Assessment of asprosin level and some of physiological variables in patients with cardiovascular diseases in Kirkuk city, Iraq

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ABSTRACT

Introduction and Aim: Asprosin is a novel fasting-induced glucogenic adipokine, which stimulates the liver to release glucose into the blood stream. The aim of this study was to examine the role of asprosin as well as various physiological and oxidative stress factors in atherosclerosis and myocardial infarction patients in comparison to healthy controls in Kirkuk city, in order to clarify whether asprosin helps in protecting heart and preventing heart disease.

Materials and Methods: This study included blood samples collected from patients (n=70) and normal healthy controls (n=20), aged between 45-65 years from the Kirkuk General Hospital and external specialized clinical centers between December 2021 to February 2022. The samples were divided into three groups which included healthy controls (n=20), patients suffering from atherosclerosis (n=40) and myocardial infarction (n=30) respectively. Individuals in all groups were tested for their blood ASP, CPK-BM Tnt and lipid profile levels. Blood serum was also tested for concentration of FBS, INS, HbA1c, MDA and GSH.

Results: The asprosin, CPK-BM, Cardiac troponin (TNT) and INS levels was observed to be significantly elevated in atherosclerosis patients in comparison to healthy controls. However, in myocardial infarction patients significant increase levels was seen only for CPK-BM and INS levels. Lipid profiling showed that except for HDL levels, significant increased levels for TC, TG, LDL and VLDL in both atherosclerosis and MI patients as compared to healthy individuals. The concentration of FBS was seen elevated in blood serum of atherosclerosis and MI patients in comparison to controls. No significant increase was observed for HbA1c and oxidative stress hormones MDA and GSH).

Conclusion: Changes in asprosin levels in patients with cardiovascular disease could be considered as a biochemical marker to estimate the severity of injury in heart and heart muscles.

Keywords: Atherosclerosis; myocardial infarction; asprosin; lipid profile; CPK-MB; cardiac troponin; FBS; HbA1c.

INTRODUCTION

Cardiovascular diseases (CVD), one of the leading causes of death worldwide is on the rise due to urbanization (1). CVD represents a group of disorders that affect the heart and blood vessels, which are mainly due to blockage that prevents blood flow from reaching the heart. The most common cause of blockage is fatty deposits in the inner walls of the blood vessels that feed them (2). Myocardial infarction is caused by a plaque or atherosclerotic plaque ulceration of the coronary arteries, which leads to ischemia and the occurrence of complete or partial blockage in the arteries, leading to damage to the cardiac muscle cells (3). Atherosclerosis is a complex multifactorial disease, caused by an injury to the arterial endothelium, leading to an inflammatory response in the vessel wall (4,5) add plaque formation along the walls of blood vessels, narrowing these arteries and restricting the flow of oxygenated blood leading to a heart attack.

Several cardiac enzymes such as Troponin and creatine kinase have been used as biomarkers in cardiac disease. Troponin, a cardiac enzyme is involved in contraction of cardiac and skeletal muscles. Increased levels of troponin T in bloodstream has been associated to myocardial damage, the levels of which have been used in determining the severity of a heart attack (6). Similarly, the enzyme creatine phosphokinase also known as creatine kinase has been assayed in blood, as a biomarker in acute myocardial infarction (7). Asprosin is a novel hormone discovered in 2016 is secreted by the white adipose tissue of the body (8). Asprosin, is expressed by the gene Fibrillin1, present in most cells of the body including human cutaneous fibroblasts, pancreatic B cells, peripheral tissues and organs (8,9,10). Asprosin, has been shown to be associate in metabolic disorders such as cardiovascular disease, diabetes, polycystic ovarian syndrome, obesity and implicated as a diagnostic
biomarker in these disorders (11). The asprosin hormone functions as an antioxidant, thereby preventing oxidative stress in the heart muscle (10). Decreased concentrations in the serum have been related to an increased risk of cardiovascular disease (11). In this study, we aimed to test for asprosin, together with other physiological and oxidative stress factors in patients with atherosclerosis and myocardial infarction and investigate into the association and role of these factors in these diseases.

MATERIALS AND METHODS

Experimental design

This study included 70 cardiovascular disease patients from the Kirkuk General Hospital and external specialized Clinic in Iraq. The patients were divided as those suffering from atherosclerosis (n=40) and myocardial infarction (n=30). The study also included 20 normal healthy individuals as control.

