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Coronary Atherosclerosis: Adiponectin and Leptin as Predictors of Disease Severity

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Abstract

Adipose tissue is known to produce and release numerous bioactive substances, known as adipokines (such as leptin and adiponectin), which have been found to be involved in various physiological processes, including the regulation of arterial tone and they are related to cardiovascular risk factors. The objective of the present study was to determine the relationship between the levels of serum leptin and adiponectin and the degree of coronary heart disease, also, to compare the sensitivity and specificity of serum circulating levels of the these two biomarkers in CAD diagnosis. Forty nine patients with established coronary artery disease (CAD) defined as old myocardial infarction and angina pectoris classified as CAD group. The control group included twenty normal healthy subjects. All patients and controls were subjected to complete clinical history taking, clinical examination including 12 lead electrocardiograms (ECG), diagnostic coronary angiography (CA) and the colorimetric measurement of serum levels of triacylglycerols (TGs), total cholesterol (total-C), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), also, ELISA for measurement of leptin and adiponectin. The predictors of coronary atherosclerosis severity include higher LDL-C, low serum adiponectin level, higher leptin level and previous myocardial infarction. Serum levels of leptin, LDL-C and total-C showed highly significant ($p< 0.0001$) increase, while, adiponectin levels showed highly significant ($p< 0.0001$) decrease in the group of patients when compared to the levels of the control group. The levels of HDL-C in the group of patients were significantly ($p< 0.05$) lower than in the control group. There was no significant difference between the levels of TGs in the patients versus the controls. The levels of leptin showed negatively significant correlation with the levels of adiponectin ($r=0.76$, $p<0.001$), it was positively significant with the levels of LDL-C ($r=0.302$, $p=0.035$), while, there was no significant correlation between the levels of leptin and HDL-C and the levels of adiponectin and HDL-C, there was a weak but significant correlation between the levels of serum adiponectin and LDL-C ($r=0.2$, $p=0.001$). The overall positive rates obtained from Receiver Operating Characteristic (ROC) curve for evolution of sensitivity and specificity of the different biomarkers is obtained. The sensitivity was 100% for both leptin and adiponectin. ROC curve results revealed that the specificity for leptin and adiponectin were 100% and 90%, respectively. The results obtained in the present study indicate that serum leptin and adiponectin might play an important pathogenic role not only in the occurrence but also in the severity of CAD. The circulating level of leptin provides highly specific biomarker for CAD more than adiponectin.

Keywords: Adiponectin, Leptin, Coronary Artery Disease

1. Introduction

White adipose tissue stores excess energy in the form of triglycerides, while brown adipose tissue is actively involved in the regulation of body temperature (Mariman and Wang, 2010). Recent studies have shown that adipose tissue is an active endocrine and paracrine organ secreting several mediators called adipokines.

Adipokines include hormones, inflammatory cytokines and other proteins (Nele and Johan, 2011). These adipokines include hormones as leptin and adiponectin, inflammatory cytokines as tumor necrosis factor α, interleukin-6 and other proteins as plasminogen activator inhibitor-1, angiotensinogen and resistin (Wozniak *et al*., 2009).

Furthermore, adipose tissue is known to release an unidentified adipocyte-derived relaxing factor (Löhn *et al*., 2002), which relaxes several arteries. Leptin is an ob

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gene-expressed protein mainly secreted by adipose tissues, with a primary role of inhibiting food intake, modulating weight balance and promoting energy metabolism (Brubeck, 2006).

Previous research has revealed that leptin is a stress mediator after injuries, and it proceeds to maintain homeostasis by accelerating oxidation of glucose and fatty acids, alleviating reactive oxygen species-induced apoptosis, and ameliorating post-septic multiple organ dysfunction (Eguchi *et al*., 2008, Lin *et al*., 2007).

Several experimental studies have shown that increased leptin level may directly or indirectly exert multiple actions at the cardiovascular level (Beltowski, 2006), where leptin receptors have been identified in various peripheral tissues, including the cardiovascular system and in human coronaries; it seems to have both vasodilatory and vasoconstrictory actions on vascular smooth muscle (Quehenberger *et al*., 2002).

Furthermore, leptin is involved in a number of diverse physiological processes, such as regulation of endocrine functions, inflammation, immune response, reproduction and angiogenesis (Otero *et al*., 2005). Several studies have found a significant association between circulating plasma leptin with insulin resistance and inflammatory markers, suggesting leptin as a risk factor for cardiovascular disease (Van Dielen *et al*., 2001).

