ASSOCIATION OF ADIPOKINE LEVELS AND INSULIN RESISTANCE IN PREDIABETES: CASE–CONTROL STUDY IN A TERTIARY CARE HOSPITAL IN NORTH KERALA

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Research Article

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Abstract

Background: Increasing evidence revealed the role of adipokines in glucose and fat metabolism. The present study was designed to evaluate the adiponectin, leptin and resistin levels in prediabetes subjects and assess the relationship between these adipokines and insulin resistance. Material and Methods: A case-control study was conducted for a period of one year from January 2021 to January 2022 in the Department of Endocrinology, Aster MIMS hospital in Calicut, Kerala, India. Totally, 200 individuals those who met the inclusion criteria were enrolled for the study. Based on oral glucose tolerance test, the study subjects were grouped into healthy controls (n =100), prediabetic non obese (n=61), and prediabetic obese (n=39). Blood glucose estimation was done by glucose oxidase peroxidase method. Chemiluminescent immunoassay was used for the measurement of insulin level and homeostasis model assessment-estimated insulin resistance was used for the assessment of insulin resistance. Serum adipokines levels were determined by ELISA. Collected data was analyzed by using SPSS Software.

Results: Serum adiponectin levels decreased significantly in obese prediabetes (7.21±2.15µg/ml) when compared to non-obese prediabetes (7.28±2.41µg/ml) and healthy control subjects (13.64 ±2.88µg/ml, p<0.001). Serum leptin levels increased significantly in obese prediabetes (13.59±2.59ng/ml) when compared to non-obese prediabetes (9.84±2.66 ng/ml) and healthy control subjects (12.28±2.65ng/ml, p<0.001). Serum resistin levels increased significantly in obese prediabetes (17.67±3.60ng/ml) when compared to non-obese prediabetes (17.2±3.93ng/ml) and healthy control subjects (14.46 ±4.16ng/ml, p<0.001). Adiponectin-leptin ratio decreased significantly in obese prediabetes (0.56±0.22) when compared to non-obese prediabetes (0.79±0.4) and healthy control subjects (1.15 ±0.37; p<0.001).

Fasting insulin resistance statistically significant (p<0.001) in all groups. Conclusion: The present study strongly suggested that the adipokine profile is an ideal diagnostic tool to predict prediabetes and metabolic syndrome especially among those with insulin resistance. Background: Increasing evidence revealed the role of adipokines in glucose and fat metabolism. The present study was designed to evaluate the adiponectin, leptin and resistin levels in prediabetes subjects and assess the relationship between these adipokines and insulin resistance. Material and Methods: A case-control study was conducted for a period of one year from January 2021 to January 2022 in the Department of Endocrinology, Aster MIMS hospital in Calicut, Kerala, India. Totally, 200 individuals those who met the inclusion criteria were enrolled for the study. Based on oral glucose tolerance test, the study subjects were grouped into healthy controls (n =100), prediabetic non obese (n=61), and prediabetic obese (n=39). Blood glucose estimation was done by glucose oxidase peroxidase method. Chemiluminescent immunoassay was used for the measurement of insulin level and homeostasis model assessment-estimated insulin resistance was used for the assessment of insulin resistance. Serum adipokines levels were determined by ELISA. Collected data was analyzed by using SPSS Software. Results: Serum adiponectin levels decreased significantly in obese prediabetes (7.21±2.15µg/ml) when compared to non-obese prediabetes (7.28±2.41µg/ml) and healthy control subjects (13.64 ±2.88µg/ml, p<0.001). Serum leptin levels increased significantly in obese prediabetes (13.59±2.59ng/ml) when compared to non-obese prediabetes (9.84±2.66 ng/ml) and healthy control subjects (12.28±2.65ng/ml, p<0.001). Serum resistin levels increased significantly in obese prediabetes (17.67±3.60ng/ml) when compared to non-
obese prediabetes (17.2±3.93ng/ml) and healthy control subjects (14.46 ±4.16ng/ml, p<0.001). Adiponectin-leptin ratio decreased significantly in obese prediabetes (0.56±0.22) when compared to non-obese prediabetes (0.79±0.4) and healthy control subjects (1.15 ±0.37; p<0.001). Fasting insulin resistance statistically significant (p<0.001) in all groups. Conclusion: The present study strongly suggested that the adipokine profile is an ideal diagnostic tool to predict prediabetes and metabolic syndrome especially among those with insulin resistance.

