EVALUATION OF SERUM RESISTIN IN OBESE TYPE TWO DIABETES MELLITUS PATIENTS IN MOSUL CITY, IRAQ

Dr. Zinah M Al-Yozbakee[a]*, Dr. Niaam N Jammil[b], Dr. Nada N Fadhil[c]

Article History: **Received:** 28.04.2022 **Revised:** 20.08.2022 **Accepted:** 03.10.2022

Abstract: Obesity is a dangerous health concern; and nowadays it becomes epidemic. It is a target factor for many diseases including type 2 diabetes mellitus (T2DM). Adepokines are factors secreted from adipose tissue that affect many organs' function. One of these adepokines is resistin. Resistin acts as a proinflammatory adepokine as it induces secretion of "tumor necrosis factor alpha and interlukin-6" and mediates insulin resistance. In this study the level of fasting serum resistin, insulin, and C-reactive protein (CRP) has been estimated, and the insulin resistance has been calculated by HOMA-IR for three groups: group one “thirty obese patients who had T2DM”, group two “thirty obese healthy persons”, and group three “thirty healthy individuals with normal body weight”. Serum resistin was higher in diabetic obese individuals than other two groups, and was positively correlated with body mass index (BMI), insulin resistance, and CRP . In conclusion serum resistin links between the T2DM and inflammation. Understanding the mechanism by which this link occurs, may help in clarification the role of resistin in the pathogenesis and treatment of T2DM.

Keywords: obesity, resistin, T2DM, inflammation, insulin resistance

[a]. Department of Microbiology, College of Medicine, University of Mosul, Iraq
[b]. Department of Microbiology, College of Medicine, Nineveh University, Iraq
[c]. Nineveh Health Department, Iraqi Ministry of Health

***Corresponding Author**

E-mail: dd.sunmar.dd@gmail.com

DOI: 10.31838/ecb/2022.11.08.023

INTRODUCTION

“Type 2 diabetes mellitus” (T2DM) is serious, common chronic disease resulting from complex interactions between genes and environmental factors (1). It has a multistage processes, the whole mark process is insulin resistance, that characterized by body inability properly using the own insulin of it, which leads to depletion of the pancreatic β cells that produce insulin, causing hyperglycaemia (2). There are several risk factors for the occurrence of T2DM including family history ,obesity, inherited factors and sedentary life style; however, the most serious one is obesity, as obese individuals are more likely to develop T2DM than non-obese persons (3). positively Central obesity is regarding with insulin resistance in T2DM patient (4). Nowadays it is clear that adipose tissue is very active endocrines that secrete a range of active substances known as ‘adipokines’ (5).Cysteine-rich resistin is one of these adipokines (6). Resistin, a new adipokine discovered in mice in 2001, and named for its ability to" resist or interfere with" insulin action (7). It belongs to the cysteine-rich proteins family known as RELMs (resistin-like molecules) and is also called ADSF (adipocyte- specific secretory factor) (8), or FIZZ3 found in inflammatory zone (9). Rodent resistin is produced predominantly in adipocytes, while human resistin is mainly expressed in inflammatory cells mainly microphages rather than adipocyte (10). In the beginning proposed that resistin was to be involved in insulin resistance and T2DM only. Recent studies found that resistin is also involved in inflammation. Resistin works as a link factor between inflammation and metabolism (11). The exact role of resistin in response to insulin resistance in T2DM patients is still obscure since the precise resistin receptor structure in different tissues is still obscure (11). Resistin acts mainly on insulin signaling pathways thus induce insulin resistance, through its effect on the response of the hypothalamus and peripheral tissues to insulin. In 2013 a study by Benomar *et al.* (12), showed that central infusion of resistin inside the ventricle of a normal rat’s brain and in human SH-SY5Y neuronal tissue cells inhibited insulin-dependent phosphorylation of insulin receptor (IR) and IR family proteins, such as protein kinase B "AKT " and extracellular kinase (the last two are connected in the pathway of insulin transduction); and this leads to a decrease in the expression of IR and upregulation of suppressors such as “cytokine signaling-3” and “phosphothyrosine phosphatase 1B,” which are two negative insulin signaling regulator. In addition, central resistin plays a role in activation of both “serine kinases” and “p38 mitogen-activated protein kinase”, those two enzymes enhance the serine phosphorylation of " insulin receptor substrate-1" which promotes the expression of the proinflammatory cytokine like "interleukin-6" in the hypothalamus and the peripheral insulin-sensitive tissues that leads to decrease insulin receptor expression (12). Therefore, resistin plays a part in the development of insulin resistance in T2DM, for these reasons founding drugs that lower serum resistin in T2DM subjects can provide better therapeutic approach (13).

