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ELEVATED LEVELS OF PLASMIN AND SOLUBLE UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR PREDICT THE RISK OF ISCHEMIC HEART DISEASES

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Abstract

Background: Ischemic heart disease (IHD) is one kind of cardiovascular disease (CVD) disorder that occurs when the heart does not get an adequate supply of blood. The Plasminogen (Plg) activator Plasmin (Pm) System is an example of a carefully controlled proteolytic system that plays an important part in fibrinolysis and thrombosis. The soluble version of the membrane-bound receptor uPAR is called soluble urokinase plasminogen activator receptor, or suPAR for short.

Aim: To determine whether the level of Plasmin and suPAR are related to the risk of IHD.

Methods: A case-control study was performed between October 2021 to July 2022. One hundred twenty individuals were enrolled, 60 patients with IHD (MI=30,angina=30) and mean age (60.7±2.06). Sixty healthy subjects served as a healthy control group, with mean age (of 48.7±0.86; 15 female, 45 male). Plasmin and suPAR serum concentrations were determined using an ELISA.

Results: The findings showed that patients with IHD had substantially higher blood levels of plasmin and suPAR than those in the control group ($P < 0.01$).

Conclusions: An increase in plasmin and suPAR levels is associated with IHD risk factors, which suggest them as biomarkers for IHD diagnosis and progress.

Keywords: Plasmin, Soluble urokinase plasminogen activator receptor, and ischemic heart diseases.

Introduction

Diseases of the coronary arteries, heart attacks, high blood pressure, and strokes are the most common forms of cardiovascular diseases(CVD) [1]. Coronary artery disease (CAD) is another name for CVD, which includes ischemic heart disease (IHD). In people with an ischemic circulatory system, fat deposits within the arteries limit the blood flow to the heart, resulting in either partial or complete heart blockage [2]. On the other hand, if the blood supply to the heart is cut off or obstructed for an extended period, pain, angina, and injury to the cardiac muscles may occur, leading to damage to the coronary arteries. In most cases, coronary heart disease is the cause of CVD. Lipid and fibrous deposits in the large and medium arteries signify coronary heart disease[3]. Two of the most significant problems that may result from a thrombus, also known as a blood clot, are myocardial ischemia and stroke [4]. Atherosclerosis is a progressive hardening of the arteries brought on by plaque accumulation. Risk factors include a diet heavy in saturated fat, high blood pressure, diabetes, smoking, obesity, and inactivity. Monocytes, T lymphocytes, and mast cells are moved into the intima of the vessel wall by

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these adhesion molecules, where they remain until they are released into the lumen [5]. Macrophages are more than just "vessels" for the accumulation of LDL cholesterol. They do this by aggressively promoting inflammation, the activation of T lymphocytes, as well as the recruitment of more macrophages. Cytokines may be involved in a variety of cardiovascular disorders [6],[7]. One of the most important mechanisms for regulating vascular health is the Plasminogen system, which plays an essential role in Atherosclerosis, myocardial infarction (MI), and re-infarction [8]. Activation of the fibrinolytic system depends on converting the plasma zymogen, Plasminogen, to the serine protease, by the physiological activators urokinase-type Plasminogen activator (uPA) or tissue-type plasminogen activator (tPA). Plasmin is the primary molecule in this system [9]. Many other critical physiological and pathological processes are impacted by the plasminogen/plasmin system, including the breakdown of the extracellular matrix, cell migration, embryonic development, tissue remodeling, wound healing, angiogenesis, inflammation, and migration of tumor cells [10]. Soluble urokinase plasminogen activator receptor (suPAR) is the soluble form of the membrane-bound receptor uPAR, which is bound to the membrane by a glycosylphosphatidylinositol (GPI) anchor. The protein consists of three domains, D1-D3, connected with a linker region between D1 and D2D3. Recently, it has been studied the link between inflammatory biomarkers and CVD in the general population, suPAR has been suggested as a biomarker for acute and chronic organ injury. In the context of cardiovascular disorders like stroke and coronary artery disease, however, it was specifically studied as a biomarker of low-grade inflammation [11] [12].

