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Detection Some Biomarkkers In Sarcopenia Patients-Basra City

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Abstract

Background: Sarcopenia is a reduction in the rate, strength, and function of skeletal muscle mass that occurs mainly during aging, reduced physical activity, inflammation, and, or as a result of oxidative stress. C-terminal agrin fragment (CAF), procollagen type 3 N-terminal peptide (P3NT), and myostatin circulate biomarkers in elderly people associated with skeletal mass. Interleukin 6 (IL-6) is also a circulating inflammation marker that contributes to the release of hs-CRP and alpha1 antichymotrypsin (AACT).

The objective of Research: To define the differences between certain biomarkers in the sarcopenic subject, the study compared the findings with those of the non-sarcopenic subject that may support awareness of the sarcopenia principles.

Materials and Methods: The study was conducted on 170 participants living in Basra city. The weight (kilogram)/height (meter) ^{2} ***equation was used in the BMI calculation. Total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, and calcium were estimated by a fully automated biochemistry analyzer using enzymatic methods. VLDL-cholesterol was tallied by using the Fridwald equation. Vitamin D, IL-6, myostatin, CAF, P3NT, AACT, and hs-CRP were measured by a fully automated ELISA analyzer.***

Results: current study parameters included (BMI, total cholesterol, triglyceride, VLDL-cholesterol, LDL-cholesterol, IL-6, myostatin, CAF, P3NT, AACT, and hs-CRP)) in the sarcopenic subject showed more than the control subject with significantly higher changes (P<0.01)., whereas the parameters included (HDL-cholesterol, calcium, and vitamin D) were demonstrated in sarcopenia group less than

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a control group with significantly higher changes (P<0.01). BMI had a direct correlation with total cholesterol, triglyceride, LDL- cholesterol, VLDL-cholesterol, IL-6, myostatin, CAF, P3NT, AACT, and hs-CRP, with highly significant (P < 0.01). BMI had an inverse correlation with HDL-cholesterol, calcium, and vitamin D, with highly statistic changes (P <0.01).

Conclusions: A findings of the study revealed that obesity serves as a health risk for sarcopenia in elderly adults. High blood levels of total cholesterol, triglyceride, VLDL-cholesterol, LDL-cholesterol, myostatin, CAF, P3NT, IL-6 AACT, and hs-CRP in blood circulation raise the risk for sarcopenia in the elderly. Decrease concentrations of vitamin D, calcium, and HDL-cholesterol increase the opportunity for the elderly to have sarcopenia.

Keywords: Sarcopenia, BMI, Aging, Biomarkers, pro-inflammatory markers, Basra

Introduction

Sarcopenia is a condition of the elderly identified with gradual and widespread skeletal muscle mass weakness, intensity, and, or function at aging, which has a major threat to quality, dependence loss of life, and lastly death [1]. Muscle atrophy can strictly debilitate the musculoskeletal system, likewise participate in the development of reducing lipid homeostasis, especially in the obese state [2].

Collagen type 3 in soft connective tissues of the skeletal muscle is synthesized from procollagen type 3 molecules which carrying elongation of C- and N-terminal peptide ends together. C- and N-terminal peptide of procollagen 3 are taken away by specific proteinases during the end steps for the synthesis of collagen and released in an equal amount into blood circulation. Procollagen type 3 N-terminal peptide (P3NP) can be measured in the serum of the human, and it is concentration explain an interesting indicator of skeletal muscle modification [3].

1, 25-hydroxy vitamin D acts physiologically as the functional source of vitamin D that binds to the skeletal muscle fibers' vitamin D receptor (VDR), enhancing their bulk, muscle strength, and biological performance. In aging, a gradual reduction in the

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number of VDR on the skeletal muscle fibers leads to reduce functional response for vitamin D, with a reduction in muscular size and intensity [4].

In healthy skeletal muscle, nerve derived protein-agrin is responsible for neuromuscular junctions, therefore, within the defect of neuromuscular, agrin splits by neurotrypsin enzyme into C-terminal agrin fragment (CAF). neurotrypsin maintains the intensity of the link between skeletal muscle cells and nerve by inactivating and splitting agrin,. The last studies have demonstrated that functional degradation of the neuromuscular junction as a result of the increase split of agrin by neurotrypsin into a CAF, and may progressively lead to sarcopenia [5].

