Inflammatory Cytokines and Sarcopenia in Iranian Adults-results from SARIR Study

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Abstract

Background

Some studies emphasize the effects of inflammatory cytokines in reducing muscle mass and muscle strength and performance. This study aimed to compare pro-inflammatory cytokines in sarcopenic and non-sarcopenic subjects.

Method

The present study used data from the "Sarcopenia and its Determinants Among Iranian Elders" (SARIR) study. Three hundred men and women aged ≥ 55 years old who lived in the 6th district of Tehran were selected using random cluster sampling. We measured all components by using standard protocols, in addition we defined sarcopenia based on former and new European Working Group on Sarcopenia in Older People (EWGSOP) guidelines. A fasting blood sample was taken from each participant to measure serum high-sensitivity C-reactive protein (hs- CRP), Interleukin 6 (IL-6), and tumor necrosis factor α (TNFα).

Results

Mean age and body mass index (BMI) of study participants were 66.7 ± 7.7 years and 27.3 ± 4.2 kg/m2, respectively. Thirty-one participants of the present study had the criteria of EWGSOP2-sarcopenia. A statistically significant difference was seen between normal and abnormal groups of muscle strength in hs-CRP (p-value =0.04). Furthermore, we did not observe a remarkable association between inflammatory biomarkers including IL-6, TNF-α and hs-CRP and risk of sarcopenia even after controlling for plausible confounders (OR: 1.15; 95% CI 0.31-4.28, OR 0.68; 95% CI 0.17-2.77 and OR 2.39; 95% CI 0.87-6.55).

Conclusion

We found that inflammatory biomarkers level was not considerably associated with odds of sarcopenia. Lack of correlation between inflammatory cytokines and sarcopenia could be due to participant's age and genetic. Future studies are required to confirm these findings.

What Is Already Known About This Topic?

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The aging population is increasing worldwide, and sarcopenia is one of the most critical complications in geriatrics. Some studies have shown that an increased level of inflammatory markers was associated with a higher degree of sarcopenia.

What does this article add?

In the present study, we revealed that the level of inflammatory biomarkers such as hs-CRP, IL-6, and TNF-α was not noticeably associated with the risk of sarcopenia.

Introduction

Aging is rapidly increasing in World's population [1] and is associated with reduced physical function with negative impacts on quality of life and independence in personal activities [2]. Sarcopenia or age-related skeletal muscle loss is one of the critical issues in geriatric medicine. Based on the new definition provided by the European Working Group On Sarcopenia (EWGSOP), sarcopenia is the presence of both low muscle mass and low muscle function (including strength or performance) [3]. Sarcopenia can result in disabilities and an increased risk of frailty, falls, fractures, and other consequences that burden society and health care services [4–6]. Some factors involved in the process of sarcopenia have been poor nutrition and physical inactivity [7].

Some physiological changes related to aging, including oxidative stress, abnormal anabolic hormone production, neuromuscular atrophy, and inflammatory factors, are associated with decreased muscle mass and strength [8]. High levels of inflammatory factors have been associated with an increased odds of disability [9,10] and mortality [11] in older adults. There are various hypotheses proposed to examine the effect of inflammation on physical performance, including the catabolic effects of inflammatory cytokines on muscle [2]. Some studies showed that increasing levels of inflammatory markers such as IL-6, TNF-a, and CRP in older adults are associated with the increased loss of muscle loss and progression of sarcopenia [12–14]. Moreover, Taaffe et al. indicated a reverse relationship between CRP level and handgrip strength, though high baseline concentrations did not predict a 7-year later decrease in performance and grip strength (4).

It is crucial to investigate the relationship between inflammatory factors and muscle performance, strength, and mass due to the high prevalence of sarcopenia in Asia. So in this cross-sectional study, we compare inflammatory factors levels, including IL6, CRP, and TNFα, between sarcopenia and non-sarcopenia in Iranian adults.