Subjects and Methods

Subjects

A case-control study involving 120 individuals, 60 had IHD (MI=30,angina=30) with mean age (60.7±2.06, 21 female, 39 male). Sixty healthy subjects (female =15 and male=45) served as a healthy control group, with average ages (48.7±0.86; 15 female, 45 male), with no history of IHD, endocrine disorders, metabolic or renal disease, acute sickness, or infection. Subjects were selected in October 2021 and July 2022. Laboratory testing was performed at Al Nasiriyah Heart Center and the Clinical biochemistry Research Lab at the college of medicine, University of Al-Qadisiyah

Methods

5 ml of blood was collected from all subjects and centrifuged (4000 xg) for 15-20 minutes at four °C to obtain serum. The serum will be kept at -80°C in Eppendorf tubes (0.3 mL)for further biochemical analysis. Plasmin and suPAR were measured by ELISA following the manufacturer's recommendations (Bioassay, China).

Statistical analysis

SPSS Statistics 23 software was used for statistical analysis. The data were expressed as Means + standard error of the mean (SEM). The Andersen-Darling test was used to check for normality. Student t-test was used to show the significance between patients and control. A one-way ANOVA, followed by Tucky, was conducted to determine whether or not there were any statistically significant

differences between the control and patient groups (MI and angina). A *P* value of < 0.05 is considered necessary throughout**.**

Results

General characteristics of the patients and control groups

One hundred twenty subjects were included, 60 patients with IHD, 30 patients with myocardial infarction (MI) with mean age (60.7±2.06 years; 8 female, 22 male), and 30 patients with angina, with mean age (57.0±0.33 years; 13 female, 17 male). Sixty healthy subjects were included in the study as a healthy control group, whose mean age was (48.7±0.86 years; 15 female, 45male). All clinical and hemodynamic variables are summarized in (table 1).

Table.1: The clinical and hemodynamic variables of the patients and control groups.

* indicates significant (P≤0.05) compared to control

parameters	Groups			p-value
	Angina	MI	Control	
Total Number	**30**	**30**	**60**	
Sex				
Females, N (%)	**13(43.3%)**	**(26.6%)**	**15(25%)**	***P*≥0.05**
Males, N (%)	**17(56.5%)**	**22(73.3%)**	**45(75%)**	***P*≤0.05**
Age(years)				
Mean ± SEM	**57±0.33**	**60.7±2.06**	**48.7±0.87**	***P*≥0.05**
Family history with diseases				
Yes	**3(10%)***	**9(30%)***	**0(0%)**	***P*≤0.05**
No	**27(90%)***	**21(70%)***	**0(0%)**	***P*≤0.05**
Other diseases				
Hypertension	**9(30%)***	**8(26%)***	**0(0%)**	***P*≤0.05**
Diabetes mellitus	**4(13%)***	**10(33%)***	**0(0%)**	***P*≤0.05**
Smoking				
Smoking	**2(6.6%)***	**3(10%)***	**0(0%)**	***P*≤0.05**
Nonsmoking	**28(93.3%)***	**27(90%)***	**60(100%)**	***P*≤0.05**

Serum Plasmin levels in IHD patients and control

The results showed high serum plasmin levels in patient groups with IHD compared to the control ($P \leq 0.01$, Figure . 1).