Myostatin is extremely strengthened as one of the transforming growth factor-beta (TGF-β) superfamily in the skeletal muscle, which would be generated and secreted as a core protein by the muscle tissue then released as a disulfide-linked homodimer that is negatively retained in the skeletal muscle mass[6].

Aging and, or obesity plays a central role in the elevating pro-inflammatory cytokines levels, including such tumor necrosis factor-alpha (TNF-alpha) and IL-6, which play a major role in the production of highly sensitive C-reactive protein (hs-CRP) and alpha 1-antichymotrypsin (AACT) from the liver[7].

Subjects

The study was conducted on 170 participants living in Basra city. The sarcopenia group was included 110 patients (55 male and 55 female) whose ages range between (51-81) years and 60 non-sarcopenic subjects as a control group (30 male and 30 female) whose age range between (53-83) years. Subjects were excluded from anyone with cardiovascular diseases, diabetes mellitus, thyroid diseases, liver diseases, systemic lupus erythematosus, renal diseases, taking steroid therapy, smokers, and/or alcohol consumption.

Blood samples were collected within the interval between April to December 2019 from participants of both sexes diagnosed with sarcopenia by using Dual Energy X-ray Absorptiometry (DEXA) scan, information included age and gender were obtained by each participant interview during fill questionnaire.

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Specimens

Five milliliters of peripheral blood were obtained from each patient and control subject by disposable syringe and without binding tourniquet on the hand at the fasting state for at least 9 hours. Whole blood was transferred into a gel tube contain clot activators. In the tube, whole blood was let stand for fifteen minutes and then centrifuged to isolated serum for 10 minutes at 3000 rotations per minute (RPM). Each serum sample was transferred and divided into three Eppendorf tubes immediately then frozen at (-70 °C) for subsequent analysis.

Materials and methods

The weight (kilogram)/height (meter) ^{2} equation was used in the BMI calculation. A fully automated biochemistry analyzer (Indiko, Thermofisher product) using enzymatic methods has been used to estimate total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, and calcium. VLDL-cholesterol concentration was calculated by using the Fridwald equation (triglyceride/5) [8]. A fully automated ELISA analyzer (ELisys Uno, Human product) was used to measured vitamin D, IL-6, myostatin, CAF, P3NT, AACT, and hs-CRP.

Analysis of statistics

The data analysis was performed using version 26 (SPSS, IBM Corporation, Chicago, USA) of the Statistical Package for the Social Sciences application. Data were viewed as a mean ± standard deviation. The coefficient of Pearson correlation (r) has been used to calculate the correlation between different quantitative variables. $P< 0.05$ was selected as the level of statistical significance. $P< 0.01$ was taken as the level of high significance change.

Results and Discussions

Table (1): represent the clinical features of patients with sarcopenia and control subjects. The mean±SD of age in the control group (67.98±6.90) was found to be non-significant ($P > 0.05$) compared to the sarcopenia group (67.98±6.90), although the mean ±SD of BMI in the control group (23.13±2.19) was highly significant ($P < 0.01$) relative to the mean ±SD of the sarcopenia group (33.65±2.44). This is an agreement

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with a study that observed that BMI was higher in the sarcopenic subject than in the control subject with a high statistical change between them [7].

In obese older persons, Starvation and obesogenic conditions associated with inactive habits and catabolic-induced elderly people can result in progressive adipocytes fat accumulation and simultaneously weakened skeletal muscle atrophy, resulting in a state known as sarcopenic obesity [9]. Also, The mean ± SD of total cholesterol, triglyceride, VLDL-cholesterol, and LDL-cholesterol in the control group were highly significant ($P < 0.01$) compared to the sarcopenia group. This finding corresponded with another study (Baek S. J., et al.) that mentions that sarcopenic obesity, in addition to sarcopenia and obesity alone, could have been regarded as a possibility of dyslipidemia [10]. In comparison, the mean±SD of HDL, calcium, and vitamin D of the control group was greater than that of the sarcopenia group with highly statically changes ($P < 0.01$). Vitamin D deficiency in the elderly is principally due to the reduced efficacy of the skin to produce cholecalciferol from 7-dehydrocholesterol, concurrently with decreased vitamin D synthesis also decreased vitamin D receptor (VDR) expression. These two phenomena are concerned with amplifying the influence deficiency of vitamin D among elderly persons. Vitamin D deficiency is already recognized to be a general characteristic of diseases commonly spread during aging, such as sarcopenia [11].