Materials And Method

This population-based cross-sectional study was carried out between May and October 2011 in Tehran, Iran. The details of the sampling method and data collection procedure were reported previously. By using random cluster sampling, a selection of 300 elderly males and females (≥55 years old) from the 6th district of Tehran was performed. The head of each 30 clusters according to a ten-digit postal code was selected. Subjects who could move without walkers, crutches, or any other assistive device and did not have any active cancers (according to self-reported data) were recruited in the present study.

The current study excluded individuals with artificial limbs or prosthesis limbs and those who had a debilitating disease such as organ failure. This study was performed based on the guidelines of Declaration of Helsinki. The study protocol was accepted by the TUMS (Tehran University of Medical Sciences) ethics committee. All subjects were confirmed the written informed consent before being data collection.

Assessment of hand grip

Handgrip test was done using a pneumatic instrument—a squeeze bulb dynamometer (manufactured by Jamar, Inc. USA: c7489-02 Rolyan) calibrated in pounds per square inch (psi)— to scale the muscle strength. Participants were requested to sit in a straight-backed chair; indeed, their shoulders adducted in neutral, arms unsupported, and elbows flexed at 90°. The measurement of maximum voluntary contraction (handgrip strength) was done three times with 30 seconds rest in between measurements for each left and right hand. The average measurements of

the subjects' hands were defined as muscle strength. To identify low muscle strength for each participant, age and sex-specific thresholds were used. These amounts differed from EWGSOP1 cutoff points; indeed, handgrip strength < 30 kg for men and < 20 kg for women were detected as low grip strength. In the recent definition, these cutoff points were altered to < 27 kg and < 16 kg for men and women, respectively.

Assessment of muscle performance

Muscle mass was measured by dual x-ray absorptiometry (DXA). DXA was used to assess fat, lean tissues, and bone as an attractive alternative method for research. This device can measure muscle mass, bone mass, fat mass, and trunk with minimal radiation exposure. We asked each individual to walk at his/her usual pace to measure the muscle performance using a 4-meter walk gait speed test. Subjects with gait speeds lower than 0.8 m/s were detected(identified) as having abnormal muscle performance.

Sarcopenia determination

The current study used both former and latter European Working Group on Sarcopenia in Older People (EWGSOP) guidelines for sarcopenia definition. EWGSOP1 suggests considering both low muscle function and mass (either performance or strength) in the definition. Based on EWGSOP2, low muscle strength has the most crucial role in sarcopenia detection. Additionally, this definition uses low muscle quantity and quality to verify the diagnosis and considers poor physical performance indicative of severe sarcopenia.

Assessment of biomarkers

Blood samples were taken after 12-hour fasting. Serums were kept at -80°C for performing the ELISA test in the Endocrinology and Metabolism Research Center of Tehran University in specialized laboratories. The ELISA method was used to measure serum IL-6 and TNF-α levels using commercial kits (I.D. labs Canadian company), and the hs-CRP test was performed using Pars Azmoon kit. All the assessments were done in duplicate for all inflammatory cytokine measures.

Assessment of other variables

A trained dietitian has collected participants' general information on age, sex, history of disease, medication use, smoking habits, socio-economic status, and alcohol consumption. To examine the dietary intake of subjects a 117-item Food Frequency Questionnaire (FFQ) was applied in the present study. The reliability and validity of this questionnaire were examined in recent studies. Furthermore, a short form of the International Physical Activity Questionnaire (IPAQ) was used to assess the physical activity level based on metabolic equivalent hours per week (MET-h/week). In addition, previously, the validity of IPAQ in the elderly population has been evaluated. A digital scale was used for weight measuring; indeed, participants' clothing was minimal. Height was assessed by using a wall tape measure in the standing position without shoes. Body mass index (BMI) was computed by dividing weight (kg) by height squared (m2).