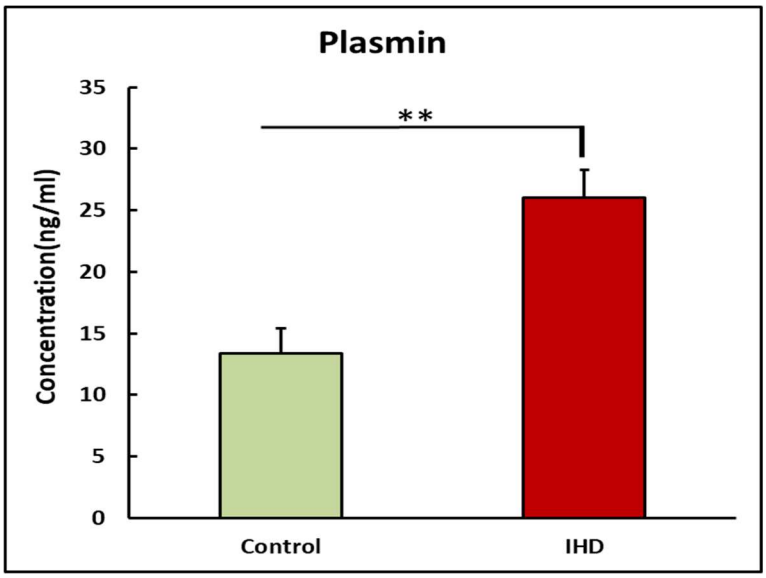

Figure 1: Serum plasmin levels in patients with IHD and control groups. Data are expressed as means ± SEM **indicates a significant change ($P \leq 0.01$) between IHD patient and control groups.

A significantly higher serum level of Plasmin was found in both angina ($P \leq 0.05$) and MI ($P \leq 0.01$) groups compared to the control, with a significant rise in the MI group. Also, significant changes were observed between patients with angina and MI ($P \leq 0.05$, figure 2)

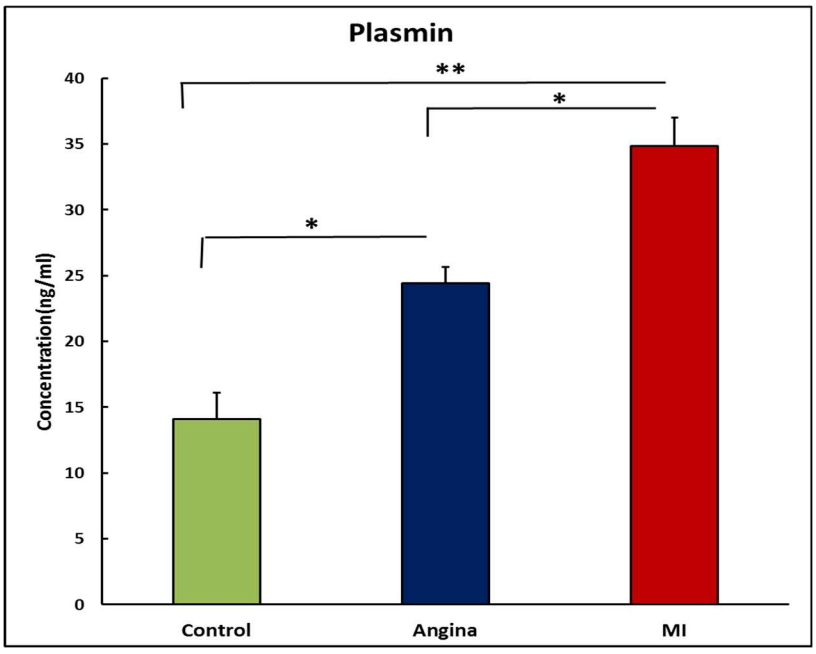

Figure 2: Serum Plasmin levels in patients with angina, MI, and control groups. Data are expressed as means ± SEM. * indicates a significant change ($P \leq 0.05$), and **indicates a significant change ($P < 0.01$ between study groups.

Serum level of soluble urokinase plasminogen activator receptor(suPAR) in IHD patients and control

The levels of released suPAR in the serum were determined using ELISA. The present study showed serum suPAR levels were significantly higher in patients with IHD than in the control group ($P \leq 0.01$, Figure 3).

