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Original Article

Association of Serum Leptin with Metabolic Syndrome in Obese Subjects

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Abstract

Background: To find out the association of circulating serum leptin levels with metabolic syndrome.

Methods: This case-control study was conducted on 100 adult subjects (consisting of 50 cases who fulfiled the WHO criteria of metabolic syndrome (Mets) along with equal number of age and sex matched controls). Enzymatic colorimetric method was used to measure fasting plasma glucose and lipid profile. Insulin resistance was calculated by using the Homeostasis Model Assessment-Insulin Resistance (HOMA-IR). Circulating leptin levels were measured using DRG Leptin (Sandwich) ELISA kit. Statistical Package for the Social Sciences (SPSS)-17 was used to analysis of data.

Results: The Mets group comprised of 59% subjects with IFT, 25% with impaired glucose tolerance (IGT), while 16% were diabetic. Mets patients were more obese, as represented by increased BMI, WC and WHR. Dyslipidemia along with elevated levels of fasting plasma glucose and fasting plasma insulin were observed in case group as measured by HOMA-IR. Patients with Mets had elevated leptin levels of 12.98 ± 2.68 (ng/ml) as compared to 5.34 ± 1.84 (ng/ml) in controls (p value < 0.001).

Conclusion: Serum leptin levels were significantly raised in subjects of Metabolic syndrome as compared to controls. Early detection of metabolic syndrome patients by measuring their circulating serum levels will be helpful in reducting the risk of various cardio-metabolic abnormalities (diabetes melitus and cardiovascular diseases) which are associated with this syndrome.

Key words: Serum leptin, Metabolic syndrome, Insulin resistance, Body-mass index, Homeostasis Model Assessment-Insulin Resistance (HOMA-IR).

Introduction

Metabolic syndrome (Mets) also known as "Multiple Risk Factor Clustering syndrome" is a combination of hyperglycaemia, hyperinsulinaemia, hypertension and dyslipidaemia in an individual.[1] Study done in 2009 estimated the prevalence of Mets in Karachi and gave the figure of approximately 35% according to International Diabetes Federation (IDF) definition and modified National Cholesterol Education Program, however the figure was 49% when Adult Treatment Panel III (NCEP ATP III) criteria was used.[2] The increased trend towards Mets was attributed due to high prevalence of obesity (46-68%) and lower levels of HDL-c (68-81%).[3] High prevalence of CVD and DM has also been observed in Pakistani population as well.[4]

The cause of Mets is a mixture of genetic and environmental factors.[5] It helps in assessing risk for developing serious complications. Data shows that Mets not only increases the risk of coronary artery disease and DM but also cerebrovascular diseases in Asia.[6] The hallmark of Mets is, indeed obesity, which is an epidemic of 21st century. It is a chronic condition, which is continuing to plague our society.[7] Although prevalence of obesity is relatively low in Asia as compared to the developed countries, it is growing into a significant public health problem.[6] General population has 17% to 25% prevalence of Mets, giving the strong evidence of interconnected link between obesity and Mets.[8] Obesity is considered as the underlying cause of this rising pandemic.[9]

Apart from inflammatory cytokines like TNF-alpha and IL-6, adipose tissue secretes several adipokines (leptin, adiponectin and resistin). Some of these, affects insulin sensitivity by acting on insulin signaling and molecules involved in glucose metabolism.[1] An elevated circulating levels of leptin has been found in obese individuals but they do not adequately respond to these increased leptin levels. This under-responsiveness to either endogenous and exogenous leptin in most forms of obesity has given rise to the idea that obesity is associated with or even caused by a state of relative leptin resistance similar to the insulin resistance of type 2 diabetes.[10] Elevated serum leptin concentration is a feature of obesity and abdominal

adiposity is a cardinal feature of Mets.[11] As the number of people suffering from this syndrome is increasing day by day, their early diagnosis will reduce the risk for developing Coronary heart disease (CHD), peripheral vascular diseases, stroke and type 2-DM.[12] Hence, Mets identifies these individuals towards whom preventive medicine should be targeted.[13]

