Association of Serum Omentin-1 Levels with Coronary Artery Disease (CAD) in a local population of Karachi - A Multicenter Study

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Abstract

Objective: To determine the association between circulating levels of omentin-1, a novel adipocytokine and coronary artery disease in the local population of Karachi.

Methods: A total of 350 subjects were included in the study from June 2016 to December 2016. About 250 patients, who had undergone coronary angiography in Karachi Institute of Heart Disease (KIHD) and Civil Hospital Karachi (CHK) and 100 served as healthy controls. Serum concentrations of omentin-1 were measured by using enzyme-linked immunosorbent assay (ELISA) at Dr. A Q. Khan Institute of Biotechnology (KIBGE) and lipid profile, fasting blood sugar were done at KIHD lab. Coronary artery disease (CAD) and cardiovascular disease risk factors were evaluated by using the software SPSS 16. Independent sample t-test and chi-square tests were used to analyse the clinical and demographic characteristics of data. Simple logistic regression and then multivariate analysis were performed to evaluate correlations between CAD and all other parameters. P value less than 0.05 was considered statistically significant. This research is part of MPhil thesis and there are two research papers; (1) Serum Omentin-1 Levels with CAD (current paper) and (2) genetic polymorphism/mutation of omentin-1 gene in developing CAD. Latter is also in process of publication.

Results: Serum omentin-1 levels were found to be lower in patients with CAD as compared to controls (456 ± 99 vs 739 ± 72; $p<0.01$). Serum concentration of omentin-1 was negatively correlated with body waist circumference (39.95 ± 3.1 vs 30.95 ± 3.7; $p< 0.05$), whereas positively associated with serum High Density Lipoprotein (HDL) levels (25.9 ± 6.2 vs 42.3 ± 10; $p< 0.05$). Moreover, multiple logistic regression analysis demonstrated that serum omentin-1 concentration was independently correlated with CAD.

Conclusion: The current study has suggested that serum concentrations of omentin-1 might be related to pathogenesis and progress of CAD.

Keywords: Adipokines, Coronary Artery disease, Omentin, HDL Lipoproteins, Cardiovascular Diseases.

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Introduction

Coronary artery disease (CAD) is a major cause of morbidity and mortality worldwide. According to World Health Organization (WHO) estimation, 20 million people around the world died of CAD in 2015, which constitutes about one-third of total deaths globally[1]. Once considered as an ailment of the affluent and developed countries, coronary artery disease is emerging as an epidemic in the developing world in general and South Asia in particular. CAD has risen greatly in the low income and middle-income countries with about 80 percent of the burden occurring in these countries[2]. Pakistani population is the part of ethnic group that have highest prevalence of CAD, manifesting at quite an

