ASSOCIATION OF PLASMA LEPTIN WITH CORONARY ARTERY CALCIUM (CAC) IN ADULTS

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ABSTRACT

Objective: To determine the correlation between leptin and CAC in scores subjects without cardiovascular disease (CVD) risk.

Study Design: Cross sectional study.

Place and Duration of Study: Aga Khan University Hospital, from Mar 2014 to Jun 2015.

Material and Methods: Total 128 subjects were included. The study was approved by ethical review committee. After informed consent a predesigned questionnaire was documented. Subjects without known cardiac disease history, coming for non-contrast CT scan for abdominal indications were included. Leptin levels were measured by Enzyme immunoassay. CAC scores were assessed on a 64 slice non-contrast CT scan. Data analyzed by SPSS version 20.

Results: Total 128 subjects were included with mean age 42.82 ± 13.1 years and 78.1% (n=100) were males. Mean BMI and waist circumference was 27.1 ± 5.4 kg/m^2 and 94.8 ± 9.0cm respectively. High median leptin levels were seen in 11.7% (n=15) of study subjects. Leptin levels were also significantly higher in female compared to male [12.5ng/ml (0.3-60.9) vs. 2.5ng/ml (0.1-50); *p*-value=0.001]. High CAC score was present in 15% (n=19) of study subjects.

Statistically significant correlation of leptin was found with waist circumference, (r 0.50; *p*=0.001), positive correlation with BMI (r 0.51, *p*<0.05) with higher levels noted in obese subjects compared to overweight and normal BMI subjects [median 7.5ng/ml (0.3-60.9) vs. 3.3ng/ml (0.1-40) & 0.1 ng/ml (0.1-0.1)]; No correlation was found between CAC score and serum leptin levels (r 0.073; *p*=0.41).

Conclusions: Leptin levels are not correlated with CAC scores in subjects with low CVD risk. However, leptin was significantly higher in females and subjects with increased waist circumference.

Keywords: BMI, Leptin, Obesity, Waist circumference.

INTRODUCTION

Leptin is a pleiotropic adipokine and plays an important role in regulating appetite and body weight[1]. Abnormalities in the production of leptin are associated with alterations in body weight leading to obesity, a known risk factor for the development of cardiovascular diseases (CVD)[2]. Leptin receptors are present in coronary arteries and increase in leptin levels is reported to be associated with significant coronary endothelial dysfunction and modulate the immune response to atherosclerosis[3,4]. The hallmark of atherosclerosis is increased vascular calcification. However, there are contradictory studies regarding leptin association with CVD, few report it to be an independent risk factor for CVD, while others report against leptin being a potential risk marker for CVD[5,6]. The reasons for contradictory results are unclear but may reflect differences in study design and populations[7,8].

For atherosclerosis assessment in high risk patients multiple biochemical markers are available. Coronary artery calcium (CAC) measured using non-contrast CT has been used for assessing CVD risks which, provides an individualized measure of atherosclerotic burden and plaque formation. The cut-off point of 10 for CAC score is considered an increased CVD risk, while, a total CAC score <10 is classified as optimal[8,9]. A Multi - Ethnic Study of

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Atherosclerosis (MESA) showed that patients with high CAC score and with no risk factor were at high risk for atherosclerotic cardiovascular disease then those with with CAC score zero and multiple risk factors[10,11].

Simultaneous evaluation of CAC scoring and leptin levels is a relatively novel concept in Pakistan. So current study was designed to determine the leptin levels in subjects without known cardiovascular disease (CVD), coming for Computed Tomography (CT) scan due to non-cardiac reason and to determine its relation with CAC scores.

PATIENTS AND METHODS

A cross sectional interdisciplinary study was conducted between March 2014 to June 2015 at the Section of Chemical Pathology, Department of Pathology and Laboratory Medicine in collaboration with Department of Radiology, AKU, Karachi, Pakistan. The study was approved by ethical review committee of the AKU. The sample size was calculated using 6.25% prevalence of CVD in Pakistan (1) with power of 80%, level of significance at 0.05 and confidence interval of 95%. Sampling strategy was non probability consecutive.

A total of 128 subjects >18 to 60 years of age, undergoing non-contrast CT scan for abdominal indications such as appendicitis and renal calculi were included, while subjects with known coronary artery disease were excluded. A questionnaire regarding any history of cardiovascular disease and anthropometric data (height, weight, BMI, waist circumference) was filled after taking informed consent. Waist circumference was considered high if >90cm in males and >80cm in females (International Diabetes Federation cut-off points for South Asians).

Five ml of blood was taken in gel tube for the measurement of serum leptin after a 12-h fast on the next day of performance of non-contrast CT after informed consent. Serum was separated and stored at -80°C for the further analysis. Leptin levels were analyzed by EIA, using Leptin-EASIA–Kit (DIA source). For quality assessment two level controls were analyzed with each batch of leptin analysis.

Coronary artery calcium scoring was assessed on a 64 slice non-contrast CT scan (Toshiba Aquilon TSX101A). After breath holding a non-contrast scan was done which extends from the pulmonary hilum to the base of the heart. Volume data was reconstructed in 3mm axial

Table: Demographic and biochemical characteristics of the study subjects (n=128).