Patients and Methods

After getting permission from the Institutional Ethics Committee of Army Medical College, this case control study was done on 100 subjects. Each participant gave the written consent. To diagnose the metabolic syndrome patient, WHO criteria was used; which is based on insulin resistance (IR) plus any 2 of following: (i) BMI>25 kg/m^2 or waist-hip ratio>0.85 in women and >0.9in men; (ii) High blood pressure≥140mm Hg systolic or ≥90 mm Hg diastolic; (iii) Serum Triglyceride≥1.7mmol/l; (iv)Serum HDL cholesterol<1.0 in women and<0.9 in men. Regarding cases, patients with a history of liver, thyroid, hematological and neoplastic diseases were excluded, while in controls, pregnant women and those who were taking oral contraceptive pills were excluded. Information regarding the demographic data (age, sex etc) and complete history was obtained.

Anthropometric indices of obesity included ,waist and hip circumference were measured in centimeters for calculating waist-to-hip ratio. Waist circumference was measured midway between the iliac crest and the lower margin of the 12th rib and height was measured to the nearest cm with a standardized measuring chart and weight was measured to the nearest kg with a standardized scale without shoes. BMI was calculated by the formula=Weight (kg)/Height(m^2).

Ten ml of fasting venous blood sample was collected under sterile conditions and equal amounts was transferred to a plain vacutainer for serum analysis and EDTA tube for plasma analysis.Serum was separated by centrifugation for 15 minutess. Routine investigations was done on the same day.The serum for leptin and insulin measurements was stored at -20 ^{0}C , until the biochemical analysis.Plasma glucose, triglyceride, total cholesterol and high density cholesterol (HDL-c) were measured by the enzymatic colorimetric method on fully automated chemistry analyzer . Low density cholesterol(LDL-c) was calculated by Friedewald formula , i.e., LDL-c(mmol/l)= [TC]-[HDL-TG/2.2]

Serum Leptin was measured in Mets patients by using DRG Leptin- ELISA (enzyme-linked immunosorbent assay) kit for research use only. Insulin was measured on Access- 2 immunoassay (Beckman Coulter) based on principle of chemiluminance immunoassay. Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) was used to measure Insulin resistance, i.e., Homa-IR=fasting plasma glucose × fasting plasma insulin/22.5 . Categorical data was compared using the chi-square test. Serum Leptin levels in the groups were compared using independent t-test. Correlation analysis of serum Leptin with the Mets components was done using Spearman correlation. A p-value < 0·05 was considered to indicate statistical significance.

Results

One hundred patients of Mets with an equal number of age and sex matched controls were included in this study. Among the 50 Mets patients, 28 (56%) were male and 22 (44%) were female . Out of 50 subjects of control group, 26 (52%) were male and 24 (48%) were female. Mean age of the cases and control was 45.24 ± 7.99 and 44.30 ± 7.88 years respectively. The Mets group comprised of 59% subjects with IFT, 25% with IGT, while 16% diabetic.

Table 1: The baseline characteristics of the cases and controls

Parameters	Cases (Mean & SD)	Controls (Mean &SD)	p-value
Age (yrs)	45.24 ± 7.99	44.30 ± 7.88	0.555
Basal metabolic rate (BMI,unit: kg/m^2)	29.40 ± 1.49	22.71 ± 1.04	< 0.001
Waist circumference (WC,unit: cm)	51.72 ± 7.12	36.20 ± 2.39	< 0.001
Waist hip ratio (WHR)	1.125 ± 0.081	0.786 ± 0.022	< 0.001
Fasting plasma Glucose (mmol/l)	6.94 ± 0.759	4.27 ± 0.608	< 0.001
Fasting plasma Insulin (µIU/ml)	28.39 ± 1.67	11.85 ± 1.14	< 0.001
HOMA-IR	79 ± 1.47	2.22 ± 0.196	< 0.001
Serum Triglyceride (TG, unit: mmol/l)	4.09 ± 1.42	1.03 ± 0.292	< 0.001
Plasma High density lipoprotein cholesterol (HDL,unit: mmol/l)	0.779 ± 0.090	2.48 ± 0.372	< 0.001
Serum Leptin (ng/ml)	12.98 ± 2.68	5.34± 1.84	< 0.001

Mets patients were more obese, as represented by increased BMI, WC and WHR. Moreover, dyslipidemia with elevated levels of serum TG, decreased levels of plasma HDL-c were seen in these patients. Elevated levels of fasting plasma glucose and fasting plasma insulin were observed in case group as measured by HOMA-IR. However, no significant difference was observed in BP readings of

the case and control groups. We have observed a significant difference in serum leptin levels among cases and controls. Patients with Mets had elevated leptin levels of 12.98 ± 2.68 (ng/ml) as compared to 5.34 ± 1.84 (ng/ml) in controls (p value < 0.001) (Table 1).