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early age[3]. The prevalence of CAD in Pakistani population is about 6%. Female to male ratio of this disease is 1:3. Changing lifestyle and moving towards urbanization are the major risk factors in this regard. However, there are many other risk factors associated with this disease including: high and uncontrolled blood pressure, deranged lipid profile, diabetes mellitus, obesity, hypertrophy of left ventricle, smoking, aging, Type A personality, hyperhomocystinemia, stress, lack of exercise, and genetic predisposition[4]. Adipose tissue is now considered as the largest endocrine organ of the body that secretes many hormone like proteins called adipokines including: adiponectin, chemerin, leptin, resistin, retinol binding protein[4], tumor necrosis factor-á (TNF-á), and interleukin-6 (IL-6) which are associated with cellular metabolism, body homeostasis, insulin resistance, vascular endothelial dysfunction, and inflammation[5-6]. Adipose tissue is dynamically involved in cell function regulation through a complex network of endocrine (signals travel through the circulatory system to reach all parts of the body), paracrine (signals sent only to cells in the vicinity of the cell station), and autocrine (only affecting cells that are the same type) signals that influence the response of many tissues, including hypothalamus, pancreas, liver, skeletal muscle, kidneys, endothelium, and the immune system, among others. This secretory nature has prompted the view of white adipose tissue as an extremely active endocrine tissue. Experimental data suggest that there are some differences, in respect to adipokine synthesis and secretion, between visceral fat and subcutaneous adipose tissue, as visceral fat appears to be more active. Both types of this tissue are characterized by production of a unique profile of adipocytokines. In the visceral tissue, for example, higher concentrations of IL-6 and PAI-1 (plasminogen activator inhibitor 1) are observed. In turn, in the subcutaneous tissue, there is a higher concentration of leptin and adiponectin[7]. Omentin-1 is a newly identified adipokine with anti-inflammatory functions[8-9]. The expression of omentin-1 is greater in visceral fats and might have an important role particularly, in coronary atherosclerosis and CAD[10,11]. Omentin-1 induces vasodilatation and inhibits endothelial cell migration, vascular inflammation and angiogenesis. It may participate in CAD with endothelial dysfunction through regulation of coronary contractility and by increasing insulin sensitivity[12]. Studies have shown that omentin-1 levels are negatively correlated with body mass index (BMI), leptin, waist circumference, fasting insulin, and homeostasis model assessment (HOMA) index, and omentin-1 levels are positively associated with adiponectin and high density lipoprotein cholesterol (HDL-C)[13-14]. In fat cells omentin-1 activate protein kinase B (PKB) also known as Akt, that in turns transport of insulin dependent glucose transporter type 4 (GLUT4) to cell membrane, demonstrate that omentin-1 has definite action in metabolism of energy and insulin resistance, insulin is a hormone that increases uptake of glucose and free fatty acids by hepatocytes, fat cells and muscle cells for consumption and accumulation[15]. Omentin-1 inhibit TNF-alpha activated nuclear factor kappa-light-chain, enhancer of activated B cells to be recruited in endothelial cells and activated protein Kinase (5'AMP) that inhibit expression of vascular adhesion molecule E-selectin. Omentin-1 cause inhibition of c-Jun N-terminal kinase (JNK) induced cyclooxy-genase-II (COX-II) expression to reduce inflammatory process in atherosclerosis[16-17].

This study aims to establish local perspective as there is paucity of local data and was designed to determine whether serum concentration of omentin-1 was independently associated with CAD in local population of Karachi because coronary artery disease and its management is emerging as a great challenge to deal with, hence, data collected from this study will help in establishing the role of omentin-1 in pathophysiology and therapy of CAD.

Materials and Methods

This research study was approved by the Institutional Ethical Committee of Dr. A Q. Khan Institute of Biotechnology (KIBGE). A total of 250 patients (males 158, females 92; mean age 51.3 ±

Table 1. Clinical and anthropometric characteristics of study group, from Civil Hospital Karachi (CHK) and Karachi Institute of Heart Diseases (KIHD).

Variables	CAD (n=250)	Control (n= 100)	p-value
Age, years	51.3 ± 6.38	49.7 ± 6.4	0.09
Gender, males	63.2%	71%	
Females	36.8%	29%	0.15
BMI, kg/m2	28.5 ± 8	25 ± 4	0.08
Waist, inches	39.95 ± 3.1	30.95 ± 3.7	0.03*
SBP, mmHg	135 ± 18	130 ± 7	0.18
DBP, mmHg	90 ± 13	87 ± 6	0.33
FBS, mg/dl	154 ± 4	135 ± 13	0.15
Cholesterol , mg/dl	239 ± 25	230 ± 21.8	0.13
Triglycerides, mg/dl	132.3 ± 40	131 ± 32	0.21
HDL, mg/dl	25.9 ± 6.2	42.3 ± 10	0.05*
LDL, mg/dl	126 ± 85	123 ± 6	0.11
Smokers	45 %	55 %	0.08
History of CAD	57.2 %	54 %	0.09
History diabetes mellitus	34 %	23 %	0.06
Exercise, yes	32 %	37 %	0.012
Junk food	55 %	57 %	0.09
Omentin-1 ng/ml	456 ± 99	739 ± 72	0.001*

Table 2. Simple logistic regression analysis of Coronary Artery Disease (CAD) patients with clinical and anthropometric variables.