Resistin participates in the inflammatory response (13, 14, 15, 16). The molecular mechanisms by which resistin enhance

inflammation are still not very well understood but studies show that resistin modulates the synthesis, secretion, and expression of key proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), IL-6, and IL-12, which are known to inhibit insulin signal transduction (17). C-reactive protein (CRP), an indicator of inflammation, was reported to be correlated with resistin concentrations in several pathophysiological conditions including T2DM (18,19,20,21). In a study done at 2005 by Bo *et al.* (22) in north-western Italy showed that serum levels of resistin are directly correlated to CRP, highly associated to metabolic abnormalities, and inversely to oxidative stress nitrotyrosine (NT) suggesting that resistin may be secreted in response to mild chronic inflammation, as that occur in T2DM and obesity, and be a part of oxidative stress.

Some studies showed that there is strong correlations between resistin and increase body weight >

30. The fundamental belief that in support of this theory is that serum resistin concentrations will elevate with the increments of adiposity (23, 24, 25). Conversely, serum resistin concentrations have been found to decrease with reducing adiposity by medical therapy as in a study done at 2003 by Valsamakis *et al.* (26). Central obesity (waistline adipose tissue) assumed considered the most important region of adipose tissue that's contributes to the elevation of serum resistin levels (27). However, other researches demonstrates that there is no significant differences between plasma levels of resistin in patients with morbid obesity have appeared, and these levels do not change after a significant reduction in bodyweight after bariatric surgery (28,29,30). This study aimed to measure the level of serum resistin, insulin and CRP in the blood of obese T2DM patients, and to calculate insulin resistance by HOMA-IR equation (31), then establish a comprehensive relationship between serum resistin, insulin resistance, and CRP of obese T2DM patients in Mosul city / Iraq.

MATERIALS AND METHODS

Ethical clearance was obtained from the Nineveh Health Institute and from Al Wafaa Health Center for Diabetes in Mosul city, Iraq. The participants were informed about the research procedures and an informed consents are signed by each individual enrolled in this study. A total of 90 subjects are involved in this study, their ages ranged from 20 to 76 years old (36 male and 54 female), and they were divided into three groups, group one consist of 30 obese subjects with T2DM who fulfilled the" American Diabetes Association criteria 2019" (28), and with body mass index (BMI) > 30. Group two consists of 30 non-diabetic obese subjects with BMI > 30. Group three consist of 30 apparently non-obese healthy control group (BMI < 25), they have no history of T2DM or any other chronic disease and matching patients group approximately by age and gender. A special Questionnaire Form of relevant clinical data was completed for each subject enrolled in this study. Under aseptic technique 5 milliliters of blood were collected from each patient that is overnight fast in the morning after an and the same for control individuals. The blood sample was then centrifuged and the sera collected then kept frozen at 20°C in labeled eppendrofes tubes.

The main inclusion criteria are: all patients with T2DM who fulfilled the" American Diabetes Association criteria 2019" (31) as having one of their criteria like their HbA1C level of 6.5 or higher or 2- hour plasma glucose level of 200 mg/dL (11.1 mmol/L) or higher after 75 g oral glucose tolerance test, or random plasma glucose level 200 mg/dL 11.1 mmol/L) or higher with classical symptoms of hyperglycemia (polyuria, polydipsia, polyphagia, weight loss). All patients should be more than 6 month diagnosed as T2DM and their BMI more than 30.While the exclusion criteria were

: any patient with type one diabetes mellitus, their BMI less than 30, and even patients with newly diagnosed T2DM less than 6 month.