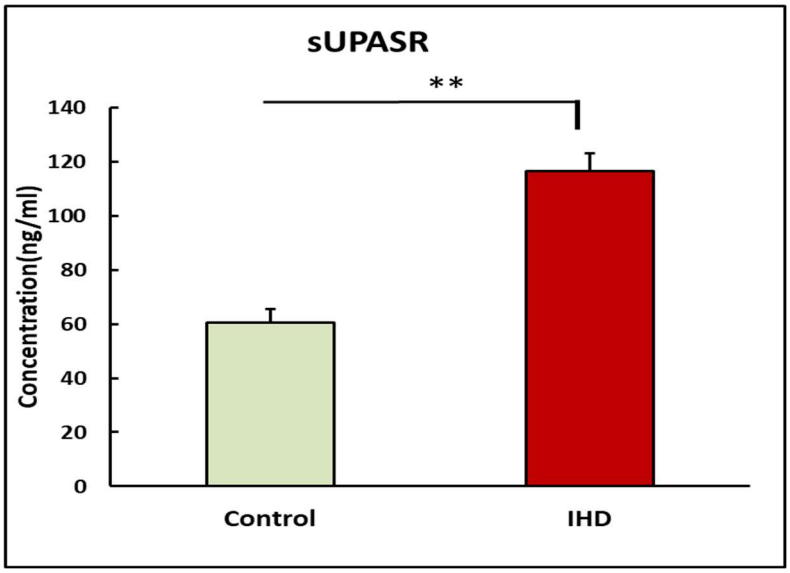

Figure 3: Serum suPAR levels in patients with IHD and control groups. Data are expressed as means ± SEM **indicates a significant change ($P \leq 0.01$) between IHD patient and control groups.

The high increase levels were reported in patients with angina($P \leq 0.01$). Non-significant changes were indicated between patient groups ($P \geq 0.05$, Figure.4).

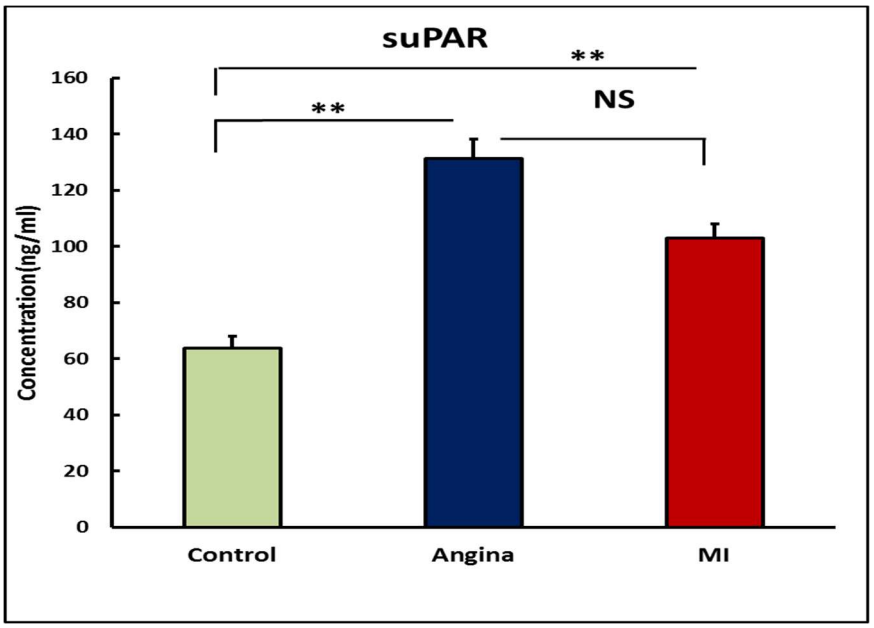

Figure 4: Serum suPAR levels in patients with angina, MI, and control groups. Data are expressed as means ± SEM. **indicates a significant change ($P \leq 0.01$) between study groups. NS non-significant

Discussion

The IHD pathogenesis is complex; that involved different biochemical factors. Thus, the investigation of cardiac biomarkers associated with IHD may open a new sight in IHD disease diagnosis and treatment.

