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Study the correlation between P-selectin and endotoxin with lipopolysaccharide-binding protein, visfatin, migration inhibitory factor, and soluble thrombomodulin in female Iraqi atherosclerosis patients?

Shakir F. T. Alaaraji*

ABSTRACT

Background: Atherosclerosis (AS), it is a prolonged inflammatory illness, including together the native and adaptive immune systems; it controls the beginning and development of the injuries, and possibly devastating thrombotic problems. We designed this study to examine the relationship of P-selectin (SELP) and endotoxin (ET) with lipopolysaccharide-binding protein (LBP), visfatin (VF), macrophage migration inhibitory factor (MIF), and soluble thrombomodulin (STM) in Iraqi AS patients, and to determine the power of this association. **Materials and Methods:** Serum levels of SELP, ET, LBP, VF, MIF, and STM were measured by ELISA technique in 42AS patients, the age range within 50–74 years and 42 healthy controls, the age within 48–71 years, matched sex, and ethnic background were selected accurately for comparison. **Results:** Serum SELP, ET, LBP, VF, MIF, and STM concentrations were raised in the AS cases than those without AS ($P < 0.01$), Pearson's correlation analysis exhibited that sera SELP and ET concentrations indicated an important positive association with all studied parameters. Receiver operating characteristic curve analysis showed that a SLEP has the best discrimination among the individual AS markers with area under the curve 0.9421 (95% confidence interval 0.8975–0.9868; standard error 0.02280; $P < 0.0001$). **Conclusion:** Serum studied parameter levels could provide additional information about the risk factors of developing AS disease and may be used as predicators for AS growth also may be used in manufactured of new therapy agents to reduce or prevent AS disease.

KEY WORDS: Atherosclerosis, Endotoxin, P-selectin, Visfatin

INTRODUCTION

Atherosclerosis (AS) is a prolonged inflammatory illness in which irritation exert a significant function at all phases, as of increase and development to diagnosis of occurrence medical happenings.[1] It is a main reason of stroke and heart attack, also is a main promoter to loss of life in the world;[2] however, it is the initiation of the inborn and acquired immune system that help in increase of disease, with modulation lipids through macrophage foam cells, atherosclerotic plates instigating by this way.[3]

We will study that the effect of cell adhesion molecule is SELP on AS disease which facilitates the linkage of inflammatory tissues to each other in vascular surfaces.[4] It is expressed on the endothelial cells and surface of stimulated platelets, initiates platelets through act together with the SELP glycoprotein ligand (PSGL)-1, which promote irritation and stimulates thrombosis, thus increase speed atherosclerotic plate creation.[5] These probable functions of SELP in AS growth are extra highlighted through its greater surface expression on platelets instable angina than other acute coronary disorders.[6] The previous study obviously demonstrated the function SELP in assistant leukocyte platelet exchanges and in leukocyte progressing on the endothelium.[7] Greater concentrations of SELP have been associated to thrombosis and AS.[8]

The causes of inflammatory reactions, and the roads through which irritation connects to vascular infection, stay not explained. The most significant cause of irritation is ET (lipopolysaccharide [LPS]), a distinctive glycolipid which involves maximum of the external leaflet of the external wall of Gram-negative bacteria (GNB).[9] All people including

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healthy persons, LPS can be noticed in their serum, ET encourages irritation through straight stimulating the macrophage/monocyte system, and vascular endothelium with producing a sequence of clear cell reactions, involving a rise in pro-inflammatory cytokine/chemokine expression, and cell adhesion molecule in endothelial cells.[10] This works on increase penetrability of recruitment and endothelium of leukocytes to increase irritations which connect to AS.[11]

Lipopolysaccharide-binding protein (LBP) of human located within family of lipid-binding proteins, which contain bactericidal penetrability elevating protein, transfer of cholesterol ester protein, and transfer of phospholipid ester protein.[12] The essential concentration of LBP in serum is small (1–15 μg/mL) then upsurge many times when inflammatory happen like infection, and like this reply is stimulated through IL-1 and IL-6.[13] LBP removes ET from membranes of bacteria through strong hook to the portion and lipid of ET.[14] LBP activate the moving of ET monomers from collections of ET to units of lipoprotein and this causes the deactivation of ET and later the diminution of the immune reply to contagion.[15]

Visfatin (VF) has been recognized as a multifunctional protein, which shows a significant function in controlling a diversity of pathological and physiological roles.[16] In case of endothelial dysfunction and irritation, the concentrations of VF are clearly increasing which suggested it as markers of AS disease, the relationship among VF levels with cardiovascular disease has been widely examined, from many studies appeared that VF serum concentrations may stimulate vascular irritation and atherosclerotic plate deterioration.[17,18] Augmented serum VF concentrations have been related with as illness.[19] Nevertheless, concentrations of circulating VF in AS plate development stay indistinct.

Migration inhibitory factor (MIF) is identified T cell marker exposed more than 40 years ago which more newly has been documented to be a main intermediary of inborn immunity and multiform inflammatory marker. It is exert essential function in the pathogenesis of prolonged and acute inflammatory illnesses like AS through stimulating and increasing included inflammatory responses such as MAPK signaling, inflammatory cytokine release, and macrophage/monocyte existence.[20] The previous paper established that MIF contributes in both human and animal AS.[21] It is expressed through macrophages and vascular endothelial cells.[22] By a deactivating MIF antibody to block of MIF led to vascular irritation decreased dramatically.[23] Straight signs for the function of MIF in deterioration of atheromatous plates originate by a previous research in apolipoprotein E-poorrats.[24]

Thrombomodulin (TM) is a thrombin receptor existing on the thrombin-TM compound and the endothelial cell surface may be stimulating protein C, which has anticoagulant elements. It is similarly exists as soluble heterogeneous parts in serum and augmented serum concentrations of this soluble TM (STM) are reflected to be related with endothelial cells damage.[25] In CAD cases, raised STM concentrations were originate to be correlated to repeated coronary actions,[26] though great STM levels have been stated to be related with a dropped danger of CVD.[27] The previous study demonstrated that a rise in the STM level through the coronary circulation was associated to the seriousness of angiographically established coronary arterial AS.[28] In the present study, we will try to explore whether inflammatory action is complicated in the grade of endothelial damage in AS illness.