The main laboratory tests done were: ELISA test to estimate serum resistin levels using a commercially available Resistin ELISA kit (Demeditec Diagnostics GmbH, Germany) according to the instructions of manufacturer. The base of the assay is the detection of circulatory homodimeric resistin by rabbit polyclonal antihuman resistin antibody. Human insulin was measured with an insulin kit (Demeditec Diagnostics GmbH, Germany) the manufacturer's instructions. Latex agglutination is used for measuring CRP. Fasting blood glucose, HbA1C taken from patients records.

HOMA insulin resistance was calculated as follows:

insulin resistance = fasting serum insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5 (31).

Statistical analysis:

For statistical analysis of the results were presented by using "statistical package for social sciences" (SPSS) version 25 for window 10 and the results were expressed as mean ± standard deviation (SD). Correlation between variables was evaluated by Persons Correlation Coefficient factor (r). One way ANOVA and post-hock Dunkan tests were used for determination of differences between variables of three groups. *P* value less than 0.05 considered significant. Pie charts, bars, figures and tables were used for data expression using Excel 2010 (32).

RESULTS

Most of the obese persons with T2DM were in the sixth and seven decade of life at a frequency (26.6 %, 36.6%) respectively as shown in Fig. (1). The majority were females (17.8 %) while the male were (15.6 %) .

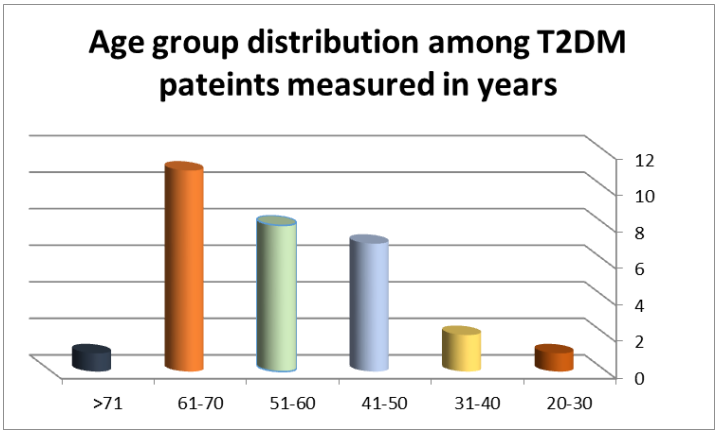

Figure 1: Age groups distribution among obese T2DM subjects.

The Mean ± Standard deviation of various parameters in obese T2DM patients and controls was shown in Table (1).

Table 1. The Mean ± Standard Deviation Of Various Parameters In The Three Studied Groups.

Parameters	Obese, diabetic patients n=30	Obese individual n=30	Non-obese, non- diabetic individual n=30	*P* value
Age (Years)	56.40 ± 12.04	44.73 ± 11.61	44.40 ± 10.59	
BMI (kg/m²)	33.60 ± 3.59	32.64 ± 1.91	22.26 ± 1.27	< 0.05
HbA1C (%)	7.72 ± 3.29			
S. resistin (ng/mL)	14.23 ± 3.79	11.79 ± 2.98	6.12 ± 2.21	< 0.05
S. insulin (мIU/mmol)	98. 67 ± 9.24	91.07 ± 7.28	42.64 ± 32.81	< 0.05
HOMA (insulin resistance)	45.31 ± 9.03	19.61 ± 3.78	8.42 ± 6.53	< 0.05
CRP	15.56 ± 8.74	*12.33 ± 7.89*	3.13 ± 1.07	< 0.05 between obese T2DM group and non- obese healthy control group > 0.05 between obese T2DM group and obese non-diabetic group

Fasting serum resistin mean level increased in increasing manner in the three groups, the highest level among obese diabetic patients (14.23 ± 3.79 ng/ mL) and lowest level among non-obese non diabetic healthy control (6.12 ± 2.21 ng/ mL). There was a significant difference in the serum resistin level by one way ANOVA test at a *P* value < 0.001 in favorable for the obese diabetic group than the other two groups and in obese non diabetic group than normal body weight group at a $P < 0.05$.
Serum resistin mean concentration was correlated positively with (BMI, FBS, serum insulin, insulin resistance, and CRP) measured by Pearson correlation factor (r) among obese, T2DM group as shown in Table (2). There was no correlation between serum resistin and HbA1C in patients group.