Discussion

Studies found raised serum leptin levels in Mets subjects.[14,15,16] Insulin resistance which is a well recognized risk factor for the development of type-2 diabetes and cardiovascular diseases had been clearly demonstrated in metabolic syndrome patients.[17-19] Previously there was controversy about the relationship between leptin and insulin resistance. In obese individuals, both the serum leptin and insulin levels are generally high, but some studies showed an inverse relation between serum leptin level and insulin resistance. In this way, the relationship between leptin and insulin resistance was less clear.[20] Associations of serum leptin and insulin levels have been reported in subjects with or without insulin resistance e.g. study showed an increase in serum leptin levels after insulin infusion.[21] Study done by Boden et al., established a dose-dependent increase in serum leptin levels during 72 hours of hyperinsulinemia.[22,23] This suggests that prolonged hyperinsulinemia enhances leptin expression in humans. However, Vidal et al. failed to increase leptin mRNA in human adipose tissues.[24]

This study was done by calculating the insulin resistance by using HOMA-IR equation as it is easy, manageable and is commonly used for population based studies.[25,26] In a study conducted by Julia Steinberger et al., the euglycaemic-hyperinsulinaemic glucose clamp technique was used which is considered the gold standard for evaluating insulin sensitivity.[27] The above technique is usually not preferable as it needs admission in hospital and there is a risk of hypoglycemia and prolonged time duration of procedure i.e three to four hours.[28] This is basis for using HOMA-IR for present study as it is less invasive and easy to use, moreover, the values of insulin resistance obtained from euglycaemic-hyperinsulinaemic glucose clamp technique were comparable to our study. Toshio et al. have compared HOMA with clamping technique, and found good correlation.[29] A cut off value of 2.5 in Mets patients was used for insulin resistance index , while in various other studies the range varies between 1.73- 2.5.[30,31] There is no universally agreed upon cut off value for HOMA-IR.[19,28] Though variations exist in the cut off values of HOMA due to variability of insulin assay procedures, however, it has good utility as a research tool in population-based studies.[31] Many studies used HOMA to determine insulin resistance in their population.[32] Shimomura et al. suggested upper limit of HOMA-IR in Japanese subjects.[33] In another study done on obese Type 2 diabetic subjects in Pakistan, mean value of HOMA-IR was 4.1 that may be due to diabetes mellitus in such patients.[34]

In present study, serum leptin levels were measured by Sandwich ELISA technique. Serum leptin levels were also measured by using radioimmuno-assay technique. Infact, Sandwich ELISA technique is better than the radioimmuno-assay as it is more specific because there are relatively much less chance of cross-reactivity to other biological products of human origin (cross-reactivity with mice leptin is 0.2% which is negligible). There is no risk of radiations as in radioimmuno-assay.[35] Also in study when leptin was measured by RIA, the mean ± SD serum leptin concentrations were 31.3 ± 24.1 ng/ml in the obese subjects and 9.3 ± 7.5 ng/ml in the normal-weight subjects (P=0.001). In our study, these levels were 12.98 ± 2.68 (ng/ml) in the obese subjects and 5.34± 1.84 (ng/ml) in the normal-weight subjects. The leptin concentration is relatively low in obese group in present study which may be due to different method of analysis and/or study population.[20] (Considine et al., 1996).

Metabolic syndrome represents a high risk population with multiple cardiometabolic risk factors, therefore, targeting this high risk group for primary prevention will greatly benefit the community both in terms of improvement in health and cost spend on treating diabetes and CVD.[36,37]

Conclusion

1. Serum leptin levels were significantly raised in subjects of Metabolic syndrome as compared to controls.
2. As leptin is secreted mainly by adipocytes in obese individuals, early diagnosis of these patients will be helpful in planning the intervention and therapeutic strategies to reduce the frequency of DM and CVDs.

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