Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i5.6106

ASSOCIATION OF CIRCULATING RESISTIN LEVELS WITH INFLAMMATORY MARKERS, GLYCEMIC AND LIPID INDICES IN PEOPLE WITH OBESITY, MORBID OBESITY AND LEAN PAKISTANI SUBJECTS

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Abstract

Obesity is a multisystem disorder and is strongly associated with metabolic impairments. Obesity is characterized by a state of altered adipose tissue functions with low-grade inflammation, insulin insensitivity and dyslipidemia. The current study aimed to explore the correlation of circulatory resistin with metabolic and inflammatory markers in adults with obesity and healthy lean Pakistani subjects. This study was carried out on 622 Pakistani subjects and anthropometric and clinical parameters were assessed, which include BMI, WHR, blood pressure manually, while resistin, insulin, inflammatory markers by enzyme-linked immunosorbent assay technique (ELISA), and lipids, and glycemic indices using the chemistry analyzer Microlab-300XL.

The analysis revealed that overall circulating resistin was markedly elevated in all categories of obesity as compared to lean, healthy subjects ($P<0.001$).The resistin outcomes were found to be strongly associated with obesity indices (BMI, $r = 0.669$ and $P<0.001$), central adiposity indices (hip, waist, and WHR, $r = 0.669$ and $P<0.001$), systolic and diastolic blood pressure ($r=0.61$, $r = 0.625$ and $P<0.001$), HOMA-IR ($r = 0.763$, and $P<0.001$), lipid indices (cholesterol with $r = 0.759$, TGs with $r = 0.755$ and HDL, $r =-0.749$ with $P< 0.001$) and IL-6 with $r = 0.849$, CRP, $r = 0.829$ and TNF-α, $r =-0.812$ with $P< 0.001$) among lean controls versus metabolically healthy, metabolically unhealthy, and subjects with obesity and diabetes. The serum resistin level was taken as dependent variable by the reduced model of multiple linear regression presented a highly significant association between elevated resistin and MHO, MUO, and DO (with $p<0.001$ and all other factors were considered insignificant in this background removal method.

In conclusion the current study uncovered that hyperresistinemia is an important indicator of several health issues, including metabolic dysregulation, insulin resistance, low-grade inflammation and obesity. These findings underscore the importance of proactive measures to effectively prevent and manage these conditions.

Key Words: Hyperresistinemia, Interleukin-6, Insulin resistance, HDL, Diabetes, Central Obesity

Introduction

Obesity is a noncommunicable disease with multifactorial etiology, characterized by excess and ectopic accumulation of fat in dysfunctional adipose tissue (1). It is associated with developmental, physiological, environmental, genetic, and socioeconomic factors (2) as well as psychosocial reasons and heterogeneity in metabolism. Central obesity is a risk factor for numerous pathologies such as insulin insensitivity, non-alcoholic fatty liver disease, type 2 diabetes mellitus, osteoarthritis, hypertension, cardiovascular diseases, obstructive sleep apnea, and several types of cancer (3). Obesity has increased dramatically since 1975 in both low and high-income countries. The onset of overweight and obesity escalation is alarming globally, which affects children and adults in developing and developed nations. World Health Organization (WHO) reported in 2016, about 1.9 billion adults, or 39%, were overweight, while 650 million were reported to have obesity (~13%) globally (4).

Pakistan is ranked as sixth most populous and developing country with 39.1% urban population in the world and is facing several socioeconomical and health crises. The Global Burden of Obesity Report revealed that Pakistan stood at ninth position regarding to obesity out of 188 nations (5). The major contributing factors in the development of obesity in Pakistan are unawareness to healthy diet and the accessibility to a diversity of delicious junk food items available at reasonable costs. It is also due to the unawareness of health effects of junk foods, lack of physical activity, the use of automobiles, increases in screen time and psychological stigma (6). Recently, overweight and obesity were not taken into consideration as illnesses, which hindered the ability of researchers to diagnose what elements, genetic as well as endocrine, are associated with the pathogenesis of overweight and obesity in the Pakistani nation (7).

Insulin resistance is a pathological state, characterized by a decreased sensitivity of insulin to accomplish its physiological role. Insulin resistance is prone to overt pathological conditions, which include metabolic syndrome (MetS), T2DM, and is associated to circumstances such as weight gain and obesity (8). Long-term obesity induces low-grade systemic inflammation, metabolic derangements, insulin resistance, diabetes, and cardiovascular events. The cornerstone factor that disturbs insulin sensitivity is the release of un-esterified fatty acids commonly observed in uncontrolled type 2 diabetes mellitus as well as in obesity, and it is linked with insulin insensitivity in both diseases (9). However, the association among inflammation, mitochondrial dysfunction, lipotoxicity, hyperinsulinemia, and glucotoxicity with insulin resistance. Oxidative stress, endoplasmic reticulum stress, ageing, lipodystrophy, hypoxia, and genetic background are also considered exaggerating factors in the pathogenesis of T2DM by inducing insulin resistance in people with obesity (10).