Background

Globally, Prediabetes is an emerging metabolic disorder, a condition characterized by slightly elevated blood glucose levels (140-199mg/dL) regarded as indicative that the individual is at the risk of progressing to type-2 diabetes (blood sugar level 200mg/dL or more). According to a report of International Diabetes Federation, its worldwide prevalence will reach 471 million by 2035 [1].

In maintaining energy homeostasis, the adipose tissue plays a key role by communicating with brain, muscle, liver and pancreas which is mediated by adipokines such as adiponectin, leptin and resistin, the bioactive peptides and proteins secreted by adipose tissue [2]. Adiponectin have anti-diabetic, anti-atherogenic and anti-inflammatory property. It promotes the insulin sensitization by reducing hepatic glucose production and increasing insulin sensitivity in the liver [3, 4]. Leptin is primarily produced by adipose tissue in proportion to the amount of body fat stores being involved in the regulation of food intake and energy homeostasis [5, 6]. Resistin, another pro-inflammatory cytokine has association with insulin resistance [7]. Disturbance in the adipokine levels provide critical clues regarding the pathophysiological mechanism of type-2 diabetes mellitus, also their secretion contribute to insulin resistance and impairment of insulin production [8, 9]. Insulin resistance is a pathological condition in which insulin action is impaired in target tissues including liver, skeletal muscle and adipose tissue. Insulin resistance is the foremost characteristic of type-2 diabetes mellitus and assist multiple organs failure along with resistance of insulin in skeletal muscle, liver, adipose tissue. Obesity is another one important phenomenon that has link between Type-2 Diabetes and it has been estimated that not less than 90% of Type-2 Diabetics are overweight or obese. Serum adiponectin level decreases in obesity [10]. The adiponectin/leptin ratio has been proposed as marker of adipose tissue dysfunction [11, 12]. Sufficient evidences are still lacking regarding the effects of adipokines in the pathogenesis of insulin resistance. Different role of adiponectin and leptin in the pathophysiology of Type-2 Diabetes mellitus still needs to be studied [10]. With this view, the present study was designed to evaluate the adiponectin, leptin and resistin levels in prediabetes subjects and assess the relationship between these adipokines and insulin resistance.

Materials And Methods

After getting necessary approval from institutional ethical committee (EC/NEW/INST/2019/406 & ECR/301/Inst/KL/2013/RR), a case-control study was conducted for a period of one year from January 2021 to January 2022 in the Department of Endocrinology, Aster MIMS, a 950 bedded super specialty
hospital in Calicut, Kerala, India. Totally, 200 individuals of both genders in out-patient section, aged between 30 to 50 years, without diabetes were enrolled for the study. Elder subjects with diabetes and other serious physical or mental illness were excluded from the study.

The participants were informed about the study and their consent was received in the prescribed format. Based on the HbA1c and OGTT report, the study subjects were categorized in to normal control group and pre-diabetes group, each had 100 participants. HPLC method was employed for HbA1c estimation and OGTT was carried out as per the WHO criteria. The participants with pre-diabetes were further grouped in to obese and non-obese on their BMI.

Initially, the demographic data was collected from all the participants in the prescribed pro forma. After overnight fasting for 8-12h, blood samples of the participants were collected in a set of evacuated tube containing sodium fluoride and potassium oxalate which is used for blood glucose estimation. Another set of blood samples collected were centrifuged for 15min at 4000rpm and the serum obtained was stored at 80°C for further evaluation.