Variable	OR	95%CI	p-value
Age	1.023	0.986 - 1.040	0.349
Gender	1.232	0.643 - 2.601	0.471
SBP	1.243	0.882 - 1.064	0.505
DBP	1.032	0.265 - 1.074	0.043
BMI	0.969	0.872 - 1.074	0.076
Waist circumference	2.322	1.023 - 1.063	0.001*
FBS	0.034	1.006 - 1.059	0.015
HDL	2.267	0.008 - 0.098	0.0001*
LDL	0.893	1.571 - 2.132	0.654
Cholesterol	0.987	0.987 - 1.543	0.098
Triglycerides	1.021	0.087 - 0.992	0.031*
Smoking	0.187	0.234 - 1.076	0.008
Family history of CAD	0.876	0.834 - 1.159	0.01*
Omentin-1	3.243	0.946 - 0.997	0.0001*

* $p < 0.05$ is significant

Table 3. Multi-variable regression analysis of the association between omentin and clinical and anthropometric variable

Variable	Model 1	Model 2	Model 3
Omentin-1 (ng/ml)	0.005	0.001	0.001
Waist circumference, (inches)		0.03	0.03
BMI			Ns
HDL, mg/dl		0.05	0.05
LDL, mg/dl			N.S.
Cholesterol, mg/dl			N.S.
Triglycerides.mg/dl			N.S.
FBS, mg/dl			N.S.
SBP, mm Hg			N.S.
DBP, mm Hg			N.S.
History of HTN			N.S.
History of Diabetes			N.S.
Family history of CAD			N.S.
Smoking			N.S.
Junk food			N.S.

N.S. (Not significant)

6.38) were enrolled in this study who had undergone coronary angiography in Karachi Institute of Heart Disease (KIHD) and Civil Hospital Karachi (CHK). The diagnosis of coronary artery disease was established on the basis of presence of prolonged chest pain of >30 min duration, Positive Troponin 1 (Trop-I), ($\geq$ 0.01ng/ml), and electrocardiographic (ECG) changes of acute myocardial infarction (appearance of significant Q waves >2 mm in more than one concurrent ECG leads for Q-wave infarction, or appearance of ST depression >2mm and symmetrical T-wave inversion changes lasting more than 48 hours without the evolution of Q waves suggestive of non-Q-wave infarction. Coronary artery disease will be defined as 50% reduction in luminal diameter proven by angiography. The patients with acute infections, malignancy, valvular heart disease, liver disease (ALT $\geq$ 59 units/L) and renal disorders (Creatinine $\geq$ 1.2 mg/dl) were excluded from the study. Age and gender matched 100 healthy controls (79 males, 21 females mean age 49.7 ± 6.4) were included in this study. The subjects participating in the study were informed about medical research, expected benefits and potential hazards of this study and written consent was obtained The history of disease was recorded in detail, body mass index (BMI) of all participants were calculated as kg/m^2. Waist circumference was measured between upper border of iliac crests and lower edge of rib cage. Morning blood samples were collected in Ethylene diamine tetra acetic acid (EDTA) tubes after 12 hour overnight fasting. Serum was separated by centrifugation and stored at 80°until used for assay at KIBGE. Routine lab tests including serum cholesterol, triglycerides (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), fasting blood sugar (FBS), and serum creatinine were analyzed in hospital laboratory. Serum omentin-1 concentrations was measured using (ELISA) Enzyme-linked immunosorbent assay kits (Bio Vender, USA). The intra-assay and inter-assay coefficients of variation of this kit were 4.1% and 4.8%, respectively. The detection limit of the assay ranged 20 to 800 ng/L. The antibodies used in this kit are specific to measurement of natural and recombinant human omentin-1 serum samples were diluted and assayed according to the manufacturer's instructions.

In this, case control study SPSS software version 16 (Chicago, IL, USA) was used. Continuous and categorical variables were presented as mean ± SD or in percentage. Independent sample t-test and chi-square tests were performed to compare the differences in means among study groups. Correlations between serum concentrations of omentin-1 and other parameters were studied by Pearson correlation analysis. We first used simple logistic regression and then multivariate analysis to analyze correlations between CAD and all other parameters. P<0.05 was considered statistically significant.