Resistin is a peptide hormone that was first reported in mice in 2001 as a thiazolidinedione-downregulated gene in mouse adipocytes and termed according to its interference in insulin and its receptor binding (11). Circulatory resistin hormone also reported to be significantly elevated in persons with diabetes and obesity. It is well established that the elevated circulatory resistin, was found linked in the development of generalized obesity and accumulation of visceral fat, which ultimately leads to insulin resistance and diabetes (12). On other hand some studies did not prove such relationships (13-15). During severe obesity, the prolonged systemic and low-grade inflammatory state enhances the macrophages infiltration in adipose tissue to secrete cytokines with pro-inflammatory properties, which contribute in the development of metabolic derangements (16). In comparison to individuals with MUO status, MHO individuals exhibit a more favourable inflammatory status (17). In contrast to metabolically healthy lean (MHL) subjects, the metabolically healthy obese may not experience complete benign conditions, as they may bear a higher risk of developing metabolic and cardiovascular pathologies (18,16).

Adipose tissue secretes proinflammatory bioactive molecules (adipokines) in a balanced manner. In the state of obesity, the adipose tissue expansion stimulates the overexpression of these proinflammatory mediators, which induce some alterations in the immune response and participates in the development of low-grade systemic inflammatory events (19). Obesity is also linked with the

infiltration of monocytes as well as macrophages in adipose tissue, which triggers the secretion of CRP and several cytokine molecules, including interleukin moieties (IL-1, IL-6, and TNF-α), in adipose tissue. Although, these cytokines, NFκB and transcription factor in these immune cells mediate the over expression of resistin gene which leads toward higher circulatory resistin (20).
The precise mechanism that connects resistin with obesity, metabolic abnormalities, and low-grade inflammation remains unclear. However, previous studies have shown a direct association between hyperresistinemia, obesity, insulin resistance, metabolic syndrome, and low-grade inflammation (12, 21). On the contrary, certain studies have not yielded conclusive evidence to establish a correlation between resistin levels, anthropometric indices, metabolic dysregulation, insulin resistance, and inflammatory markers (22). Based on national and international reports, it has been identified that Pakistan has the highest prevalence of obesity and its comorbidities (5). Our study aimed to quantify circulating resistin levels in obese and lean Pakistani subjects and investigate their associations with obesity indices, insulin sensitivity, lipid profiles, glycemic indices, and inflammatory markers. For the first time, our study reported such a potential association with a large sample size.

Materials and Methods

Study subjects

The study was conducted on 622 individuals obtained from the outpatient door of medicine at a tertiary care hospital in Lahore (Lahore General Hospital). However, the controls were selected from the general population. The duration of study was from June 2022 to July 2023. All of the subjects were pre-informed about the purpose of our study, and written consent was taken from them regarding the demographic data, including lifestyle, age, sex, exercise habits, family history of diabetes and obesity. The physical data from each subject, including height, weight, waist, and hip circumferences, were recorded by standard procedures. Further, the clinical diagnosis, medical, and treatment records were taken from the hospital (23).
The inclusion criteria for study subjects involved a BMI >30 kg/m^2, considered as an indication of obesity and individuals with BMI <25 kg/m^2 were taken as lean controls. The healthy controls were selected after a careful review of their medical history and outcomes of anthropometric and biochemical outcomes (24). Among 650 participants of study, the subjects who did not fulfil the inclusion criteria and had any history of other known genetic disorders or any type of malignancy, recurrent infections, autoimmune disorders or taking medications for any disease such as hormonal preparations (n=05), steroids (n=04), immunosuppressants (n=05), antibiotics (n=03), suffering from autoimmune disorders (n=02), or positive for viral hepatitis B or C (n=07) and those who refused to participate (02) were excluded. Eventually, 622 volunteers were recruited for this study. The study was approved by the ethics committee of the University of Central Punjab, Lahore, Pakistan. All the study procedures were carried out according to the principles of the Declaration of Helsinki.

Anthropometric measurements

For anthropometric study, the physical measurements of the subjects were assessed under the supervision of trained medical staff. Height (without headwear and bare feet) was recorded by using an audiometer with a precision of 0.1 cm. Body weight (in bare feet and excluding massive clothes) was determined using a digital weighing scale with an accuracy limit of 0.1 kg, while BMI, was calculated using the formula: body weight in kg divided by height of person in metre^2. Waist circumference (a narrow diameter between the iliac crest and xiphoid process) and hip circumference (a wide diameter above the larger trochanters) were obtained to find the waist-hip ratio (WHR) (25). Blood pressure was recorded twice from the right arm of each subject in a comfortable sitting position using a mercury sphygmomanometer (26).

