Serum Resistin and Anthropometric parameter in lean and Overweight Insulin -Dependent Diabetic Children (IDDM)

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Abstract
Background: Type 1 diabetes mellitus is a multifactorial syndrome due to a body's inability to synthesis insulin. In patients with type 1 DM, the beta cells are attacked by the immune system (specifically by macrophages and T lymphocytes) and killed, so insulin can no longer be synthesised. Resistin is a newly identified adipocyte secreted hormone belonging to a cysteine-rich protein family. Human resistin gene is expressed in pancreatic islets, pre-adipocytes and bone marrow. It is of relevance for inflammation processes as well as for lipid metabolism. The body mass index BMI a measured of relative weight, adjusted for height. Resistin serum levels were found to be related to body mass index (BMI) in human subjects. Waist circumference is minimally related to height, so correction for height (as in waist to height ratio) dose not improve its relation with intra-abdominal fat or ill health

Objective: The aim is to determin the role of Resistin in lean and overweight.
Subjects and methods: sixty diabetic children (33 males and 27 females), aged (1.3-13) years (mean± SD) (5.6±2.8) years, were enrolled in this study. All had T1DM with no serious long-term complications and C-reactive protein for all patients was negative. All patients used short- and intermediate-acting subcutaneous insulin injections (two daily injections) and no other medication. Duration of disease varied from (0.13-84) months. A matching group of thirty healthy volunteer children with age range of (1.3-13) years, (18 females, 12 males) were included as a control without any family history of diabetes. Serum Resistin was measured by (enzyme linked immunosorbant assay, ELISA). Anthropometric determined are measured as BMI and WHR. BMI (weight in kg divided by the square of the height in meters), Waist and hip circumferences were measured to determine waist-hip ratio (WHR) following standardized procedures.

Results: Resistin values showed no significant difference when comparing both groups (patients and controls). A significant decrease in the level of fasting serum Resistin was found in overweight patients when compared to control, while no such significance was shown for serum Resistin in lean patients when compared to controls. However a significant correlation was found when comparing serum Resistin and BMI in controls but the correlation was not significant in patients.

Conclusion: Resistin levels was found to be highly associated with obesity in healthy controls with a significant elevation in overweight when compared to lean sunjects. However the opposite was found in patients under treatment with insulin. The latter was found to have a depleting effect on Resistin gene expression.
Key words: Resistin, Lean and, Overweight type 1 diabetes mellitus

Introduction
Diabetes is a group of diseases characterized by elevated blood glucose concentration. It may be a consequence of either diminished or totally exhausted insulin secretion from pancreatic beta cells, weakened glucose uptake (insulin resistance), or both (1).

Type 1 diabetes (juvenile onset diabetes): Characterized by total exhaustion of insulin secretion [insulin dependent diabetes IDDM]. In type 1 diabetes, the pancreas is unable to synthesis insulin. However glucose maintained from food cannot be utilized by cells leading to a high elevated blood glucose which untimely cause health problems. Insulin shots are required as insulin pump (2). Type 1 diabetes, scientists think it has something to do with genes. Genes are the instructors for how the body should look and work and are passed on from parents to kids (3). The principal treatment of type 1 diabetes, even from the earliest stages, is the replacement of insulin. Without insulin, ketosis and diabetic ketoacidosis develops and coma or death will result (4).

Resistin is a 12.5 kD cysteine-rich peptide secreted from adipocytes and present in the circulation (5). It belongs to a protein family known as resistin-like molecules that are probably involved in the inflammatory process, but various studies in rodent models have shown that resistin impairs glucose tolerance and insulin action and inhibits adipogenesis (6) (7).

The level of Resistin protein was found to be high in adipocytes in a variety of rodent models of obesity both genetic and diet-induced. Circulating Resistin levels in mouse serum decreased with the administration of the antidiabetic drugs, rosiglitazone and other TZDs (8).

