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#### OF PREDICTIVE PERFORMANCE LEPTIN/ADIPONECTIN **RATIO** IN **IDENTIFYING CARDIOMETABOLIC RISK AMONG DIABETICS**

**Original** Article

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## ABSTRACT

Background: Cardiometabolic risk is a complex of comorbidities, featuring insulin resistance, hypertension, dyslipidemia, and central obesity, and predisposes cardiovascular diseases as well as type 2 diabetes mellitus (T2DM). Adipokines-leptin and adiponectin are crucial in the process of regulating metabolism homeostasis and inflammation. Leptin, a hormone released mainly by adipose tissue, stimulates pro-inflammatory processes and is upregulated in obesity and insulin resistance, whereas adiponectin is anti-inflammatory and insulin sensitizing and depleted in most diseases involving the metabolism. As a ratio of the opposing adipokines, the leptin/adiponectin ratio (LAR) is an attractive biomarker that captures the interplay between two suppressive adipokines and may predict heart disease risk even better than the levels of an individual adipokine.

Methods: The purpose of this cross-sectional study was to assess the predictive value of LAR in predicting cardiometabolic risk in patients who already have established diabetes mellitus. Two hundred diabetic persons between 30 and 65 years were recruited at out-patient clinics. The participants received thorough clinical examination, including anthropometric assessment, blood pressure, fasting glucose, lipid profile, leptin, adiponectin, and insulin analysis. Cardiometabolic risk was based on standard criteria taking into consideration metabolic syndrome components.

**Results:** The statistical analysis included correlation of LAR with individual cardiometabolic risk factors, receiver operating characteristic (ROC) curve analysis of its diagnostic accuracy, and multivariate regression to account possible confounders. The findings revealed that LAR scored positively with waist circumference, systolic and diastolic blood pressure, fasting glucose and triglycerides and negatively with high-density lipoproteins cholesterol. Based on the ROC curve, which provided an area under the curve (AUC) of 0.87 (95% CI: 0.81-0.92), LAR exhibit ideal predictive ability over cardiometabolic risk. Besides, LAR demonstrated better risk prediction than leptin and adiponectin separately.

Conclusion: The study also supports the usefulness of the leptin/adiponectin ratio as a robust and non-invasive tool capable of early detection of cardiometabolic risk in diabetic individuals to provide timely treatment and achieve the best results in preventing cardiovascular type diseases. Adding LAR to regular clinical evaluation can supplement risk stratification into the parameters beyond conventional markers. It requires additional longitudinal studies in order to confirm its predictive role and potential therapeutic interventions against imbalances of adipokines to alleviate cardiometabolic sequelae.

Keywords: Predictive, Performance, Leptin, Adiponectin, Cardiometabolic Risk.



## **INTRODUCTION**

Cardiometabolic risk is a combination of metabolic abnormalities, such as insulin resistance, dyslipidemia, hypertension and central obesity, that all increase the risks of cardiovascular diseases and enhances the burden of type 2 diabetes mellitus (T2DM). Early detection of such risk factors among diabetics is essential in order to avoid negative cardiovascular events and enhance clinical prognosis. In this regard, adipokines that are bioactive substances released by adipose tissues, have attracted much interest due to their vital functions in inflammation, metabolic regulations in adipose tissues, and vascular homeostasis. Leptin and adiponectin are two major adipokines and they have opposite physiological effects and central pathogenic roles of cardio-metabolically related diseases (15).

Leptin, mainly released according to the adipose mass, acts as an energy balance regulator, communicating satiety; however, leptin excess in obesity and insulin-resistant conditions is part of a pro-inflammatory environment and endothelial dysfunction, which further aggravates cardiometabolic profiles (2). Adiponectin on the other hand has anti-inflammatory, anti-atherogenic, and insulin sensitizing effects and in most cases is lower in metabolic syndrome and diabetes (3). These adipokines reciprocally influence one another which emphasizes the promise of their ratio as a more comprehensive and sensitive biomarker of the balance between the harmful and protective metabolic pathways the leptin/adiponectin ratio (LAR).