This study aimed to explore the association between sera SELP and ET levels with LBP, VF, MIF, and STM in an Iraqi men population with AS illness and also, to analyze the differences of SELP, ET, LBP, VF, MIF, and STM serum levels between AS patients and healthy controls (HCs).

MATERIALS AND METHODS

The current study included 42 patients with AS from the Region Al-Fallujah in Al-Anbar Governorate, as a control group, 42 sex- and age-matched healthy volunteers were enrolled from same city. Rejection principles for AS patients: Earlier history of cerebrovascular accident or CHD, and other autoimmune and inflammatory illness. Rejection principles for HCs: Cerebrovascular accident or earlier history of CHD, new infectious, pregnancy, or inflammatory illnesses. The methods and objective were completely clarified to every contributor before to contribution and every contributor donated printed informed agreement preceding registration, and samples were processed and analyzed in a blinded fashion. This study was approved by the ethics board of University of Anbar and our inquiry was directed consistent to the standards of the statement of Helsinki (1964). Sera concentrations of SELP, ET, LBP, VF, MIF, and STM were determined by an enzyme-linked immunosorbent assay kits provided from (MyBioSource Co., USA).

Statistics

Statistical investigates of our results were done by GraphPad Prism 7.04 provided from (GraphPad Software, La Jolla, CA, USA). The results are stated as mean, standard deviation (SD), and standard error of mean (SEM). The statistical importance of the differences among the subjects with and without AS was verified with *t*-test, bivariate associations were tested through a Pearson correlation investigates with two-tailed, while the precision of the investigation was measured through the area

under the curve (AUC) of the receiver operating characteristic (ROC) curve. $P < 0.05$ was measured to be statistically important.

RESULTS

In Table 1, the standard experimental features of the subjects are described. With a mean age of 62.23 years in HCs and 63.45 years in AS patients ($P = 0.442$), AS patients had importantly greater SELP, ET, LBP, VF, MIF, and STM serum concentrations than in HCs with $P < 0.0001$ for all parameters except VF which has $P = 0.0056$, as shown in Table 1 and Figures 1-6, respectively.

Important positive relationships were detected among SELP and LBP, STM, ET, MIF, and VF concentrations established on grade of association descending [Table 2 and Figures 7-11, respectively]. ET also had important positive relationships with MIF, STM, LBP, and VF established on grade of association descending [Table 3 and Figures 12-15, respectively].

Table 1: Baseline clinical and laboratory characteristics of the subjects

Parameter	Healthy controls			Patients (atherosclerosis)			
	Mean	SD	SEM	Mean	SD	SEM	*P*-value
Age years	62.23	14.76	2.34	63.45	13.53	2.19	0.4240
SELP ng/mL	21.53	7.583	1.143	50.16	17.23	2.598	<0.0001
ET pg/mL	3.255	1.159	0.1747	5.777	3.605	0.5435	<0.0001
LBP ng/mL	3.584	1.429	0.2155	6.316	2.016	0.3039	<0.0001
VF ng/mL	1.734	0.714	0.1076	2.618	0.1229	0.8151	0.0056
MIF ng/mL	12.51	4.588	0.6917	20.19	6.023	0.9081	<0.0001
STM ng/mL	1.88	0.69	0.104	3.214	1.238	0.1866	<0.0001

Table 2: Correlations between SLEP and ET with other studied variables

	SELP	ET	LBP	VF	MIF	STM
SELP ng/mL						
r		0.381	0.548	0.232	0.288	0.399
p		0.0003	0.000009	0.030	0.007	0.001
ET pg/mL						
r			0.348	0.312	0.437	0.379
p			0.00090	0.0031	0.00002	0.00027
LBP ng/mL						
r				0.174	0.320	0.357
p				0.1055	0.002385	0.00063
VF ng/mL						
r					0.277	0.175
p					0.0089	0.103
MIF ng/mL						
r						0.387
p						0.00012

Table 3: Correlations between SLEP and ET with other studied variables

	SELP	ET	LBP	VF	MIF	STM
SELP ng/mL						
r		0.381	0.548	0.232	0.288	0.399
p		0.0003	0.000009	0.030	0.007	0.001
ET pg/mL						
r			0.348	0.312	0.437	0.379
p			0.00090	0.0031	0.00002	0.00027
LBP ng/mL						
r				0.174	0.320	0.357
p				0.1055	0.002385	0.00063
VF ng/mL						
r					0.277	0.175
p					0.0089	0.103
MIF ng/mL						
r						0.387
p						0.00012