Adiponectin is a protein hormone secreted by adipocytes; it binds to two different seven transmembrane domain receptors called AdipoR1 and AdipoR2. AdipoR1 is predominantly expressed in skeletal muscles, whereas AdipoR2 is predominantly expressed in liver and throughout the brain (Bjursell *et al*., 2007). Many other cells have adiponectin receptors as macrophages, osteoblasts, adipocytes, endothelial and muscular cells of the vascular wall, pancreatic cells and central nervous system (Zhou *et al*., 2005).

Adiponectin has been considered an anti-inflammatory and antioxidative adipokine that protects against cardiovascular disease (Antoniades *et al*., 2009). Plasma adiponectin has been correlated with endothelium-dependent vasorelaxation in humans (Tan *et al*., 2004). These results were confirmed by other studies that have shown an increase in NO production as well as NO-mediated and potassium channel-mediated (voltage-dependent) vasorelaxation in rats by adiponectin (Greenstein *et al*., 2009, Xi *et al*., 2005, Fésüs *et al*., 2007). Increased NO production inhibits platelet aggregation, leucocyte adhesion to endothelial cells and vascular smooth muscle cell proliferation. Furthermore, it reduces oxidative stress by decreasing ROS production in endothelial cells. All of these effects protect the vascular system against endothelial dysfunction (Antoniades *et al*., 2009).

The aim of the present study is to determine the relationship between the levels of serum leptin & adiponectin and the degree of coronary heart disease; also, to compare the sensitivity and specificity of serum circulating levels of these two biomarkers in CAD diagnosis.

2. Materials and Methods

2.1. Patients And Study Protocol

The criteria for the diagnosis of CAD include myocardial infarction and angina pectoris based on the clinical history, ECG and diagnostic coronary angiography (CA) was carried out on forty nine consecutive patients with age ranging between 50-65 years with mean ±SD of 59.175±3.112 years (31 males and 18 females) who were selected from the Interventional Cardiology Department, Istishari Hospital to participate in the current study, the duration between the onset of disease and the time of performing the assay of the biomarkers was ranging between 90-270 days with mean ±SD of 136.48±4.96 day. The control group included 10 normal healthy subjects with age ranging between 54-61 years with mean ±SD of 57.200±2.573 years (17 males and 3 females) who were non-diabetic, non-hypertensive, with no history of previous CAD; having normal ECG and normal (CA). A written informed consent was obtained from each participant. All patients and the control groups were subjected to diagnostic coronary angiography (CA) in Cath-Lab of Interventional Cardiology Department, Istishari Hospital and the biochemical analyses were carried out in the Biochemistry and Molecular Biology Department, Faculty of Medicine, Mu'tah University.

2.2. Diagnostic Coronary Angiography (CA)

It was done for all participants using a flat-panel imaging system. All subjects were fasting and sedated. It was performed from the femoral artery approach. After local groin infiltration of 10-20 ml xylocaine 2% using modified seldinger's technique and injection of 5000 IU of Heparin, 6F JL then JR coronary catheters were used to engage the corresponding arteries. The study was conducted with a General Electric Innova 2000 angiographic unit (GE medical system Milwaukee, WI, USA). The selection criteria of the patients were presence of more than 50% of coronary lesions in their angiographic projections and normal (CA) to be used as a control group.

2.3. Laboratory Measurements

Blood samples were drawn after an overnight fast from each patient of the test group and each healthy subject of the control group. Each blood sample was centrifuged to collect serum, which was stored at -20oC till the time of analysis. Total-C, HDL-C and TGs were measured by enzymatic colorimetric methods as described by Richmond (1973), Gordon et al. (1977) and Jacobs and Vandemark (1960), respectively, using reagents from (Human Gesellschaft fur Biochemica Diagnostica mbH, Germany).

LDL-C was calculated by Friedewald's formula (Friedewald *et al.*, 1972). Leptin was measured using Human Leptin ELISA kit (SRL, Tokyo) and adiponectin was estimated using Human Adiponectin ELISA kit (Otsuka Pharmaceutical Inc., Tokyo), as described by Engvall *et al.* (1971).

2.4. Statistical Analysis

All data were analyzed using analysis of variance (ANOVA) test for the comparison between the different means of variables and the data summarized as mean and standard deviation (mean± SD). Correlation between different numerical variables was done using Spearman correlation test (r). Differences were accepted as significant at $p<0.05$. ROC curve analysis was done using MedCalc software for evolution of sensitivity and specificity of the different biomarkers.

