DEREGULATION OF SERUM LEPTIN LEVELS IN ACUTE CORONARY SYNDROME

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ABSTRACT

Objective: To test whether leptin is a risk factor for acute myocardial infarction.

Study Design: A case control study.

Place and Duration of Study: The study was carried out at Shifa International Hospital Islamabad for one year.

Patients and Methods: Serum leptin levels of 86 patients of acute coronary syndrome (ACS) were compared with 86 age and sex matched controls. Leptin levels were measured by enzyme linked immunosorbent assay (ELISA) technique.

Results: Our study indicated that patients of ACS have higher body mass index (BMI), systolic and diastolic blood pressure and blood sugar levels as compared to the controls (normal subjects free of any cardiac disease). Moreover serum leptin levels were also significantly higher in the patients of ACS (47.87 ± 6.16ng/ml) as compared to the control group (14.97 + 1.90ng/ml).

Smoking, higher systolic and diastolic blood pressure, blood sugar and leptin levels were significant risk factors for ACS in univariate analysis. Whereas smoking, higher systolic blood pressure, blood sugar and leptin levels also remained significant risk factors for ACS in the multivariate model. Multivariate analysis indicated that increased leptin levels lead to 7.9 % increase in the odds ratio of ACS.

Conclusions; The current study has indicated that patients of ACS have significantly higher leptin levels as compared to controls, and this leads to 7 % increase in the odds ratio of ACS. This study showed that in addition to the traditional risk factors like diabetes mellitus, smoking and hypertension, increased leptin levels is also an important link in the development of cardiovascular disease. Therefore development of therapeutic agents primarily directed against increased leptin levels could contribute in reducing the mortality and morbidity associated with CAD.

Keywords: Acute coronary syndrome, Coronary Artery Disease, Leptin

INTRODUCTION

Leptin is a 16 K Da (167-amino acid) protein expressed mainly by adipocytes and released in the blood in proportion to the size of adipose tissue and relays a satiety signal to the hypothalamus [1]. In the physiological range leptin serves as a physiological regulator of cardiovascular functions whereas elevated plasma leptin levels act as a pathophysiological trigger and/or marker for cardiovascular diseases due to tissue leptin resistance [2].

Coronary artery disease (CAD) is the most common form of heart disease and the single most important cause of death in the developed World [3]. Leptin has been implicated in many atherogenic processes common to the pathogenesis of cardiovascular disease and plaque rupture. Interestingly, it has been reported that mice lacking leptin (ob/ob) or leptin receptor (db/db) had reduced atherosclerotic lesion areas compared with controls. Because leptin regulates inflammation and immunity, it is intriguing to speculate that the reduced lesion areas are due to the absence of leptin and its proinflammatory effects in the mutant mice [4].

Increased leptin levels can lead to the generation, propagation and rupture of the atherosclerotic plaques by causing endothelial dysfunction [5], stimulation of mitochondrial superoxide production in the endothelial cells [6], generation of reactive oxygen species (ROS) [7], proliferation and activation of the

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circulating monocytes, [8] modulation of T-cell immune response [9] and production of inflammatory cytokines, e.g., tumor necrosing factor (TNF) and interleukin 6 (IL-6) [10]. Increased leptin levels also promote platelet aggregation and thrombosis on the ruptured plaque and can therefore lead to acute coronary syndrome (ACS) [11]. Furthermore, the presence of the leptin receptors on calcifying vascular cells suggest that leptin regulates the osteoblastic differentiation and calcification of vascular cells, therefore further contributing in the development of atherosclerosis [12].

Evidence has suggested that leptin plays a specific role in the intricate cascade of cardiovascular events, in addition to its well-established effects on plaque rupture and development of ACS. Increased leptin levels have also been demonstrated to correlate with the predisposing effects of CAD including insulin resistance, obesity, hyperlipidemia, metabolic syndrome and hypertension [13].

In the light of the above mentioned observations, the present study was designed to find out a relationship between increased leptin levels and acute coronary syndrome. By establishment of such a link, increased leptin levels may be used as a routine part of global risk assessment for ACS and restoration of normal leptin levels may help to reduce the mortality and morbidity associated with ACS.

