale clinical study (Bian et al., 2017). Another limitation of our study is the clinical-based setting where patients who come to the polyclinic with various comorbids, and hope that

further community-based research can be carried out within healthy individuals in the future with few or no comorbids so as not to bias IL-6 levels.

5. CONCLUSION

In the elderly subject, IL-6 levels increases according to the severity of sarcopenia. IL-6 levels also increases with age, and the number of comorbids.

Source of Fundling

This study used private funds.

Ethical Clearance

Ethical permission has been obtained from the Health Research Ethics Committee of the Hasanuddin University Medical Faculty / Wahidin Sudirohusodo Hospital with letter number: LB.02.01/2.2/4071/2020.

Competing Interests Statement

No potential conflict of interest relevant to be declared.

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