Table (1): clinical characteristics of the patients with sarcopenia and control subjects

Parameters	Study Group	N	Mean±SD	t-test	P-Value	C.S.
Age (years)	Control	60	67.98±6.90	1.341	.182	P>0.05 (NS)
	Sarcopenia	110	66.41±7.53			
BMI (kg/m^2)	Control	60	23.13±2.19	27.826	.000	P<0.01 (HS)
	Sarcopenia	110	33.65±2.44			
Total cholesterol (mg/dl)	Control	60	188.98±28.12	11.075	.000	P<0.01 (HS)
	Sarcopenia	110	275.81±57.01			
Triglyceride (mg/dl)	Control	60	145.37±36.93	6.052	.000	P<0.01 (HS)
	Sarcopenia	110	194.39±56.47			
VLDL cholesterol (mg/dl)	Control	60	29.03±7.43	5.873	.000	P<0.01 (HS)
	Sarcopenia	110	38.56±11.30			

LDL cholesterol (mg/dl)	Control	60	111.93±30.13	9.501	.000	P<0.01 (HS)
	Sarcopenia	110	195.18±63.51			
HDL cholesterol (mg/dl)	Control	60	47.82±9.32	6.146	.000	P<0.01 (HS)
	Sarcopenia	110	38.05±10.20			
Calcium (mg/dl)	Control	60	9.83±0.95	17.795	.000	P<0.01 (HS)
	Sarcopenia	110	5.66±1.67			
Vitamin D (ng/ml)	Control	60	18.62±9.14	15.215	.000	P<0.01 (HS)
	Sarcopenia	110	4.54±2.44			

The mean±SD of the control group for IL-6, AACT, and hs-CRP is lower than that in the sarcopenia group with highly significant variations (P<0.01) presented in the table (2). This result is compatible with another study (Schaap L. A., et al.) which observed that higher serum IL-6 and CRP levels correlate with muscle strength deterioration in the elderly, but disagree with those who noted that a higher AACT concentration reduces muscle strength deterioration and is aimed at reducing sarcopenia [12]. The following are the potential pathways of IL-6 leading to sarcopenia: (1) inflammatory factors may inhibit muscle protein formation, promote protein degradation, and increase myostatin muscle factor inhibitor and muscle atrophy protein production, increase protein catabolism and accelerate skeletal muscle damage. The largest quantities of proteins in the human body are found in muscle tissue, and IL- 6 will cause a decrease in muscle mass by interrupting protein synthesis and leading directly to protein breakdown. Besides, a high IL-6 level can suppress muscle fibers anabolism in IGF-1[13]. (2) Alterations in body composition associated with age can rapidly lead to enhance cytokine products. In elderly people, Adipose cells, IL-6, TNF-alpha, are increasing that can quickly produce, causing inflammation. Moreover, it will lead to a drop in lean muscle mass, progressing to sarcopenia [14]. (3) Sarcopenia's other cause is insulin tolerance. Insulin does not only minimize glucose in the body but also activates the cell growth of the target where the fibers of the muscle are located. Simultaneously, insulin is affecting the cells in the absorption of calcium. Insulin resistance, which does not encourage muscle contraction, can cause a decrease in calcium uptake [15].

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Elevated circulating IL-6 and hs-CRP concentrations can therefore contribute to insulin resistance and sarcopenia.

The mean ±SD of CAF in the control group was lower than the sarcopenia group with extremely significant ($P < 0.01$) in the table (2). This is in accordance with (Francesco Landi et al.) who found that serum CAF concentrations in the sarcopenic subject were significantly higher than in non-sarcopenic subjects [16].

Agrin is an extracellular proteoglycan originating from nerve cells, then brought along on the nerve fibers and eventually penetrated the synaptic basal lamina, wherever postsynaptic differentiation is activated (which would include clustering of acetylcholine receptor). It is hence necessary for the creation and relaxation of nerve terminals [17]. CAF can be observed as a free soluble in human serum.

Table (2) shows that the sarcopenia community had myostatin significantly higher relative to healthy control ($P < 0.01$). This outcome is consistent with (Ryan A. S. et al.) who observed that in cases of permanent impairment (such as stroke paretic limb) Myostatin is elevated. Myostatin will, therefore, be increased with sarcopenia in the elderly. Myostatin, which has a positive and negative influence on atrophy and hypertrophy metabolic regulation, is n significant negative switch of lean mass in people and other animals, and as such can affect physical function and health [18].