Blood glucose estimation was done by glucose oxidase peroxidase method. Chemiluminescent immunoassay was used for the measurement of insulin level and homeostasis model assessment-estimated insulin resistance (HOMA-IR) was used for the assessment of insulin resistance calculated by glucose (mg/dl) x insulin (μU/ml)/405.

All biochemical investigations were done by using COBAS 6000, auto analyzer Roche. Bio-Rad D-10 HbA1c analyzer was used for the HbA1c estimation. Adiponectin, Leptin and Resistin ELISA kits of Krishgen BioSystems were used in the estimation of Adiponectin, Leptin and Resistin levels. Sandwich ELISA was employed for the estimation.

IBM SPSS Statistics V21.0 was used for the data analysis. Descriptive statistics represented by mean and standard deviation was used for numerical variables. One way ANOVA followed by multiple comparison Bonferroni test was applied to analyze the statistical significance of the comparison of Adiponectin, Leptin and Resistin level between obese prediabetic, non-obese prediabetic and control group. The relationship of Adiponectin, Leptin and Resistin level with the fasting insulin resistance was analyzed by Pearson Correlation and graphical representation of scatter plot has done. P<0.05 was considered statistically significant.

**Results**

In this a yearlong study, various significant results were obtained. Totally 200 patients were enrolled in the present study. Based on the HbA1c and OGTT report, the study subjects were grouped in to normal control group (N=100) and pre-diabetes group (N=100). Based on their BMI, the pre-diabetes group was further divided in to obese (N=39) and non-obese (N=61). Initially, the socio-demographic data of study subjects were compared with biochemical parameters which is shown in Table 1. In this analysis, it was found that the mean age of participants in the control group was 39.03±5.72, but it was 38.39±6.31 in
case of pre-diabetic non-obese group and 39±5.20 in pre-diabetic obese group. However, the age was not significant with a p value of 0.78. The mean comparison of other demographic details such as BMI (Kg/m²), Systolic BP (mmHg), Diastolic BP (mmHg), HbA1c (%), Fasting blood sugar (mg/dl), Post prandial blood sugar (mg/dl), fasting insulin (µIU/ml) and fasting insulin resistance results among three groups found to be significant with p value of <0.001.

Table 1. Comparison of socio-demographic data and biochemical parameters of study subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (N=100) Mean ± SD</th>
<th>Pre-diabetic; Non-obese (N=61) Mean ± SD</th>
<th>Pre-diabetic; Obese (N=39) Mean ± SD</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(years)</td>
<td>39.03±5.72</td>
<td>38.39±6.31</td>
<td>39±5.20</td>
<td>0.78</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>23.80±2.08</td>
<td>24.15±1.63</td>
<td>26.13±0.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>123.40±7.41</td>
<td>131.80±8.06</td>
<td>126.41±7.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>81.10±4.90</td>
<td>92.46±12.86</td>
<td>84.10±6.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FBS (mg/dl)</td>
<td>86.83±7.27</td>
<td>117.05±4.04</td>
<td>118.51±4.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PPBS(mg/dl)</td>
<td>93.76±12.5</td>
<td>152.64±9.32</td>
<td>167.26±14.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.28±.37</td>
<td>6.03±0.96</td>
<td>6.15±0.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting insulin (µIU/ml)</td>
<td>7.01±1.53</td>
<td>12.26±3.83</td>
<td>18.42±3.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting insulin resistance</td>
<td>1.49±0.35</td>
<td>3.53±1.12</td>
<td>5.38±0.93</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Mean comparison (Mean ± SD); p-value of <0.05 considered to be significant

The results of mean comparison of adiponectin, leptin, resistin levels and Adiponectin-Leptin (A/L) ratio between all three groups analyzed showed a statistically significant difference (p value <0.001). It was found that the serum adiponectin levels decreased significantly in obese prediabetes (7.21±2.15µg/ml) when compared to non-obese prediabetes (7.28±2.41µg/ml) and healthy control subjects (13.64±2.88µg/ml, p<0.001). The results indicated that serum leptin levels increased significantly in obese prediabetes (13.59±2.59ng/ml) when compared to non-obese prediabetes (9.84±2.66ng/ml) and healthy control subjects (12.28±2.65ng/ml, p<0.001). Serum resistin levels increased significantly in obese prediabetes (17.67±3.60ng/ml) when compared to non-obese prediabetes (17.2±3.93ng/ml) and healthy control subjects (14.46±4.16ng/ml, p<0.001). The A/L ratio decreased significantly in obese prediabetes (0.56±0.22) when compared to non-obese prediabetes (0.79±0.4) and healthy control subjects (1.15±0.37, p<0.001) (Table 2).

