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Assessment of follistatin level in atherosclerosis patients and its relation with some anthropometric criterion as predictor survival.

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Abstract

Follistatin (FST) is glycoprotein, otherwise called activin restricting protein, is an endogenously created protein that ties activin A with high fondness and restrains its bioactivity. The essential capacity of FST is the authoritative and bioneutralization of individuals from the TGF-β superfamily and activin A specifically. FST advances adipogenic separation of begetter cells. FST has been embroiled in aggravation and renovating, any adjustment in the level of FST, which might be a determinant of the seriousness of irritation, or tissue phenotypic change. Atherosclerosis is a dynamical procedure that rises up out of the interchange between lipid digestion, irritation and inborn insusceptibility. The blood vessel area of atherosclerosis makes it strategically and morally hard to think about in vivo. To enhance our comprehension of the infection, we should discover elective approaches to research its movement. The points of this investigation; we need to know the connection between the serum FST level with atherosclerosis malady and its relationship with some deliberate rule, for example, sex, age, BMI, waist circumference and smoking.

Keywords: Follistatin, Atherosclerosis, Gender, Age, Waist circumference.

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Introduction

Atherosclerosis is the main source of death in created nations, and regardless of best current therapeutic treatments, it keeps on being a noteworthy general medical problem. The reaction to the maintenance theory of atherosclerosis recommends that atherosclerosis starts in the sub endothelial space of the vessel divider when atherogenic lipoproteins from the dissemination are held through ionic associations with vascular proteoglycans [1,2].Clinical investigations exhibit that diffuse intimal thickening with an expansion in proteoglycan content happens before the inundation of incendiary cells [3,4]. Atherosclerosis is a ceaseless provocative condition prompting different cardiovascular and in addition cerebrovascular occasions and fringe vascular malady (PVD). Nearness of critical atherosclerosis prompts perioperative unfriendly occasions, dismalness and mortality in the high hazard careful populace. The typical techniques for assessing the like hood of unfavourable cardiovascular or cerebrovascular occasions postoperatively in these arrangement of patients depends principally on scoring framework and utilitarian status of the cardiovascular framework amid preoperative evaluation [5]. Atherosclerosis is an unending incendiary process in the veins that outcomes in the development of atheromatous plaque over the endothelial coating of veins prompting firmness and loss of versatility of the vessel, stenosis of the supply route, aneurysm arrangement, plaque burst and brokenness of endothelial cell lining [6]. Previous atherosclerosis may prompt intense a foreseen myocardial localized necrosis amid the perioperative period. A foreseen arrhythmia, congestive cardiovascular disappointment, intense coronary disorder and cerebrovascular mischances are some other perioperative unfavourable occasions, which can cause major postoperative grimness and mortality. Aside from the different non-biochemical tests accessible for chance stratification, estimation of biomarkers in the blood amid perioperative period can give some insight for postoperative [5,7-9]. Three principle factors represent the aggregation of lipoprotein buildings at central zones of the blood vessel tree which at that point can prompt plaque improvement: (a) veering stream of the blood vessel tree which is punctuated at blood vessel bifurcations (b) the blood vessel divider is a semi-penetrable layer with enduring mass stream of liquid, through the interstices of the intimal divider, and (c) shear subordinate diffusivity of macromolecules which depends firmly on nearby shear rate. These causative factors specifically identify with clinical information, which joins smoking to expanded divider penetrability and therefore penetration rate, to frequency and seriousness of atherosclerotic plaques [10,11]. FST was first confined from ox and porcine like follicular liquid [12,13] was at first depicted

as a protein associated with the control of the discharge of folliclular empowering hormone (FSH).This glycosylated polypeptide monomeric chain is encoded by a solitary quality situated on the chromosome 5q11.2 long arm [14] which through elective grafting might be translated into the forerunners of mRNA, FST344 and FST317. FST isoforms might be created, to be specific FST288 from pre-FST317, FST315 from pre-FST344 and a third FST isoform, FST303 delivered from the post-translational truncation of the FST315 C-end. These three primary FST isoforms can likewise be glycosylated to yield six further FST isoforms that were already distinguished in cow-like [15] and porcine [16] and follicular liquid [17].

Material and Methods

Human FST (follistatin) ELISA kit

It was supplied by Elabscience, Catalog No: E-EL-H0076 (96T), Specific kit for measuring human follistatin level in serum.

