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ORIGINAL ARTICLE

Association of adipokine levels and insulin resistance in prediabetes: hospital-based descriptive study in a tertiary care hospital in North Kerala

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Abstract

Objective Increasing evidence revealed the role of adipokines in carbohydrates and fat metabolism. The present study was designed to evaluate the adiponectin, leptin, and resistin levels in prediabetes subjects and evaluate the relationship between these adipokines and insulin resistance.

Methods A hospital-based descriptive study was conducted for one year. 200 individuals who met the inclusion criteria were enrolled in the study. Based on the 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 the 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. Statistical analysis was performed by using SPSS Software.

Results Serum adiponectin levels decreased significantly in obese prediabetes (7.21 ± 2.15 µg/ml) when compared to non-obese prediabetes (7.28 ± 2.41 µg/ml) and healthy control subjects (13.64 ± 2.88 µg/ml, $p < 0.001$). Serum leptin levels increased significantly in obese prediabetes (13.59 ± 2.59 ng/ml) when compared to non-obese prediabetes (9.84 ± 2.66 ng/ml) and healthy control subjects (12.28 ± 2.65 ng/ml, $p < 0.001$). Serum resistin levels increased significantly in obese prediabetes (17.67 ± 3.60 ng/ml) when compared to non-obese prediabetes (17.2 ± 3.93 ng/ml) and healthy control subjects (14.46 ± 4.16 ng/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 was statistically significant ($p < 0.001$) in all groups.

Conclusion The present study strongly suggests that the adipokine profile is an ideal diagnostic tool to predict prediabetes and metabolic syndrome, especially among those with insulin resistance.

Keywords Prediabetes · Adipokines · ELISA · Insulin resistance · HOMA-IR

Introduction

Globally, Prediabetes is an emerging metabolic disorder, a condition characterized by slightly elevated blood glucose levels (140–199 mg/dL) regarded as indicating that the individual is at risk of progressing to type-2 diabetes (T2DM) (blood sugar level of 200 mg/dL or more). According to a report from the International Diabetes Federation, the worldwide prevalence of prediabetes will reach 471 million by 2035 [1].

In maintaining energy homeostasis, the adipose tissue plays a key role by communicating with the brain, muscle, liver, and pancreas which is mediated by adipokines such as adiponectin, leptin, and resistin, the bioactive peptides

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and proteins secreted by adipose tissue [2]. Adiponectin has anti-diabetic, anti-atherogenic, and anti-inflammatory properties. It promotes 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 involved in the regulation of food intake and energy homeostasis [5, 6]. Resistin, another pro-inflammatory cytokine has an association with insulin resistance [7]. Disturbance in the adipokine levels provides critical clues regarding the pathophysiological mechanism of T2DM, also their secretion contributes 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 the liver, skeletal muscle, and adipose tissue. Insulin resistance is the foremost characteristic of T2DM and assists multiple organ failure along with the resistance of insulin in skeletal muscle, and liver, adipose tissue. Obesity is another important phenomenon that has a link to T2DM, and it has been estimated that not less than 90% of T2DM are overweight or obese. Serum adiponectin level decreases in obesity [10]. The adiponectin/leptin ratio has been proposed as a marker of adipose tissue dysfunction [11, 12]. Evidence is still lacking regarding the effects of adipokines in the pathogenesis of insulin resistance. The different role of adiponectin and leptin in the pathophysiology of T2DM 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

Study design

After getting the necessary approval from the institutional ethical committee (EC/NEW/INST/2019/406 & ECR/301/Inst/KL/2013/RR), a descriptive hospital-based 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. 200 individuals of both genders in the outpatient section, aged between 30 to 50 years, without diabetes, were enrolled in the study. Elderly subjects with diabetes and other serious physical or mental illnesses were excluded from the study.

The participants were informed about the study and their consent was received in the prescribed format. Based on the Glycated hemoglobin (HbA1c) and 75gm oral glucose tolerance test (OGTT) report, the study subjects were categorized into a normal control group and a 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 into obese and non-obese based on their Body mass index (BMI).