The body mass index BMI which is a measure of the relative weight, adjusted for height. This allows comparisons both within and between populations. The BMI is calculated in both men and women as follows:

\[
BMI = \frac{\text{weight in kg}}{\text{height in meters}^2} = \frac{\text{weight in lb}}{\text{height in inches}^2 \times 703}
\]

The healthy range for the BMI is between 19.5 and 25.0. Individuals with a BMI between 25 and 29.9 are considered overweight, that who’s BMI is equal to or greater than 30 are defined as obese. Resistin serum levels were found to be related to body mass index (BMI) in human subjects (9), (10), but other studies did not reveal a correlation between body mass and resistin levels in blood (11). A considerable number of studies failed to detect an association between resistin concentration and markers for insulin sensitivity (9), (11). Waist circumference was developed initially as a simpler measure and a potentially better indicator of health risk than BMI to use in health promotion. Waist circumference is at least a good indicator of total body fat as BMI or skin fold thicknesses, and is also the best anthropometric predictor of visceral fat. People with increased fat around the abdomen or wasting of large muscle groups, or both, tend to have a large waist circumference relative to that of the hips (high waist to hip ratio). Waist circumference alone, however, gives better prediction of visceral and total fat and of disease risks than waist to hip ratio. Waist circumference is minimally related to height, so correction for height (as in waist to height ratio) dose not improve its relation with intra-abdominal fat or ill health, (e.g., WHR ≥ 0.90 for men or WHR ≥ 0.80 for women was coded as high by gender (12).

This paper is aimed to determine the function of Resistin in overweight and lean patients and controls.
Subjects and methods:

Sixty patients (33 males and 27 females), age range (1.3-13) years (mean± SD) (5.6±2.8) years were enrolled in this study. All were consecutively admitted to the pediatric clinic at AL-Mansour Hospital. All patients had Type 1 Diabetes Mellitus T1DM as diagnosed by the physician and used short-and intermediate acting subcutaneous insulin injections (two daily injections only) with no other medications. Duration of the disease varied from (0.13- 84) months. the disease duration is defined as the day of initial diagnosis of diabetes to the day of blood collection for analysis. Only subjects without hypertension, microalbuminuria, retinopathy, neuropathy or signs of ketoacidosis were recruited. Patients with disorders affecting metabolic parameters such as hypercortisolism, thyroid disease, abnormalities in sex hormone regulation and patients with positive C-reactive protein were excluded. Informed consent was obtained from the parents of the children and the study was approved by the Departmental Committee. For statistical analysis, the following anthropometric variables were used: weight, height, and BMI (weight in kg divided by the square of the height in meters). Waist and hip circumferences were measured to determine waist-hip ratio (WHR) following standardized procedures. All patients were separated families and were investigated after an overnight fast and without the morning insulin injection. 64.7% of patients family history of type 2 diabetes mellitus, while 35.3% had no family history. But all patients had history of stress prior to presentation.

Thirty non diabetic healthy children, similar in age and sex were selected as control subjects (12 females and 18 males).

All subjects were divided according to:

Diabetes (n=60)

Overweight BMI ≥24.9 n= 10
Lean < 24.9 n= 50

Controls (n=30)

Overweight BMI ≥24. n =8
Lean < 24.9 n=22

Eight milliliters (ml) of venous blood sample were collected; using plastic disposable syringes then were separated by centrifugation at (300 rpm) for 15 min. The sera separated were stored frozen at (-20 OC) until assayed. Serum resistin was measured by enzyme linked immunosorbent assay. (ELISA, Sandwich assay). The quantitative determination of glucose was done by the enzymatic colorimetric method.