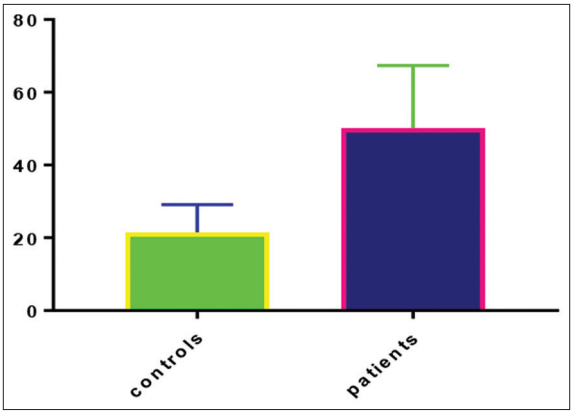

Figure 1: Mean + standard deviation for SELP (ng/ml) in control and AS patients

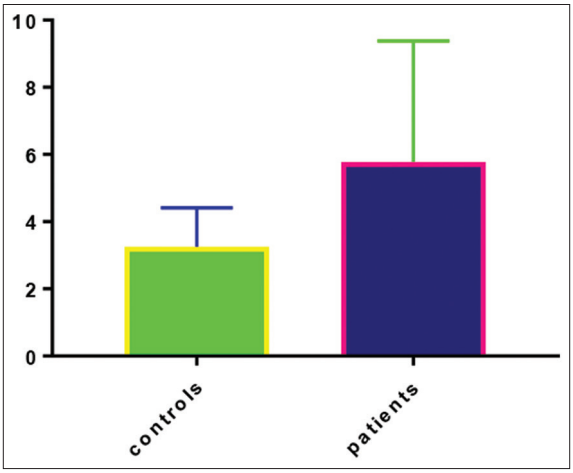

Figure 2: Mean + standard deviation for ET (pg/ml) in control and AS patients

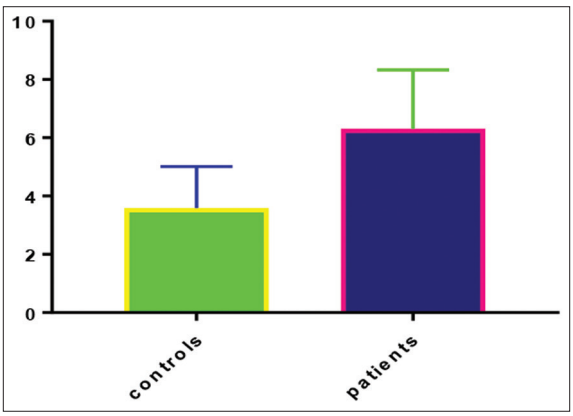

Figure 3: Mean + standard deviation for lipopolysaccharide-binding protein (ng/ml) in control and AS patients

Comparisons of the areas and confidence intervals (CI) for the studied parameters and plots resulting from ROC curve investigation are displayed in Table 4 and Figures 16-21. Established on the areas and CI values in Table 4, SLEP displays the best discrimination among the individual AS markers with AUC 0.9421 (95% CI 0.8975–0.9868; SE 0.02280;

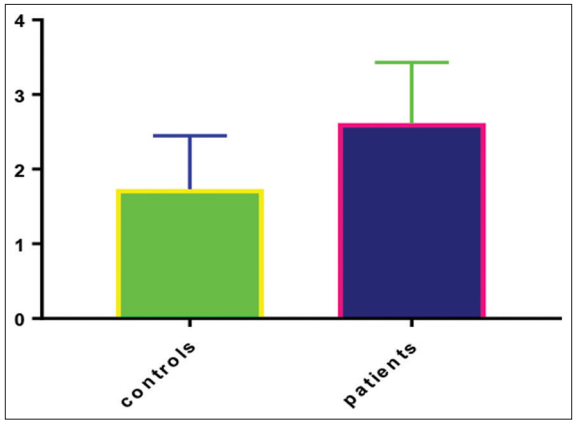

Figure 4: Mean + standard deviation for visfatin (ng/ml) in control and AS patients

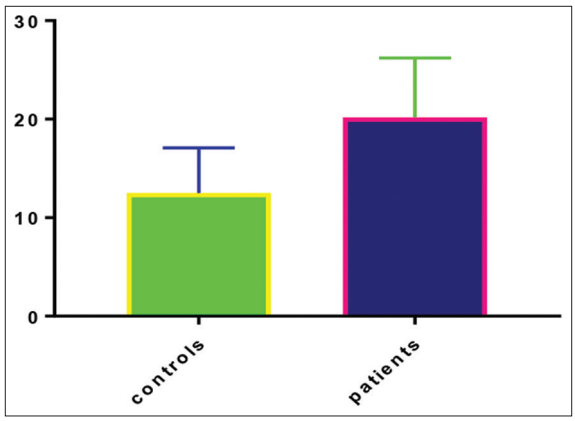

Figure 5: Mean + standard deviation for migration inhibitory factor (ng/ml) in control and AS patients

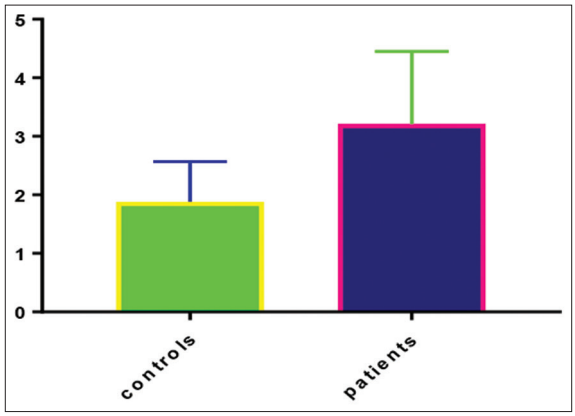

Figure 6: Mean + standard deviation for soluble thrombomodulin (ng/ml) in control and AS patients

$P < 0.0001$); [Figure 16], value for AUC here is considered excellent. In the second place came LBP, MIF, ET, and STM with AUC values 0.8430 (95% CI 0.7633–0.9227; SE 0.04067), 0.8298 (95% CI 0.7481–0.9116; SE 0.04171), 0.8254 (95% CI 0.7389–0.912; SE 0.04416), and 0.8042 (95% CI 0.7109–0.8976; SE 0.04763); [Figures 17-20], respectively, and $P < 0.0001$ for all these parameters. The poorest marker was VF with AUC value 0.7843 (95% CI