3. Results

The biochemical parameters of the patients' group versus the control group are presented in Table 1, in the form of mean ±SD. The results showed highly significant ($p< 0.0001$) increase in the levels of leptin, LDL-C and total-C of the CAD group versus the control group, also, there was highly significant ($p< 0.0001$) decrease in the levels of adiponectin of patients when compared to the controls. HDL-C values revealed a significant ($p< 0.05$) decrease for CAD group in respect to the control group, while, the values of TGs showed insignificant difference ($p=0.0871$).

Table 1. Baseline biochemical parameters of CHD and control groups

Parameter	CAD group (n=49)	Control group (n=20)
Leptin (ng/mL)[a]	27.72±4.28*	12.75±1.72
Adiponectin (µg/dL)[a]	7.23±1.01*	12.12±1.14
TGs (mg/dL)[a]	298.562±30.34**	237.95±8.73
LDL-C (mg/dL)[a]	148.24±6.16*	105.09±5.32
HDL-C (mg/dL)[a]	32.10±2.07***	37.18±3.24
Total-C (mg/dL)[a]	289.37±23.68*	187.31±2.38

[a] Values were expressed as mean ± standard deviation (SD), *=*P*<0.0001is highly significant, **=*P*>0.05is insignificant and ***=*P*<0.05is significant when compared with the values of the control group.

In CAD group, the obtained results revealed a negatively significant correlation between the levels of serum leptin and adiponectin (r=0.76, $p<0.001$), positively significant correlation between the levels of serum leptin and LDL-C (r=0.302, $p=0.035$), while, there was no significant correlation between levels of leptin and HDL-C (r=0.011, $p=0.94$) & the levels of adiponectin and HDL-C (r=0.007, $p=0.96$), there was a weak significant correlation between the levels of serum adiponectin and LDL-C (r=0.2, $p=0.001$) (Figure 1).

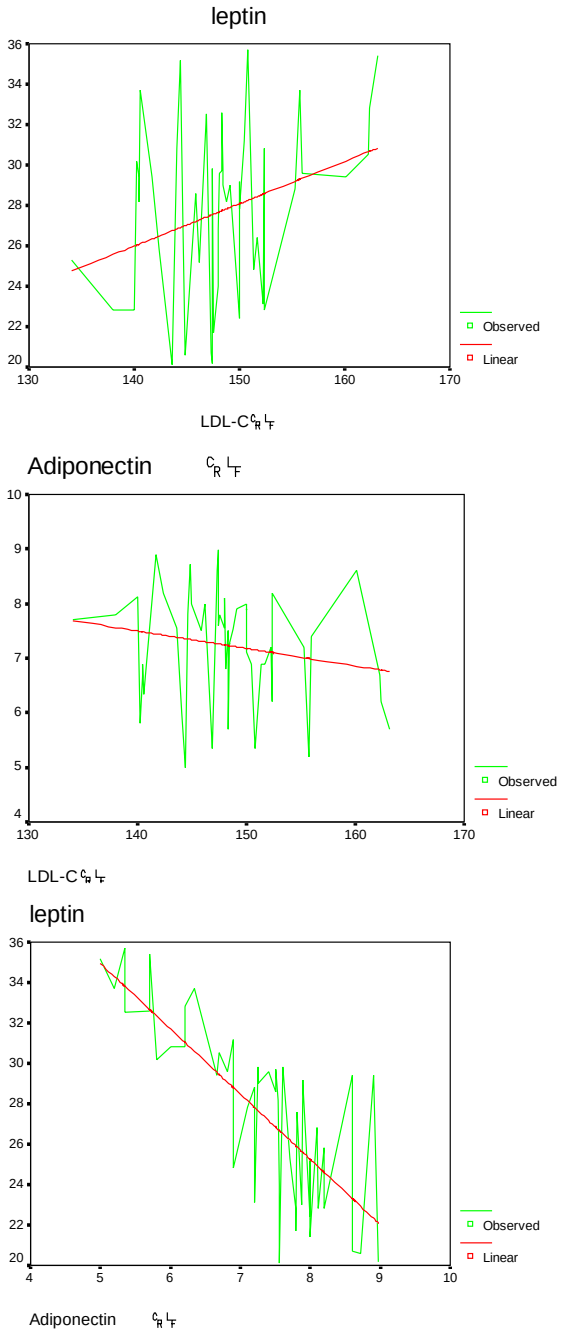

Figure 1. Correlations between some of the biochemical parameters of the study (CAD) group