Results:

As shown in the table1 serum Resistin levels had no significant difference in both groups. One other hand there was a significance difference in the levels of serum Resistin in both genders when compared in each group as shown in table(1) and Fig (1).
Table (1) : The levels of Resistin (ng/ml) in non diabetic controls and in patients with type I diabetes:

<table>
<thead>
<tr>
<th></th>
<th>Type I Diabetic</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistin total</td>
<td>3.5±2.09</td>
<td>3.8±2.02</td>
</tr>
<tr>
<td>Resistin- Male</td>
<td>2.9±1.66</td>
<td>3.31±2.23*</td>
</tr>
<tr>
<td>(mean± SD)</td>
<td>N=30</td>
<td>N=15</td>
</tr>
<tr>
<td>Resistin- Female</td>
<td>3.8±2.0</td>
<td>4.25±1.8*</td>
</tr>
<tr>
<td>(mean± SD)</td>
<td>N=30</td>
<td>N=15</td>
</tr>
<tr>
<td>Resistin Median</td>
<td>3.2</td>
<td>3.1</td>
</tr>
<tr>
<td>Resistin Range</td>
<td>1.0-10.0</td>
<td>1.09-9.21</td>
</tr>
</tbody>
</table>

* P< 0.05 Significant

Fig. (1): The mean levels of Resistin (ng/ml) in male, females and total in patients with diabetes type 1 and healthy controls. Serum Resistin level was weak negatively correlated in patients type I diabetes and control (r=0.13), p>0.05.
Fig. (2): The correlation of Resistin levels (ng/ml) in patients of diabetes type 1 and control. (r= 0.13)

Table (2) shows the anthropometric data of patients and controls. Each group was then subdivided first: according to BMI into overweight and lean groups. Second: according to WHR subjects who have BMI $\geq 24.9$ kg/m² and they have WHR $> 0.86$ which their called upper-body obesity (anterior obesity) or (overweight) and subjects who have BMI $\leq 25$ kg/m² and they have WHR $< 0.86$ called lean.

Mean values of hormone Resistin and biochemical parameters measured for patients and control groups are shown in Table (2). The table shows a significantly high level of glucose in overweight patients when compared to controls, p< 0.05. Also shows a significantly high level of glucose in lean patients when compared to control, p<0.05. But no significance in glucose within groups.

The table shows a highly significant level of fasting Resistin in overweight patients when compared to controls, p< 0.05. However no significance obtained when comparing serum level of fasting Resistin in lean patients and controls, P>0.05. Also no significant levels of serum fasting Resistin was exist in overweight patients when compared to lean ones. However the high significant levels of serum fasting Resistin in overweight controls when compared to leans Fig (3).
Fig(3): The mean levels of Resistin of obese and lean in patients type 1 diabetes and in controls.

Table (2): Anthropometric data and biochemical parameter of overweight and lean children in type 1 diabetic and controls of:

<table>
<thead>
<tr>
<th></th>
<th>Diabetic overweight</th>
<th>Lean</th>
<th>Control overweight</th>
<th>Lean</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (male/female)</td>
<td>(10) 4/6</td>
<td>50(26/24)</td>
<td>(8) 3/5</td>
<td>22(12/10)</td>
</tr>
<tr>
<td>Age (yr.)</td>
<td>9.8±1.9</td>
<td>7.88± 3.11</td>
<td>4.48±3.22</td>
<td>6.1± 2.7</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>35.6±17.3</td>
<td>24.1± 7.57</td>
<td>25.59±0.99</td>
<td>19.6± 0.44</td>
</tr>
<tr>
<td>BMI (Kg/m2)</td>
<td>33.2± 16</td>
<td>16.2± 1.8</td>
<td>25.3±1.04</td>
<td>15.64± 1.14</td>
</tr>
<tr>
<td>WHP</td>
<td>0.89± 0.033</td>
<td>0.85± 0.059</td>
<td>0.93± 0.2</td>
<td>0.86± 0.12</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>199±47.3*</td>
<td>228.12± 97.9*</td>
<td>89.7±11.8</td>
<td>87.22±21.5</td>
</tr>
<tr>
<td>Resistin ng/ml</td>
<td>3.6±2.0*</td>
<td>3.4± 1.9</td>
<td>5.57±1.28</td>
<td>3.14± 1.9#</td>
</tr>
</tbody>
</table>

* Compared between groups  P<0.05
# Compared between same group  P<0.05

Serum Resistin level had a very weak negative correlation with BMI in patients (r=0.033), p>0.05 as shown in Fig (4)
Fig. (4) : Correlation between BMI (Kg/ m2) and Resistin (ng /ml) in patients with diabetic \((r=0.033)\).
However serum Resistin levels was positively correlated with BMI in controls \((r=0.44)\), \(p<0.05\) as shown in (Fig 5).

