Original Article

Association of Circulating Resistin with Metabolic Risk Factors in Indian Females Having Metabolic Syndrome

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ABSTRACT

Role of resistin in insulin sensitivity and metabolic syndrome (MetS) is controversial till date. Increased serum resistin levels are associated with MetS and insulin resistance. The aim of this study was to investigate the relationship between serum resistin levels with markers of the MetS in females. In a cross-sectional study, a total of 170 healthy female subjects were selected for the study. Out of which 71 (age 31.59 ± 4.88 years) were with MetS and 99 (age 31.75 ± 6.34 years) were without MetS. Different parameters of MetS and serum resistin level were measured according to the standard protocols as given in NCEP ATP III 2001 guideline. Serum resistin levels were significantly higher in subjects with MetS when compared with subjects without MetS [13.54 ± 4.14 ng/ml (n = 71) vs. 7.42 ± 2.31 ng/ml (n = 99); $P \leq 0.001$]. Resistin levels were positively associated with waist circumference, systolic and diastolic blood pressure, plasma glucose, waist/hip ratio, serum triglycerides, serum cholesterol, serum VLDL, plasma insulin, and insulin resistance, while it was negatively associated with high-density lipoprotein. This study demonstrates a positive correlation between resistin and factors of MetS except high-density lipoprotein which was found to be negatively correlated in Indian female subjects.

Key words: Insulin resistance, metabolic syndrome, resistin

INTRODUCTION

Resistin is a circulating protein of 114 amino acids which belongs to the resistin-like family.[1,2] Resistin is regulated by insulin, glucose, growth hormone, and thiazolidinediones.[3-6] The role of resistin in humans is not very clear till now. Studies with rodents have suggested resistin protein as a link between obesity, insulin resistance, and diabetes.[7-9] In humans, data on the role of resistin in insulin sensitivity and obesity are still controversial.[10] Increased resistin levels were found in mice with diet-induced obesity and in ob/ob mice.[1] Studies suggested that mice injected with recombinant resistin or overexpressing resistin protein had impaired glucose tolerance and insulin action.[11-13] It was found that human hepatic cells overexpressing resistin had impaired glucose uptake and glycogen synthesis.[14] It was positively correlated with proinflammatory factors in adults with pathophysiological conditions such as atherosclerosis, renal disease, and inflammation of respiratory tracts.[15-22] Resistin is clearly involved in inflammation but its specific function in that situation remains to be clarified. Because of its link with obesity, inflammation, and insulin resistance, resistin has been tagged as a potential metabolic syndrome (MetS) marker. Supporting this theory, adults with MetS tend to have higher resistin levels than their healthy counterparts.[23] However, the correlation between insulin resistance and resistin in adult humans remains

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controversial, supported by some studies[24-26] while opposed by some other workers,[27-29] therefore, proven weak correlation between resistin and MetS. Some authors indicated that increased serum resistin levels were associated with increased obesity, visceral fat,[1,30,31] insulin resistance, and type 2 diabetes,[15] while other groups failed to observe such correlations.[22,32-34] Furthermore, the contribution of resistin to the MetS is still under investigation.[35,36] The aim of this study was to investigate the correlation between serum resistin levels with the markers of MetS in Indian female subjects.

MATERIALS AND METHODS

Study design

This is a cross-sectional case–control study, conducted in north Indian adult females with age between 20 and 40 years. We enrolled 170 adult women for this study. A structured proforma was filled to collect the information regarding their medical, personal, family, and dietary history. This study was approved by the ethical committee of our institute and "we certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research." Written informed consent was obtained from all the participants. This study was conducted under the principles of the Declaration of Helsinki.

All the subjects were diagnosed according to the NCEP ATP III criteria for MetS.[37] Samples were divided into two group: study group and control group. Study group composed of 71 human female subjects having MetS (mean age 31.59 ± 4.88 years) and control group composed of 99 healthy human female subjects without MetS (mean age 31.75 ± 6.34 years) who were non-alcoholic, non-diabetic, and without any kind of cardiac, respiratory, inflammatory, and metabolic diseases. All samples were collected from Lucknow and nearby areas.

Anthropometric measurements

All the subjects were evaluated; in addition to waist hip ratio (WHR) (a good marker for measuring central/visceral obesity), the following anthropometric parameters, body mass index (BMI), height (Ht.), weight (Wt.), waist circumference (WC), blood pressure (BP), and pulse rate (PR), were also measured. BMI was calculated as weight (in kilograms) divided by square of height (in meters).