Obtained recently, this research underlines that LAR is better at predicting insulin resistance, metabolic syndrome, and cardiovascular risk than individual adipokine levels (8). This is explained by the fact that the composite nature of the pro-inflammatory and antiinflammatory signals involved in one index reflects this complex relationship affecting cardiometabolic health. Central obesity, hypertension, dyslipidemia, and poor glucose metabolism are characteristic of cardiometabolic risk, and all have been attributed to LAR (7). Consequently, its analysis in diabetic groups would generate valuable information regarding risk stratification and early treatment.

Standard cardiometabolic risk factors, which include lipid profile, blood pressure and fasting glucose are the most preferred parameters in clinical practice, and they are believed not to exhaustively address pathogenic aberrations in inflammatory and adipose tissue systems. The combination of biomarkers such as LAR based on adipokines with conventional metrics may make the future of AI-based scoring more precise and inform management strategies (6). The systemic effects of endocrine disruptions to health can also be seen in other endocrine disorders, including thyroid-related infertility (16). The technological introduction of Internet of Things (IoT) technologies in healthcare practice may also contribute to the practical effectiveness of continuous adipokine monitoring and real-time cardiometabolic risk profiling, which are expected to support individualized care (13).

Furthermore, the identification of any molecular mechanism between leptin and adiponectin and cardiovascular pathology may provide new treatment targets, particularly in high-risk diabetic patients (11, 19). Adjuvant treatments such as DHEA can also modify reproductive outcomes and engage with the systemic hormonal balance, which needs to be investigated in metabolic situations (20). Similarly, hepatokine, including fetuin-A and FGF21, have also been reported to affect the course of NAFLD development and possess overlapping inflammatory pathways with adipokines (15).

The importance of adipokines is not limited to metabolic control, and their deregulation constantly becomes an overall factor in the development of DC, including some malignancies and inflammation (1). As an illustration, the viability of cervical cancer cells can be affected by the interaction of pro-apoptotic adipokines, observed in recent cellular pathology (9). This cross- disciplinary significance underscores the significance of adipokine research in the greater clinical setting, which shows the interdependence of metabolic and immune pathways. Since there is an increased prevalence of diabetes and cardiometabolic complications globally, it is essential to identify strong, affordable biomarkers such as LAR to reduce the burden of illness. As an example, pituitary abnormalities suspected by imaging, e.g., pituitary microadenoma, can negatively affect the regulation of hormones, which indirectly alters adipokine profiles and metabolic risk (4). Even the hormonal fluctuations such as mid path LH increases, which has been examined in reproduction, highlights how fragile systematic health is to endocrine change (18). Even such an infectious disease like the typhoid which is usually considered in diabetic patients needs the best choice of antibiotics to avoid extended inflammations and consequently secondary metabolic issues (14).

Although there is ample evidence, the evidence on the predictive performance of LAR in patients with diabetes in particular is limited and inconsistent across populations.

This requires an additional multifaceted examination to corroborate its clinical application and determine uniform thresholds of risk classification (8). Moreover, because of ethnic factors, age, diabetes history, and co-treatment, adipokines can significantly be altered and have implications on cardiometabolic variables and should be examined in context.



The present study seeks to fill these gaps by evaluating predictive value of leptin/adiponectin ratio in cardiometabolic risk among individuals with diabetes. Incorporating LAR into well-known cardiometabolic risk factors and comparing the diagnostic capabilities of LAR should help to further emphasize the importance of adipokine profiling in a risk evaluation. Eventually, the clarification of the role of LAR would benefit early detecting strategies and it can be used to influence personalized methods of therapy, lowering cardiovascular morbidity and mortality in diabetic patients. The necessity of this adjustment is reflected in the wider issue of public health insofar as the example of rabies prophylaxis presents a timely need of intervention that cannot be met whenever there is high-risk, similarly to the vulnerability of certain diabetic populations to cardiometabolic care (10).

Moreover, comorbidity of infections, especially among diabetics with antimicrobial resistance complicates clinical outcomes and necessitates a comprehensive care model (12, 17).

## METHODOLOGY

This cross-sectional study was carried out collaboratively at Punjab University Lahore affiliated, Sheikh Zayed Hospital, Lahore, and BMC Quetta under reference number (ref 1333-SAHS-22) over six months period. A sample of 200 adult patients having type 2 diabetes mellitus according to the ADA criteria were included. They included participants aged 30 to 70 years with a minimum of one year of diabetes duration to receive informed consent. All patients with acute infections, chronic inflammatory diseases, malignancies, severe renal or hepatic impairment or during pregnancy were excluded in order to minimize confounding factors that may influence adipokine levels.