Statistical analysis

Our statistical analyses were performed using SPSS software Version 16(SPSS Inc., Chicago, IL, USA). Normally distributed variables were described using mean and standard deviation. The general characteristics of the study individuals were compared using one-way analysis of variance and chi-squared tests. The distribution of inflammatory markers was examined using the Kolmogorov-Smirnov test since plasma levels of inflammatory

markers were not normally distributed. The analysis was performed using their log values. T-test and Mann Whitney test were used to compare two groups' hs-CRP, IL6 and TNFα mean, respectively. multivariate logistic regression was applied to find the relationship between inflammatory cytokines and risk of sarcopenia. In the first statistical model, we adjusted for age (years) and sex (male/female). Further controlling was performed for physical activity smoking (yes/no), (MET-h/week), alcohol consumption (yes/no), medication use (yes/no), and history of the disease (yes/no) in the second model. hs-CRP, IL-6, and TNF-α were categorized based on detection limits (5 mg/l for hs-CRP, 10 pg/ml for IL-6, and 8 pg/ml for TNF-α), which levels above the detection limits assumed in the high category. Our statistical analyses were performed using SPSS software Version 16 (SPSS Inc., Chicago, IL, USA). P values less than 0.05 were considered statistically significant.

Results

General characteristics of participants have been demonstrated in Table 1. 50% of participants in the sarcopenia group and 48% of participants in the non-sarcopenia group were female. 27.5% of the sarcopenia group had a medical history versus 40% in the non-sarcopenia group. Regarding taking medication, 5% of sarcopenia and 2.5% of the non-sarcopenia group were using Sexual hormones, and 2.5% of both groups were taking corticosteroids. In the sarcopenia group population, handgrip strength was significantly lower than the control group.

Table 1
baseline Characteristic of the study participants, Mean ± S.D.

	Sarcopenia (N=40)	Non-sarcopenia (N=80)	P-value
Age (year)	67.1±7.9	66.6±8/1	0.67**
Weight (Kg)	62.4±11.2	73.8±12.1	0.76
Height (Cm)	160.4±9.5	161±9	0.50
Body mass index (Kg/m2)	26.8±17.7	28.2±4.4	0.18
Handgrip strength (Psi)	9.6±2.9	11.4±3.8	0.03
4-m gait speed test (m/s)	0.78±0.2	0.84±0.2	0.08
Appendicular skeletal muscle (Kg)	15.6±4.6	17.8±3.8	0.54
Muscle Index (Kg/m2) *	8.2±14.9	6.7±0.9	0.38
physical activity (met. Hour/week)	1260.7±1450.5	1140.6±1034.1	0.60
Marital status (%married)	85	78.8	0.08
Gender (%female)	50	48.8	0.89
Education status (%)			
History of alcohol use in past six months			0.55
Never (%)	85	88.8	
Sometimes (%)	15	11.3	
History of cigarette smoking use in past six months			0.44
Never (%)	85	83.3	
Always (%)	15	12.5	
Medical history			0.17
No (%)	72.5	60	
Yes (%)	27.5	40	
Drug history			
Sexual hormone use (%)	5	2.5	0.47
Statin use (%)	42.5	32.5	0.28
Corticosteroid use (%)	2.5	2.5	1.00

*Muscle index =appendicular skeletal muscle/height^2, **Pvalue <0.05 significant

Comparison of inflammatory cytokines between normal and abnormal components of sarcopenia, including handgrip strength, muscle mass, and gait speed test, is shown in Table 2. These results showed a statistically

significant difference in serum hs-CRP between normal and abnormal handgrip strength groups (P-Value = 0.04). There was no statistically significant difference in other groups. Linear regression analysis of the relationship between inflammatory biomarkers and risk of sarcopenia is represented in Table 3. In the crude model, there was no association between hs-CRP levels and risk of sarcopenia (OR: 1.02; 95% CI 0.32, 3.24). In addition, after adjustment for plausible confounders, this association remained non-significant (OR: 1.15; 95% CI 0.31, 4.28). Moreover, we did not observe considerable association between IL-6 and TNF- and risk of sarcopenia either before (OR 0.90; 95% CI 0.25, 3.13, OR 1.60; 95% CI 0.73, 3.48) or after controlling for potential confounders (OR 0.68; 95% CI 0.17, 2.77, OR 2.39; 95% CI 0.87, 6.55).