Sample Collection

Blood samples were drawn from overnight fasting subjects (8–12 Hrs.), 8 ml of blood was drawn from the right cubital fossa by aseptic technique, of which 2 ml was added into sodium fluoride-

containing vacutainers (for glucose estimation) and 2 ml in EDTA vacutainers for other analysis. The rest of the blood sample was added to gel vacutainers and placed in racks to clot for 5 minutes. After clotting, serum was isolated on the spot using a centrifuge machine from both clot-enhancing vacutainers at 5000 r.p.m. for 5 minutes. Serum was aspirated and aliquoted (100µL) into autoclaved eppendorf tubes to avoid repeated thawing that may cause degradation of molecules of interest (27). All the serum samples were screened for the presence of any viral markers (HBV, HCV, and HIV). Positive samples were excluded, and the rest of the samples were transported into an ice box to the research laboratory and stored at -20^{o}C for further analysis. The biochemical analysis conducted on batches of samples weekly to avoid decomposition of sensitive biomarkers (28).

Biochemical analysis

Biochemical parameters were estimated using a semiauto-chemistry analyzer (Microlab-300XL) and commercially available reagent kits (Human Diagnostics Germany). Serum glucose was estimated by the glucose oxidase method, and the lipid profile, including serum cholesterol total and triglycerides by the enzymatic method (per-oxidase method). The estimation of HDL-c is carried out by the precipitation method, while VLDL and LDL were taken by the calculation by the Friedewald formula and the quantification of CRP is done by the turbidimetric method (Bioactive Diagnostics. GmbH.). Serum insulin, (Calbiotech Inc.) serum resistin, TNF, and IL-6 (Thermo. Fisher Sci. Co.) were determined by sandwich ELISA method using a microplate reader (Bio-Tek XLx-800). Fasting blood sugar and insulin outcomes were used to analyze homeostasis model assessment of insulin resistance (HOMA-IR) the formula is as below (27).

$$\text{HOMA-IR} = \text{Fasting plasma sugar (in mmol/L)} \times \text{plasma insulin (µIU/mL)} /22.5$$

Statistical Analysis

Metabolically healthy (MHO), and MUO status was diagnosed according to WHO criteria. MHO was labeled in those who have BMI ≥30 kg/m^2, fasting circulatory triglycerides ≤150 mg/dL HDL circulatory cholesterol >40 mg/dL (in males) >50 mg/dL (in females). Fasting blood glucose ≤100 mg/dl. Systolic blood pressure (SBP) ≤130 mmHg Diastolic blood pressure ≤85 mmHg. The alteration of any three parameters was labeled as metabolically unhealthy obesity. A sample size of 59 in each group was estimated by using a 95% confidence level and 90% power with expected mean levels of resistin for subjects with obesity and non-obese subjects of 5.3 and 3.6 ng/ml, respectively, with a combined standard deviation of 2.84. It was ensured that there would be a minimum of 59 cases in each group. The analysis was done using SPSS version 20.0. Data for age, all anthropometric measures, blood pressures, glucose, insulin, HOMA-IR, lipid profiles, and resistin level were described by using the median and interquartile range for each group.

Comparisons among groups for all these measures were made by using Kruskal-Wallis ANOVA, and post-hoc comparison were taken by using the Mann-Whitney U test. Those with common letters as superscript were insignificant, and those with no common letter were significantly different at the level of significance (≤0.05). The Spearman correlation coefficient was used to see the association of all measures with Resistin, in the complete sample as well as within each group. Multiple linear regression analysis with the backward removal method was applied to see the effect of anthropometric measures, lipids, blood pressures, and inflammatory markers as independent variables by taking resistin levels as a dependent variable, and then by taking patient status (groups) into consideration in addition to the above-mentioned variables. The ROC curve was used to determine cutoffs for resistin levels for distinguishing different groups pairwise and a P-value ≤ 0.05 was considered as significant.

Results

The study was conducted on 622 subjects, according to the medical history and clinical outcomes of our study, these participants were divided into four groups, of which 71 were with metabolically healthy obesity (MHO) and labeled as Group 2, 230 were in Group 3 with metabolically unhealthy obesity (MUO), and 120 were with diabetes and obesity (DO) labeled as Group 4, as well as 201 were

healthy lean taken as a control (HC) and considered as Group 1, Pakistani subjects of both genders. Analysis revealed that out of 622 participants the 276 (44.4%) were males. Group 1, Group 3, and Group 4 consisting more female members, while Group 2 had more (64.8%) male participants. The distribution of gender was significantly different among four Groups with a p-value <0.001, which may be due to the division into groups (Table 1). All measures found a highly significant difference among the four groups overall. The age was lowest for group 2, with a median of 25 (21-30) years, while it was highest for group 4. BMI was lower for group 1 (control subjects) and highest for group 4 (DO), while groups 3 and 4 showed insignificant differences. On waist/hip ratio, group 1 had a median of 0.8 (0.8–0.9), group 2 and 3 had a majority around 1.1 (1.1-1.1), while group 4 had a waist/hip ratio of 1.1 (1.1-1.2).

Table 1: Gender distribution of cases in four groups and its comparison among groups.

Gender	Groups									
	Group 1 HC (n=201)		Group 2 MHO (n=71)		Group 3 MUO (n=230)		Group 4 DO (n=120)		Total	
	n	%	n	%	n	%	n	%	n	%
Male	92	45.8	46	64.8	96	41.7	42	35.0	276	44.4
Female	109	54.2	25	35.2	134	58.3	78	65.0	346	55.6
Total	201	100.0	71	100.0	230	100.0	120	100.0	622	100.0

Chi-square test=17.07 and significance considered at P-value <0.001.