Characteristics		Mean ± SD	>Cutoff	Frequency (%)	Mean ± SD
Mean BMI (kg/m^2)		27.1 ± 5.4	>25 *	45 (35%)	27.0 ± 1.4
			>30 *	37 (28.9%)	33.4 ± 3.2
Mean Waist circumference (cm)	Males	94.0 ± 9.0	>90 **	16 (12.5%)	109 ± 3.5
	Females	97.6 ± 8.8	>80 **	25 (19.5%)	99.4 ± 7.5
Median leptin (ng/ml)	Males (n=100)	2.5 (0.1-50)	High***	4 (4%)	19.5 (15.3-43.3)
	Female (n=28)	12.5 (0.3-60.9)	High***	11 (39%)	27.3 (20.1-52.2)
Median CAC score		0.0 (0.0-1825)	>10	19 (15%)	161 (11-1825)

Values are expressed in frequency (%) and Mean ± SD

Source: *WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. 2004; 363(9403): 157-63.

**International Diabetes Federation cut-off points for South Asia

*** Cutoffs in Female (18-24 yrs 0.5-0.7ng/ml), (25-29 yrs 4.1-14.5ng/ml), (30-56 yrs 5.5-40.4ng/ml)

***Cutoffs in Male (18-24 yrs 0.5-3.2ng/ml), (25-29 yrs 0.5-14.6ng/ml), (30-56 yrs 2.5-42.1 ng/ml)

images. Data obtained was then sent to a Vitrea 2 Workstation (Vital Images, USA) and the coronary artery calcium was entered manually. The calcium score was calculated by means of the Agatston scoring system[12]. This scoring was completed by skilled technologist and radiologist, blinded to clinical and laboratory characteristics.

Data was analyzed by SPSS version 20 for Windows (IMB, Chicago, IL, USA). Mean and standard deviation for age, BMI and waist circumference, while median with interquartile value for leptin levels and CAC were calculated. Frequency and percentage were calculated for qualitative variables. Mann-Whitney Test was done to compare leptin levels in different genders, while Kruskal-Wallis Test to compare leptin levels in subjects with different waist circumference and BMI groups.

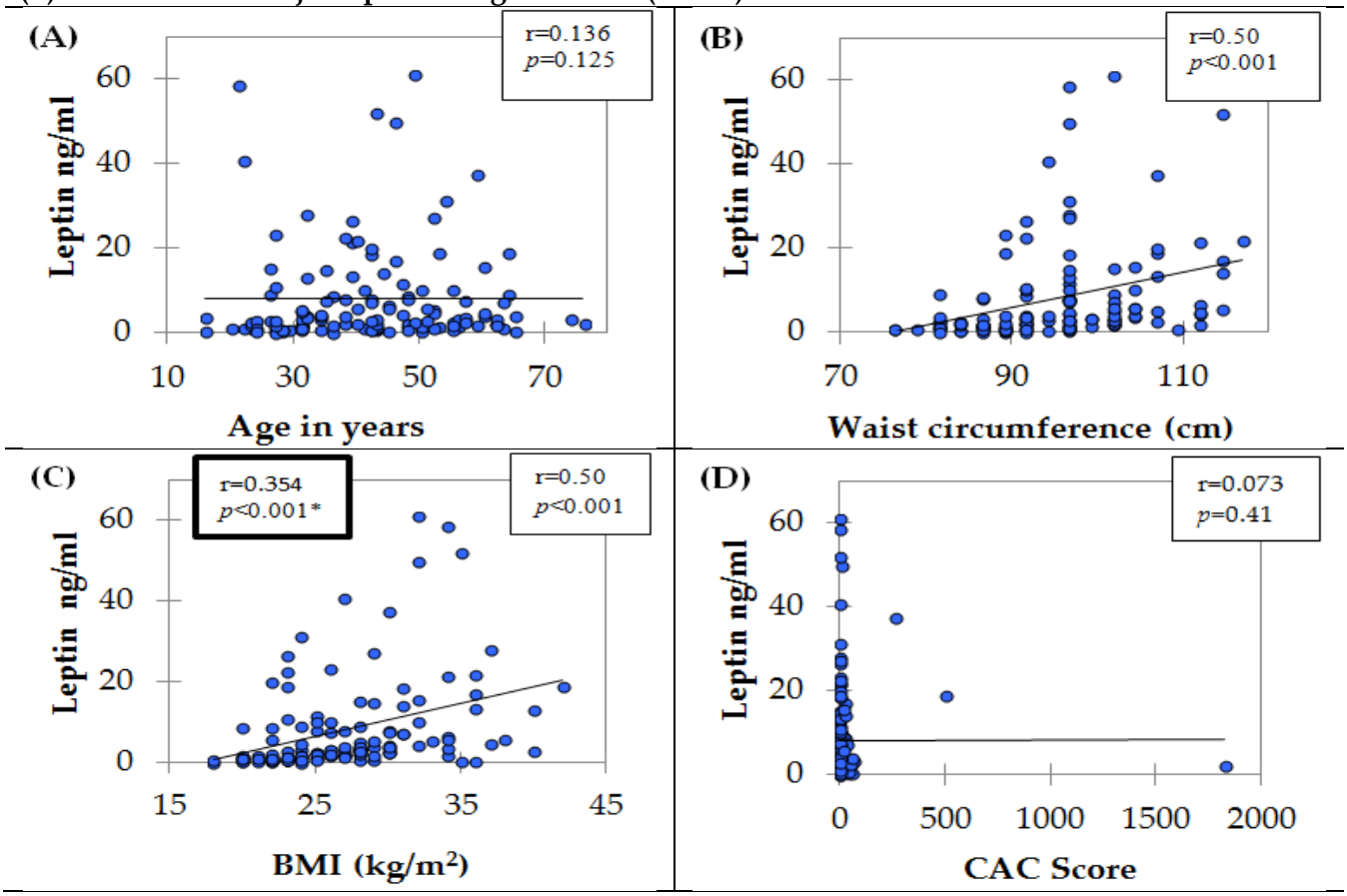
CAC scores were categorized into optimal (<10) and high (>10). Correlation between leptin, BMI, waist circumference and CAC scoring was determined by spearman's correlation.