Assessment of serum follistatin level

Principle of the test: ELISA kit uses Sandwich-ELISA. The micro plate of Elisa presented in this kit have been pre-coated with specific an antibody to FST of human. Samples or Standards are added to appropriate micro plate wells and combined with the specific antibody, the biotinylated detection antibodies specific for FST of human and Avidin Horseradish Peroxidase (HRP) conjugate are put successively to each micro plate well and incubated. During incubation, free components were washed away. Then the reagent of substrate is added to each well, those wells that contain human of FST only, blue colour will appear the biotinylated detection antibody and Avidin-HRP conjugate. The reaction of enzyme substrate will be terminated by put stop solution and detects yellow colour. The optical density (OD) measured with spectrophotometry at 450 nm ± 2 nm a wavelength; the concentration FST of human is proportional to the OD value. The concentration FST of human in samples was calculated by comparing the optical density of the samples by the standard curve.

Procedure of assay:

1. From standard or sample add 100 µL to each well for 90 min. incubate at 37℃.
2. The liquid must remove and 100 µL of biotinylated detection Ab add. For 1 hour at 37℃ Incubate.
3. Aspirate and wash three times.
4. 100 µL of HRP conjugate add. For 30 min at 37℃ incubate.
5. Aspirate and wash five times.
6. 90 µL of substrate reagent add. For 15 min at 37℃ incubate.
7. 50 µL of stop solution add. At 450 nm read immediately. Calculation the results.

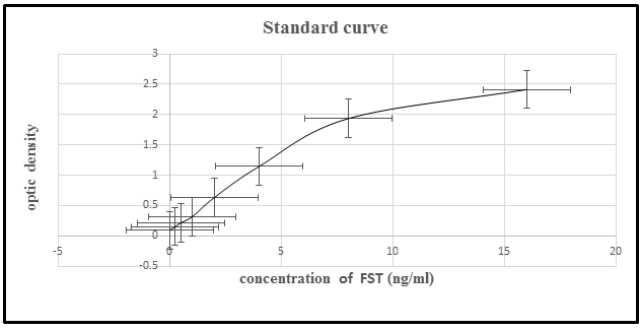

Figure 1. *Standard curve of FST.*

Patients and healthy group collection

The study was applied on 88 people aged 30-69 years, included 65 patients suffering from atherosclerosis disease, which are diagnostic by specialist physician in AL-Sader Teaching Hospital in Holy-Najaf province /Iraq. The samples were collected during the period from December 2017 to May 2018. The patients group was divided into subgroups according to gender, age, body mass index (BMI) types, waist circumference types and smoking. The healthy group was composed of 23 appear healthy; they were divided into subgroups according to gender, age, body mass index types, waist circumference types and healthy nonsmoking.

Ethical statement

The ethics committee for human of AL-Sader Teaching Hospital in Holy-Najaf province /Iraq approved protocol.

Collection of blood samples

2 ml of venous blood were drawn from patients and healthy group from ante cubital venipuncture using a disposable needle and plastic syringes. Blood was left at room temperature for 10 minutes to clot in the gel tube. The serum was isolated after centrifugation at 3000 rpm for 15 minutes and then serum was separated and transported into new disposable tubes Eppendorf tube by micropipette and stored at -20℃.

Body mass index (BMI)

A person weight with kilograms divided by the square of height with meters called BMI.

BMI = Weight (kg)/Height (m)

The person who has BMI ranges from (18.5-24.9 kg/m²) is normal and (25-29.9 kg/m²) is overweight while more than (30 kg/m^2) is obese [18]

Waist circumference (WC)

The normal measurement for men is 102 cm (40 inch) while for women is 88 cm (35inch).The measuring of (WC) mast be from the top of the iliac crest and lower margin of the least palpable rib at the midpoint by tape stretch-resistant [19].

Statistical analysis

Graphpad prism v6 windows software packages were used to analyze data of the present study (Version 6.01, 2016) for windows 2010. Data were ordered as Mean ± Standard deviation (SD), unpaired sample t-test was used for the comparison between two groups and one way ANOVA test was used for the comparison among subdivided groups in the measured parameters. Figure 1 had been constructed using EXEL program of Microsoft Office 2010. P value<0.05 was used as a level of statistically significant.