Among other parameters, SBP, DBP (hypertension), fasting glucose, insulin levels, HOMA-IR (glycemic indices), and TGs, all variants of cholesterol except HDL (lipid indices) were lowest in the healthy control group (group 1), highest in DO (group 4), and significantly higher in each group in order as compared to the previous group. The HDL-C was exactly in inverse order, i.e. higher in the group 1 and lower in the group 4. The three inflammatory markers (TNF-α, CRP, and IL-6) were also significantly different for all four groups as well as from each other when compared pairwise. The circulatory resistin levels for group 1 were 7.2 (6.2-8.2), for group 2, 11.6 (10.2-14.8), for group 3, 16.5 (14.1-18.8), and that for group 4, 20 (17.1-22.8) ng/mL (Table 2).

Table 2. Distribution of all measures for each group and their comparison among groups.

Traits	Group 1 HC (n=201)	Group 2 MHO (n=71)	Group 3 MUO (n=230)	Group 4 DO (n=120)	P-value
Age	40 (27 - 46)^{a}	25 (21 - 30)^{b}	39 (35 - 43)^{c}	43 (39.5 - 45)^{cd}	<0.001
Wt (Kg)	58 (54 - 62)^{a}	90 (85 - 95)^{b}	89 (84 - 93)^{bc}	86 (80 - 91)^{d}	<0.001
Ht (Ft)	5.3 (5.1 - 5.6)^{a}	5.6 (5.4 - 5.7)^{b}	5.4 (5.3 - 5.6)^{c}	5.4 (5.2 - 5.5)^{acd}	<0.001
BMI (Kg/m^2)	22.2 (21.0 – 22.8)^{a}	31.5 (30.9 – 32.2)^{b}	31.8 (30.9 – 33.1)^{c}	31.8 (31.0 – 32.8)^{cd}	<0.001
Wst (Inches)	29 (28 - 30)^{a}	42.5 (40 - 46)^{b}	43 (40 - 46)^{bc}	42.5 (40 - 45)^{bcd}	<0.001
Hip (Inches)	34 (33 - 36)^{a}	39 (36 - 42)^{b}	38.3 (36 - 42)^{bc}	38 (35.9 - 40.1)^{bcd}	<0.001
WHR	0.83 (0.82 - 0.89)^{a}	1.10 (1.08 - 1.13)^{b}	1.12 (1.09 - 1.14)^{bc}	1.12 (1.10 - 1.15)^{cd}	<0.001
S-BP (mmHg)	110 (105 - 120)^{a}	120 (115 - 125)^{b}	135 (120 - 140)^{c}	150 (133 - 165)^{d}	<0.001
D-BP (mmHg)	75 (65 - 80)^{a}	80 (80 - 90)^{b}	95 (80 - 100)^{c}	100 (90 - 105)^{d}	<0.001
Glucose mmol/L	4.2 (3.9 - 4.5)^{a}	5.3 (4.9 - 5.6)^{b}	5.6 (5.4 - 6.2)^{c}	8.3 (7 - 9.6)^{d}	<0.001
Insulin µU/L	4 (3.2 - 4.6)^{a}	8 (7 - 8)^{b}	16 (13 - 18)^{c}	19 (17 - 20)^{d}	<0.001
HOMA-IR	0.7 (0.7 - 0.8)^{a}	1.8 (1.7 - 1.9)^{b}	4 (3.4 - 4.7)^{c}	6.9 (5.6 - 7.7)^{d}	<0.001
CHOL (mg/dL)	160 (152 - 167)^{a}	185 (172 - 192)^{b}	226 (198 - 252)^{c}	265 (249 - 285)^{d}	<0.001
TG (mg/dL)	142 (138 - 147)^{a}	165 (158 - 176)^{b}	199 (187 - 219)^{c}	221 (200 - 264)^{d}	<0.001
VLDL-c (mg/dL)	28 (28 - 29)^{a}	33 (32 - 35)^{b}	40 (37 - 44)^{c}	44 (40 - 53)^{d}	<0.001
HDL-c (mg/dL)	54 (50 - 58)^{a}	38 (36 - 40)^{b}	34 (31 - 38)^{c}	31 (29 - 33)^{d}	<0.001
LDL-c (mg/dL)	78 (68 - 86)^{a}	113 (98 - 121)^{b}	152 (125 - 177)^{c}	187 (173 - 208)^{d}	<0.001
CRP (mg/dL)	1.9 (1.6 - 2.2) ^{a}	4.5 (4.2 - 4.8) ^{b}	7.3 (6.8 - 7.8)^{c}	10.7 (9.6 - 11.9)^{d}	<0.001
IL6 (pg/mL)	3.0 (2.0 – 4.0) ^{a}	7.0 (6.0 – 9.0) ^{b}	11.0 (10.0 – 13.0)^{c}	18.0 (16.0 – 21.0)^{d}	<0.001
TNF-α (pg/mL)	3.0 (2.3 – 4.0) ^{a}	7.0 (6.0 – 8.0) ^{b}	12.0 (11.0 – 14.0)^{c}	17.0 (15.0 – 19.0)^{d}	<0.001
S/Resistin (ng/mL)	7.2 (6.2 - 8.2)^{a}	11.6 (10.2 - 14.8)^{b}	16.5 (14.1 - 18.8)^{c}	20 (17.1 - 22.8)^{d}	<0.001