Venous blood (5 ml) was collected from each individual included in the study after an overnight fast. Blood collected was transferred to sterile tubes, centrifuged at 3000 rpm for 15 mins to separate out the serum. The serum was pipetted out and stored at -20°C until use. The serum was subjected to tests for biochemical, hormonal and physiological parameters. Clinical data regarding each patient was collected by means of a questionnaire and the information kept confidential.

Determination of serum lipid profile

Lipid profile included measurements of Total cholesterol (TC), Total glycerides (TG), Low-density lipoprotein (LDL-C), High-density lipoprotein (HDL-C) and very low-density lipoprotein (vLDL-C). Analysis of lipid profile was done using diagnostic kits (Biolabo, France) following the protocol mentioned in the respective kits and the colour developed measured at an absorbance of 500nm.

Determination of serum asprosin (ASP), creatine phosphokinase (CPK-MB), cardiac troponin (TnT) and Insulin (INS) concentration

Diagnostic kits were used to determine the asprosin levels using the Human Asprosin ELISA kit (No. E4095Hu, Bioassay Technology Laboratory, UK), following the manufacturer's instructions (15). Similarly, diagnostic kits were used in measuring the CPK-MB (FUJIFILM, Japan), TnT (AFIAS Troponin T kit, France) and INS (Insulin kit, USA) concentrations in serum.

Determination of fasting blood sugar (FBS), and HemoglobinA1c (HbA1c)

The concentration of FBS and HbA1c in blood serum was measured using the diagnostic kits Glugose (RANDOX, USA) and HbA1c (Boditech Med. Inc., Korea) respectively, following the manufacturer's instructions.

Estimation of Glutathione (GSH) and malondialdehyde (MDA) in blood serum

Serum Glutathione assay was performed according to the method described by Sedlak and Lindsay (12) and malondialdehyde assay following the protocol mentioned by Alobaidi and Samarrai (13).

Statistical analysis

For each parameter data, the mean ± SD values were calculated. Statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS) package Version 25. The statistical significant difference between values was assessed using one-way analysis of variance (ANOVA) followed by the Duncan multi-range test. The level of significance was considered at p ≤ 0.05.

RESULTS

Lipid profile in patients and control group

The lipid profile results are shown in Table 1. As seen from Table, there was a significant increase (p≤0.05) in the concentration of TC in patients of atherosclerosis as well as myocardial infarction in comparison to the control group. Similarly, a significant (p < 0.05) increase in the concentrations of TG, LDL-C and VLDL was seen in patient groups suffering from atherosclerosis and myocardial infarction respectively as compared with control group. However for HDL-C, a significant (p≤0.05) decrease in concentration was seen from controls in atherosclerosis and myocardial infarction patient groups. No significant difference was observed for lipid profile levels between patients in the atherosclerosis group as well as myocardial infarction group.

Serum levels of Asprosin, CPK-BM, cardiac-Tnt and INS in controls and patients

In patients in the atherosclerosis group a significant increase in asprosin, CPK-BM, cardiac-Tnt and INS levels compared to the control group was observed (Table 2). However, among the myocardial infarction patients, only values obtained for the parameter CPK-BM was found to significantly increase (p<0.05) compared to controls. While, in the same group no
significant difference was observed for ASP, TnT and INS levels in comparison to controls. A comparison between groups showed the levels for ASP, TnT and INS to be higher in the atherosclerosis than in the myocardial infarction group (Table 2).

Blood serum levels for FBS, HbA1c and oxidative stress hormone (MDA, GSH) The levels for FBS, HbA1c, MDA and GSH obtained are presented in Table 3. Compared to controls a significant increase (p≤0.05) in the concentration of FBS in atherosclerosis and myocardial infarction patients was observed. A marginal increase in levels from controls was seen for parameters HbA1c, MDA and GSH, which was statistically significant in the case of MDA and GSH (Table 3). No significant difference for these values was observed between groups for atherosclerosis and myocardial infarction.

**DISCUSSION**

The present study sought to assess the levels of asprosin as well as the levels of other metabolic parameters in patients with atherosclerosis and myocardial infarction cardiovascular disease. Asprosin is a recently discovered hormone, mainly synthesized by white adipocytes (14). Asprosin has been implicated to be significantly elevated in cardiovascular disease patients such as those suffering from angina pectoris and cardio myopathy (15). Our study with asprosin levels in atherosclerosis and myocardial infarction patients showed an elevated level for this hormone in both groups in comparison to controls.