Biochemical measurements

Blood samples for biochemical parameters were collected from subjects in the morning having 12 h fast. Serum and plasma were separated from 3.0 ml of blood. Estimation of plasma glucose was done by GOD-POD method (Randox Laboratories Ltd., Antrim, UK) and serum lipid profile was done by enzymatic method (Randox Laboratories Ltd.). Plasma insulin was estimated by Immuno-radiometric assay method (Immunotech Radiova, Prague). Subsequently, insulin resistance was calculated by homeostatic model assessment index[38] using the equation:

HOMA Index = [fasting Insulin (μU/I) × fasting glucose (mmol/l)/22.5]

Determination of serum resistin levels

Resistin concentration in plasma was measured by using sandwich ELISA assay kit (Biovendor Research and Diagnostic Products, Czech Republic), according to the manufacturer's protocol. In brief, plasma samples were diluted into 1:3 ratios with dilution buffer. The lowest detectable amount of the human resistin protein was 0.1 ng/ml having interassay variability of 5.1% and intraassay variability of 2.8%. For this analysis, aliquots of plasma kept at -20°C were thawed and processed in single time.

Statistical analysis

All the clinical data and anthropometric values are presented as mean ± SD. Statistical analysis was conducted by using SPSS Version 11.0 for windows. Comparisons between groups were made by using Student's *t*-test. For all analyses, *P* value <0.05 was considered as statistically significant.

RESULTS

Characteristics of subjects with and without MetS are shown in Table 1. The subjects with MetS were well overweight (BMI 28.93 ± 3.85 kg/m^2) when compared with the subjects without MetS (22.63 ± 4.05 kg/m^2, $P \leq 0.001$; Table 1). Serum resistin levels were found significantly ($P < 0.05$) higher in subjects with MetS when compared with subjects without MetS (13.53 ± 4.11 ng/ml vs. 7.42 ± 2.31 ng/ml, $P \leq 0.001$; Table 1). Serum resistin levels were not correlated with the age of the subjects. We observed a significant difference in levels of all the biochemical parameters and anthropometrical measurements [Tables 1 and 2]. Factors of MetS were significantly higher in the cases compared with controls, viz., WC (91.69 ± 13.41 vs. 75.63 ± 12.46, $P \leq 0.001$), systolic and diastolic blood pressure (128.33 ± 10.76 vs. 119.26 ± 8.2, $P \leq 0.001$ and 87.18 ± 7.19 vs. 80.95 ± 6.71, $P \leq 0.001$), triglyceride (TG) levels (171.86 ± 70.59 vs. 106.35 ± 40.98 mg/dl, $P \leq 0.0001$), high-density lipoprotein (HDL) level (40.48 ± 6.85 vs. 50.08 ±.6.67 mg/dl, $P \leq 0.001$), and glucose concentration (107.89 ± 20.41 vs. 91.47 ± 15.68 mg/dl, $P \leq 0.001$) [Table 2]. Anthropometrical measurements were also significantly higher in subjects with MetS when compared with the subjects without MetS (BMI 28.93 ± 3.85 vs. 22.63 ± 4.05 kg/m^2, $P \leq 0.001$; weight 68.68 ± 8.09 vs. 53.72 ± 9.64 kg, $P \leq$

Table 1: Demographic characteristics and biochemical parameters summary (Mean ± SD) of control and cases

Variables	Control (n=99)	Cases (n=71)	*P* value
Age (yrs)	31.75± 6.33	31.59 ± 4.88	NS
Height (cm)	154.16 ± 6.33	154.29 ± 6.02	NS 0.89
Weight (kg)	53.72± 9.64	68.68 ± 8.09	<0.001
BMI (kg m^{-2})	22.63 ± 4.05	28.93 ± 3.85	<0.001
HC (cm)	90.56±9.1	96.52 ± 7.88	<0.001
WHR	0.83±.08	0.94± 0.10	<0.001
FPI (µU/ml)	6.52 ± 1.73	12.53±4.55	<0.001
HOMA-IR	1.47±0.46	3.44±1.77	<0.001
Resistin (ng/ml)	7.42 ± 2.31	13.53 ± 4.11	<0.001
TC (mg/dl)	155.28 ± 45.01	173.83 ± 41.34	<0.0001
VLDL (mg/dl)	21.27 ± 8.21	34.37 ± 14.11	<0.0001
LDL (mg/dl)	94.89 ± 52.89	98.97 ± 41.61	NS
TC/ HDL	3.56 ± 1.32	4.43 ± 1.35	<0.0001
HDL/ LDL	0.60 ± 0.36	0.47± 0.22	<0.009
LDL/HDL	2.22± 1.48	2.55± 1.20	NS

BMI- Body Mass Index; HC- Hip Circumference; WHR- Waist to Hip Ratio; IR-Insulin Resistance; TC-Total Cholesterol; VLDL- Very Low Density Lipoprotein; LDL- Low Density Lipoprotein; FPI- Fasting Plasma Insulin; HOMA-Homeostasis Model Assessment

Table 2: Summary of parameters of metabolic syndrome (Mean ± SD) of control and cases