Table (2) indicates that P3NT concentrations in the sarcopenia category were greater than those in the control category, with a greater statistical difference ($P< 0.01$). P3NT is a fragment that, by proteolytic breaking, is published by collagen formation inside the muscle. For muscle fibers regeneration research, it represents an interesting indicator. An indicator indicates that serum P3NT concentrations exhibit muscle mass [19].

Table (2): Compare Mean ± SD of Biomarkers among Study Groups

Biomarkers	Study Groups	N	Mean±SD	t-test	P-Value	C.S.
IL-6 (ng/L)	Control	60	18.99±8.81	15.077	.000	P<0.01 (HS)
	Sarcopenia	110	51.79±15.53			
Myostatin (ng/ml)	Control	60	0.69±0.42	13.778	.000	P<0.01 (HS)
	Sarcopenia	110	2.029±0.69			
CAF (pmol/L)	Control	60	3.505±3.91	5.418	.000	P<0.01 (HS)

	Sarcopenia	110	8.20±6.05			
P3NT (pg/ml)	Control	60	239.35±83.97	11.774	.000	P<0.01 (HS)
	Sarcopenia	110	496.47±157.22			
AACT (ng/ml)	Control	60	8.48±9.83	14.008	.000	P<0.01(HS)
	Sarcopenia	110	28.70±7.1858			
hs-CRP (ng/ml)	Control	60	1.64±0.93	8.429	.000	P<0.01 (HS)
	Sarcopenia	110	3.77±1.83			

Table (3): shown Correlations (r) between BMI and lipid parameters in the sarcopenia group. BMI had an intermediate direct correlation with total cholesterol, triglycerides, VLDL-cholesterol, and LDL-cholesterol with highly significant ($P < 0.01$). Although the BMI had a major significant ($P < 0.01$) and an intermediate inverse association between it and HDL-cholesterol. These findings are close to the results of another Korean study, which clarified that there was a direct correlation among total cholesterol and LDL-cholesterol with BMI [20]. Also, the weak inverse association of BMI with HDL-cholesterol did describe by the study carried out in Nigeria [21]. The intermediate direct correlations of triglycerides and VLDL-cholesterol with total cholesterol were also highly statistical changes ($P < 0.01$), and there was a strong direct correlation between total cholesterol and LDL-cholesterol with extreme significantly ($P < 0.01$), although there was an intermediate negative correlation between total cholesterol and HDL-cholesterol with a highly significant ($P < 0.01$). There was a perfect direct correlation and highly significant ($P<0.01$) between triglyceride and VLDL-cholesterol, otherwise there was a week-long direct association between triglyceride and LDL-cholesterol with a highly change ($P<0.01$), an intermediate inverse correlation between triglyceride and HDL-cholesterol with a high difference ($P <0.01$), A week-long direct correlation between VLDL-cholesterol and LDL-cholesterol with an extremely significant ($P< 0.01$), an intermediate inverse association between VLDL-cholesterol and HDL-cholesterol and a high difference ($P<0.01$) association between HDL-cholesterol and LDL-cholesterol.

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Table (3): Correlations (r) between BMI and lipid parameters in sarcopenia group

Parameters	Correlations	BMI	Total cholesterol	Triglyceride	VLDL-cholesterol	LDL-cholesterol	HDL-cholesterol
BMI	r	1					
	Sig.						
Total cholesterol	r	.687**	1				
	Sig.	.000					
Triglyceride	r	.361**	.317**	1			
	Sig.	.000	.000				
VLDL-cholesterol	r	.351**	.310**	1.000**	1		
	Sig.	.000	.000	.000			
LDL-cholesterol	r	.646**	.958**	.217**	.212**	1	
	Sig.	.000	.000	.005	.006		
HDL-cholesterol	r	-.452**	-.441**	-.428**	-.426**	-.522**	1
	Sig.	.000	.000	.000	.000	.000	
** Correlation is significant at the 0.01 (2-tailed) level. [P<0.01 (HS)]							

Correlations (r) between BMI, calcium, and vitamin D in the sarcopenia group, as shown in Table (4). BMI had intermediate inverse associations with calcium and vitamin D with a strongly significant variation (P<0.01 Whereas, calcium and vitamin D had a strongly directed correlation with a high statistic difference (P < 0.01). This finding is following another recent study in the city of Hillah, which confirms there had been a strong inverse relation between excess body weight and vitamin D levels in the elderly Iraqi population [22].