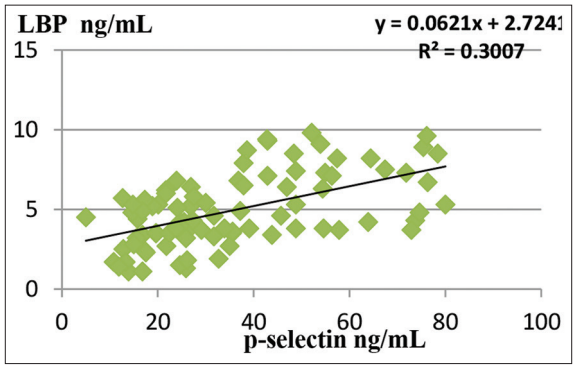

Figure 7: Relationship between SELP with lipopolysaccharide-binding protein

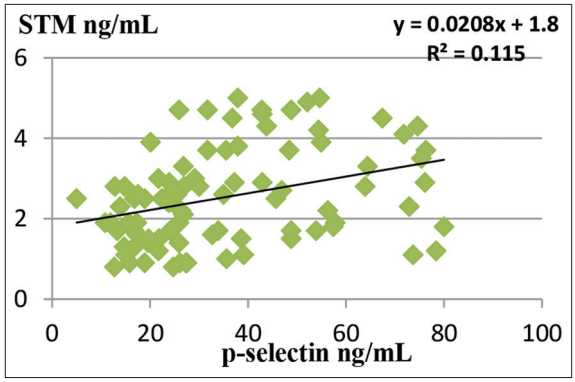

Figure 8: Relationship between SELP with soluble thrombomodulin

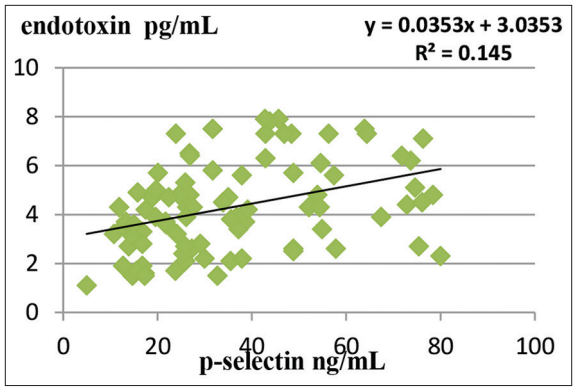

Figure 9: Relationship between SELP with ET

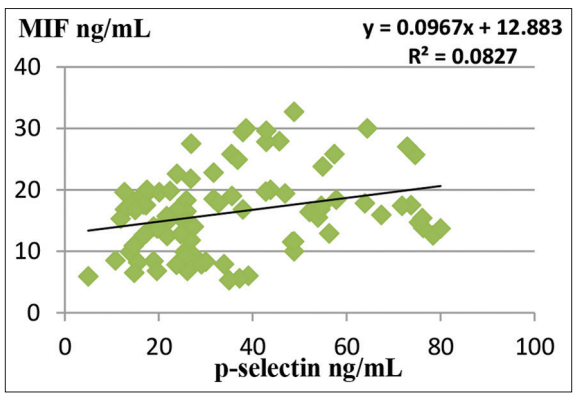

Figure 10: Relationship between SELP with migration inhibitory factor

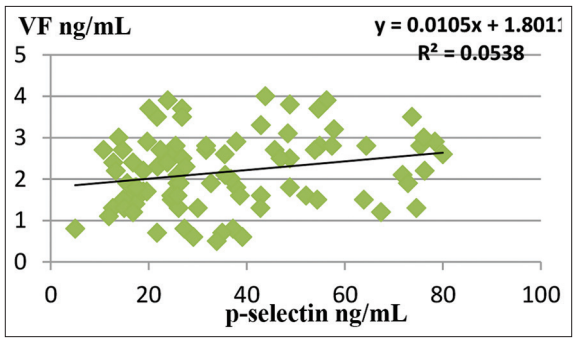

Figure 11: Relationship between SELP with visfatin

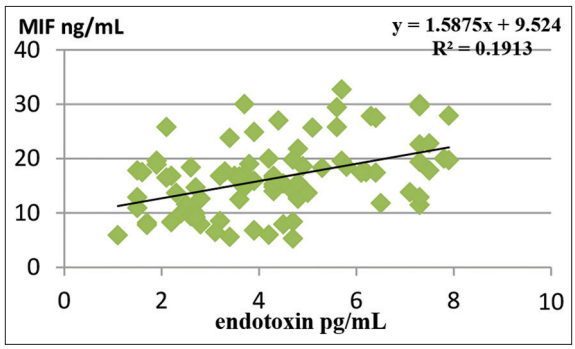

Figure 12: Relationship between ET with migration inhibitory factor

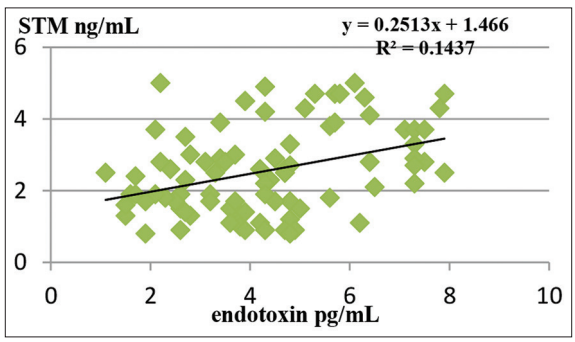

Figure 13: Relationship between ET with soluble thrombomodulin

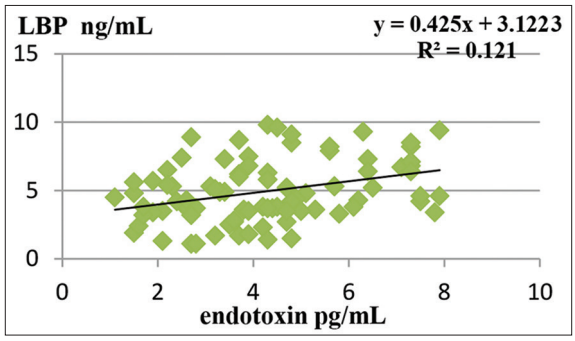

Figure 14: Relationship between ET with lipopolysaccharide-binding protein

0.6912–0.8775; SE 0.04753; $P < 0.0001$), [Figure 21]. On the basis of the ROC areas, SLEP appears to be the best predictor for AS disease; its area is so close to 1, while VF appears to be the poorest marker for AS disease; its area <0.8.