Serum Resistin levels was positively correlated with WHR in patients \((r=0.17), P<0.05\) and positive correlated between Serum Resistin levels and WHR in controls \((r=0.14), P<0.05\) as shown in Figs (6), (7) respectively.
Fig. (6) : Correlation between WHR and Resistin levels (ng /ml) in T1DM (r=0.17), P<0.05.

\[ y = 3.1451x + 1.087 \]
\[ R^2 = 0.037 \]

Fig. (7) : Correlation between WHR and Resistin levels (ng /ml) in control (r=0.19), P<0.05.

Discussion:
Patients have lower levels of fasting serum Resistin than healthy controls with (r=0.13). However, this trend did not reach a statistical significance in total population (controls or patients) with type 1 diabetes P>0.05 as shown in table (1) and Fig (2).

Based on an overall interpretation of the results of this study, there is a relationship between diabetic status and adipocytokines. The fact that insulin treatment has an effect on the various tissue (with the exception of its physiological effects) must also be taken into consideration due to reduced insulin activity in the liver, as opposed to chronic hyperinsulinaemia. Hyperliptineamia in IDDM subjects was attributed to chronic hyperinsulineamia resulting from insulin treatment(13). This finding is in agreement with previous studies (14,15).

This might be due to the fact that resistine gene expression is regulated in response to insulin and glucose. Insulin administration can suppress Resistin gene expression in adipocyte(16), (17).

Serum Resistin levels in both genders (males and females) in patients group (2.9±1.66), (3.8±2.0) were significantly lower than in their corresponding controls (3.31±2.23), (4.25±1.8) respectively as shown in table (1). Our data found that serum Resistin levels was weakly positive correlated in patients with IDDM and control (r=0.13), p>0.05 as shown in figure (2).

However studies concerning the relationship of serum levels of resistin on gender furnished contrasting results(14), (18).

Youn BS, et al, and McTernn et al observed a decreased in serum Resistin levels in diabetic children with no gender influence (8, 11).

These studies oppose our findings in which male serum Resistin levels in patients were significantly lower than in females. Same was observed in the control group as shown in table (1) and fig (3), i.e. females tend to have higher Resistin levels than males. These results are in agreement with other researchers(15), (19). However we believe that our data are more significant because of the greater number of cases involved in the study.
This can be attributed to the fact that Resistin increases ovarian androgen production by directly stimulating the ovarian theca cell steroidogenesis by enhancing 17-hydroxylase mRNA expression activity in the presence of insulin, or indirectly by augmenting pancreatic β-cell production of insulin(21). Resistin increases both basal and human chorionic gonadotropin – stimulated testosterone secretion, indicating a potential role of Resistin in the reproductive endocrine function(21).

In normal : Upper body obesity has high lipolytic activity releasing FFA into blood circulation. FFA compete with glucose uptake in muscle and fat cells, resulting in increased FAA oxidation and impaired insulin mediated glucose utilization (glucose oxidation and glycogen deposition) in skeletal muscle and acceleration of glycogenlysis in liver (22). Upper body obesity is thought to induce insulin resistance by expressing and secreting several peptide hormone (adiponectin, leptin, resistin and cytokines which interestingly reported in high in obese (23).

Alternatively, the distribution of body fat could play a role in determination of Resistin plasma levels as proposed by Mc- Ternan et al (8) who found higher Resistin mRNA expression in abdominal fat than in thigh. Therefore, the weak association that was found between fasting serum Resistin and waist or hip p>0.05 circumference, but significant correlation in controls in overweight children may reflect different amounts of abdominal fat in morbidly obese individuals.