Result

Comparison of FST serum level between atherosclerosis patients group and healthy group

The results exhibited significant increase (P>0.05) in FST serum level of atherosclerosis patients (11.92 ± 2.522 ng/ml) compared with healthy group (0.6703 ± 0.1012 ng/ml) as shown in Table 1.

Table 1. *Comparison of FST serum level between atherosclerosis patients group and healthy group.*

Follistatin Serum (ng/ml) Mean ± SD		P.value
Healthy group	**Patients group**	0.0001
0.6703 b ± 0.1012	11.92 a ± 2.522	
The dissimilar letters represent significant differences (P>0.05) between means.		

Comparison of FST serum level between male and female of atherosclerosis patients group and healthy group

The result in Table 2 exhibited significant increase (P>0.05) in FST serum level of female atherosclerosis patients group (10.912 ± 0.5823 ng/ml) compared with male atherosclerosis patients group (2.892 ± 0.3849 ng/ml) and also compared with male and female healthy group (0.136 ± 0.0721 ng/ml, 0.799 ± 0.2023 ng/ml) respectively. In addition, there was significant increase (P>0.05) in FST serum level of the healthy female group compared with the healthy male group.

Comparison of FST serum level among different ages of atherosclerosis patients group and healthy group

The results of Table 3 indicated there was significant increase (P>0.05) in FST serum level of ages group (60-69 years) of atherosclerosis patients have (10.822 ± 1.143 ng/ml) compared with ages (50-59 years), (40-49 years) and (30-39 years) have (7.659 ± 1.322 ng/ml, 4.386 ± 1.531 ng/ml and 1.989 ± 0.012 ng/ml) respectively, also ages (50-59 years) compared with ages (40-49 years and 30-39 years) and ages (40-49 years) compared with ages (30-39 years). There was non-significant differences in ages of the healthy group (60-69 years), (50-59 years), (40-49 years) and (30-39 years) have (0.7341 ± 0.0123 ng/ml, 0.6213 ± 0.1311 ng/ml, 0.6115 ± 0.1214 ng/ml and 0.597 ± 0.213 ng/ml) respectively Table 3.

Comparison of FST serum level among atherosclerosis patients group and healthy group according to BMI (normal weight, over weight and obese weight)

The results indicated there was significant increase (P>0.05) in FST serum level in the obese weight of the atherosclerosis patients group have (8.286 ± 2.112 ng/ml) compared with (over weight and normal weight) have (4.266 ± 1.012 ng/ml and 2.221 ± 0.568 ng/ml) respectively, and overweight compared to normal weight. There was non-significant difference in FST serum level among healthy group (obese weight, over weight and normal weight) have (0.7685 ± 0.0121 ng/ml, 0.6615 ± 0.1311 ng/ml and 0.5251 ± 0.2324 ng/ml) respectively Table 4.

Table 2. *Comparison of FST serum level between male and female of atherosclerosis patients' group and healthy group according to the gender.*

Gender	Follistatin Serum (ng/ml) Mean ± SD		P-value
	Healthy group	**Patients group**	
Male	0.136 d ± 0.0721	2.892 b± 0.3849	0.0001
Female	0.799 c ± 0.2023	10.912 a± 0.5823	0.0001
P-value	0.0016	0.0309	

Table 3. *Comparison of FST serum level among different ages of atherosclerosis patients group and healthy group [Note: The dissimilar letters represent significant differences (P>0.05) among different ages. The similar letters represent non-significant difference].*

Ages (year)	Follistatin serum (ng/ml) Mean ± SD		P-value
	Healthy group	**Patients group**	
30-39y	0.597 e ± 0.213	1.989 d ± 0.012	0.0001
40-49y	0.6115 e ± 0.1214	4.386 c ± 1.531	0.0001

50-59y	0.6213 e ± 0.1311	7.659 b ± 1.322	0.0001
60-69y	0.7341 e ± 0.0123	10.822 a ± 1.143	0.0001
P-value	0.7948	0.0001	

The dissimilar letters represent significant differences (P>0.05) among different ages. The similar letters represent non-significant difference.

