ORIGINAL ARTICLE

RELATIONSHIP OF SERUM RESISTIN WITH INSULIN RESISTANCE AND OBESITY

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Background: Adipokines have been implicated in the modulation of insulin sensitivity and glucose tolerance and have thus gained importance in the study of Type 2 diabetes mellitus (T2DM). Resistin, a unique signalling molecule, is being proposed as a significant factor in the pathogenesis of obesity-related insulin resistance. However, its relevance to human diabetes mellitus remains uncertain and controversial. This study was therefore planned to compare and correlate the potential role of resistin in obese patients with T2DM and obese non-diabetic controls and also to evaluate the correlation between resistin and marker of obesity and glycaemic parameters. **Methods**: Fasting serum resistin, glucose and insulin were measured in forty obese diabetics (mean±SD BMI 35±5 kg/m2) and forty obese non-diabetics (mean±SD BMI 33±3 kg/m2). Insulin resistance was assessed using the HOMA-IR formula derived from fasting insulin and glucose levels. **Results:** Serum resistin levels (38±8 ng/ml) were significantly higher in type 2 diabetic patients as compared with the controls. Fasting blood glucose (164±46 mg/dl), serum insulin (37±7 μU/ml) and insulin resistance (19±8), were considerably higher among the studied diabetics than in the controls. Pearson's correlation analysis revealed positive correlation between serum resistin and BMI (p=0.001) and HOMA-IR (p=0.561) in diabetic subjects. Similarly, a correlation also existed between serum resistin and BMI (p=0.016) and HOMA-IR (p=0.307) in control obese subjects. However, it was highly significant in diabetics as compared to non-diabetic controls. **Conclusion**: A significant BMI-dependent association exists between resistin and insulin resistance in patients with T2DM. It appears that resistin may play a role in the pathogenesis of obesity and insulin resistance and that both of these may contribute to the development of T2DM.

Keywords: Diabetes mellitus, Insulin Resistance, Resistin, Obesity

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INTRODUCTION

Diabetes Mellitus is the most common endocrine disorder. According to WHO, an increase in incidence of diabetes is occurring worldwide. At least 171 million people are currently suffering from this menace and this figure is going to increase to 366 million by 2030. Around 3.2 million deaths are occurring annually mainly due to cardiovascular diseases which is one of the major complication of diabetes mellitus.[1] Shaw, ranked Pakistan seventh on the diabetes prevalence list, with 7.1 million people affected and predicted that this number will grow to 11.5 million by 2030 unless measures are taken to control the disease.[2]

One of the major predisposing factors for developing diabetes mellitus is obesity. Shah projects that by 2015, approximately 2.3 billion adults will be overweight and more than 700 million will be obese.[3] Obesity is now so common within the world's population that it is beginning to replace malnutrition and infectious diseases as the most significant contributor to ill health. Once considered a problem only in high-income countries, obesity is now on the rise in urban areas of low and middle income countries.[4] Although the worldwide epidemic of obesity has caused a dramatic increase in the prevalence of insulin resistance and T2DM, yet factors causing this altered state of metabolism are still not clear. Obesity leads to accumulation of lipid in non adipose tissue and may contribute to insulin resistance and diabetes.[5] A large number of endocrine, inflammatory and cell intrinsic pathways have been shown to be dysregulated in obesity. It is most likely that their dynamic interplay underlies the pathophysiology of insulin resistance.[6] White adipose tissue is the physiological site of energy storage in the form of lipids. Now it is also recognized as an active endocrine organ which produces hormones like leptin, resistin and adiponectin which regulate metabolic homeostasis like lipid and glucose metabolism.[7,8] The increase in adipocytes leads to an increase in resistin secretion leading to impaired lipid storage in adipocytes and ectopic fat accumulation in non-adipose tissue like skeletal muscle and liver thus leading to insulin resistance.[9]

Resistin, an adipose-macrophage-derived hormone leads to development of diet-induced insulin resistance in rodents. Neutralization of resistin by injection of antibodies in these mice leads to decreased blood glucose levels and improved insulin sensitivity.[10] Although the role of resistin causing insulin resistance in humans is still conflicting, it

is commonly believed that resistin interferes with insulin signalling by inhibiting the ability of the insulin receptor to recruit and activate insulin receptor substrate-1.[11] Emanuelli noted that resistin induces the expression of suppressor of cytokine signaling-3 which has been implicated as a mediator by which insulin negatively regulates its own signalling cascade.[12] Resistin has been implicated in increasing free fatty acids by increasing activity of hormone sensitive lipase and decreasing lipoprotein lipase in humanized resistin mice.[13]

The information available leaves lacunae to be identified and studied, as the role of resistin in the pathophysiology of T2DM is still unclear and its role vis-a-vis obesity is confusing as well as controversial. Our study aims to measure correlation, if any, between serum resistin and insulin resistance, while also measuring the resistin levels in obese non-diabetics and obese type 2 diabetics.