PATIENTS AND METHODS

This was a case control study carried out at Shifa College of Medicine and Shifa International Hospital Islamabad. Non probability convenience sampling technique was used to recruit patients of ACS.

Subjects

A total of 172 subjects were included in this study, which were divided into two groups. Group A included 86 patients of ACS (Acute myocardial ionfarction and unstable angina). All the patients underwent a detailed history, complete physical examination and relevant laboratory investigations to document the presence of ACS. The criteria for diagnosis of ACS were, typical history of chest pain, ST elevation or depression of more than 0.1 mV in two consecutive ECG leads. Patients with clinical picture of unstable angina (pain at rest for >10 minutes or of new onset with a crescendo pattern with ST depression or T wave flattening or inversion and normal cardiac enzymes). In addition the patients with biochemical changes in troponin I (> 0.4 ng/ml) and CK-MB (> 9.3 ng/ml). Group B included 86 age and sex matched controls. The control group had no evidence of CAD or of any other cardiovascular diseases on the basis of history and physical examination.

Exclusion criteria included the subjects from whom the written consent could not be taken and the patients with acute inflammatory disease in the last 15 days (as Increased leptin levels are considered to be an acute phase reactant).

The protocol was approved by the ethics review committee and Institutional review board of Shifa College of Medicine.

Biochemical Measurements

Five milliliters (ml) of venous blood was drawn from the patients of ACS in the morning after an overnight fast of at least 10 hours. Same amount of the blood was also drawn from the control group.

Leptin levels were determined by ELISA (enzyme-linked immunosorbent assay) method, using DRG Leptin (sandwich) ELISA; EIA-2395 kit Germany. CK-MB was determined by microparticle enzyme immuno-assay (MEIA) based on 'sandwich' principle using AxSYM system from Abbott Laboratories USA. Troponin I was determined by Microparticle Enzyme immuno-assay (MEIA) based on 'sandwich' principle and cascade of alkaline phosphatase using AxSYM system from the Abbott Laboratories USA. Blood glucose levels were measured with the help of glucose test strips (Lot No. 23202) which were inserted into the Medisense optium Glucometer (Lot No.12345). Test was started as soon as the

blood sample was detected. The result was displayed after a lapse of about twenty seconds.

Estimation of Traditional Risk Factors

The recorded risk factors were hypertension, diabetes mellitus, obesity and cigarette smoking.

Hypertension: Hypertension was considered to be present if the patient was on antihypertensive medication at the time of admission, or blood pressure measured at three separate occasions at hospitalization was greater than 140/90 mm/Hg.

Diabetes mellitus: Diabetes mellitus was considered to be present if the patient was on ant diabetic medicine or had fasting blood glucose > 100 mg/dl or two random plasma glucose levels > 140 mg/dl.

Obesity: BMI of the subjects was calculated as weight (kg) divided by height (m2). The subjects with BMI greater than 25kg/m2 were considered overweight, whereas the subjects with body mass index (BMI) greater than 30kg/m2 were considered obese.

Cigarette smoking: subjects were defined as smokers if they were current smokers or

Table-1: Univariate analysis of showing the comparison between leptin, traditional risk factors, and ACS risk

Variables	Patients of ACS	Controls	Odds Ratio	95% CI	p value
Body mass index (kg/m^2)	25.82 ± 0.50	24.70 ± 0.42	1.25	0.0007 to 2.45	0.065
Systolic Blood Pressure (mm Hg)	141.40 ± 2.13*	127.30 ± 1.56	13.41	6.01 to 29.73	< 0.001
Diastolic Blood Pressure (mm Hg)	92.79 ± 1.51*	80.93 ± 1.39	7.69	4.04 to 15.88	< 0.001
Random Blood Sugar (mg/dl)	169 ± 8.98*	118 ± 4.26	4.54	2.3 to 8.91	< 0.001
Serum Leptin (ng/ml)	47.82 ± 6.16*	14.97 ± 1.92	13.65	65 3.47 to 16.63	< 0.001
Smoking %	38%*	9%	6.01	2.06 to 14.16	< 0.001

Values are expressed as Mean ± SEM.
*p value < 0.05 is taken as statistically significant as compared to controls.