Result

The clinical characteristics and laboratory data of the study groups are shown in (Table 1). There were no differences in term of age, BMI, sex, smoking, family history of CAD and creatinine between patients and controls. However, patients had increased waist circumference (39.95 ± 3.1 vs 30.95 ± 3.7; p<0.005), and lower serum omentin-1(456 ± 99 vs 739 ± 72; p<0.001), and HDL-C levels (25.9 ± 6.2 vs 42.3 ± 10; p<0.05) as compared to controls.Initially, a simple regression analysis was performed (Table 2) and the incidence of CAD was correlated with its major risk factors including age, smoking, family history HDL (p<0.05), LDL, FBS, waist circumference (p<0.05), BMI and omentin-1 level (p<0.05).We performed a multivariable linear regression analysis to test whether level of omentin-1 is significantly associated with laboratory and anthropometric parameters in CAD patients (Table 3). Using waist circumference, a dependent variable, in a uni-variable linear regression model 1, we found that omentin-1 is significantly correlated with waist circumference among cases and controls (p<0.005). We used a multi-variable linear regression in model 2, after adjusting for clinical variables associated with reduced levels of omentin-1 in Table 1, whereas all clinical and anthropometric variables were analysed in regression model 3, the correla-

tion between omentin-1 and waist circumferences was still significant. Moreover omentin-1 is negatively associated with HDL levels. We did not find any association between serum omentin-1 levels and other variables such as plasma glucose levels, blood pressure, age, smoking, sex, BMI, family history of CAD, junk food, exercise and hypertension.

Discussion

The present study has demonstrated that serum omentin-1 levels were reduced in patients with CAD as compared to control subjects, whereas positive correlation of circulating omentin-1 levels were found with HDL levels and negative correlation with waist circumference was found. Studies have shown that omentin-1 induces vasodilation via endothelium-derived nitric oxide through phosphorylation of endothelial nitric oxide synthase (eNOS) in rat isolated aorta[12]. CAD may also be associated with impaired endothelium-dependent coronary dilatation; therefore, omentin-1 may have a role in the pathogenesis of CAD through regulation of contraction of coronary artery[16]. Omentin-1 has been found to be associated with insulin resistance[17]. Rodrigues et al. made it known that insulin resistance is the most significant risk factor for subclinical atherosclerosis and is linked with coronary artery atherosclerotic changes in patients with type 1 diabetes mellitus (T1DM)[18]. Bertoluci et al. has indicated that increased HOMA of insulin resistance was positively associated with angiographic coronary artery disease[19]. These results showed that decreased omentin-1 levels may contribute to development of CAD by modulating insulin action. It was reported that plasma omentin-1 was decreased in DM type 1 subjects[20], Crohn's disease[23], and rheumatoid arthritis[15]. Our present data is consistent with other studies and demonstrating that omentin-1 might be involved in the development of CAD via endothelial dysfunction, insulin resistance and inflammation. Omentin-1 is reported to be expressed from visceral adipose tissue (AT) including: epicardial fat and omental fat depot.

In the present study, for the first time, to the best of our knowledge, we demonstrate that serum omentin-1 levels were negatively correlated with waist circumference but no significant association of omentin-1 was found with BMI in patients with CAD and controls in our local population. This data has revealed that individuals in Pakistani population, having increased waist circumference even with normal BMI have decreased levels of circulating omentin-1 protein and are at more risk of developing atherosclerosis and CAD, in contrast to several reports in different populations that have been shown a negative association of omentin-1 with waist circumference and BMI simultaneously[21-24]. Further investigation is needed to determine the mechanism and pathogenesis.

In current study, positive correlation between omentin-1 and HDL levels was found but with no association with LDL and total cholesterol levels in both study groups illustrated that lipid profile is deranged not only in patients but also in healthy individuals of our local population, so, these statistics have suggested that abnormal lipid profile along with decreased levels of anti-inflammatory protein omentin-1 may contribute to develop ischemic cardiac episodes in apparently healthy subjects with subclinical atherosclerotic changes of coronary arteries.

The mechanism and exact biological function of omentin-1 in development of CAD is not well understood. Further studies may address this question. Studies on different ethnic groups are required to confirm our findings.

Conclusion

Our study is the first study conducted in patients from two centres of Karachi, Pakistan that has demonstrated the association of omentin-1 and prevalence of coronary artery disease. Our study has concluded that lower levels of omentin-1 may be associated with pathogenesis of CAD and it may act as biochemical marker for detection of subclinical atherosclerosis of coronary arteries.

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Conflict of Interest

Authors have no conflict of interests and no grant or funding from any organization

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