Table 2. Comparison of adipokines level and A/L ratio of study subjects
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (N=100) Mean ± SD</th>
<th>Pre-diabetic; Non-obese (N=61) Mean ± SD</th>
<th>Pre-diabetic; Obese (N=39) Mean ± SD</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>13.64±2.88</td>
<td>7.28±2.41</td>
<td>7.21±2.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>12.28±2.65</td>
<td>9.84±2.66</td>
<td>13.59±2.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Resistin (µg/ml)</td>
<td>14.46±4.16</td>
<td>17.2±3.93</td>
<td>17.67±3.60</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A/L ratio</td>
<td>1.15±0.37</td>
<td>0.79±0.40</td>
<td>0.56±0.22</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Mean comparison (Mean ± SD); p-value of <0.05 considered to be significant

A weak positive correlation in all three groups with no statistical significance was found on correlating the adiponectin level and fasting insulin resistance with p value of 0.143 in control group, 0.119 in prediabetic non-obese and 0.812 prediabetic obese group (Table 3 and Fig. 1)

Table 3. Correlation between adiponectin level and fasting insulin resistance in study subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (N=100)</th>
<th>Pre-diabetic; Non-obese (N=61)</th>
<th>Pre-diabetic; Obese (N=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting insulin resistance</td>
<td>R</td>
<td>p-value</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>0.148</td>
<td>0.143</td>
<td>0.031</td>
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<td></td>
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</tr>
</tbody>
</table>

In case of leptin level and fasting insulin resistance, the results showed a weak positive correlation among prediabetic obese group (p value 0.299), and prediabetic non-obese group (p value 0.091) and an intermediate positive correlation among control group (p value 0.182). These results were statistically not significant (Table 4 and Fig. 2).

Table 4. Correlation between leptin level and fasting insulin resistance in study subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (N=100)</th>
<th>Pre-diabetic; Non-obese (N=61)</th>
<th>Pre-diabetic; Obese (N=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting insulin resistance</td>
<td>R</td>
<td>p-value</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>0.173</td>
<td>0.182</td>
<td>0.275</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Table 5. Correlation between resistin level and fasting insulin resistance in study subjects
## Discussion

Previous literatures indicated that the fasting insulin level seems to be reliable and promising tool for the diagnosis and the management of prediabetes. Moreover, insulin resistance is the main determinant of developing prediabetes whereas beta cell function is the main determinant of type-2 DM \([13, 14]\). The results of the present study indicated that the state of insulin resistance may be a key point in the development of normal glucose tolerance to prediabetic. It was found that the mean fasting insulin resistance score among samples in experimental group (obese) \((5.38 \pm 0.93)\) was higher than the Fasting insulin resistance score among samples in experimental group (non-obese) \((3.53 \pm 1.12)\) and control group \((1.49 \pm 0.35)\). Fasting insulin resistance in all three groups was statistically significant \((p < 0.001)\).