Table-2: Serum Leptin levels in male and female patients of acute coronary syndrome in comparison with controls.

Gender	Leptin levels in patients of ACS (ng/ml)	Leptin levels in controls (ng/ml)	P value
Females	77.84 ± 13.62*	26.19 ± 5.52	n = 22
Males	37.57 ± 6.44*	10.98 ± 1.32	n = 64

Values are expressed as Mean ± SEM.
*p value < 0.05 is taken as statistically significant as compared to controls.

Table-3: Comparison of serum Leptin levels and BMI of patients and controls.

Body mass index	Leptin levels in Patients of ACS	Leptin levels in controls	p value
Normal weight Individuals, BMI $\leq$ 25 kg/m^2	33.80 ± 5.51*	9.42 ± 1.46	< 0.001
Over weight and obese individuals, BMI > 25 kg/m^2	62.20 ± 10.82*	21.84 ± 3.31	< 0.001

Values are expressed as Mean ± SEM.
*p value < 0.05 is taken as statistically significant as compared to controls.

Table-4: Multivariate conditional Logistic regression analysis incorporating leptin

Variables	Odds ratio	95 % CI	P value
Systolic B.P (mmHg)	11.01	4.46 to 27.71	0.001*
Random blood sugar (mg/dl)	3.12	1.29 to 7.54	0.011*
Leptin (ng/ml)	7.94	2.97 to 21.07	< 0.001*
Smoking	5.23	1.85 to 14.8	< 0.002*

*p value < 0.05 is taken as statistically significant.

had smoked (10 cigarettes/day) within the last ten years. All others were classified as non smokers.

Statistical Analysis

Data and the results of the study were analyzed on computer software SPSS version 10.0. Descriptive statistics were presented as mean ± standard error of mean comparing the patients of acute coronary syndrome (cases) and the healthy individuals (controls). Major risk factors of the ischemic heart disease including systolic and diastolic blood pressure, smoking, and diabetes and serum leptin levels were compared between cases and controls and univariate analysis was carried out by conditional logistic regression, by the application of t-test. Results were presented in the form of odds ratio, 95% confidence interval and p value. Multivariate conditional regression model was also applied to the factors which were proven significant in univariate analysis. The p-values less than 0.05 were regarded as statistically significant.

RESULTS

Results of the present study showed that the mean age of the patients with ACS was 60 + 2. Moreover, categorization of the age showed that 89% of the patients of ACS were older than 45 years of age. Number of male patients was 74% whereas females represented 26% of the patients. Cases and controls were matched for age and sex.

The univariate analysis of comparison between leptin, traditional risk factors and ACS risk is shown in table 1.

Comparison of the serum leptin levels was also done separately for males and females in experimental and control groups and values were found to be significantly different in both the groups (Table 2).

Further analysis of the data revealed that mean leptin levels in overweight and obese patients were significantly higher in ACS patients as compared to controls (Table 3).

The importance of leptin as a risk factor of coronary artery disease is also explored via Conditional Logistic regression analysis. Multivariate model was applied to only those risk factors which were considered to be significant in the univariate analysis (Table 4).

DISCUSSION

Data of the present study demonstrate that serum leptin levels are significantly elevated in the patients of ACS (47.87 ± 6.16ng/ml) as compared to the control group (14.97 + 1.90ng/ml). Similar findings have been reported in several other studies [14]. In a study conducted by Taneli et al [14]. leptin concentrations were found to be significantly higher in the patients of ST elevation myocardial infarctio (85.23 ± ng/ml) as compared to the controls (26.54 ± 3.2 ng/ml). Soderberg et al [15] also indicated the existence of a strong relationship between Increased leptin levels and ACS. Yan et al [16] also showed that serum leptin levels of patients with AMI are higher (53.45 ± 4.21 ng/ml) as compared to the controls (22.43 ± 1.90 ng/ml). Quebec cardiovascular study is in opposition of our findings, but the reason of this difference is not clear. However it is noteworthy that our analysis included only the cases with events (MI and unstable angina) whereas the Quebec study included the patients with effort angina and coronary insufficiency and these may not be strongly associated with increased leptin levels [17].