Table 2
Comparison of inflammatory cytokine between normal and abnormal muscle mass, handgrip strength, and gait speed

	Muscle mass			Handgrip strength			Gait speed test		
Inflammatory factor	normal	abnormal	p*	normal	abnormal	p*	normal	abnormal	p*
hs-CRP (mg/dl)	2.7±0.3	11.0±1.4	0.1	2.6±0.4	3.7±1.2	0.04	2.6±0.4	11.2±1.5	0.6
TNFα (Pg/dl)	33.2±9.2	34.8±9.6	0.8	21.4±4.5	42.1±10.4	0.5	36.5±10.0	31.0±8.5	0.6
IL6 (Pg/dl)	10.7±0.3	10.6±0.3	0.3	10.6±0.4	10.7±0.2	0.8	10.6±0.3	10.8±0.3	0.2
*P-value <0.05 significant									

Table 3

Multivariable-adjusted odds ratios (95% C.I.s) for sarcopenia* and inflammatory biomarkers.

	Crude		Model 1		Model 2	
	OR** (95% CI)	P_{value}	OR (95% CI)	P_{value}	OR (95% CI)	P_{value}
CRP	1.02 (0.32, 3.24)	0.96	1.00 (0.31, 3.17)	0.99	1.15 (0.31, 4.28)	0.82
IL-6	0.90 (0.25, 3.13)	0.87	0.91 (0.26, 3.17)	0.88	0.68 (0.17, 2.77)	0.59
TNF-α	1.6 (0.73, 3.48)	0.23	1.69 (0.76, 3.75)	0.19	2.39 (0.87, 6.55)	0.08
*Sarcopenia was defined based on the European Working Group on Sarcopenia in Older People 2 (EWGSOP2) definition						
**The analysis of binary logistic regression was used to determine the OR and 95% confidence interval.						
Model 1: Adjusted for age (continuous), sex (male/female)						
Model 2: Further adjusted for physical activity, smoking (yes/no), alcohol consumption (yes/no), drug history (yes/no), and positive history of the disease (yes/no).						

Discussion

In the current study we investigated the association between sarcopenia and control groups on inflammatory markers like TNF-a, il-6, and hs-CRP. The findings showed that the levels of TNF-a and IL-6 between normal and abnormal groups of muscle mass and muscle performance were not significant. However, a significant difference in hs-CRP level between normal and abnormal muscle strength groups was observed. Several previous studies showed that inflammation plays a vital role in the development of sarcopenia. A longitudinal aging study in Amsterdam revealed a positive association between higher levels of IL-6 and CRP and elevated risk of muscle strength loss but not muscle mass [13].

Moreover, in another prospective cohort study, this author found that increased levels of inflammatory markers were significantly correlated with a more 5-year decrease in the thigh muscle area. Higher TNF-a and its soluble receptors investigated the most consistent relationship with lower grip strength and muscle mass [12]. Zhao et al. In a cross-sectional study on middle-aged and older adults indicated a higher platelet-to-lymphocyte ratio (PLR), neutrophil-to-lymphocyte ratio (NLR), and systemic immune-inflammation index (SII), were associated with increased odds of sarcopenia [15]. In support of these findings, a systematic review and meta-analysis that evaluated the association of markers of inflammation with muscle strength and muscle mass found a significant relationship between increased levels of circulating TNF-α, CRP, and IL-6 with a decline in muscle mass, handgrip and knee extension strength [16]. On the other hand, a systematic review and meta-analysis of cross-sectional studies investigated that subjects with sarcopenia compared with controls have higher levels of serum hs-CRP. However, levels of IL-6 and TNF-α were not significantly different [17]. TNF-α, CRP, and IL-6 are inflammatory markers that are suitable indicators to show the association of inflammatory status with sarcopenia and its components.