Table 2. Serum Resistin Mean Concentration For The Three Groups Was Correlated With (BMI, FBS, serum insulin, HOMA, and CRP).

Variables	r value	*P* value
BMI	0.5	< 0.05 (*P*=0.0049)
CRP	0.6	< 0.05 (*P*=0.000457)
FBS	0.6	< 0.05 (*P*=0.000457)
S. insulin	0.7	< 0.05 (*P*=0.000017)
HOMA	0.2	< 0.05 (*P*=0.00289)

CRP is significantly greater in T2DM and obese group than normal body weight group at a $P < 0.05$, but there was no significant difference between CRP of T2DM and obese group. Insulin resistance measured by HOMA- IR equation was significantly higher in T2DM and in obese non diabetic group than normal body weight group at a $P < 0.05$ as shown in Table (3).

Table 3. Insulin Resistance Measured by HOMA-IR Equation In The Three Studied Groups

Studied groups	Mean	Standard Deviation (SD)	Minimum	Maximum
T2DM patient No.= 30	45.31	9.034	30	61
Obese non- diabetic No.= 30	19.61	3.780	15	31
Healthy normal body weight group	8.42	6.530	1	23

No.= 30				

DISCUSSION

This case – control study reports serum resistin level in three groups: obese subjects with T2DM, non-diabetic obese group, and healthy subjects with normal BMI from Mosul city / Iraq. There was a significant differences in serum resistin level in between the three groups at a *P* value < 0.05. In diabetic obese subjects serum resistin level is elevated and directly correlated to insulin resistance their mean value (14.23 ± 3.79 ng/ mL). This was in agreement with other studies like that of Zaidi and Sherwani (23) 2015, who discover human resistin-insulin resistance correlation in diabetic group and obese non diabetic subjects , People with resistance levels are higher in people with severe insulin resistance compared to people with normal insulin action. Therefore, this study may add another approve that obesity may be a predictor of higher serum resistin level and subsequently more insulin resistance (23,24,25,27,33). Nerveless some other studies showed no correlation between obesity, insulin resistance and serum resistin level like that of Heilbornn *et al* 2004 (28). The differences in the results of the various researches may be due to the differences in the duration of T2DM disease, presence of complications from T2DM or not, BMI and even the way for measuring serum resistin with different kit specific cutoff levels.

This study demonstrated a positive correlation between high CRP (in both obese diabetic mean value 15.56 ± 8.74 and obese non diabetic groups mean value12.33 ± 7.89) and serum resistin at a *P* value < 0.05, suggesting resistin may play a role in obesity related inflammation and insulin resistance, this is due to the fact that obesity and T2DM are both had chronic inflammation and resistin enhance secretion of IL-6 and even TNF-α (17,18). The results are consistent with reports which indicates that resistin level is higher in T2DM patients with high CRP (17,18,19,20,21,33) so this will conform strong correlation.

In conclusion, hyperresistinemia occurs in T2DM and obese subjects, and serum resistin level is significantly related with insulin resistance and inflammatory marker CRP.

Further studies are recommended to be done about the effect of serum resistin and other adepokines like (leptin and adeponectine) in other obesity related diseases like (hypertension, hyperlipidemia, cardiovascular diseases) as resistin may preclude these diseases occurrence. Also it is recommended to evaluate resistin gene expression in T2DM obese patients to know the exact role of this adepokine in T2DM pathogenesis.

Conflict of interest: The authors have no conflict of interest.

Consent of Ethics: Administrative approval was taken from all places where samples were collected, as well as written and oral consent was taken for all participants in the research

Financial Support: No external fund was received.