We also compared serum leptin levels in male and female patients of ACS separately and compared them with male and female control groups. The female group was having three times higher leptin levels as compared to the male group. Similar findings were reported by Wang et al [18] and AL-harithy who indicated that the adipose tissue of female secretes significantly higher amounts of leptin than that of men. Moreover, estrogen is also involved in the regulation of circulating leptin levels, therefore resulting in the higher leptin levels in females as compared to males [19].

Our study also showed that Increase in the leptin levels is a risk factor for the development of ACS independent of BMI. This finding is consistent with several other studies. As several other studies have

indicated that Increased leptin levels are associated with other risk factors of the CAD including hypertension, insulin resistance, hyperlipidemia, and CRP levels independent of BMI and these risk factors can therefore be the contributors of coronary events in patients with increased leptin levels but normal BMI [13, 20].

Relationship between Increased leptin levels and hypertension is addressed in several studies. It has been observed that leptin causes increased sympathetic activity to the kidneys, lower limbs and adrenals, and can therefore lead to vasoconstriction, hypertension and atherosclerosis resulting in increased incidence of CAD [21]. Moreover, Quehenberger demonstrated that there is an influence of leptin on the production of endothelin-1 (a potent vasoconstrictor) by the endothelial cells and can therefore act as a vasoconstrictor and promoter of HTN [22].

Investigators have also reported correlations between plasma leptin levels and insulin resistance independent of BMI [26], so leptin seems to predict subsequent development of type 2 diabetes and of course, associations between diabetese mellitus, insulin resistance and cardiovascular risk are well established [23].

A positive correlation between increased leptin levels and hyperlipidemia is also observed in some studies [24].

Another important finding of our results is that leptin retained its significance as a risk factor for ACS even in the multivariate logistic regression analysis. This analysis indicated that hypertension, smoking, diabetes mellitus as well as Increased leptin levels are the major risk factors for the acute coronary syndrome.

There are several studies providing an explanation for leptin as an independent risk factor for the coronary events. It has been shown that leptin is involved in atherogenic processe common to the pathogenesis of cardiovascular disease and plaque rupture. An important study supporting this finding is that mice lacking leptin (ob/ob) or leptin receptor (db/db) have reduced atherosclerotic lesion areas as compared to the controls [4]. Moreover, leptin receptors are present in the coronary vasculature and increased leptin levels cause direct endothelial cell damage. This direct damage to the coronary vasculature is considered to be the first step in the initiation of atherosclerosis [5]. Increased leptin levels also cause stimulation of mitochondrial superoxide production in endothelial cells and lead to the generation of ROS [6], proliferation and activation of the circulating monocytes [8], modulates T-cell immune response [9] and causes the production of inflammatory cytokines [10]. So by the above mentioned mechanisms there is an increased inflammatory activity in the plaque, which leads to the degradation and thinning of the fibrous cap, causes the plaque rupture and results in MI [25].

Another contribution of leptin in the development of ACS is that increased leptin levels promote platelet aggregation and thrombosis on the ruptured plaque and both of these phenomenon's are the major contributors of the acute coronary events [26].

All the above mentioned observations indicate the existence of a positive relationship between Increased leptin levels and ACS.

CONCLUSIONS

Results of our study demonstrate the existence of a strong relationship between Increased leptin levels and ACS, as the patients of ACS have shown significantly higher serum leptin levels as compared to the controls. Moreover Increased leptin levels act as a risk factor for ACS independent of BMI as the serum analysis revealed Increased leptin levels in the normal weight as well as in the overweight individuals.

The current study has also indicated that Increased leptin levels leads to 7 % increase in the odds ratio of ACS and can therefore serve as a stronger risk factor than diabetese mellitus smoking and hypertension. Further studies may be required to probe the mechanism of action by which increased

leptin levels can lead to ACS, and restoration of normal leptin levels may therefore help to reduce the mortality and morbidity associated with ACS.

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