CRP is an indicator of acute and chronic phase inflammation and increases chronic diseases such as type 2 diabetes, cardiovascular disease, and sarcopenia. Muscle mass, muscle strength, and physical performance decline [17–19]. Increased level of CRP influences muscle cell size by suppression of muscle protein synthesis pathway [20]. High levels of CRP are associated with obesity and insulin resistance [21]. It seems that there is a direct relation between the mechanical and metabolic performance of aged muscles. Increasing CRP levels can increase insulin resistance, which disturbs muscles' metabolic function, and as a result, mechanical function is also impaired [22]. Although the reason is unknown, the mechanism of cellular and molecular changes in sarcopenia and insulin resistance are the same. In both cases, there is an accumulation of fat in muscle fibers which can affect the insulin pathway [23]. Furthermore, disruption of a critical protein in muscles, such as myosin heavy chain, can be seen in insulin resistance and aging muscles [24,25]. However, the exact effect of CRP on muscle atrophy is still unknown [16].

IL-6 is an Inflammatory cytokine secreted by immune cells in tissue damage or infection conditions [16]. Also, IL-6 is known as a myokine, which is produced by skeletal muscle and regulates muscle contraction and metabolism [26,27]. Some studies showed that temporal and low levels of IL-6 could be beneficial [28]; however, it is well understood that chronic exposure to IL-6 may result in muscle atrophy and facilitate muscle catabolism [29]. TNFα is a pro-inflammatory cytokine and has been associated with muscle pathology [30]. Muscle catabolism in various inflammatory diseases, including congestive heart failure [31], cancer [32], and chronic obstructive pulmonary disease (COPD) [33], has been attributed to TNF-α. Studies investigate that TNF-α interferes with the muscle differentiation process and can elevate catabolism in mature cells. Also, TNF-α, through another pathway mediated by reactive oxygen species (ROS) and nuclear factor-kappa B, promotes muscle wasting [34]. In the present study, we found no significant association between inflammatory markers and sarcopenia and its components, including muscle mass

and muscle performance. We have just seen a significant association between normal and abnormal muscle strength groups evaluated based on handgrip strength. Our findings could be related to the limited number of participants in this study, and more longitudinal studies are needed to clarify our findings. Also, this inconsistency between our results and previous studies may be due to racial and age differences in the current study.

It may be because of the way that cytokines were measured. The measurement of plasma inflammatory cytokines may not suffice to determine the differences, and cellular cytokines must be measured. Inflammation statuses such as disability, neurodegenerative processes, and aging-related hormonal changes can be other causes of sarcopenia.[35]

Our study has some limitations that need to be considered: Insufficient budget, which affected the sample size, and this limited number of participants affects the validity of the results and should be considered to interpret our findings. The causality association between inflammatory markers and sarcopenia cannot be identified due to the study's cross-sectional design. Moreover, because of using FFQ for dietary assessment, recall bias and measurement error were inevitable. Finally, we conducted the present study on older adults, so generalization of these results to other age groups should be cautious.

Conclusion

There was no significant association between TNF-a, il-6, and hs-CRP with sarcopenia, muscle mass, and performance. However, we found a significant difference between serum level of hs-CRP and muscle strength. More prospective studies are needed to provide further insights into the association between these inflammatory markers and sarcopenia.

Declarations

Author contributions

F.A., F.D., SR, RHe, MB, Z.J., and RHa contributed to the design, conception, data acquisition, analysis, and interpretation of the data, manuscript writing, and review of the final version of the manuscript. All authors approved the submitted version.

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Declaration of interest

None

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Research ethics and patient consent

The study protocol was approved by the Tehran University of Medical Sciences ethics committee. We explained the study's aims to the participants at first and then requested all participants to complete a written informed consent before data collection.

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