Table 4: Diagnostic criteria of the receiver operating characteristic curve for tested variables in as cases

Parameter	AUC	Std. Error	95% confidence interval	*P*-value
SELP ng/mL	0.9421	0.02280	0.8975–0.9868	<0.0001
ET pg/mL	0.8254	0.04416	0.7389–0.912	<0.0001
LBP ng/mL	0.8430	0.04067	0.7633–0.9227	<0.0001
VF ng/mL	0.7843	0.04753	0.6912–0.8775	<0.0001
MIF ng/mL	0.8298	0.04171	0.7481–0.9116	<0.0001
STM ng/mL	0.8042	0.04763	0.7109–0.8976	<0.0001

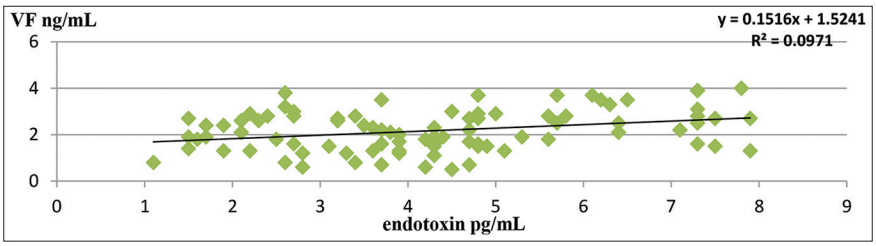

Figure 15: Relationship between ET with visfatin

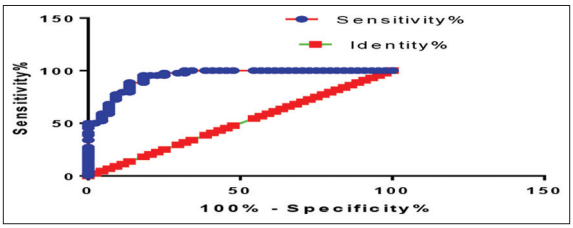

Figure 16: Receiver operating characteristic curve displaying area under the curve of SELP in AS patients

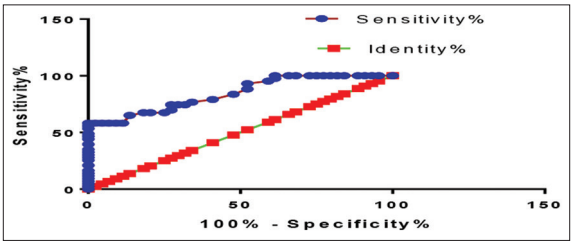

Figure 17: Receiver operating characteristic curve displaying area under the curve of lipopolysaccharide-binding protein in AS patients

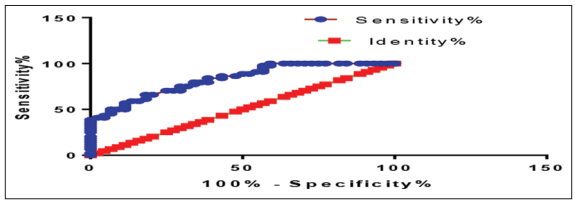

Figure 18: Receiver operating characteristic curve displaying area under the curve of migration inhibitory factor in AS patients

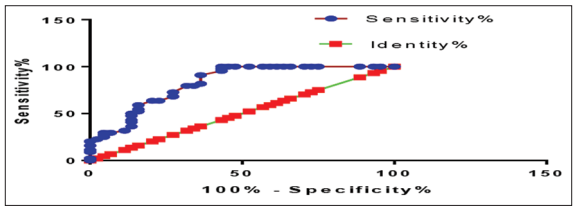

Figure 19: Receiver operating characteristic curve displaying area under the curve of ET in AS patients

DISCUSSION

The signs for AS as an inflammatory illness are very clear. The significance of immune stimulation in AS is established in numerous animal labs, was elimination of cell kinds or essential inflammatory intermediaries have been displayed to broadly decrease plate growth.[29] The present results showing that the concentration of SELP exerts a central function in together initial and late phases of AS progress. This outcome is built on the subsequent annotations, prior paper had showed that the main role of SELP is to facilitate monocyte, lymphocyte, and neutrophil platelet developing along the wall of venular and their effects in controlling inflammatory developments appear to be interacting.[30] *In vitro,* data propose that the SELP similarly change the biological roles of monocyte, mainly in the manufacture of pro-inflammatory markerslikeIL-6 and TNF-α.[31] Hence, it is probable that deficiency of SELP inhibits lesion development by alteration the role of macrophage in cytokines production.

Macrophages in the subendothelium may provide protection from AS through removal of harmful elements, like OX-LDL, however it is similarly postulated that they help in formation OX-LDL and the process of fibro proliferative by excretion of several factors of growth.[32] SELP is greatly complicated in the process of atherosclerotic which leads to accumulation of platelet, encouraging the up-controlling of growth factor which exerts a central function in estimating thrombosis of arterial.[33]

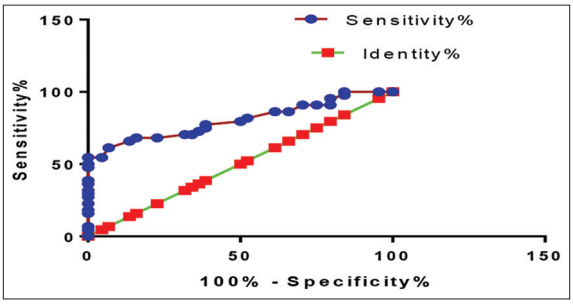

Figure 20: Receiver operating characteristic curve displaying area under the curve of W.W in AS patients

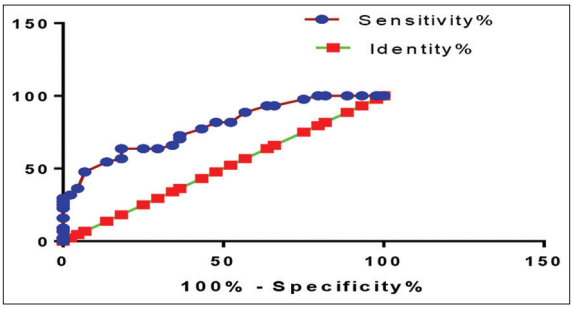

Figure 21: Receiver operating characteristic curve displaying area under the curve of visfatin in AS patients

In the present study, SLEP was positively related with a number of AS risk issues such as ET, LBP, VF, MIF, and STM. The biggest part of SLEP measurable in serum is believed to elevate from platelet origin as stated in former paper.[34] As a result, we and others have been recommended that serum SLEP levels may be being a marker of *in vivo* activation of platelet.[35] The present data showed, SLEP extremely associated with LBP is kept in the endothelial tissue and is excreted on stimulation of endothelial tissues, thus the strong association of LBP shows that together endothelial tissues and platelets involved importantly to serum SELP concentrations. Our results recommend that either raised concentrations of SELP rise as a result of the existence of public fundamental issues involve to up-controlling of every of these CV danger issues or AS.