Table (4): Correlations (r) among BMI, Calcium, and Vitamin D in the sarcopenia Group

Parameters	Correlations	BMI	Calcium	Vitamin D
BMI	r	1		
	Sig.			
Calcium	r	-.741**	1	
	Sig.	.000		
Vitamin D	r	-.687**	.761**	1
	Sig.	.000	.000	
** The correlation at the 0.01 (2-tailed) level is significance. [P<0.01 (HS)]				

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Table (5) represents the correlations (r) among BMI and biomarkers in the sarcopenia group. With high significant variations (P < 0.01), BMI had an intermediate direct correlation with IL-6, myostatin, CAF, P3NT, AACT, and hs-CRP. These outcomes had been consistent with other studies that concluded levels of IL-6 in the blood can demonstrate the severity of chronic inflammation, which rises with high rates of obesity [23]. In overweight patients, myostatin concentrations were significantly increased and were positively associated with BMI in the Chinese North Han population [24]. In patients with cardiovascular disease, serum P3NT concentrations increased significantly and correlated positively with BMI [25]. As well as IL-6 had intermediate direct correlations with myostatin, CAF, P3NT, AACT, and hs-CRP, with a highly significant difference (P < 0.01). So, the weekly direct association between myostatin and CAF with a non-significant difference (P > 0.05), whereas myostatin had intermediate direct correlations with P3NT, AACT, and hs-CRP extreme significant variations are included (P<0.01), a weekly direct association between CAF and P3NT with a highly significant difference (p<0.01).

There was also a weekly direct correlation between CAF and AACT with a statistical difference (P < 0.01), but there was a strong direct correlation between CAF and hs-CRP with a significant difference (P < 0.05). CAF is introduced as an essential indicator of sarcopenia induced by the degradation of the neuromuscular junctions in older persons [26]. An intermediate positive correlation was among P3NT with AACT and hs-CRP with highly significant differences (P < 0.01). An intermediate direct association with a gap of high significance (P<0.01) between AACT and hs-CRP was also observed.

Table (5): Correlations (r) among BMI and Biomarkers in the Sarcopenia Group

Biomarkers	Correlations	BMI	IL-6	Myostatin	CAF	P3NT	AACT	hs-CRP
BMI	r	1						
	Sig.							
IL-6	r	.682**	1					
	Sig.	.000						
Myostatin	r	.685**	.541**	1				

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	Sig.	.000	.000					
CAF	r	.411**	.411**	.144	1			
	Sig.	.000	.000	.061				
P3NT	r	.639**	.435**	.511**	.252**	1		
	Sig.	.000	.000	.000	.001			
AACT	r	.670**	.537**	.576**	.188*	.479**	1	
	Sig.	.000	.000	.000	.014	.000		
hs-CRP	r	.457**	.353**	.487**	.839*	.338**	.439**	1
	Sig.	.000	.000	.000	.016	.000	.000	
** Correlation at 0.01 level (2-tailed) is significant [P<0.01 (HS)]								
* The correlation at the 0.05 (2-tailed) is significant level. [P<0.05 (S)]								

Conclusions

A study's findings indicate that obesity in elderly people serves as a causal factor for Sarcopenia. High blood concentration of total cholesterol, triglycerides, VLDL-cholesterol, and LDL-cholesterol, although low levels of HDL-cholesterol, calcium, and vitamin D in the blood raise the risk of sarcopenia in the elderly. High blood concentration of IL-6, AACT, and hs-CRP significantly raise the chance of sarcopenia in the elderly. These remarks propose an inflammatory-associated component included in the age-associated deterioration of muscle health. High levels of myostatin, CAF, and P3NT in blood circulation increase the risk of sarcopenia in the elderly. BMI had a direct correlation with total cholesterol, triglyceride, LDL- cholesterol, VLDL-cholesterol, IL-6, myostatin, CAF, P3NT, AACT, and hs-CRP. BMI had an inverse correlation with HDL-cholesterol, calcium, and vitamin D. More studies are required to explain whether these parameters and biomarkers can indicate skeletal muscle qualitative changes with more comprehensive and more extended studies.

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