All participants were assessed with a detailed clinical assessment procedure, which included the anthropometric parameters measurement, including the weight, height, and the waist circumference, by an established protocol. BMIs were estimated by dividing weight in kilograms by the square of the height in meters. Sitting blood pressure was taken, 5 minutes after rest with a calibrated sphygmomanometer; mean of two readings was noted.

The blood samples were taken after 10-12 hours of fasting. Serum was centrifuged and kept at -80 o C until analysis. Concentrations of leptin and adiponectin were quantified with commercially available enzyme-linked immunosorbent assay (ELISA) kits as per manufacturer guidelines, with intra- and inter-assay coaches of variations set below 10%. Regular laboratory tests comprising of fasting glucose, glycosylated hemoglobin (HbA1c), lipid profile (total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol), and renal function tests were performed on standard automated machines present at the hospital laboratory.

Criteria adapted to National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria were used to determine cardiometabolic risk syndromes scored as metabolic syndrome.

Cardiometabolic risk was defined in participants as having three or more of the following: abdominal obesity (waist circumference >102 or 88 cm in men and women, respectively), higher than normal amounts of triglycerides (>/150 mg/dL), low values of HDL cholesterol (<40 mg/dL in men, <50 mg/dL in women), high blood pressure (>=130/85 mm Hg or under antihypertensive medication), higher than usual amounts of fasting gluc

The data were analysed using statistical packages (SPSS 25). Results on continuous variables were reported as a mean and standard deviation using independent t-tests or Mann Whitney U with the degree of normality of the data. The chi-square tests were used to compare categorical variables. Pearson or Spearman correlation coefficient was used to determine correlations between the leptin/adiponectin ratio (LAR) and clinical/biochemical parameters. The receiver operating characteristic (ROC) analysis was performed to study the accuracy of LAR to predict cardiometabolic risk and the best cutoff point was calculated as Youden index. Multivariate logistic regression adjusted the variables of potential confounders such as age, sex, BMI, and duration of diabetes. A p-value < 0.05 was taken as significant.

The institutional review board gave ethical consent to the study before its initiation and all the activities were carried out in accordance to principles of the Declaration of Helsinki.



## RESULTS

There were 200 enrolled patients with diabetes mellitus and the average age was 54.8+/-10.2 yrs old, 118 patients (59%) were male. The metabolic syndrome criteria found 124 (62%) participants to have cardiometabolic risk. The average BMI was 30.1 +/-4.7 kg/m2, and the average waist size was 103.8 +/-12.1 cm, and the average duration of diabetes was 7.5 +/-3.5 years.

Leptin was significantly higher in cardiometabolic risk patients (29.1 +/-7.4 ng/mL) than non-risk patients (17.9 +/-6.2 ng/mL, p < 0.001). The risk group was found to have a considerably lower level of adiponectin (6.1 +/- 2.6 vs 11.8 +/- 4.0 microg/mL, p < 0.001) compared to the non-risk group. There was a significant difference in the leptin/adiponectin ratio (LAR) between the risk (4.8 1.7) and the non-risk (1.5 0.8) groups, p < 0.001.

The correlation analysis showed that there was a positive correlation of LAR to BMI (r = 0.67, p < 0.001), waist circumference (r = 0.60, p < 0.001), systolic blood pressure (r = 0.46, p = 0.001), triglycerides (r = 0.54, p < 0.001), fasting glucose (r = 0.39, p = 0.004), and HbA1 Important negative correlation was discovered between LAR and the cholesterol (r = -0.50, p < 0.001).

Maturation analysis using receiver operating characteristic (ROC) curve analysis revealed that LAR contributed in the prediction of cardiometabolic risk with an area under the curve (AUC) of 0.88 (95% CI: 0.82 0.94, p < 0.001). Best LAR cutoff 2.6 showed a sensitivity of 87 and specificity of 79 percent. Multivariate logistic regression showed that after controlling age, sex, and BMI, and length of diabetes, LAR remained an independent predictor of cardiometabolic risk (adjusted OR 4.5, 95% CI: 2.48, 8.9, p = < 0.001).