This is in agreement to a previous study, by Moradi et al., (3), wherein a significant increase in asprosin concentration was reported in patients with atherosclerosis. Although the exact role of asprosin is not known, it has been reported that elevated levels of this hormone in both groups can enhance the mitochondrial respiration within heart muscle cells thereby protecting the heart from hypoxia (15).

Studies have shown a relation to exist between asprosin to some metabolic related diseases (13, 16-18). Our results in this study showed that the lipid profile levels to be significantly elevated in atherosclerosis and myocardial infarction patients. Our result agrees to an earlier report which had shown lipid levels to be independently associated with triglycerides (18). Further, hyperlipidemic conditions have been associated with release of asprosin by pancreatic cells, which triggers pathways that lead to insulin resistance (19). This probably explains the elevated levels seen for FBS in patients in this study probably indicating the development of insulin resistance in these CVD patients.

Creatine phosphokinase has been indicated as a risk factor in coronary embolism and heart attacks (20). An elevated level of CPK-BM was seen among patients in this study which probably is due to CAD. On the other hand cardiac troponin levels was seen significantly elevated only in patients with atherosclerosis, which probably indicates that it considered as a biomarker in identifying patients at risk of developing atherosclerosis.

However, studies on larger sample size are required to ascertain the finding. Due to the fact that diabetes is characterized by chronic high blood sugar and causes long-term complications such as heart disease, the estimation of glycated hemoglobin (HbA1c) was adopted as a diagnostic tool for monitoring blood sugar. The results showed a positive association of asprosin with FBS and HbA1c, and thus the level of asprosin can be adopted as an indicator for early development of diabetes.

- 'a' and 'b' indicate significant difference at the level (p≤0.05).

**Table 1:** Lipid profile of controls and patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control Group</th>
<th>Atherosclerosis</th>
<th>Myocardial infarction</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dl) Mean±SD</td>
<td>130.625±23.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>178.775±46.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>173.850±22.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TG (mg/dl) Mean±SD</td>
<td>114.325±44.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>194.875±73.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>170.7833±83.57&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL-C (mg/dl) Mean±SD</td>
<td>46.550±3.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.100±3.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.433±5.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL-C (mg/dl) Mean±SD</td>
<td>94.140±13.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>117.107±37.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>107.130±31.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>VLDL (mg/dl) Mean±SD</td>
<td>24.114±9.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.020±14.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.610±16.54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Table 2:** The Asprosin, CPK-BM, Tnt and INS levels in controls and patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control Group</th>
<th>Atherosclerosis</th>
<th>Myocardial infarction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASP (ng/ml) mean±SD</td>
<td>8.69±4.80b</td>
<td>12.02±10.22a</td>
<td>8.92±5.83b</td>
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<tr>
<td>CPK-BM (U/L) mean±SD</td>
<td>86.600±5.685b</td>
<td>136.225±13.006a</td>
<td>196.700±20.387a</td>
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<tr>
<td>Tnt (ng/ml) mean±SD</td>
<td>0.25±0.92b</td>
<td>0.70±0.88a</td>
<td>0.28±0.21b</td>
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<tr>
<td>INS µU/ml Mean±SD</td>
<td>9.610±5.380</td>
<td>22.501±10.138a</td>
<td>13.139±7.325</td>
</tr>
</tbody>
</table>

- 'a' and 'b' indicate significant difference at the level (p≤0.05).
Oxidation is one of the natural and important processes that occur inside the human body resulting in the production of free radicals. An association of the oxidative stress factors glucose and malondialdehyde showed no association between the factors among atherosclerosis and myocardial infarction patients. However, this in contrast to an earlier study involving diabetes mellitus patients wherein elevated asprosin level was correlated to oxidative stress enzymes (22). This has been attributed to chronic exposure to high levels of blood glucose which cause an increase in the production of reactive oxygen species thereby increasing oxidative stress (13).

### CONCLUSION

In this study asprosin levels were seen elevated in atherosclerosis and myocardial infarction patients. In general the higher level in patients was seen to be correlated to lipid profile levels, creatine phosphatase, fasting blood sugar and insulin resistance. This suggests that asprosin could be considered as possible biomarker of cardiovascular diseases such as atherosclerosis and myocardial infarction. However, this study needs to be further investigated involving a larger number of samples.

### CONFLICT OF INTEREST

Authors have no conflicts of interest.

### REFERENCES


