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Association of Fibronectin, Leptin and LDL-Oxide Serum Levels With Coronary Artery Disease in Non-smoker and Non-diabetic Patients

Ali Reza Yaghoubi[1], Farank Kargar[1], Fatemeh Khaki-Khatibi[2*]

Abstract

Objective: Coronary artery disease (CAD) is a leading cause of death of women and men worldwide. Endothelial dysfunction, smoker, diabetic, cell adhesion and oxidative stress may be considered as novel risk factors of CAD. These materials are cooperative events involved in atherosclerosis development. In the present study the serum levels of fibronectin, leptin and LDL-oxide were investigated in patients of CAD including non-smoker and non-diabetic with control group. Measurement of these parameters helps to prevent and treat the disease.

Materials and Methods: In this study we measured serum levels of fibronectin, leptin and LDL-oxide in 200 individuals including 100 patients with CAD and 100 individuals as control group. Also patients with malignancy, renal and liver diseases and other disease were excluded from the study. Serum leptin and fibronectin were measured by enzyme-linked immunosorbent assay (ELISA) method using kits from German Imediagnostic and Chinese Crystal Day companies, respectively. Moreover, ELISA procedures were used to determine the serum LDL-oxide.

Results: The serum levels of fibronectin, leptin and LDL-oxide were increased significantly as compared to control group ($P \leq 0.05$ in all cases). It seems that there was strong (+) correlation between fibronectin, leptin and LDL-oxide in CAD.

Conclusion: It was concluded that endothelial dysfunction, cell adhesion and stress oxidative are cooperative events involved in atherosclerosis development. Fibronectin, leptin and LDL-oxide have become greatly important in pathogenesis of CAD. Association between fibronectin, leptin and stress oxidative suggest that their involvement in development of atherosclerosis can be used as detective measure.

Keywords: Coronary artery disease, fibronectin, Leptin, LDL-oxide, Non-diabetic, Non-smoker

Introduction

Coronary artery disease (CAD) is one of the leading causes of morbidity and mortality in developed countries (1) which results in 7.2 million deaths per year in human society in the world (2). The disease is affected by factors like age, gender (3), diabetes, lipid profile, body mass index (BMI), oxidative stress, homeostasis disorder, metabolic syndrome, smoking and heredity (4).

Blood platelets, coagulation factors, endothelial cells and fibrinolytic system play an important role in homeostasis (5), which its disruption makes body susceptible to CAD. Also, it is interesting to know that changing in endothelial cells phenotype and fibrinolytic system is more common than the other factor in atherosclerosis (6,7). Alteration in fibrinolytic systems, gene expression, response to growth factors, and size of Golgi apparatus and rough endothelium reticulum increase changes of endothelial cells phenotype (7,8).

Endothelial cells are the main source of fibronectin (6). Fibronectin is a high molecular weight glycoprotein presenting in body fluids and tissue's extracellular matrix in soluble and insoluble forms, respectively (8,9). It acts as a bridge between cells and collagen network and is also involved in various processes such as cell growth, adhesion, migration and wound healing process (9). Increased expression of fibronectin makes changes in muscle cell of intimae layer in coronary artery. This event leads to atherosclerotic lesions (7).

Metabolic syndrome is one of the most important public health issues and its prevalence increases with diabetes and obesity (10). Obesity is associated with adipose tissue inflammation and its progress stimulates adipose tissue to secrete adipokines like leptin, adiponektin, and resistin (11). Obesity (OB) gene expresses leptin as a 160 kDa hydrophobic protein in adipose tissue (10). Leptin receptor (OB-R) belongs to the first class of cytokine receptors and is expressed by diabetes gene (12). It acts through JAK/STAT and AMPK signaling pathway (13). OB-R is present on the surface of cells such as monocytes, atherosclerosis plaques, platelets, endothelial cells, neointima cells and