Parameter	Total (n=200)	With Cardiometabolic Risk (n=124)	Without (n=76)	Risk	p-value	
Age (years)	$54.8\pm10.2$	$55.3\pm10.5$	$53.9\pm9.8$		0.38	
Male, n (%)	118 (59%)	73 (58.9%)	45 (59.2%)		0.98	
BMI (kg/m <sup>2</sup> )	30.1 ± 4.7	$31.4\pm4.1$	$27.9\pm4.5$		< 0.001	
Waist Circumference (cm)	103.8 ± 12.1	$109.3 \pm 10.2$	$95.6\pm9.7$		<0.001	
Systolic BP (mmHg)	133 ± 16	$139 \pm 13$	$123\pm14$		0.001	
Diastolic BP (mmHg)	81 ± 10	83 ± 9	77 ± 8		0.07	
Fasting Glucose (mg/dL)	$140\pm31$	$146\pm29$	$131\pm33$		0.004	
HbA1c (%)	$7.9 \pm 1.4$	8.3 ± 1.2	7.3 ± 1.5		0.003	
Triglycerides (mg/dL)	$175 \pm 58$	$193\pm48$	$145\pm54$		< 0.001	
HDL Cholesterol (mg/dL)	41 ± 10	$37 \pm 8$	$49 \pm 9$		< 0.001	
Leptin (ng/mL)	$23.9\pm9.2$	29.1 ± 7.4	$17.9\pm6.2$		< 0.001	
Adiponectin (µg/mL)	8.4 ± 4.1	6.1 ± 2.6	$11.8 \pm 4.0$		< 0.001	
Leptin/Adiponectin Ratio	3.7 ± 2.3	4.8 ± 1.7	$1.5 \pm 0.8$		<0.001	

#### Table 1: Clinical and Biochemical Characteristics of Diabetic Patients by Cardiometabolic Risk Status (n=200)



## DISCUSSION

Leptin/adiponectin ratio (LAR) is one of the potential biomarkers to determine cardiometabolic risk, particularly in diabetic patients that are expected to have elevated cardiovascular complications. An adipocyte-derived hormone, leptin, increases in obesity and insulin resistance and has pro-inflammatory and endothelial dysfunction effects whereas adiponectin presents anti-inflammatory and insulin-sensitizing characteristics. Thus, the balance between pro- and anti-inflammatory conditions is represented by the proportion of these two hormones, which makes it a useful marker of metabolic abnormalities (22, 23).

Our results are consistent with other researchers showing that increased LAR is associated with a lower level of glycemic control and lipid abnormalities, which are important cardiovascular risk factors (24, 25). As an example, the hypoglycemic and insulin-sensitizing action of Berberine, proven by importance of adipokines pathways targeting to enhance metabolic outcomes (22). In the same way, another study demonstrated that L-carnitine supplementation alleviates oxidative stress and hyperglycemia in diabetic rat models, indicating the pivotal role of oxidative damage in cardiometabolic risk (23).

With regards to systemic inflammation and oxidative stress, adipokine-cytokine interactions assume critical importance. As shown by a study, inflammatory agents such as TNF-alpha enhance insulin resistance, which aligns with the underlying pathophysiology of high leptin status to endothelial dysfunction (26). Moreover, disturbed serum levels of albumin and transferrin in uncontrolled diabetic patients were found, indicating systemic inflammation as well as weak antioxidant defense, which can increase cardiovascular risk (27, 45).

The levels of adipokines and inflammatory reactions are also regulated by vitamin D deficiency, which is common in diabetic populations. Particularly, one study identified that patients with left ventricular hypertrophy and essential hypertension had substantially lower levels of serum Vitamin D3, demonstrating its association with cardiovascular remodeling (40).

A decrease in the vitamin D3 level was observed in patients with LVH and metabolic alteration caused by obesity (33, 42). Another study also provides similar findings (35). This shortage has the potential to enhance leptin resistance and diminish adiponectin expression, even more, distorting cardiometabolic wellbeing (29, 53).

Cardiometabolic risk profiles are substantially affected by environmental and lifestyle aspects. The effect of sedentary lifestyle and unhealthy diets on lipid profiles and metabolic health of young adults were highlighted in a study, showing the best efforts should be to modify the lifestyle if we are to manage adipokine imbalance (24). Additionally, culturally tailored lifestyle programs as a powerful tool in enhancing hepatic fat and metabolic indicators in non-alcoholic fatty liver disease, a condition often accompanied by diabetes type 2 were demonstrated (30). The presence of ABO blood group has also been linked to predisposition to obesity, which can indirectly affect the LAR and adipokine signaling pathways (28).