Adiponectin may be useful marker in the identification of individual with an elevated risk of prediabetes and coronary artery disease. Serum adiponectin concentration have been shown to be inversely correlated with the severity of insulin resistance in patient with type-2 diabetes mellitus. A decreased level of serum adiponectin can be a risk factor for the progression of prediabetes and type-2 diabetes mellitus \([15–17]\). In the present study, serum adiponectin levels decreased significantly in obese prediabetes \((7.21 \pm 2.15\mu g)\) when compared to non-obese prediabetes \((7.28 \pm 2.41 \mu g)\) and healthy subjects in the control group \((13.64 \pm 2.88 \mu g, p < 0.001)\). The adipose tissue is not only an inert storage depot for lipids, but also it secretes a variety of bioactive molecules, known as adipokines, which affect whole-body homeostasis. Adiponectin is the most abundant of these adipocytokines and is known to have a regulatory effect on the metabolism of glucose and lipid \([14]\). In the present study, the correlation of adiponectin level and fasting insulin resistance, showed a weak positive correlation in all three groups with no statistical significance.

Leptin, another on adipokine may represent a predictor of obesity and type-2 diabetes mellitus \([18]\). Plasma leptin levels were associated with insulin resistance and prediabetes. Leptin may be an additional biomarker for screening individuals at high risk for prediabetes \([19]\). In the present study, serum leptin levels increased significantly in obese prediabetes \((13.59 \pm 2.59ng)\) when compared to non-obese prediabetes \((9.84 \pm 2.66ng)\) and healthy subjects in the control group \((12.28 \pm 2.65ng, p < 0.001)\). The correlation between leptin level and fasting insulin resistance showed positive weak correlation among prediabetic obese samples and among prediabetic non-obese samples. But a positive intermediate correlation among control group were found.

In the present study, serum resistin levels increased significantly in obese prediabetes \((17.67 \pm 3.60\mu g)\) when compared to non-obese prediabetes \((17.2 \pm 3.93\mu g)\) and healthy subjects in the control group.
(14.46 ± 4.16µg, p < 0.001). On correlating resistin level with fasting insulin resistance, they showed positive weak correlation in control group and positive intermediate correlation in obese group i.e. as fasting insulin resistance increases, the resistin level increases and negative weak correlation in non-obese group i.e. as fasting insulin resistance increases, the resistin level decreases.

According to previous data, it was considered that an Adiponectin-Leptin ratio equal or higher to 1.0 (with adiponectin concentrations expressed in µg/mL and leptin levels in ng/mL) can be considered normal, a ratio between 0.5 and 1.0 can indicate moderate-medium increased risk, and a ratio below 0.5 suggests a severe increase in cardio metabolic risk [12]. Findings of the present study indicated that adiponectin-leptin ratio decreased significantly in obese prediabetes (0.56 ± 0.22) when compared to non-obese prediabetes (0.79 ± 0.4) and healthy subjects in the control group (1.15 ± 0.37, p < 0.001). The correlation between adiponectin-leptin ratio and fasting insulin resistance showed negative weak correlation in both obese and non-obese groups and positive weak correlation in control group. The p values of all correlation appeared as not statistically significant.

**Conclusion**

Our study suggests that adipokine profile is an ideal diagnostic tool to predict the underlying prediabetes and metabolic syndrome especially in the individuals with insulin resistance. Our findings suggested that, there is a link between adipokines and insulin resistance in patients with prediabetes, adipocytokine (leptin, resistin, and adiponectin) concentration get differed between patients who had normal BMI and those who were obese. Individuals with prediabetes who were obese also, exhibited a disturbed adipocytokine profile in the form of a significantly increased leptin concentration and reduced adiponectin level, compared with prediabetic individuals with normal BMI. However, further studies are needed to identify the causal relationships involved and to determine whether treatment regulating adipocytokine levels could aid in personalized approaches for the management of diabetes and its prevention.

**Declarations**

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Conflict of interest: Nil.

Author contribution:
References


Figures
On correlating the Adiponectin level and Fasting insulin resistance, showed a weak positive correlation in all three groups with no statistical significance.

Figure 1
Figure 2

The correlation between Leptin level and Fasting insulin resistance showed positive weak correlation among Pre diabetic obese patients, positive weak correlation among pre diabetic non obese and positive intermediate correlation among control group were found to be not statistically significant.

Figure 3

On correlating resistin level with fasting insulin resistance, they showed positive weak correlation in control group and positive intermediate correlation in obese group and negative weak correlation in non-obese group.