Table 4. *Comparison of FST serum level among atherosclerosis patients group and healthy group according to BMI (normal weight, over weight and obese weight).*

BMI (wt/m^2)	**Follistatin serum (ng/ml) Mean ± SD**		**P-value**
	Healthy group	**Patients group**	
Normal weight	0.5251 d ± 0.2324	2.221 c ± 0.568	0.0001
Over weight	0.6615 d ± 0.1311	4.266 b ± 1.012	0.0001
Obese weight	0.7685 d ± 0.0121	8.286 a ± 2.112	0.0001
P-value	0.9212	0.0001	

The dissimilar letters represent significant differences (P>0.05) between different groups. The similar letters represent non-significant difference.

Table 5. *Comparison of FST serum level among atherosclerosis patients group and healthy group according to waist circumference.*

Waist Circumference (cm)	**Follistatin serum (ng/ml) Mean ± SD**		**P-value**
	Healthy group	**Patients group**	
70-80	0.497 f ± 0.115	2.541 e ± 0.104	0.0001
81-90	0.5017 f ± 0.151	3.766 d ± 1.034	0.0001
91-100	0.5218 f ± 0.1375	5.861 c ± 0.121	0.0001
101-110	0.5302 f ± 0.021	8.282 b ± 1.022	0.0001
111-120	0.545 f ± 0.028	14.769 a ± 1.021	0.0001
P-value	0.3134	0.0001	

The dissimilar letters represent significant differences (P>0.05) between different groups.The similar letters represent non-significant difference

Table 6. *Comparison of FST serum level between healthy group (non-smokers) and atherosclerosis patients' group (non-smokers and smokers) according to smoking.*

Follistatin serum (ng/ml) Mean ± SD			**P-value**
Healthy group	**Patients group**		
	Non smokers	**Smokers**	
0.6703 c ± 0.1012	5.949 b ± 2.101	13.974 a ± 1.433	0.0001

The dissimilar letters represent significant differences (P>0.05) between different groups.

Comparison of FST serum level among atherosclerosis patients group and healthy group according to waist circumference

The results in Table 5 exhibited significant increase (P>0.05) in FST serum level of patients group according to waist circumference (111-120 cm) have (14.769 ± 1.021 ng/ml) compared with (101-110 cm, 91-100 cm, 81-90 cm and 70-80 cm) have (8.282 ± 1.022 ng/ml, 5.861 ± 0.121 ng/ml, 3.766 ± 1.034 ng/ml and 2.541 ± 0.104 ng/ml) respectively and waist circumference (101-110 cm) compared with (91-100 cm, 81-90 cm and 70-80 cm) and (91-100 cm) compared with (81-90 cm and 70-80 cm) and (81-90 cm) compared with (70-80 cm), while there was non-significant difference in FST serum level among healthy group of waist circumference (111-120, 101-110, 91-100, 81-90 and 70-80) have (0.545 ± 0.028 ng/ml, 0.5302 ± 0.021 ng/ml, 0.5218 ± 0.1375 ng/ml, 0.5017 ± 0.151 ng/ml and 0.497 ± 0.115 ng/ml) respectively.

Comparison of FST serum level between healthy group (nonsmokers) and atherosclerosis patients' group (nonsmokers and smokers) according to smoking

The results showed significant increase (P>0.05) in FST serum level in smokers atherosclerosis patients' group have (13.974 ± 1.433 ng/ml) compared to non-smokers of the atherosclerosis

patients' group and healthy group non-smokers have (5.949 ± 2.101 ng/ml and 0.6703 ± 0.1012 ng/ml) respectively and non-smokers of the atherosclerosis patients' group compared with the healthy group non-smokers Table 6.

Discussion

The results exhibit significant increase in FST serum level of atherosclerosis patients compared with healthy group. Previous study was considered in atherosclerosis disease smooth muscle cells (SMCs) in vivo proliferation plays a central role in the pathogenesis of this disease. follistatin was greatly expressed in the diseased artery, then abnormal proliferation of SMCs occurred. Suggest that vascular SMCs is produced follistatin and its involved in the course of atherogenesis [20].