Study procedure

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

Blood glucose estimation was done by the 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 and was calculated by the formula:

$$\text{Homeostasis Model Assessment for Insulin Resistance} = \left(\frac{\text{glucose(mg/dl)x insulin}(\mu\text{U/ml})}{405}\right)$$

Biochemical parameters (Blood glucose and Plasma insulin) estimation was done on a fully automated biochemistry analyzer Cobas 6000. HbA1c estimation was done in a BIORAD-D10 analyzer (Hercules, California, United States). Adiponectin, Leptin, and Resistin levels were quantitatively assessed by Sandwich ELISA kits (Krishgen BioSystems, Mumbai).

Statistical analysis

Data are presented as mean ± standard deviation (SD). One-way ANOVA followed by the Bonferroni test was applied to assess differences between the selected groups. The correlation between two variables was computed by Pearson's correlation coefficients (*r)* and graphically represented by scatter plots. The analyses were performed using SPSS 21.0 (SPSS, Chicago, IL, USA). A *p*-value less than 0.05 was considered statistically significant.

Results

In this yearlong study, various significant results were obtained. Totally 200 patients (100 healthy controls, prediabetic non-obese 61, prediabetic obese 39) were enrolled

in the present study with a mean comparison age of 39.03 ± 5.725 in healthy controls, 38.39 ± 6.312 in the pre-diabetic non-obese group, and 39 ± 5.206 in the prediabetic obese group. However, the age was not significant with a *p*-value of 0.781. On comparison of other demographic and biochemical parameters such as BMI (Kg/m^2), SBP (mmHg), DBP (mmHg), HbA1C (%), FBS (mg/dl), PPBS (mg/dl), fasting insulin(μIU/ml) and fasting insulin resistance we observed a significant difference in the mean among the three groups with a *p*-value < 0.001. Based on the HbA1c and OGTT report, the study subjects were grouped into the normal control group (*N* = 100) and the pre-diabetes group (*N* = 100). Based on their BMI, the pre-diabetes group was further divided into 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.

The adiponectin, leptin, resistin, and the A/L ratio were compared among the three groups. The results of the 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 ± 2.15 μg/ml) when compared to non-obese prediabetes (7.28 ± 2.41 μg/ml) and healthy control subjects (13.64 ± 2.88 μg/ml, *p* < 0.001). The results indicated that serum leptin levels increased significantly in obese prediabetes (13.59 ± 2.59 ng/ml) when compared to non-obese prediabetes (9.84 ± 2.66 ng/ml) and healthy control subjects (12.28 ± 2.65 ng/ml, *p* < 0.001). Serum resistin levels increased significantly in obese prediabetes (17.67 ± 3.60 ng/ml) when compared to non-obese prediabetes (17.2 ± 3.93 ng/ml) and healthy control subjects (14.46 ± 4.16 ng/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).

A weak positive correlation in all three groups with no statistical significance was found on correlating the

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

Parameters	Control (*N* = 100) Mean ± SD	Pre-diabetic; Non-obese (*N* = 61) Mean ± SD	Pre-diabetic: Obese (*N* = 39) Mean ± SD	*p*-Value
Age(years)	39.03 ± 5.72	38.39 ± 6.31	39 ± 5.20	0.78
BMI (Kg/m^2)	23.80 ± 2.08	24.15 ± 1.63	26.13 ± 0.54	< 0.001
Systolic BP	123.40 ± 7.41	131.80 ± 8.06	126.41 ± 7.77	< 0.001
Diastolic BP	81.10 ± 4.90	92.46 ± 12.86	84.10 ± 6.37	< 0.001
FBS (mg/dl)	86.83 ± 7.27	117.05 ± 4.04	118.51 ± 4.36	< 0.001
PPBS (mg/dl)	93.76 ± 12.5	152.64 ± 9.32	167.26 ± 14.33	< 0.001
HbA1c (%)	5.28 ± 0.37	6.03 ± 0.96	6.15 ± 0.20	< 0.001
Fasting insulin (μIU/ml)	7.01 ± 1.53	12.26 ± 3.83	18.42 ± 3.30	< 0.001
Fasting insulin resistance	1.49 ± 0.35	3.53 ± 1.12	5.38 ± 0.93	< 0.001

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

Table 2 Comparison of adipokines level and A/L ratio of study subjects

Parameters	Control (*N* = 100) Mean ± SD	Pre-diabetic: Non-obese (*N* = 61) Mean ± SD	Pre-diabetic: Obese (*N* = 39) Mean ± SD	*p*-Value
Adiponectin (μg/ml)	13.64 ± 2.88	7.28 ± 2.41	7.21 ± 2.15	< 0.001
Leptin (ng/ml)	12.28 ± 2.65	9.84 ± 2.66	13.59 ± 2.59	< 0.001
Resistin (μg/ml)	14.46 ± 4.16	17.2 ± 3.93	17.67 ± 3.60	< 0.001
A/L ratio	1.15 ± 0.37	0.79 ± 0.40	0.56 ± 0.22	< 0.001