MATERIAL AND METHODS

This cross sectional study was carried out in the Department of Physiology, Postgraduate Medical Institute Lahore in collaboration with the Department of Medicine, Lahore General Hospital, Lahore. Eighty male and female obese subjects with BMI ≥30, at ages between 35–55 yrs, who were not taking any medicines like corticosteroids, phenytoin, insulin and oral hypoglycaemics, that may affect their glycaemic status, were selected. They did not have any endocrinological problems like Cushing syndrome, acromegaly, pheochromocytoma and Type 1 diabetes mellitus or any acute and chronic inflammatory disease**,** and no family history of diabetes and gestational diabetes. Among them were forty newly diagnosed cases of diabetes taken from the Diabetic Clinic of Lahore, General Hospital, Lahore and forty non-diabetic controls selected from the attendants of the patients visiting the hospital. The female subjects were not pregnant.

Informed written consent to participate in the study was obtained from each subject. All measures were adopted to fulfil the requirement of Helsinki's convention. Body weight and height were recorded in all subjects before breakfast while they were wearing light clothes without shoes. General physical and systemic examination was conducted.

Five ml of fasting blood sample was taken from antecubital vein under strict aseptic technique between 8:00–9:00 a.m. Three ml of the blood sample was placed in a plain serum tube and 2 ml was added to sodium fluoride plasma tube, which was centrifuged immediately. Plasma sample was analyzed for glucose estimation on the same day while serum was drawn and stored in aliquots at minus 80 $^{\circ}$C until used.

Plasma glucose was estimated by glucose oxidase method using kit of Fortress Diagnostic, Antrim Technology Park, United Kingdom, (Lot no) 110818. Human insulin was estimated by solid phase Enzyme Amplified Sensitivity Immunoassay using kit of BioSource INS-EASIA (Lot No 063904/C). Human Resistin was estimated by ELISA using kit of Creative Diagnostics, USA (Lot No XQDEK 05181G).

Arithmetic mean and standard deviation of mean (SDM) of each parameter were determined. The significance of differences among the diabetic and non-diabetic group was analyzed by student's t test. Pearson correlation coefficient was used to determine correlation between resistin and BMI, blood glucose, insulin and HOMA-IR. p value ≤0.05 was considered statistically significant. All calculations were carried out with SPSS version 16 (SPSS, Inc. Chicago IL, USA).

RESULTS

Eighty diabetic and non diabetic obese subjects (40 each) were included in this study. Diabetic group included 28 females (70%) and 12 males (30%), with a mean±SD age of 44±7 years. Likewise, the non-diabetic group consisted of 28 females (70%) and 12 males (30%),) with a mean±SD age of 40±6 years.

BMI mean±SD of diabetics was 35±5 kg/m^2 and of controls was 33±3 kg/m^2. A statistically significant difference was observed between the two groups.

Fasting blood glucose concentration was significantly higher in cases (164±46 mg/dl) than in controls (83±8 mg/dl). Similarly, serum insulin levels were significantly higher in diabetic subjects (37±7μU/ml) than in non diabetics (26±6 μU/ml). Moreover, HOMA-IR calculations showed that T2DM subjects had a highly significant increase in insulin resistance (19±8) as compared to normal subjects (5±1). Mean±SD serum resistin in diabetics was 38±8 ng/ml and in non-diabetic subjects it was 25±5 ng/ml.

A significant positive correlation was found between serum resistin and BMI (r=0.509, p=0.001) in diabetic subjects and in obese controls. (r=0.378, p=0.016). Serum resistin was significantly correlated with serum insulin (r=0.308, p=0.053) in non- diabetic patients and in diabetic patients (p=0.980, r=0.000). Correlation of serum resistin with HOMA-IR in non diabetic subjects was significant (p=0.307 r=0.054) and similar significant results in diabetic patients (p=0.561, r=0.000) were found.