The function of the bacterium in AS may be multi-roles with ET as one a limited participant. Microorganisms may exert a function in the AS pathogenesis at more than one step: (i) Atherosclerotic plate contaminating; (ii) causing severe actions through stimulating the coagulation cascade or affecting on weak plate; (iii) increase speed of primary AS through cytokine encouragement leading to topical rises in OX-LDL and LDL; and (iv) direct or indirect damage of endothelial vascular.[36] Serum ET action associated positively with serum LBP, VF, MIF, and STM concentrations, which proposes that ET is clarified to certain degree through contact to essential constituents of the GNB. ET stimulates MCP1 expression, which induces and recruits monocytes from the circulation to the location of irritation. It is an indicator of gram negative bacterial irritation, while Gram-positive bacteria are not contributed to ET inflammatory reply.[37] As a result, the knowledge of pathologic effects of ET could be beneficial in deal with it as biomarker that is together specialist and careful to AS cases.

LBP was recognized as an adipokine manufactured in large quantities through human adipose tissues of human in situations of irritation, positive energy equilibrium and insulin resistance *in vitro* and *in vivo*.[38] Inside the body LBP manufactured through intestinal epithelial tissues, adipose tissues and liver cells in reply to translocation of intestinal microbial, that means in reply to the their produces through barrier of intestinal mucosal without overt bacteremia or gut microbial species movement[39] earlier data had shown that HDL is the main ET removal lipoprotein in serum or full blood and that HDL-related ET is then transported to VLDL and LDL in a period dependent method.[40] Our results in agreement with former paper which show that LBP is able to moving ET from HDL to LDL and, to a smaller amount, to VLDL which importantly raises the danger of AS disease.[41] Actually, the present results propose that LBP may be a pivotal link among the ET rise in pro-inflammatory effectors and their harmful effects on AS progress.

In the current study, we demonstrated that serum VF concentrations are importantly increased in AS cases than in those without AS. In the past 9 years, there has been significant growth and wide interest in the possible function of VF in the pathological causes of metabolic linked CV problems. Augmented VF serum concentrations were stated to be associated with vulnerability of carotid plate in carotid stenosis cases,[42] and VF concentrations may be a predictor of morbidity in severe ischemic stroke and CV mortality.[43] VF is a pro-inflammatory marker, it can uncontrolled the manufacture of TNF-α, IL-6, and IL-1β, in vascular endothelial tissues and monocytes of human.[44] Greater VF serum level may clearly shows a greater formula of inflammatory, thus causative to the development of carotid plates. Furthermore, in the chronic kidney illness development, VF serum concentrations were originated to be strictly related with TNF-α and IL-6 in AS disease.[45]

The cytokine MIF was the major one which used in defined autoimmune illnesses and prolonged inflammatory and was diagnosed to be a central regulator in AS disease, pathological causes of prolonged irritation of the arterial wall and AS described through main function of chemokine regulated invasion of leukocytes.[46] MIF is greatly expressed in endothelial tissues and macrophages of diverse kinds of atherosclerotic plates, like to

chemokines; MIF activate movement of T cells and monocytes.[47] Recent paper demonstrate that irritation is powerfully linked to manufacture of inflammatory markers and dysfunction of endothelial cells, and these considered a preface to thrombotic and AS illness.[48] The high levels of inflammatory markers and the adhesion molecules expression by definite pathways may stimulate adhesion of lymphocyte and accumulation of mononuclear tissue to stimulate foam cell construction, macrophages, and encouraging the development of atherosclerotic plate.[49]

Our findings show that STM concentrations increased significantly in AS than in HCs and show significant correlations were detected with SELP and ET. Elevated STM levels may indicate augmented expression of STM on surfaces endothelial cells, or reveal indirect and direct damage to the endothelial tissues. Augmented level of STM was related with impairment of endothelial cells in former research.[50] Impairment of endothelial cell is a state that increases thrombotic and AS events as submitted through the outcomes of epidemiological data.[51] Usually inflammatory responses are the chief reasons of AS, and the effects of different subtypes of lymphocytes, neutrophils, mononuclear tissues, and further immune and inflammatory tissues on the AS pathological development should be study extensively.[52]

We think that the most of the studied parameters resulting from damaged endothelial tissues, not just excreted through endothelial tissues and that augmented their concentrations in the coronary circulation reveal endothelial injury resultant from inflammatory responses of peroxide and protease manufactured through arteries of atherosclerotic coronary. These damages can progress great concentrations of studied variables in serum of AS cases than in HCs.

Our study suffers from a number of limitations. First, the case–control project used in this study with a fairly small sample size of female Iraqi AS patients makes our results weak, second, subjects were of an older age (62.84 years of age), and had greater contact to main danger issues such as: Smoking, hypertension, and diabetes. These data should therefore be checked through prospective research with bigger samples and in further ethnic collections are necessary to examine the biological functions of serum studied variables in AS cases and should be accomplished before these variables are established as confident danger issues for AS disease. Third, we did not assess TIMP-1, OX-LDL, MMP-9, TGF-B, MCP-1, GM-CSF, VEGF, and sICAM-1 perhaps further unfamiliar confusing elements that may involve with the influence on AS occurrence and diagnosis.

CONCLUSION

These data submitted that the effect of augmented studied variables concentration should be clarified based on the history of AS patients since it decreases the main danger of AS between cases free of prior history vascular disease and rises the danger of loss of life in AS cases.