Serum level of plasmin in IHD

The plasminogen is converted into plasmin. Blood plasma proteins such as fibrin clots may be degraded by the enzyme plasmin found in the bloodstream. The plasminogen gene is responsible for producing plasmin in humans. The natural activators of Plasminogen, tPA, and uPA cause a bond cleavage at a particular point in the plasminogen molecule, resulting in a two-chain molecule connected by two disulfide bonds. There are many ways that plasmin can be stopped, but one of the main ways is by binding to plasmin inhibitors, which form a stable complex that has little effect on proteins [13].

The current study showed that the biomarker plasmin in serum increased significantly in patients with IHD compared to the control group ($P \leq 0.05$, Figures 1 and 2). This finding was consistent with Drinan, M. C., et al. [14]. They found that patients with CAD have higher plasmin levels in their serum than healthy control. Plasmin. Besides fibrin, it is involved in various physiological and pathological processes. Its primary target is fibrin. As part of embryonic development, cell migration, tissue remodeling, wound healing, angiogenesis, and inflammation, this

enzyme can turn pro-hormones and cytokines into active forms. It can also be used to contest cancer and spread it to other body parts [15].

Plasmin is formed especially on the fibrin surface, which provides binding sites for plasminogen and its primary activator in the blood, tPA. This binding enhances plasminogen activation and directs plasmin activity to areas of fibrin formation, promoting effective clot breakdown. Plasma inhibitors, such as the plasmin inhibitor and the plasminogen activator inhibitor 1 (PAI-1), also have a role in keeping regulation under control. Plasmin deficiency may result in thrombosis because clots are not adequately destroyed. Plasminogen deficiency causes defective liver repair, wound healing, and reproductive problems [16]

Serum level of Soluble urokinase plasminogen activator receptor (suPAR) in IHD

suPAR stands for soluble urokinase plasminogen activator receptor. Smooth muscle cells Immune cells, and endothelial cells, are the most common cells that express suPAR. It is also recognized as vitronectin, or urokinase, a membrane-bound receptor for uPA. During inflammation or immunological activation, membrane-bound uPAR cleaved, resulting in the soluble variant of uPAR, which is known as suPAR [17]. The immune system's activation is positively associated with the concentration of suPAR. Thus it is a measure of disease severity, aggressiveness, and a predictor of morbidity and death in various acute and chronic disorders [18]. suPAR may be found in the following bodily fluids: urine, plasma, serum, blood, and cerebrospinal fluid [19]; the current study showed that the serum biomarker suPAR increased significantly in patients with IHD compared to the control group ($P \leq 0.05$, Figures 3 and 4). A survey by Persson, Margaretha, et al. declared that high levels of suPAR aren't linked to established cardiovascular risk factors but are linked to a higher risk of CVD in elderly subjects [20]. Also, it was found those who have high levels of plasma suPAR have a heart attack more than those who don't, and these levels are also linked to the presence and severity of CAD, whether suspected or confirmed [21].

Compared to healthy people, suPAR is higher in those with cardiovascular disorders. suPAR is a biomarker that measures the immune system's activity in response to inflammatory stimuli. suPAR levels are linked to inflammatory indicators like C-reactive protein, tumor necrosis factor, and other variables like leukocyte numbers. It's also linked to organ damage in several conditions. suPAR levels beyond a certain threshold have been related to an improved risk of systemic inflammatory response syndrome and heart disease in the general population. In the general population, suPAR predicts cardiovascular morbidity and death. Healthy people have suPAR plasma levels of less than four ng/mL, about 4–6 ng/mL in emergency rooms, and more than six ng/mL in critically ill people. A low level of suPAR indicates a good prognosis and supports the decision to let the person go home. suPAR is cleared by both cardiac and renal mechanisms [22],[18],[23]

Conclusions

The serum levels of plasmin and suPAR were increased significantly in IHD patients, which suggests the ability of these biomarkers to predict the risk of IHD. Further studies are needed to confirm the applicability of these biomarkers in IHD diagnosis and treatment.

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