Table-1: BMI, resistin and glycaemic parameters in the diabetic and non diabetic groups

Variables	Diabetics Mean±SD	Non Diabetics Mean±SD	*p*-value
BMI kg/m^2	35±5	33±3	0.032
Resistin ng/ml	38±8	25±5	0.000
Blood sugar fasting mg/dl	164±46	83±8	0.000
InsulinIU/ml	37±7	26±6	0.000
HOMA-IR	19±8	5±1	0.000

Table-2: Pearson correlation between serum resistin and BMI and glycaemic parameters

Variables	Diabetics	Non Diabetics
BMI	r=0.509 *p*=0.001	r=0.378 *p*=0.016
Blood Sugar Fasting	r=-0.097 *p*=0.552	r=-0.021 *p*=0.898
Insulin	r=0.980 *p*=0.000	r=0.308 *p*=0.053
HOMA-IR	r=0.561 *p*=0.000	r=0.307 *p*=0.054

DISCUSSION

The present study compared plasma resistin level between non diabetic and T2DM obese subjects. It showed that plasma resistin levels were significantly increased in diabetic subjects as compared to non diabetic obese. This finding is in consistence with other studies linking resistin with the degree of adiposity and diabetes.[14,15] Contrarily, Mohammedzadeh and Sinorita did not find any significant increase in serum resistin level in obese diabetic patients.[15,16] This could be explained on the basis of specificity of ELISA. Human ELISA have potential to cross react with circulating resistin like molecules (RELMs) and studies using resistin ELISA did not check RELM cross reactivity before the analysis**.** Therefore the use of different ELISA may result in varying resistin concentrations in serum.

BMI of the obese diabetic subjects was significantly higher as compared to obese controls. There was a significant positive correlation between serum resistin and BMI in both diabetic and non diabetic control subjects but this correlation was highly significant in diabetics. Similar results were noted by Gharibeh and Asano who showed a positive correlation between serum resistin levels and BMI thus supporting a possible link between resistin and obesity.[14,17] A possible explanation is that obesity being a common factor, there could be a cause-effect- relationship which needs further exploration.

Glycaemic control was significantly impaired in diabetic subjects when compared with non diabetic obese as shown by significant increase in fasting blood sugar level in diabetic subjects. However, no correlation was found between resistin and fasting blood glucose in both groups. Similar results were shown by Gharibeh and Mohammadzadeh, who were unable to show any association between resistin with blood glucose.[14,15] Moreover, Chanchay**,** Al Harithy and Al-Ghamadi showed a positive correlation between resistin and blood glucose in diabetic population.[13,18] Ethnic variations, genetic and environmental influences or differences in using standardized assessment methodology could be the reason for the different findings. Further studies are required to elaborate the relationship between hyperglycaemia of diabetes and any change in resistin level concomitantly.

Current study showed that serum resistin level was positively correlated with insulin and HOMA-IR in diabetic subjects. It is in line with Gharibeh, Al Harithy and Al-Ghamadi, who showed a significant correlation between resistin and HOMA-IR.[14,18] Thus our data added to the growing body of evidence that serum resistin is strongly correlated with insulin resistance. It has been shown that an increase in human obesity is associated with increased serum resistin level and is directly correlated with insulin resistance.[19] An interesting and important question that arises here is, whether resistin is a factor which influences insulin resistance? Our data showed that in diabetics there were increased levels of serum resistin, glucose and insulin resistance but obese non-diabetics did not show similar significant values. This suggests that resistin could cause induction of insulin resistance or vice versa when insulin reaches a certain critical level. However, possibly other factors may contribute to significantly high insulin resistance in diabetics. Several studies have shown that production of other adipokines, like leptin and adiponectin, is altered in T2DM and these might be involved in development of insulin resistance.[20] The role of these adipokines was not investigated in this study. Overall, our data shows a possible link between obesity, resistin and insulin resistance despite inconsistent results of other studies. The correlation is significant both in diabetics and non-diabetics, although weaker in the latter as there is always predisposition of obese to develop insulin resistance.[21]

CONCLUSION

Resistin could be a potential link between obesity and diabetes with a functional role as a pathogenic factor contributing to disruption in insulin signalling pathway that may lead to development of insulin resistance and diabetes.

Treatment formulations which specifically target hormones like resistin produced within adipose tissue are likely to deliver immense therapeutic effects.

AUTHOR'S CONTRIBUTION

Both authors contributed equally to the study.

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