REFERENCES

i. Hameed I, Masoodi SR, Mir SA, Nabi M, Ghazanfar K, and Ganai BA. Type 2 diabetes mellitus: From a metabolic disorder to an inflammatory condition. World J Diabetes. 2015; 6(4): 598–612.

ii. Cantley J, Ashcrof FM. Q&A: insulin secretion and type 2 diabetes: why do β-cells fail?. BMC Biol. 2015; 13: 33

iii. Aravinda J. Risk factors in patients with type 2 diabetes in Bengaluru: A retrospective study. World J Diabetes. 2019; 10(4): 241–248.

iv. Papaetis GS, Papakyriakou P, Panagiotou TN. Central obesity, type 2 diabetes and insulin: exploring a pathway full of thorns. Arch Med Sci. 2015; 11(3): 463–482.

v. Oakhill JS, Galic S, Steinberg GR. Adipose tissue as an endocrie organ. Molecular and Cellular endocrinology. 2019; 316(2):129-139.

vi. Dasari R, Raghunath V. Obesity and Type II diabetes mellitus: Is resistin the link?. Journal of Diabetes and Endocrine Practice. 2018; 1(1):1-8.

vii. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. Nature. 2001; 409(6818):307–12.

viii. Kim KH, Lee K, Moon YS, Sul HS. A cysteine-rich adipose tissue-specific secretory factor inhibits adipocyte differentiation. J Biol Chem. 2001; 276(14):11252–6.

ix. Pandey R. Resistin is there is any role in mediation of obesity, insulin resistance and diabetes mellitus. Juniper online journal of case studies. 2018; 6(3):555686.

x. ParkHK, Kwak MK, Kim HJ, Ahima RS. Linking resistin, inflammation, and cardiometabolic diseases. Korean J Intern Vandana Med 2017; 32(2):239-247.

xi. Ren Y, Zuo Zc, Wan TM. Resistin: its role in insulin resistance and mechanism of action. Sheng Li Xue Bao. 2016; 68(1): 65-74.

xii. Benomar Y, Arieh Gertler, Pamela De Lacy, Delphine Crépin, Hassina Ould Hamouda,Laure Riffault, and Mohammed Taouis. Central Resistin Overexposure Induces Insulin Resistance Through Toll-Like Receptor 4. Diabetes. 2013; 62(1): 102–114.

xiii. Nicholson T, Church C, Baker D.J, Jones S.W. The role of adipokines in skeletal muscle inflammation and insulin sensitivity. Journal of Inflammation. 2018; 15(9) available from https://doi.org/10.1186/s12950-018-0185-8.

xiv. Malyszko J, Malyszko JS, Pawlak K, Mysliwiec M. "Resistin, a new adipokine, is related to inflammation and renal function in kidney allograft recipients". Transplant. Proc.2006; 38(10): 3434–6.

xv. Nagaev I, Bokarewa M, Tarkowski A, Smith U. Valcarcel J, ed. "Human Resistin Is a Systemic Immune-Derived Proinflammatory Cytokine Targeting both Leukocytes and Adipocytes". PLoS ONE.2006; 1(1): e31. doi:10.1371/journal.pone.0000031. PMC 1762367 Freely accessible. PMID 17183659.

xvi. Ren Y et al. Resistin increases the expression of NOD2 in mouse monocytes. Exp Ther Med. 2017; 13(5): 2523–2528.

xvii. Ellulu M, Patimah I, Khaza'ai H, Rahmat A,3 and Abed Y. Obesity and inflammation: the linking mechanism

and the complications. Arch Med Sci. 2017; 13(4): 851–863.

xviii. Shetty GK, Economides PA, Horton ES, Mantzoros CS, Veve.A. Circulating adiponectin and resistin levels in relation to metabolic factors, inflammatory markers, and vascular reactivity in diabetic patients and subjects at risk for diabetes. Diabetes Care. 2004; 27:2450-2457

xix. Pane E, Acquarone F, Monace R, Borghi A, Odetti NP. Resistin: A reappraisal. Mechanisms of Ageing and Development. 2019; 187:46-6

xx. Panayoula C. Tsiotra, Eleni Boutati, George Dimitriadis, and Sotirios A. Raptis. High Insulin and Leptin Increase Resistin and Inflammatory Cytokine Production from Human Mononuclear Cells. BioMed. 2013, Article ID 487081, 10 pages available from https://doi.org/10.1155/2013/487081.