Group 1- Healthy lean controls (HC); Group 2- Subjects with metabolically healthy obesity (MHO); Group 3- Subjects with metabolically unhealthy obesity (MUO) and Group 4- Subjects with diabetes and obesity (DO). Group numbers, 1,2,3,4 considered as a, b, c, d respectively group of digits (ac, cd. etc.) showing the similarity with each other.

All measures showed a significant association with resistin levels, when studied overall, height had the weakest correlation with resistin (r value 0.081), and age, (r = 0.159). The BMI showed a good correlation (r = 0.679) and a waist/hip ratio (r = 0.669) also. HOMA-IR and insulin had the strongest correlations, with r = 0.821 and r =-0.789, respectively. The HDL was shows significant but inverse relation to resistin levels, with r =-0.749. When these correlations were correlated with each other, only a very few markers showed significant correlations, and those that were found to be significant but weak or very weak in nature. This weak correlation may be due to the age and duration of the onset of obesity among groups. For instance, group 1 had a significant correlation (r = 0.159, 0.145, and 0.152) with weight, height, and HOMA-IR, respectively. In group 2, the triglyceride and VLDL had insignificant correlations with coefficients of -0.257 each as compared to groups 3 and 4 (TG for both groups= -0.017 and VLDL for both groups= -0.060). In group 3, the resistin had a significant negative correlation with SBP, DBP, cholesterol, and LDL. In group 4, the resistin levels were significantly correlated with age, but that correlation was too weak with a coefficient of 0.184 ng/mL (Table 3).

Table 3. Correlation of serum resistin levels with all measures, overall and within each group.

Variables	Overall		Group1 HC (n=201)		Group 2 MHO (n=71)		Group 3 MUO (n=230)		Group 4 DO (n=120)	
	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value
Age	0.159**	<0.001	-0.075	0.288	-0.154	0.200	-0.067	0.313	0.184*	0.044
Wt (Kg)	0.618**	<0.001	0.159*	0.024	0.094	0.437	-0.005	0.944	-0.024	0.797
Ht (Ft)	0.081*	0.043	0.145*	0.039	0.106	0.380	-0.048	0.469	-0.054	0.561
BMI	0.669**	<0.001	0.015	0.835	0.053	0.661	0.048	0.465	-0.006	0.948
Wst (Inches)	0.648**	<0.001	0.031	0.665	0.22	0.065	0.077	0.247	-0.081	0.378
Hip (Inches)	0.427**	<0.001	0.094	0.182	0.227	0.056	0.029	0.66	-0.016	0.866
WHR	0.669**	<0.001	-0.017	0.812	-0.053	0.661	0.071	0.284	-0.053	0.563
S-BP (mm/Hg)	0.614**	<0.001	0.014	0.84	0.041	0.736	-0.167*	0.011	0.020	0.828
D-BP (mm/Hg)	0.625**	<0.001	-0.033	0.64	0.227	0.056	-0.137*	0.038	0.099	0.284
Glucose mmol/L	0.763**	<0.001	-0.039	0.583	0.065	0.588	0.051	0.440	0.041	0.659
Insulin µU/L	0.789**	<0.001	0.130	0.067	-0.105	0.383	0.002	0.975	-0.080	0.386
HOMA- IR	0.821**	<0.001	0.152*	0.031	-0.111	0.357	0.008	0.901	0.019	0.838
Cholesterol (mg/dL)	0.759**	<0.001	0.063	0.377	0.057	0.635	-0.144*	0.029	0.059	0.524
TGs (mg/dL)	0.755**	<0.001	0.085	0.228	-0.257*	0.030	-0.017	0.797	-0.060	0.513
VLDL (mg/dL)	0.754**	<0.001	0.072	1.000	-0.257*	0.030	-0.017	0.797	-0.060	0.513
HDL (mg/dL)	-0.749**	<0.001	-0.030	0.677	-0.100	0.406	0.075	0.257	0.018	0.846
LDL (mg/dL)	0.765**	<0.001	0.072	0.310	0.127	0.291	-0.144*	0.029	0.110	0.232
CRP (mg/dL)	0.829**	<0.001	-0.099	0.161	-0.084	0.484	0.126	0.057	-0.034	0.709
IL6 (pg/mL)	0.849**	<0.001	-0.106	0.133	-0.084	0.487	0.059	0.374	0.074	0.420
TNF-α (pg/mL)	0.812**	<0.001	0.080	0.261	-0.137	0.254	0.017	0.795	0.002	0.982

Highly Significant correlation at the 0.01 level ** (2-tailed). Significant correlation is at the 0.05 level* (2-tailed).