Table 2 shows the area under the ROC curves for leptin and adiponectin in (1.00 and 0.00 for the two parameters, respectively). Also, the optimal cutoff value of leptin (27.7 ng/mL) (sensitivity 100% and specificity 100%) (Figure 2), of adiponectin (7.6µg/dL) (sensitivity 100% and specificity 90%) (Figure 3)

Table 2. Area under the (ROC) curves for the two parameters

Test Result Variable(s)	Area	Asymptotic 95% Confidence Interval	
		Lower Bound	Upper Bound
Leptin	1.000	1.000	1.000
Adiponectin	0.000	0.000	0.000

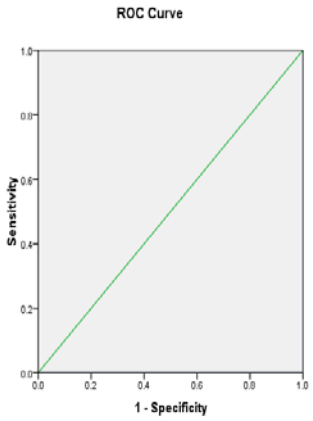

Figure 2.ROC curve for leptin.

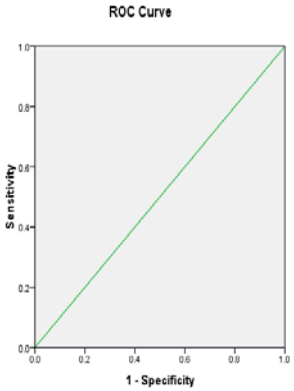

Figure 3. ROC curve for adiponectin

4. Discussion

Leptin and adiponectin differ from almost all other adipocytokines in being secreted exclusively by adipocytes, the details of all the factors regulating their synthesis, secretion and clearance remain incomplete (Finucane *et al*., 2009).

Adiponectin is a 244 amino acid protein (Sattar *et al*., 2009). It has been shown to have several beneficial effects in the cardiovascular system including an essential role in the maintenance of heart architecture, as the cytokine may attenuate angiotensin II-induced cardiac hypertrophy (Shibata *et al*., 2004**)**. Also, it represses atherosclerotic lesions in a mouse model of atherosclerosis and adiponectin-deficient mice exhibit an accelerated vascular remodeling response to injury (Ouchi *et al*., 2003).

In addition, adiponectin stimulates nitric oxide production in endothelial cells through AMPK-dependent and AMPK-independent phosphorylation of endothelial nitric oxide synthase (eNOS) (Cheng *et al*., 2007) and hypoadiponectinemia is associated with the progression of left ventricular hypertrophy (LVH), which is accompanied by diastolic dysfunction (Hong *et al*., 2004).

Through the induction of cAMP-activated protein kinase, adiponectin can stimulate glucose uptake by muscles, fatty acids oxidation in muscles and liver, also, decrease hepatic glucose production, cholesterol and triacylglycerols synthesis and lipogenesis (Ouchi *et al*., 2000). Therefore, increased blood lipid concentrations in this study may be explained by our results which showed decreased adiponectin concentrations in the CAD group of patients.

Although whether low levels of adiponectin predict hypertension remains controversial (Asferg *et al*., 2010) and whether adiponectin levels in hypertension are decreased (Adamczak *et al*., 2003), low adiponectin levels might contribute to the pathogenesis of obesity-related hypertension.

This study confirms the previous reports (Hara *et al*., 2007, Selcuk *et al*., 2008) that plasma adiponectin levels are lower in patients with CAD and correlated significantly to the severity of disease. However, Lim *et al*. (2005) found no significant relation between serum adiponectin and the severity of coronary atherosclerosis. Studies in experimental animals have shown that adiponectin has the potential to inhibit neointimal formation (Jaleel *et al*., 2006), which is supported by the report of (Kubota *et al*., 2002) who stated that adiponectin-deficient mice have severe neointimal thickening and increased proliferation of vascular smooth muscle cells in mechanically injured arteries that can be attenuated by adenovirus-mediated adiponectin administration (Matsuda *et al*., 2002). Our findings show that the levels of adiponectin are correlated positively and negatively with the values of HDL-C and LDL-C values, respectively, in CAD group which is in agreement with the results obtained by Yutaka *et al*. (2011)**.**