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[1]Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran. [2]Drug Applied Research Center and Department of Clinical Biochemistry, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.
***Corresponding author:** Fatemeh Khaki-Khatibi, Tel: +98-41-33364666, Fax: +98-41-33364666, Email: fatemehkhakikhatibi@yahoo.com

vascular smooth muscle cells (12,14). Leptin secretion is motivated by insulin and its concentration is associated with obesity, BMI, and food intake. Leptin plays an important role in angiogenesis, osteogenesis, immune system and pregnancy (15,16). Its major roles in immune system includes macrophage differentiation and phagocytosis, neutrophil chemotaxis, free radicals releasing by monocytes, activating NK cells, and MCP-1 production (12,13,17). Leptin increases mitochondrial super oxide dismutase products, fatty acid oxidation, protein kinase A and reactive oxygen species (ROS) (18). Increased ROS, more than antioxidant capacity, causes oxidative stress involved in CAD by damaging biomolecules such as lipids, proteins and nucleic acids (19,20).

Oxidative stress may have an important role in pathogenesis of atherogenesis. Production of excessive ROS in oxidative stress conditions results in proxidation of polyunsaturated fatty acids which are the main components of lipoproteins. Among lipoproteins, LDL comparably undergoes more lipid proxidation (21,22). Elevated levels of oxidized low-density lipoprotein (Ox-LDL) have previously been detected in the plasma of CAD patients (23). Oxidative Ox-LDL penetrates into the artery wall at the earliest stage of atherosclerosis and leads to generation of foam cells, as the main factor of atherosclerosis. It also undergoes further oxidative or enzymatic modifications. Ox-LDL and related compounds are also observed in lesion formation at the later stages of atherosclerosis. Therefore, Ox-LDL could play a role both in atherogenesis and in plaque complications (24,25).

This article discusses association between serum levels of fibronectin, leptin, and LDL-oxide with CAD in non-smoker and non-diabetic patients. Measuring these parameters helps in prevention and treatment of the disease.

Materials and Methods

The study investigated 200 people by analytical descriptive method. The patients were 100 individuals. The control group included healthy people with no sign of CAD, diabetes, smoking, hypertension, and hyperlipidemia (n = 100). All of the volunteers were checked for the presence of diseases such advanced liver disease, renal failure, autoimmune diseases, etc.

After project description and satisfaction of volunteers, 10 ml venous blood was collected at 10 AM after one night rest and fasting. Blood pressure was measured after 5 minutes in prone position. Normal and hypertensive subjects were divided into two groups based on blood pressure greater or less than 140/90 mm Hg.

Venous blood samples were centrifuged at speed of 2000 RPM for 10 minutes and then frozen at -80°C until testing. Serum leptin and fibronectin levels were measured by enzyme-linked immunosorbent assay (ELISA) method using kits from German Imediagnostic and Chinese Crystal Day companies, respectively. Moreover, ELISA procedures were used to determine the serum LDL-oxide (Glory Scince co. Ltd Cat. No: 93614).

Data are presented as mean ± SD. Statistical analysis was performed using SPSS version 16. Comparison of continuous parameters was performed using the Student *t* test or analysis of variance (ANOVA), where appropriate. *P* values equal to or less than 0.05 were considered significant.

Results

The results of serum fibronectin analysis are shown in Table 1. As the table shows, mean serum fibronectin levels in patients were significantly higher than the control group ($P < 0.05$; Table 1). Mean levels of serum leptin in CAD patients were significantly higher than control group ($P < 0.05$; Table 1). As the table shows mean serum LDL-oxide levels in patients were significantly higher than the control group ($P < 0.05$; Table 1).

Discussion

CAD is one of the biggest causes of death worldwide (26). It is believed that the disease is an ongoing and continuing process which causes tissue damage and heart failure (27). Fibronectin gene expression is distinctly associated with vascular smooth muscle cell and leptin expression is affected by ERK1/2. ERK1/2 is a MAP kinase with 42 up to 44 kDa molecular weight. Its activation is one of the major pathways in regulating proliferation of the vascular smooth muscle cells. Fibronectin participates in the processes which changes the form of vascular smooth muscle cells during atherosclerosis and causes accumulation of cells (8,14,28). Fibronectin with fibrinogen/fibrin can contribute to the growth and sustainability of thrombosis. Cross connection of fibronectin to fibrin is established by FXIII which increase platelet adhesion and thrombus development as well as atherosclerosis (28). Several studies found a positive association between the expression of fibronectin and atherosclerosis. Ekmekci et al showed that the mean plasma fibronectin levels in patients with atherosclerosis were significantly higher than healthy people (29). Another study by Matuskova et al revealed that reduction of plasma fibronectin delays extension of thrombosis in arterial trauma (30). This study approved the positive association between fibronectin level in serum and atherosclerosis progression and moreover showed fibronectin level in serum increased by developing atherosclerosis. In our research serum levels of fibronectin in CAD patients were higher than control group. Taken together, these data indicate that fibronectin expression is closely associated with development of CAD.