Finally, a multiple linear regression model was fitted by taking all variables as independent but not including group status information. This model included eight variables among the 16 included as significant contributors, with an adjusted R^2 value of 0.778. Here, the gender being female, BMI, glucose, insulin, and all inflammatory markers were causing an increase in serum resistin levels, and HDL-C was causing a significant decrease in resistin of 0.08 ng/mL with a one mg/dL HDL-C increase. Later, the status of cases defined as groups was transformed into three dummy variables presenting status as MHO, MUO, and DO, taking HC as the reference category, in addition to the previous sixteen variables. This model overshadowed the performance of all 8 significant variables by bringing in the status of patients as highly significant contributors, with an adjusted R^2 of 0.789 and those 8 variables removed as insignificant.

The serum resistin level was taken as dependent variable. Here, the reduced model (by the backward removal method) presented MHO, MUO, and DO as significant factors with $p<0.001$ and all other factors were considered insignificant. It can be concluded through a regression model that the healthy control group may have an average resistin level of 7.36 ng/mL, and if the group has metabolically healthy obesity, it may face a rise of 4.97 ng/ml. Similarly, the average rise in resistin levels was 9.09 ng for metabolically unhealthy obesity and 12.53 ng/mL for subjects with obesity and diabetes (Table 4).

Table 4. Multiple linear regression model presenting effect of all measures on resistin level (without considering status; model 9 & considering status: model 17).

Regression model without considering group status (Adjusted R^2 = 0.778)						
Models		**Unstandardized Coefficients**		**Standardized Coefficients**	**t**	**P-value**
		B	Std. Error	Beta		
9	(Constant)	4.54	2.04		2.22	0.027
	Gender	0.58	0.26	0.05	2.28	0.023
	BMI	0.18	0.04	0.16	4.13	<0.001
	Glucose mmol/L	0.18	0.10	0.05	1.70	0.091
	Insulin µU/L	0.10	0.04	0.12	2.57	0.011
	HDL-C (mg/dL)	-0.08	0.03	-0.15	-3.04	0.002
	CRP (mg/dL)	0.34	0.10	0.21	3.50	<0.001
	IL6 (pg/mL)	0.12	0.04	0.13	2.87	0.004
	TNF (pg/mL)	0.13	0.05	0.13	2.57	0.010
Regression model by considering the group status as independent variables (Adjusted R^2 = 0.789)						
17	(Constant)	7.36	0.18		41.81	<0.001
	MHO (n=71)	4.97	0.34	0.29	14.43	<0.001
	MUO (230)	9.04	0.24	0.80	37.52	<0.001
	DO (120)	12.53	0.29	0.91	43.53	<0.001
a. Dependent Variable: Serum resistin ng/mL						