Adiponectin suppresses lipid accumulation in macrophages, resulting in markedly decreased uptake of oxidized LDL and inhibition of foam cell formation which provides vasculoprotection through improvement of lipid metabolism (Ouchi *et al*., 2001), which is supporting the results obtained in the present study. The group of patients showed increase in the levels of total cholesterol, LDL-C and triacylglycerols, while, the levels of HDL-C is decreased with the decrease in the levels of adiponectin. The mechanism by which adiponectin influences lipid metabolism suggests that the positive effects of adiponectin on HDL levels which might result from its significant positive relationship with lipoprotein lipase activity. Furthermore, the discussion about the mechanism of adiponectin in atherosclerosis is inappropriate because of a lack of direct data regarding this issue. Nevertheless, these reported findings, with the present results, indicate that lower levels of adiponectin may provide certain information for predicting CAD (Yutaka *et al*., 2011)**.**

Leptin is a 26 kDa (Von *et al*., 2004), almost exclusively secreted by white and brown adipocytes (Buyse *et al*., 2001). Its expression and secretion are also regulated by a variety of other factors; for example, leptin is increased by insulin, glucocorticoids, TNF-α, and estrogen (Ouchi *et al*., 2001). Under normal conditions; leptin contributes to blood pressure homeostasis by its vasorelaxing and vasocontractile effects (Lembo *et al*., 2000). While the contractile effect of leptin is attributed to sympathetic nervous system activation (Frühbeck, 1999). Various mechanisms seem to be responsible for leptin-induced vasorelaxation. This latter effect can be

endothelium-dependent, either through the release of NO (Vecchione *et al*., 2002) or by other mechanisms (Matsuda *et al*., 2003). The vascular effects in an isolated preparation are independent of any neutrally mediated actions of leptin. They are consistent with several previous researches demonstrating leptin-induced vasodilatation of coronary artery in humans and activation of endothelial nitric oxide production in human aortic endothelial cells (Matsuda *et al*., 2003).

The administration of leptin may increase oxidative stress *in vitro* cultured human endothelial cells (Bouloumie *et al*., 2002). The increase in oxidative stress may interact with nitric oxide to form peroxy nitrite and thereby, decrease the bioavailability of nitric oxide, which is associated with an impairment of endothelium-dependent vasodilatation (Cooke and Oka, 2002).

Leptin stimulates synthesis of endothelin-1, a potent vasoconstrictor and mitogen (Quehenberger *et al*., 2002). Also, under effect of leptin, there is increase in the secretion of lipoprotein lipase enzyme in macrophages (Maingrette and Renier, 2003), and accumulation of cholesterol esters in the foam cells especially at high plasma glucose concentration (O' Rourke *et al*., 2001). There is a positive correlation between leptin and plasma concentration of fibrinogen and von Willebrand factor (Thogersen *et al*., 2004) and leptin promotes ADP-platelet aggregation (Corsonello *et al*., 2003**)**.

Leptin may also activate adult human progenitor cells and promote angiogenesis (Wolk *et al*., 2005), protect macrophages from cholesterol overload (O' Rourke *et al*., 2002). The apparent discrepancy between the protective actions of leptin and its association with impaired cardiovascular outcome in the epidemiological studies can be explained by: first, the broad spectrum actions of leptin on the cardiovascular system; second, dose dependent effects of leptin; and third, the concept of selective leptin resistance (Wolk and Somers, 2006).

In the present study, the mean value of serum leptin levels of CAD group were higher when compared to the control group and inversely correlated to the levels of serum adiponectin and correlated positively with the severity of CAD. Our findings are in agreement with the reported results of Yutaka *et al*. (2011) and Wolk *et al*. (2004), also, leptin levels show positive insignificant correlations with values of HDL-C and LDL-C. However, other investigators emphasized a potential protective role in CAD (Matsuda *et al*., 2003, Couillard *et al*., 1998**,** Piemonti *et al*., 2003).

From the results of Receiver Operating Characteristic (ROC) curve for the studied parameters, it is shown that leptin and adiponectin have the same sensitivity (100%) as biomarkers for CAD, also, leptin is more specific than adiponectin. A previous report showed that leptin levels were the most sensitive marker for predicting the accumulation cardiovascular risk factors in the general population of elementary school children (Yoshinaga *et al*., 2008). Nakatani *et al*. (2008), reported that serum leptin was a useful biomarker of metabolic abnormalities than high molecular weight adiponectin in general male adolescents.

5. Conclusion

Serum leptin and adiponectin are biomarkers for and correlated to CAD not only in the role they might play in the pathogenesis of the disease but also in their severity and leptin is a more specific biomarker than adiponectin.

The limitation to the present study is the relatively small patients' number included in the study.

The future plan will be directed towards leptin receptor gene polymorphisms and their effects on the circulating levels of leptin and the signaling capacity of leptin.

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