Previous study suspected that activin-A has a mitogenic effect on vascular SMCs from rat aorta and that this effect of activin-A is different from that of TGF-β. Activin-A increased the number of vascular SMCs after 30 hours of incubation [21]. Activin-A is produced by monocyte/macrophage lineage cells, [22] which are abundant in arteriosclerotic lesions.[23] he suspected that a mitogenic effect of activin-A on vascular SMCs might be involved in neointimal proliferation. Moreover, because activin and follistatin are involved in the morphogenesis of early development, [24,25,26] they suspected that they might be involved in the regeneration or morphogenesis which occurs in arteriosclerotic lesions. Follistatin, known as activin-binding protein, is produced an endogenously protein that binds activin A with high affinity and inhibits its bioactivity. The primary function of follistatin is the binding and bioneutralization of members of the TGF-β superfamily, and activin-A in particular [27]. There is some evidence indicating that FST may play a promotory role in the progression of tumors angiogenesis [11]. High level of follistatin may be to neutralized or inhibited activin action. Therefor the role of the high level of FST in atherosclerosis disease is may be due to neutralize the activin action that is increase proliferation, differentiation of the target cells in the blood vessels, as a cellular respond in the body; FST increased in serum, thus we can consider FST as marker for atherosclerosis disease.

The results exhibited significant increase in FST serum level of atherosclerosis patients group of female compared with male atherosclerosis patients group and compared with male and female healthy group. Follistatin was initially identified and isolated from follicular fluid on the basis of its inhibition of pituitary FSH secretion [28]. It is expressed in multiple tissues including the ovary, pituitary, adrenal cortex and pancreas [29]. It has been isolated from human placenta [11,30].

High secretion of FST in ovary compared to other organs of the body may be resulting in increased FST level in female than male. In addition to atherosclerosis, smooth muscle cells of artery affected produced FST also; therefore, highest concentration was found in female.

The results indicated there was significant increase in FST serum level of different ages group of atherosclerosis patients compared with the healthy group for the ages (60-69 years), (50-59 years), (40-49 years) and (30-39 years) respectively. The ages (60-69 years) show highest significant increase in FST serum level compared with the other ages while the results showed non-significant differences between the healthy groups in all ages. As the body ages the risk for atherosclerosis increases and genetic or lifestyle factors cause plaque to gradually build in the arteries [31]. FST increased in elder age than younger age may be because smooth muscle cells of arteries in atherosclerosis disease produce more FST for long period in elder than younger age.

The results indicated there was significant increase in FST serum level in atherosclerosis patients group compared to healthy group according to BMI (obese weight, over weight and normal weight). There is non-significant difference in FST serum level between healthy groups. Risk factor for atherosclerosis obesity is having extra body fat with a high amount; over weight is having extra body weight from muscle, bone, fat, and/or water [32] these tissues may be produce more FST therefore FST serum level increase in obesity weight compared to other weights. Obesity shows an inverse correlation between the body mass index and atherosclerosis of the aorta [33]. Despite the adverse relationship between BMI and arteriosclerosis, however, we find high FST when increasing BMI due to increase body mass, which results in increased FST production compared to the weak. Follistatin promotes adipogenic differentiation of progenitor cells in adipose tissue [34]. Follistatin's mode of action is likely due to its ability to block myostatin and enhance neovascularization [35].

The results exhibited significant increase in FST serum level of patients group compared with healthy group according to waist circumference (111-120, 101-110, 91-100, 81-90 and 70-80). The highest significant increase in FST serum level of patients group is for (111-120) compared with other waist circumferences of patients group. Visceral fat increase in high waist circumferences, increase FST serum could affect weight gain in mice [36] these increase may be effect on increase waist circumference. Increased in waist circumferences consider a marker for risk health. Good evidence that central obesity carries more health risks compared with total obesity assessed by BMI. It has been suggested that waist circumference (WC), a proxy for central obesity [36].

The results showed significant increase in FST serum level in smoker's atherosclerosis patients' group compared to non-smokers atherosclerosis patients' group and healthy group non-smokers. One of the factors increase risk atherosclerosis disease is tobacco smoking, smoking increase oxidative stress in arteries of atherosclerosis patients which increase inflammation this lead to more monocyte and macrophage to produce FST [10,32,37-39]. Increased serum level of FST in smokers atherosclerosis patients may be due to oxidative stress of tobacco in arteries lead to more monocyte, macrophage and smooth muscle cells to produce FST [10,40].

Conclusion

Follistatin is the new biomarker for prognostic, staging and detection of atherosclerosis disease.

Significance Statement

This study is the first clinical study in Iraq.

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