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

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

Variable	Control (*N* = 100)		Pre-diabetic; Non-obese (*N* = 61)		Pre-diabetic; Obese (*N* = 39)	
Fasting insulin resistance	R	*p*-value	R	*p*-value	R	*p*-value
	0.148	0.143	0.031	0.119	0.471	0.812

adiponectin level and fasting insulin resistance with a -*p*-value of 0.143 in the control group, 0.119 in the prediabetic non-obese and 0.812 in the prediabetic obese group (Table 3).

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

The Resistin levels and the fasting insulin resistance were correlated. On correlating resistin level with fasting insulin resistance also showed a positive weak correlation in control group (*p*-value 0.833) and positive intermediate correlation in the prediabetic-obese group (*p*-value 0.792) and a negative weak correlation in the prediabetic-non-obese group (*p*-value 0.167) (Table 5).

Discussion

Previous literature indicated that the fasting insulin level seems to be a reliable and promising tool for the diagnosis and management of prediabetes. Moreover, insulin resistance is the main determinant of developing prediabetes whereas beta cell function is the main determinant of T2DM [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 in prediabetics. It was found that the mean fasting insulin resistance score among samples in experimental group (obese) (5.38 ± 0.93) was higher than the Fasting insulin resistance score among samples in experimental group (non-obese) (3.53 ± 1.12) and control group (1.49 ± 0.35). Fasting insulin resistance in all three groups was statistically significant ($p < 0.001$).

Adiponectin may be a useful marker in the identification of individuals with an elevated risk of prediabetes and coronary artery disease. Serum adiponectin concentration is inversely correlated with the severity of insulin resistance in patients with T2DM. A decreased level of serum adiponectin can be a risk factor for the progression of prediabetes and T2DM [15–17]. In the present study, serum adiponectin levels decreased significantly in obese prediabetes (7.21 ± 2.15 µg) when compared to non-obese prediabetes (7.28 ± 2.41 µg) and healthy subjects in the control group (13.64 ± 2.88 µg, $p < 0.001$). 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 lipids [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 T2DM [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 ± 2.59 ng) when compared to non-obese prediabetes (9.84 ± 2.66 ng) and healthy subjects in the control group (12.28 ± 2.65 ng, $p < 0.001$). The correlation between leptin level and fasting insulin resistance showed a positive weak correlation among prediabetic obese samples and prediabetic non-obese samples. However, a positive intermediate correlation among the control group was found.

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

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

Variable	Control ($N=100$)		Pre-diabetic; Non-obese ($N=61$)		Pre-diabetic; Obese ($N=39$)	
Fasting insulin resistance	R	*p*-value	R	*p*-value	R	*p*-value
	0.173	0.182	0.275	0.091	0.105	0.299

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

Variable	Control ($N=100$)		Pre-diabetic; Non-obese ($N=61$)		Pre-diabetic; Obese ($N=39$)	
Fasting insulin resistance	R	*p*-value	R	*p*-value	R	*p*-value
	0.021	0.833	- 0.179	0.167	-0.044	0.792

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 cardiometabolic risk [12]. The findings of the present study indicated that the 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 a negative weak correlation in both obese and non-obese groups and a positive weak correlation in the control group. The *p* values of all correlations appeared as not statistically significant.

Conclusion

Our study suggests that the adipokine profile is an ideal diagnostic tool to predict the underlying prediabetes and metabolic syndrome, especially in 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 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.

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Author contribution Study design (A), Data collection (B), Statistical analysis (C), Data interpretation (D), Manuscript preparation (E), Literature search (F), Fund collection. (ABCDEFG)- 1 Bineesh C P, (ACDEF)- 2 Pranav Kumar Prabhakar, (ACD)- 3 M. V. Vimal and (BEF)- 4 Vipin Viswanath.

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Declarations

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest Authors declare no conflict of interests.