Similarly, AS needs a long time disease to development with a multifaceted pathological manner, for that reason, a distinct bio-variable could not be adequate to identify or guess atherosclerotic plates. Serum studied marker concentrations may give further data about the risk elements of increasing AS illness. A strong signs in AS propose that parameters in this study are therapeutic goals for AS disease. The presence of these variables may provide a potential mechanism for the augmented atherogenesis detected in AS illnesses. Similarly, support the postulate that studied variables facilitate the increasing of AS.

Nevertheless, manufacture inhibitors of studied variables have appeared as an encouraging pharmacological goal in the situation of cardiovascular problems which be a novel category of orally vigorous direct anticytokine medications with possible applications not only in classic inflammatory illnesses, such as AS, but also, moreover in the common public complications of atheroma, in which the usage of biological anticytokine treatments may be quite expensive. It stays to be seen whether such compounds can be industrialized, but important efforts are in progress in numerous test center toward this aim.

Higher serum concentrations of inflammatory markers, such as studied variables, are great problem in AS illnesses. In the current study, serum SELP and ET were correlated positively with all parameters in the study, which proposes that the “low-grade inflammation” is clarified to certain level through contact to fundamental constituents of the Gram-negative bacteria. Finally, serum studied parameters may serve as predictors of AS progress.

REFERENCES

1. Teague HL, Ahlman MA, Alavi A, Wagner DD, Lichtman AH, Nahrendorf M, *et al*. Unraveling vascular inflammation: From immunology to imaging. J Am Coll Cardiol 2017;70:1403-12.
2. World Health Organization. Cardiovascular Diseases (CVDs). Geneva: World Health Organization; 2017. Available from: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds). [Last accessed on 2019 Jul 12].
3. Legein B, Temmerman L, Biessen EA, Lutgens E. Inflammation and immune system interactions in atherosclerosis. Cell Mol Life Sci 2013;70:3847-69.
4. Vestweber D, Blanks JE. Mechanisms that regulate the function of the selectins and their ligands. Physiol Rev 1999;79:181-213.
5. Panicker SR. Mehta-D'souza P, Zhang N, Klopocki AG,