Resisten level was found to be related to BMI(9, 24). Studying the inter relationship between Resisten and obesity in patients and controls, produce the results shown in table (2) and fig (3). It can be shown clearly that the overweight upper body obese diabetic children have serum Resistin levels (3.2±1.5) which is lower than in the controls (5.5±1.28) which is a statistically significant depletion.

Regional variation in the expression of Resistin has also been observed. Excessive adiposity, particularly abdominal adiposity is undoubtedly one of the determining factors leading to the clustering of metabolic disturbances observed in metabolic syndrome. A recent study suggests an increase in Resistin mRNA expression in abdominal depots compared with thigh providing one explanation for the increase in metabolic abnormalities in abdominal obesity. The same group has also confirmed the increased expression of Resistin in abdominal fat(8).

However, lean diabetic children showed to have higher serum Resistin levels than in their corresponding controls but without any statistical significance. This might be explained by the fact of insulin treatment of the patients group only.

Resistin gene expression has also been investigated in adiposities in response to insulin and glucose in both in vitro and in vivo and was found that insulin administration can suppress Resistin gen expression in adipoite. However lean subjects (patients and controls) are not affected by such influence and thus serum resistin level was augmented in patients rather than control due to inflammatory causes.

Patients serum Resistin levels were weakly negatively correlated with BMI (r=-0.033), p>0.05 as shown in fig (4). But serum Resistin levels was highly positively correlated with BMI in controls (r=0.46), p<0.05 as shown in fig. (5).

The highly positive correlation with BMI in controls reflects that levels of Resistin is associated with obesity in both groups. But insulin treatment in patients group affects its significance.

This finding is in agreement with Schaffler A, et al and Rajala MW etal (15), (26) but apposes that of Lee JH et al (19).

Comparing the value of serum Resistin in control group subdivided according to their BMI table (2). Overweight control had a well defined and significantly elevated serum
Resistin levels when compared to lean subjects. This may attribute to the present accumulated adiposye that are considered to be a source of Resistin(24).

However resistin was not different in non obese and obese, diabetic groups despite variations in insulin sensitivity. Previous studies in mice have shown that Resistin administration impairs insulin sensitivity (7). Rajala et al., observed that this was the result of impaired suppression of hepatic glucose production, rather than peripheral insulin resistance in rats. However, resistin decreased glucose uptake in skeletal muscle cells, although this effect was independent of insulin signaling pathways (glucose transporter -4 translocation, insulin receptor substrate -1 tyrosine phosphorylation, or phosphoinositol 3- kinase activity)(26).

This study shows a good significant correlation between Resistin levels and WHR in patients and controls \( r = 0.17, \ r = 0.19, \ p < 0.05 \) respectively in fig (6), Fig (7). That indicates WHR better indicator of health risk than BMI.

Also in this study there is no significant correlation between fasting serum Resistin level and fasting blood glucose as shown in table (2). The reason is that blood glucose of the selected patients with IDDM, had not reached a certain level to exert an effect on Resistin although the patient had higher blood glucose than normal controls. Studies on Pima Indians have reported no association of serum Resistin levels with fasting glucose and insulin levels, although they were proportional to the degree of adiposity(27).

Rajala et al recently demonstrated that circulating Resistin levels were significantly elevated and positively concordant with rising levels of insulin, glucose and lipids in mice(26).
Conclusions:
Resistin serum levels was found to be highly associatd with obesity in healthy controls with a significant elevation in overweight when compared to lean subjects. However the opposite was found in patient subjects, under treatment with insulin. Thw latter was found to have a depleting effect on Resistin gene expression.
Gender type was found to have noticeable effect on inflammatory marker (Resistin). female were found to have higher levels than males in both studies groups. Sex hormones are involved in the mechanism of such variation but the rout has not yet been established.

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