xxi. Toan NL, Hoan NV, Cuong DV, Dung NV, Dung PT, Hang NT, et al. Adipose tissue-derived cytokines and their correlations with clinical characteristics in Vietnamese patients with type 2 diabetes mellitus. Diabetology & Metabolic Syndrome. 2018; 10(41) available from https://doi.org/10.1186/s13098-018-0343-4

xxii. Bo S, Gambino R, Pagani A, et al. Relationships between human serum resistin, inflammatory markers and insulin resistance. Int J Obes (Lond). 2005; 29:1315-1320.

xxiii. Zaidi SI, Shirwany TA. Relationship of serum resistin with insulin resistance and obesity. J Ayub Med Coll Abbottabad. 2015; 27(3):552-5.

xxiv. Asensio C, Cettour-Rose P, Theander-Carrillo C, Rohner-Jeanrenaud F, Muzzin P. "Changes in glycemia by leptin administration or high-fat feeding in rodent models of obesity/type 2 diabetes suggest a link between resistin expression and control of glucose homeostasis". Endocrinology. 2004; 145(5): 2206–13. doi:10.1210/en.2003-1679. PMID 14962997.

xxv. Lee JH, Bullen JW, Stoyneva VL, Mantzoros CS. "Circulating resistin in lean, obese, and insulin-resistant mouse models: lack of association with insulinemia and glycemia". Am. J. Physiol. Endocrinol. Metab. 2005; 288(3): E625–32.

xxvi. Valsamakis G, McTernan PG, Chetty R, Al Daghri N, Field A, Hanif W, Barnett AH, Kumar S. "Modest weight loss and reduction in waist circumference after medical treatment are associated with favorable changes in serum adipocytokines". Metab. Clin. Exp. 2004; 53(4):430–4.

xxvii. Elena Parreño Caparrós, Fátima Illán Gómez, Manolo Gonzálvez Ortega, Isabel Orea Soler, Maria Luisa Lozano Almela, Elena Arjonilla Sampedro and Maria Soledad Alcaraz Tafalla. Resistin and Obesity: Obesity and Inflammation. Endocrine Society's 96th Annual Meeting and Expo, 2014 – Chicago.

xxviii. Heilbronn LK1, Rood J, Janderova L, Albu JB, Kelley DE, Ravussin E, Smith SR. J Clin Endocrinol Metab. 2004 Apr;89(4):1844-8. Relationship between serum resistin concentrations and insulin resistance in non obese, obese, and obese diabetic subjects. J Clin Endocrinol Metab. 2004;89(4):1844-8

xxix. Acquarone E, Monacelli F, BorghiA R, Odetti NP. Resistin: A reappraisal. Mechanisms of Ageing and Development. 2019; 178: 46-63.

xxx. Nordström A, Hadrévi J, Olsson T, Franks P.W, and Nordström P. Higher Prevalence of Type 2 Diabetes in Men Than in Women Is Associated With Differences in Visceral Fat Mass. J Clin Endocrinol Metab, October 2016; 101(10):3740–3746.

xxxi. Riddle MC et al. Standeres of medical care in diabetes. Diabetes care.2019; 42(1): S1-S187 available at https://care.diabetesjournals.org.

xxxii. Ramayah T. Note for data analysis workshop. Ramayah T. published at school of management universiti Sains Malaysia;2011.50-89 available from http://ramayah.com/wp-content/uploads/2011/04/Data-Analysis.pdf

xxxiii. Azab N, Abdel-Aziz T, Ahmed A,El-DeenI.M. Correlation of serum resistin level with insulin resistance and severity of retinopathy in type two diabetes mellitus. Journal of Saudi Chemical Society 2016; 20: 272-277.

xxxiv. Schulze M.B, Mansn J.E, Ludwig D.S, Colditz G.A, Stampfer M.J, Willett W.C, Hu F.B. Sugar- Sweetened Beverages, Weight Gain, and Incidence of Type 2 Diabetes in Young and Middle- Aged Women. JAMA. 2004; 292(8):927-34.