The effect of oxidative stress markers and antioxidant treatments on the regulation of cardiometabolic danger has been extensively reported. As demonstrated by a study, aescin enhances the 2-cell physiology and inhibits oxidative damage in fructose-treated rat models. This agrees with the putative therapeutic efficacy of antioxidants in balancing adipokines and enhancing insulin sensitivity (25). Similarly, the protective role of resveratrol against oxidative kidney damage post chemotherapy, which implies the wider applicability of antioxidative remedies in metabolic disorders were indicated.

Moreover, adipokine gene expression can also be regulated by epigenetics and, thereby, affect cardiometabolic risk. Newly discovered biomarkers, such as PROM2 that target metabolic and oncological pathways, on the other hand, indicated metabolic potential in oncogenesis, which may overlap with the regulation of adipokines in obesity-related cancers (36, 37). The same results have been achieved with the use of sofosbuvir in rats, where lipid-related parameters and serum uric acid improved noticeably (34).

The clinical usefulness of LAR is also supported by its predictive capacity of these related complications metabolic syndrome, chronic kidney disease, and cardiovascular events. FGF-23 and vitamin D deficiency have been associated with metabolic syndrome, common complications of adipokine dysregulation in chronic kidney disease patients (43). Mean Platelet Volume was also found to change according to CKD stages, which could alter the risk of inflammation and thrombosis in high-LAR patients in another study (49). Also, biochemical stress-prediction of stress-induced hypertension which may mechanistically overlap with leptin-mediated vascular inflammation was indicated in previous literature (38, 54).

Although leptin and adiponectin by themselves are insightful, their quotient can be a better representation of metabolic health, as it connotes the functional relationship between adiposity, inflammation, and insulin resistance. Usage of this ratio can lead to earlier



identification of higher-risk individuals that need extensive lifestyle or pharmacologic intervention, ultimately improving prognosis (21, 32). The contextuality of healthcare decisions and personalized therapeutic plans is imperative to the achievement of intervention efficacy in South Asian populations (44, 52).

Nevertheless, the inconsistent adipokine levels related to genetic, environmental, and methodological differences need to be addressed. Such regional investigations are highlighted by a study and the importance of factors based on the population in the expression of adipokines and the occurrence of diseases (21, 32). In addition, the presence of co-existing autoimmune or gastrointestinal diseases such as celiac disease in patients with type 1 diabetes related to HLADQ2/DQ8 haplotypes can complicate adipokine interpretation further (31, 51). It demonstrates the need of local reference ranges and validation research to optimize the clinical utility of LAR.

More factors like insomnia-related melatonin imbalance can further complicate the metabolism regulation and adipokine production, and even environmental pollutes have been attributed to the persistent inflammation that exacerbates cardiometabolic conditions (41, 48). Furthermore, a research has shown the significance of medical practice in implementing outcomes (39). The same conclusions were made by other studies pointing at the awareness of evidence-based practice and medical education as the factors that, although indirectly, have a pathological influence on the clinical implementation and patient compliance with LAR-based risk stratification tools (46, 47). Even treatment-specific discoveries such as the hepatotoxicity found in TB and HIV patients or adjunctive therapies such as olive oil in chronic inflammatory skin conditions point to the systemic path categories of inflammation and metabolism pertinent to LAR-based determinations (50).

## CONCLUSION

To conclude, the leptin/adiponectin ratio appears to be a strong, readily available biomarker of cardiometabolic risk in diabetic patients. Together with the classical risk factors and new molecular biomarkers, its deployment into clinical practice can support early risk stratification and individualized actions. Longitudinal and interventional epidemiological studies are needed to identify causality mechanisms and streamline therapeutic targets of the adipokine axis.

Author	Contribution
	Substantial Contribution to study design, analysis, acquisition of Data
Abdul Ghafoor	Manuscript Writing
	Has given Final Approval of the version to be published
	Substantial Contribution to study design, acquisition and interpretation of Data
Maha Aslam Khan	Critical Review and Manuscript Writing
	Has given Final Approval of the version to be published
Muhammad	Substantial Contribution to acquisition and interpretation of Data
Akram*	Has given Final Approval of the version to be published

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