RESULTS

One hundred and twenty-eight subjects were included in study with mean age 42.82 ± 13.1 years, 78.1% (n=100) were males and 22% (n=28) females. Mean BMI and waist circumference was 27.1 ± 5.4 kg/m² and 94.8 ± 9.0cm respectively. Out of 128 subjects, 28.8% (n=37) were obese and 32% (n=41) had high waist circumference (94.8 ± 9 cm) table. Krusak off Willis test showed *p*-value for BMI is *p*-value=1 and for waist circumference is *p*-value=0.000.

Median serum leptin levels was 8.16 ng/ml with range of 0.1-60.9. High median leptin levels

Figure: Correlation between Median Serum Leptin & (a) Age, (b) Waist circumference, (c) BMI, (d) CAC score in subjects presenting at AKUH (n=128).



were seen in 11.7% (n=15) of study subjects. Leptin levels were also significantly higher in female compared to male [12.5ng/ml (0.3-60.9) vs. 2.5ng/ml (0.1-50); *p*-value <0.001]. High CAC score was present in 15% (n=19) of study subjects.

Statistically significant correlation of leptin was found with waist circumference, (r 0.50; *p*<0.001), with higher leptin levels in subjects with high waist circumference compared to the rest (median 2.3 (1.2-3.9) *p*<0.001); positive correlation with BMI (r 0.51, *p*<0.05) with higher levels noted in obese subjects compared to overweight and normal BMI subjects [median 7.5ng/ml (0.3-60.9) vs. 3.3ng/ml (0.1-40) & 0.1 ng/ml (0.1-0.1)]; No significant correlation was found between CAC score and serum leptin levels (r 0.073; *p*=0.41), shown in figure.

DISCUSSION

In this study, we wanted to determine the association between serum leptin and coronary artery calcuim, a measure of subclinical coronary atherosclerosis and risk factor for CVD. We did not find any association with CAC scores as compared to other studies that showed positive correlation of plasma leptin levels and CAC score[13,14]. Only 15 subjects have high leptin levels and 14 out of them had CAC scores of 0. While 24 subjects had high CAC scores and only 1 of these subjects had high leptin levels.

Previous studies have reported contradictory results; only some studies have shown positive relations between plasma leptin and clinical cardiovascular diseases while some reported no association between the two tools[15,16]. The reasons for these contradictory results are unclear. Although experiments done to study cellular effects of leptin have reported it to induce the inflammatory markers like TNFα, IL6 and reactive oxygen species etc and reported it to be correlated with CAC scores as an indicator of subclinical atherosclerosis. No association between leptin and CAC score observed in this study could be due to the patient population included; as the patients included in current study had history of appendicitis or renal calculi and may have been on anti-inflammatory treatment which could have led to lower leptin levels[17]. Further research is necessary to shed light on the role of leptin in the development and progression of CAC in our population.

Furthermore, higher plasma leptin in females compared to males were observed in current study. Similar findings were reported by previous studies which are attributed to the higher percentage body fat in women and high androgens in male leading to low leptin levels[18,19].

Moreover, in current study there was a positive association of leptin with BMI and waist circumference; A measure of central obesity. Similar findings are reported by other studies from Pakistan. Leptin is released by adipose tissue so these findings are comprehensible[20]. Worldwide as well as in Pakistan incidence of obesity is on rise[21,22] and it is considered independent risk factor for developing diseases associated with obesity like CVD. In this context understanding role of leptin in these diseases becomes important, so that application of these biomarkers as measureable tools to detect CVD risk assessment can be understood.

Limitations of the current study were small sample size and unequal gender distribution and cross sectional study design.

CONCLUSION

Our results suggest that there was no association between CAC and leptin in subjects with low CVD risk. However significantly higher leptin levels were observed in females and subjects with obesity, especially central obesity.

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CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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