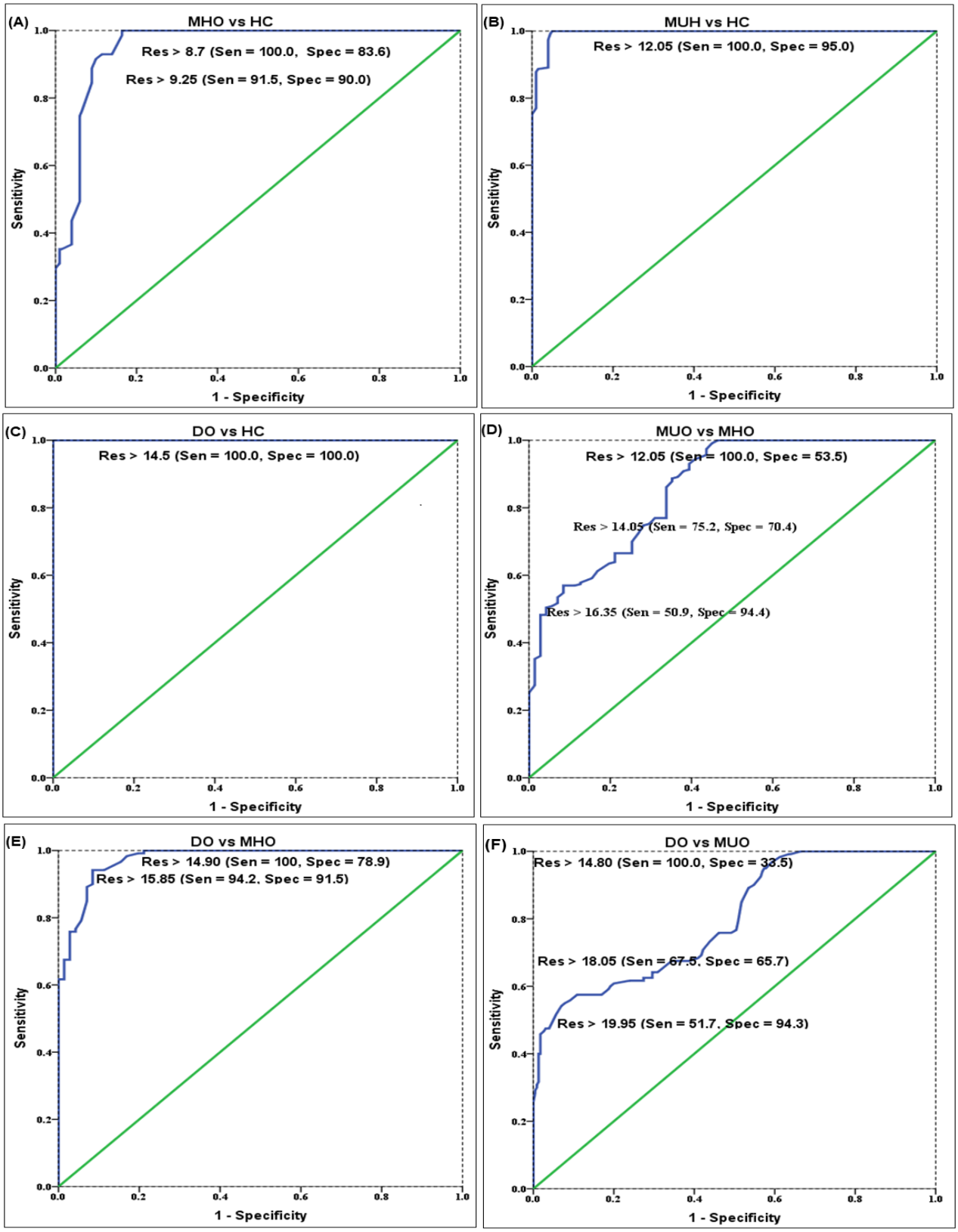

Figure 1. Receiver Operative Characteristic (ROC) curves to determine cutoffs of serum resistin levels for identification of status for cases in each group.

Discussion

The outcomes of our current study revealed that the circulatory resistin levels significantly altered among all study subjects including subjects with metabolically healthy obesity, subjects with unhealthy obesity and obesity with diabetes as compare to healthy lean control group of Pakistani

subjects. Over the past decade, numerous studies conducted on human groups of different nations to examined the association between circulatory resistin and adiposity as well as diabetes pathobiology. The interpretation of previous findings has been difficult and conflicting as a consequence of variations in several ethnic groups and the medical histories of the study subjects, or the target epitopes used in the determination of resistin.

In our study, the findings exhibited that overall circulating resistin concentrations were significantly higher in all categories of obesity as compared to healthy lean subjects, and resistin levels were observed to be highest in subjects with obesity and diabetes simultaneously. These findings of our study are in line with the outcomes of a previous study in which authors revealed that the resistin was positively correlated with overall adiposity (BMI) and truncal obesity (WHR) among subjects with obesity (29). Similarly, in another study, the authors stated their findings, which are consistent with our outcomes, as they observed that resistin levels were significantly higher in subjects with metabolically unhealthy obesity when compared to subjects with obesity that have healthy metabolic parameters and lean controls (30). On the other hand, a study opposed our findings, in which authors reported a negative correlation in their study conducted on subjects with obesity (25).

The traditional diagnostic parameters of central obesity (Hip and waist circumferences, or WHR) have been used as reliable measures of the health status of a person or their alteration as a risk factor in the development of serious health events. Recently published research demonstrated that apple-shaped bodies (with higher waist circumferences) are more likely to develop health risks than those with pear-shaped morphology (with higher hip circumferences). In our current study, body weight, BMI, waist circumferences, and WHR were significantly higher in all the groups with obesity (MHO, MUO, and DO) in comparison to lean, healthy controls (HC). This is in accordance with the study conducted by Mabrouk and his colleagues, who stated that the resistin levels were considerably elevated in persons with obesity and markedly increased in subjects with obesity and diabetes as compared to healthy subjects (31). Similarly, in another study it was observed that the resistin levels were higher in study subjects with obesity and found to be considerably associated with obesity indices, especially central obesity indices such as BMI, waist, and WHR, respectively (32).

Likewise, a population-based study conducted on Indians by the authors revealed that people with high BMI and obesity with T2DM in both classes showed significantly higher resistin concentrations and were strongly linked with anthropometric indices than healthy control participants with a normal BMI (33). In contrast several studies opposed our outcomes in all these studies conducted on different ethnic populations did not show any alterations in resistin concentration in subjects with obesity in any study group as compare to healthy lean subjects (14).

Hypertension is considered a consequence of obesity and diabetes and a key factor for developing cardiac diseases. Some human studies have reported that high levels of the resistin hormone might contribute to develop hypertension. The outcomes of our study were higher in metabolically unhealthy obese subjects and considerably more elevated in obesity with diabetes when compared to metabolically normal obesity as well as non-obese healthy control subjects. These outcomes of our study are in agreement with the outcomes of several studies conducted by other workers and reported by a review and meta-analysis, as they stated that raised plasma resistin levels are linked to hypertensive events. They also reported that the resistin was significantly linked to hypertension and comorbidities (34). Conversely, some recent studies reported opposing outcomes they stated a negative correlation between serum resistin levels and recently diagnosed hypertension in obesity and in lean, non-hypertensive controls (35).