Leptin is an adipokine with 167 amino acid residues

Table 1. Levels of Serum of Fibronectin, Leptin and LDL-Oxide in Patients With (Non-smoker and Non-diabetic) and Control Group

Parameter	Patient (n=100) (Mean±SD)	Control (n=100) (Mean ±SD)
Fibronectin (mg/ml)	262.45±23.09	240.04±25.08
Leptin (ng/ml)	6.96±1.76	1.47±0.05
LDL-oxide (ug/ml)	2.98±0.83	0.02

Abbreviation: CAD, coronary artery disease; LDL, low-density lipoprotein.

which is the obesity gene in adipose tissue. Leptin secretion affects hypothalamus to reduce food intake and also modulates carbohydrate and lipid metabolism to increase energy consumption (31). Serum levels of leptin are highly related to BMI, diabetes and genetic disorders which is variable by dietary changes (13,14). Based on the data obtained from animal and human studies, hyperleptinemia is associated with increased mortality in CAD. Increased serum leptin levels in humans are associated with atherosclerosis, obesity, myocardial infarction, insulin resistance, inflammation and homeostasis disruption. Leptin increases the production of MCP-1 and SOD products in aorta endothelial cells. Therefore, it plays an important role in the recruitment of macrophages, the formation and development of atherosclerotic plaque (13). According to recent reports, leptin plays a major role in the development of atherosclerotic lesions. Gormez et al reported that leptin gene expression in subcutaneous, epicardial and pericardial adipose tissue in CAD disease is significantly higher than other people (10). Mcmahon and colleagues stated in their study that high leptin levels could help identify SLE disease associated with atherosclerosis. They reported significant high serum leptin levels in patients with both SLE and CAD component in comparison to CAD patients alone (32). In the United States, Johnson et al reported that myocardial infarction (MI) patients have high serum level of leptin compared to control group (33). Karakas et al (34), Ekmekci et al (29), Wolk et al (35), and Payne et al (36) reported leptin as CAD development novel marker. Our research shows serum levels of leptin in CAD patients are higher than control group. According to this data, two hypotheses can be concluded; first, there is a positive association between CAD disease and serum levels of leptin, and second, serum levels of leptin increase with development of atherosclerosis.

It is postulated that oxidative stress is primarily mediated through the Ox-LDL and that the oxidation of other molecules (lipid, protein, and DNA) are also involved (37). Our results which confirmed data from previous studies, found that lipid peroxidation could potentially be more sensitive and highly correlated to large stenotic vascular lesion changes (38-40). In contrast, Kotur-Stevuljevic et al (41), Bridges et al (42) and Stranger et al (43) did not find any significant correlation between MDA and the angiographic diagnosis that indicated the severity of CAD.

In agreement with our findings the results from a prospective 3-year study show that circulating Ox-LDL is associated with the progression of ultrasound-assessed atherosclerosis in CAD patients (44). In another study the titer of auto antibodies to Ox-LDL was introduced as an independent predictor of the progression of carotid atherosclerosis in Finnish men (45). The similar conclusions were proposed by others (46-48).

Conclusion

It was concluded that endothelial dysfunction, cell adhesion and stress oxidative are cooperative events involved in atherosclerosis development. Fibronectin, leptin and LDL-oxide have become greatly important in pathogenesis of CAD and association between fibronectin, leptin and stress oxidative suggest that their involvement in atherosclerosis development can be used as a detective measure. Measuring these parameters helps to prevent and treat the disease. Although smoking and diabetes are important factors in causing CAD, in our study the patients were non-smoker and non-diabetic.

Ethical Issues

Ethics of this research work was approved by local ethics committee.

Conflict of interests

The authors declare no conflict of interests.

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