References

1. Magliano DJ, Boyko EJ; IDF Diabetes Atlas 10th edition scientific committee. IDF DIABETES ATLAS [Internet]. 10th ed. Brussels: International Diabetes Federation; 2021.
2. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab. 2004;89(6):2548–56.
3. Herder C, Carstensen M, Ouwens DM. Anti-inflammatory cytokines and risk of type 2 diabetes. Diabetes Obes Metab. 2013;15(s3):39–50.
4. Schottker B, Herder C, Rothenbacher D, Roden M, Kolb H, Muller H, Brenner H. Pro-inflammatory cytokines, adiponectin, and increased risk of primary cardiovascular events in diabetic patients with or without renal dysfunction: results from the ESTHER study. Diabetes Care. 2013;36(6):1703–11.
5. Bluher M, Mantzoros CS. From leptin to other adipokines in health and disease: facts and expectations at the beginning of the 21st century. Metab Clin Exp. 2015;64(1):131–45.
6. Rodriguez A, Ezquerro S, Mendez-Gimenez L, Becerril S, Fruhbeck G. Revisiting the adipocyte: a model for integration of cytokine signaling in the regulation of energy metabolism. Am J Physiol Endocrinol Metab. 2015;309(8):E691-714.
7. Vrachnis N, Belitsos P, Sifakis S, Dafopoulos K, Siristatidis C, Pappa KI, Iliodromiti Z. Iliodromiti, Role of Adipokines and other inflammatory mediators in gestational diabetes mellitus and previous gestational diabetes mellitus. Int J Endocrinol. 2012;2012:549748. https://doi.org/10.1155/2012/549748
8. Lee SH. Adipokine profiles and metabolic health. Endocrinol Metab. 2015;30(2):175–6.
9. Kocot J, Dziemidok P, Kiełczykowska M, Hordyjewska A, Szczesniak G, Musik I. Adipokine profile in patients with type 2 diabetes depends on the degree of obesity. Med Sci Monit. 2017;23:4995–5004.
10. Choudhary R, Nagtilak S, Shukla SK. Association of adiponectin & leptin with insulin resistance in type-2 diabetes. Int J Clin Biochem Res. 2020;7(4):446–50.
11. Vega GL, Grundy SM. Metabolic risk susceptibility in men is partially related to adiponectin/leptin ratio. J Obes. 2013;2013:409679. https://doi.org/10.1155/2013/409679
12. Fruuhbeck G, Catalan V, Rodriguez A, Gomez-Ambrosi J. Adiponectin-leptin ratio: A promising index to estimate adipose tissue dysfunction. Relation with obesity-associated cardiometabolic risk. Adipocyte. 2018;7(1):57–62.
13. Altemani A, Alamri A, Ahmed M, Al Garbo M, Alharbi T, Al-Rasheed R, Alshagraawi S, Alzilai I, Alotaibi F, Al-shahrani A. Prediabetes and serum insulin levels. Int J Community Med Public Health. 2018;5(5):1684–9.
14. Cai X, Xia L, Pan Y, He D, Zhu H, Wei T, He Y. Differential role of insulin resistance and β-cell function in the development of prediabetes and diabetes in middle-aged and elderly Chinese population. Diabetol metab syndr. 2019;11(1):1–8.
15. Stojanovic S, Deljanin-Ilic M, Ilic S, Petrovic D, Dukic S. The significance of adiponectin as a biomarker in metabolic syndrome and/or coronary artery disease. Vojnosanit Pregl. 2015;72(9):779–84.
16. Aleidi S, Issa A, Bustanji H, Khalil M, Bustanji Y. Adiponectin serum levels correlate with insulin resistance in type 2 diabetic patients. Saudi Pharm J. 2015;23(3):250–6.
17. Banerjee A, Khemka VK, Roy D, Poddar J, Roy TK, Karnam SA. Role of serum adiponectin and vitamin D in prediabetes and diabetes mellitus. Can J Diabetes. 2017;41(3):259–65.

18. Ghadge AA, Khaire AA. Leptin as a predictive marker for metabolic syndrome. Cytokine. 2019;121:154735. https://doi.org/10.1016/j.cyto.2019.154735
19. Wang G, Liu X, Christoffel KK, Zhang S, Wang B, Liu R, Li Z, Liu X, Brickman WJ, Zimmerman D, Xu X. Prediabetes is not all about obesity: Association between plasma leptin and prediabetes in lean rural Chinese adults. Eur J Endocrinol. 2010;163(2):243–9.