Variables	Control (n=99)	Cases (n=71)	*P* value
WC (cm)	75.63± 12.46	91.69 ± 13.41	<0.0001
Systolic BP	119.26 ± 8.2	128.33 ±10.76	<0.0001
Diastolic BP	80.95 ± 6.71	87.18 ± 7.19	<0.001
Glucose	91.47 ± 15.68	107.89 ± 20.41	<0.0001
TG	106.35±40.98	171.86 ± 70.59	<0.0001
HDL cholesterol	50.08±.6.67	40.48± 6.85	<0.0001

0.001; WHR 0.94 ± 0.10 vs. 0.83 ± 0.08 m, $P \leq 0.001$). On performing Pearson correlation to quantify that how biochemical parameters like TG, WC, HDL and Glucose levels are correlated to resistin, we obtained that TG, WC and Glucose were positively where as HDL was negatively correlated with resistin [Figure 1].

DISCUSSION

Our present investigation showed the higher serum resistin levels in the Indian females with MetS (cases) when compared with controls (females without MetS) and well correlated with the factors of MetS such as fasting plasma glucose, TG, systolic and diastolic blood pressure and WC [Figure 1]. Further, these findings were also correlated with various biochemical parameters such as total cholesterol and VLDL levels as well as physiological parameters such as BMI, hip circumference, and WHR in the female subjects. Serum resistin level negatively correlated with HDL level.

Most of the anthropometric parameters, factors of MetS, and biochemical parameters as depicted in Table 1 and 2, have shown significant differences between cases and controls. Serum resistin levels were also significantly higher in the subjects with MetS when compared with subjects without MetS (13.53 ± 4.11 ng/ml vs. 7.42 ± 2.31 ng/ml, $P \leq 0.001$; Table 1). We also found a significant correlation of between serum resistin levels with BMI. Same types of the results were also reported by some workers[21,22]; however, some showed negative findings.[39-43] It has been reported that resistin is associated with low HDL in a smaller number of healthy and T2DM subjects.[44,45] The serum resistin levels were also found associated with serum concentrations of TG and BMI.[46] The serum resistin levels were inversely

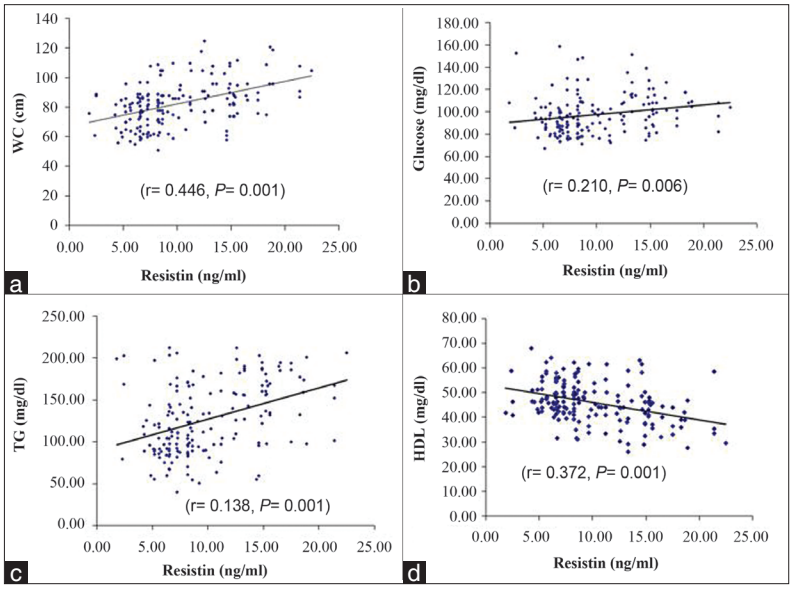

Figure 1: Relationship (Pearson correlation) of serum resistin (ng/ml) with (a) WC (cm), (b) fasting plasma glucose (mg/dl), (c) serum TG level, and (d) with serum HDL (mg/dl) level in cases of Indian females aged between 20 and 40 years. It is positively correlated with TG, fasting plasma glucose and WC.

associated with serum concentrations of HDL-cholesterol which was in agreement with the previous report of Osawa *et al.* who have reported an inverse correlation of resistin with HDL in the Japanese general population.[47] Furuhashi *et al* revealed that circulating resistin was not correlated with blood pressure. These resistin levels were not very different among controls and cases with essential hypertension with or without insulin resistance; however, our results suggest a positive correlation between serum resistin levels with SBP and DBP.[48] Recently, Norata and their colleagues reported that serum resistin levels were higher in females with MetS in a population-based study including 1090 subjects and mimicking our results.[49] Although, our study was mainly confined toward the physical measurements and biochemical parameters. Menzaghi and their colleagues have reported the genetic correlations of resistin with BMI, WC, and homeostasis model assessment of insulin resistance (HOMA-IR) index.[24]

In conclusion, we found that resistin levels were associated with metabolic and anthropometric parameters in cases with MetS. Furthermore, an extensive study is needed to establish these correlations between resistin and factors of MetS.

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