Shao B, McEver RP. Circulating soluble P-selectin must dimerize to promote inflammation and coagulation in mice. Blood 2017;130:181-91.
6. Ridker PM, Buring JE, Rifai N. Soluble P-selectin and the risk of future cardiovascular events. Circulation 2001;103:491-5.
7. Norman KE, Katopodis AG, Thoma G, Kolbinger F, Hicks AE, Cotter MJ, *et al*. P-selectin glycoprotein ligand-1 supports rolling on E-and P-selectin *in vivo*. Blood 2000;96:3585-91.
8. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: The leukocyte adhesion cascade updated. Nat Rev Immunol 2007;7:678-89.
9. Raetz CR, Whitfield C. Lipopolysaccharide endotoxins. Annu Rev Biochem 2002;71:635-700.
10. Bierhaus A, Chen J, Liliensiek B, Nawroth PP. LPS and cytokine-activated endothelium. Semin Thromb Hemost 2000;26:571-87.
11. Cohen J. The immunopathogenesis of sepsis. Nature 2002;420:885-91.
12. Tobias PS, Mathison JC, Ulevitch RJ. A family of lipopolysaccharide binding proteins involved in responses to gram-negative sepsis. J Biol Chem 1988;263:13479-81.
13. Grube BJ, Cochane CG, Ye RD, Green CE, McPhail ME, Ulevitch RJ, *et al*. Lipopolysaccharide binding protein expression in primary human hepatocytes and HepG2 hepatoma cells. J Biol Chem 1994;269:8477-82.
14. Tobias PS, Soldau K, Ulevitch RJ. Isolation of a lipopolysaccharide-binding acute phase reactant from rabbit serum. J Exp Med 1986;164:777-93.
15. Wurfel MM, Kunitake ST, Lichenstein HS, Kane JP, Wright SD. Lipopolysaccharide (LPS)-binding protein is carried on lipoproteins and acts as a cofactor in the neutralization of LPS. J Exp Med 1994;180:1025-35.
16. Carbone F, Liberale L, Bonaventura A, Vecchie A, Casula M, Cea M, *et al*. Regulation and function of extracellular nicotinamide phosphoribosyltransferase/visfatin. Compr Physiol 2017;7:603-21.
17. Yu PL, Wang C, Li W, Zhang FX. Visfatin level and the risk of hypertension and cerebrovascular accident: A systematic review and metaanalysis. Horm Metab Res 2019;51:220-9.
18. Li B, Zhao Y, Liu H, Meng B, Wang J, Qi T, *et al*. Visfatin destabilizes atherosclerotic plaques in apolipoprotein Edeficient mice. PLoS One, 2016;11:e0148273.
19. Zhong M, Tan HW, Gong HP, Wang SF, Zhang Y, Zhang W. Increased serum visfatin in patients with metabolic syndrome and carotid atherosclerosis. Clin Endocrinol (Oxf) 2008;69:878-84.
20. Morand EF, Leech M, Bernhagen J. MIF: A new cytokine link between rheumatoid arthritis and atherosclerosis. Nat Rev Drug Discov 2006;5:399-410.
21. Lin SG, Yu XY, Chen YX, Huang XR, Metz C, Bucala R, *et al*. *De novo* expression of macrophage migration inhibitory factor in atherogenesis in rabbits. Circ Res 2000;87:1202-8.
22. Burger-Kentischer A, Goebel H, Seiler R, Fraedrich G, Schaefer HE, Dimmeler S, *et al*. Expression of macrophage migration inhibitory factor in different stages of human atherosclerosis. Circulation 2002;105:1561-6.
23. Chen Z, Sakuma M, Zago AC, Zhang X, Shi C, Leng L, *et al*. Evidence for a role of macrophage migration inhibitory factor in vascular disease. Arterioscler Thromb Vasc Biol 2004;24:1-8.
24. Schober A, Bernhagen J, Thiele M, Zeiffer U, Knarren S, Roller M, *et al*. Stabilization of atherosclerotic plaques by blockade of macrophage migration inhibitory factor after vascular injury in apolipoprotein E-deficient mice. Circulation 2004;109:380-5.
25. Ishii H, Majerus PW. Thrombomodulin is present in human plasma and urine. J Clin Invest 1985;76:2178-81.
26. Blann AD, Amiral J, McCollum CN. Prognostic value of increased soluble thrombomodulin and increased soluble E-selectin in ischaemic heart disease. Eur J Haematol 1997;59:115-20.
27. Salomaa V, Matei C, Aleksic N, Sansores-Garcia L, Folsom AR, Juneja H, *et al*. Soluble thrombomodulin as a predictor of incident coronary heart disease and symptomless carotid artery atherosclerosis in the atherosclerosis risk in communities (ARIC) study: A case-cohort study. Lancet 1999;353:1729-34.
28. Nakagawa I, Matsubara T, Hori T, Imai S, Ozaki K, Mezaki T, *et al*. Significance of soluble thrombomodulin in the coronary circulation of patients with coronary artery disease. J Cardiol 2001;38:145-52.
29. Libby P. Inflammation in atherosclerosis. Arterioscler Thromb Vasc Biol 2012;32:2045-51.
30. Frenette PS, Johnson RC, Hynes RO, Wagner DD. Platelets roll on stimulated endothelium *in vivo*: An interaction mediated by endothelial P-selectin. Proc Natl Acad Sci U S A 1995;92:7450-4.
31. Weyrich AS, McIntyre TM, McEver RP, Prescott SM, Zimmerman GA. Monocyte tethering by P-selectin regulates monocyte chemotactic protein-1 and tumor necrosis factor-alpha secretion. Signal integration and NF-kappa B translocation. J Clin Invest 1995;95:2297-303.
32. Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, Edwards PA, *et al*. Atherosclerosis: Basic mechanisms. Circulation 1995;91:2488-96.
33. Merten M, Thiagarajan P. P-selectin in arterial thrombosis. Z Kardiol 2004;93:855-63.
34. Jilma B, Dirnberger E, Eichler HG, Kapiotis S. Sex differences in circulating P-selectin, E-selectin and thrombomodulin. Br J Haematol 1996;95:575-6.
35. Blann AD, Lip GY. Hypothesis: Is soluble P-selectin a new marker of platelet activation? Atherosclerosis 1997;128:135-8.
36. Honarmand H. Atherosclerosis induced by *Chlamydophila pneumoniae*: A controversial theory. Interdiscip Perspect Infect Dis 2013;2013:941392.
37. Pal GD, Shaikh M, Forsyth CB, Ouyang B, Keshavarzian A, Shannon KM. Abnormal lipopolysaccharide binding protein as marker of gastrointestinal inflammation in Parkinson disease. Front Neurosci 2015;9:306.
38. Moreno-Navarrete JM, Escote X, Ortega F, Camps M, Ricart W, Zorzano A, *et al*. Lipopolysaccharide binding protein is an adipokine involved in the resilience of the mouse adipocyte to inflammation. Diabetologia 2015;58:2424-34.
39. Patel PN, Shah RY, Ferguson JF, Reilly MP. Human experimental endotoxemia in modeling the pathophysiology, genomics, and therapeutics of innate immunity in complex cardiometabolic diseases. Arterioscler Thromb Vasc Biol 2015;35:525-34.
40. Levels JH, Abraham PR, Ende AV, Van Deventer SJ. Distribution and kinetics of lipoprotein-bound endotoxin. Infect Immun 2001;68:2821-8.
41. Levels JH, Marquart JA, Abraham PR, van den Ende AE, Molhuizen HO, van Deventer SJ, *et al*. Lipopolysaccharide is transferred from high-density to low-density lipoproteins by lipopolysaccharide-binding protein and phospholipid transfer protein. Infect Immun 2005;73:2321-6.
42. Kadoglou NP, Sailer N, Moumtzouoglou A, Kapelouzou A, Gerasimidis T, Kostakis A, *et al*. Adipokines: A novel link between adiposity and carotid plaque vulnerability. Eur J Clin Invest 2012;42:1278-86.
43. Kadoglou NP, Fotiadis G, Lambadiari V, Maratou E, Dimitriadis G, Liapis CD. Serum levels of novel adipokines in patients with acute ischemic stroke: Potential contribution to diagnosis and prognosis. Peptides 2014;57:12-6.
44. Tilg H, Moschen AR. Role of adiponectin and PBEF/visfatin as regulators of inflammation: Involvement in obesityassociated diseases. Clin Sci 2008;114:275-88.
45. Tang X, Chen M, Zhang W. Association between elevated visfatin and carotid atherosclerosis in patients with chronic kidney disease. Zhong Nan Da Xue Xue Bao Yi Xue Ban 2013;38:553-9.
46. Halaris A. Inflammation-associated comorbidity between depression and cardiovascular disease. Curr Top Behav Neurosci 2017;31:45-70.
47. Schober A, Bernhagen J, Weber C. Chemokine-like functions of MIF in atherosclerosis. J Mol Med (Berl) 2008;86:761-70.
48. Li C, Xu MM, Wang K, Adler AJ, Vella AT, Zhou B.

Macrophage polarization and meta-inflammation. Transl Res 2018;191:29-44.
49. Dogan HO, Büyüktuna SA, Kapancik S, Bakir S. Evaluation of the associations between endothelial dysfunction, inflammation and coagulation in Crimean-Congo hemorrhagic fever patients. Arch Virol 2018;163:609-16.
50. Ishii H, Uchiyama H, Kazama M. Soluble thrombomodulin antigen in conditioned medium is increased by damage of endothelial cells. Thromb Haemost 1991;65:618-23.
51. Blann AD, McCollum CN. von Willebrand factor and soluble thrombomodulin as predictors of adverse events among subjects with peripheral or coronary atherosclerosis. Blood Coagul Fibrinolysis 1999;10:375-80.
52. Yao BC, Meng L, Hao M, Zhang Y, Gong T, Guo Z. Chronic stress: A critical risk factor for atherosclerosis. J Int Med Res 2019;47:1429-40.

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