Circulatory lipid indices, which include triglycerides (TGs), cholesterol, LDL, HDL, and VLDL, may all be influenced by diabetes and obesity, and their associations with elevated resistin hormone remain unclear. Consequently, in our study findings, lipid indices were observed to be parallel to resistin, as all these were found to be altered along with the metabolic status of morbid and diabetic obese subjects. The current study outcomes explored the significant correlation among two categories of morbid obesity subjects when compared to subjects with healthy obesity and lean subjects. The circulatory TGs, LDL, VLDL, and total cholesterol levels were found to be markedly increased in

MUO and DO groups in comparison to MHO and healthy lean groups (HC) while having a strong and significant correlation with circulatory resistin levels. Similarly, HDL levels were found to be significant but negatively correlated with hormonal levels. All these findings are consistent with the previous study conducted by Asibey and his team, as they revealed that their outcomes of resistin found to be higher and significantly correlated with adiposity and dyslipidemia than in control subjects (36). On the other hand, our outcomes are inconsistent with the findings of study conducted on female subjects (32). Similarly, findings of another study conducted by group of workers and revealed inverse correlation while in both of these studies authors fail to prove the correlation of elevated resistin levels with lipid indices in subjects with obesity (37).

Resistin is one of the adipokines produced by numerous cell types, and immune cells are a major source of resistin secretion in the state of human adiposity, which has been reported in previous studies as a biomarker linked with obesity, insulin insensitivity, and the development of diabetes, although the exact pathobiological mechanism is unknown. In our current study, the indices of insulin resistance (fasting insulin, glucose, and HOMA-IR) in persons with obesity alone or diabetes and obesity simultaneously were markedly increased along with resistin levels and strongly correlated with resistin levels as compared to metabolically healthy obese as well as healthy lean subjects. It is suggested that higher circulatory resistin is linked with metabolic dysregulation. Our findings are in line with other studies conducted in recent past (38, 39) both of these groups reported positive correlation with metabolic markers.

In spite of these studies and a meta-analysis in which author reported the findings of numerous studies who reported opposing outcomes in their studies, they stated no correlations among insulin sensitivity, other indices of metabolic syndrome, or circulatory resistin levels (40). The resistin hormone is predominantly secreted by immune cells (monocytes and macrophages) in humans, while in rodents, resistin expression is limited to adipocytes (41). Human macrophages are activated to express resistin by cytokines, which include TNF-α, LPS, IL-1, and IL-6. In the human body, resistin activates peripheral mononuclear cells to produce IL-6 and TNF-α via the NF-κB pathway, and rosiglitazone, which is a PPAR agonist, inhibits resistin gene expression in adipose tissues, leading to a decrease in the inflammatory response (42).

In our study, inflammatory markers were altered significantly in the both groups (MUO and DO) as compared to the MHO and lean healthy groups. Of these markers, the CRP status is in line with a study of Aquilante and colleagues (43) and another study conducted on Chinese subjects (44). In both of studies, authors stated a significant correlation among resistin, inflammatory markers and metabolic derangements in morbid obesity states. While the outcomes of our study are inconsistent with the findings of a recent study in which authors described the negative correlation with obesity indices (45). Other potential markers responsible for inflammatory events, which include TNF-α and IL-6 concentrations recorded in our study, are highly significant in the MUO and DO groups as compared to the MHO and lean healthy groups, which are in agreement with a study conducted in the recent past as the reported positive correlation with morbid obesity and adiposity with diabetes (46). However, in recent past, a study group reported their findings, which were inconsistent with the outcomes of our study (47).

Conclusion

The outcomes of the current study propose that the discrepancies in resistin concentrations are strongly linked to the predisposition to metabolic dysregulation, insulin resistance, low-grade inflammation, obesity, and diabetes type 2. Hyperresistinemia may be the primary cause of obesity and its comorbidities. Future prospective studies on different ethnic groups and on larger sample size are essential to establish the cutoff value of the circulating resistin levels in the early extrapolation of insulin resistance in the test population, which seems to be at greater risk of developing overweight or obesity and related comorbidities. Further work is needed to understand the genetic changes can also be helpful to uncover the causes of hyperresistinemia and in the development of interventions

that can targets resistin secretion and therefore, treat obesity and its comorbidities at the molecular level.

Limitations of the study

The study had some limitations that need to be acknowledged. Ensuring balanced gender representation was crucial, but financial constraints made it challenging to recruit more participants. A larger sample size that includes both urban and rural populations is essential to investigate the impact of lifestyle factors on hyperresistinemia. Genetic polymorphism analysis can enhance the study's reliability and gain insights into the underlying mechanisms of hyperresistinemia.

Acknowledgements

We want to thank to Prof. Dr. Farah Deeba Head of the Biochemistry Department at AL-Aleem Medical College Lahore, Ms. Sonaina Ehsan PhD. scholar and Dr. Sumera Zaib Associate professor and Head of department of Basic and Applied Chemistry at University of central Punjab, Lahore, Prof. Dr. Ghias-Un-Nabi Tayyab Head of Medicine Department Lahore General Hospital Lahore to facilitate this work and unconditional support and all the study subjects for their participation in this study.

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