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ORIGINAL ARTICLE

The correlation between serum resistin and toll-like receptor-4 with insulin resistance in hypertensive subjects with or without type 2 diabetes mellitus

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ABSTRACT

Background: The most chronic disease prevalence in the Iraqi population are type-2 diabetes mellitus (T2DM) and hypertension (HT). One of the important causes of these chronic diseases is obesity. Resistin (RETN) is a major link between obesity and insulin resistance (IR) or T2DM (which induces IR). The action of RETN on IR is mediated by toll-like receptor-4 (TLR4). TLR4 is a putative RETN receptor that has been suggested to participate in RETN-inducing inflammation and IR.
Objectives: To study the association between serum RETN/TLR4 and IR in hypertensive patients with or without T2DM subjects.
Methods: This cross-sectional study was conducted on 120 men that classified into four different groups. These groups consist of the following: 30 apparently control group, 30 patients with hypertension, 30 patients with T2DM but without HT and 30 hypertensive patients with T2DM. For all the subjects, serum RETN, TLR4 and serum insulin was estimated by using the ELISA technique.
Results: Our results showed that mean levels of the serum RETN and TLR4 were significantly elevated in all patient groups when compared with the control group. Also, a positive correlation between serum RETN and TLR4 was found in hypertensive patients with T2DM patients.
Conclusions: Serum RETN and TLR4 were higher in all patient groups when compared with the control group. In addition, a positive correlation between RETN and IR in all study groups was noted. Then, we suggested a close association between RETN and TLR4 and their positive correlations with IR.

Keywords hypertension, insulin resistance, resistin, TLR4, type 2 diabetes mellitus

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INTRODUCTION

Obesity is defined as a chronic disease that is indirectly related to serious medical illnesses, including type2 diabetes mellitus (T2DM), hypertension (HT), insulin resistance

(IR) and cardiovascular diseases.[1] It is also defined as an abnormal or excessive fat accumulation that represents a risk to health.[1] A previous study reported significant association between obesity and IR with elevated serum resistin (RTEN) in humans. RTEN is a newly identified hormone that links obesity to T2DM, its name comes from the induction of IR, and chemically is a 12.5-kDa peptide hormone secreted by adipocytes, immune cells, and epithelial cells and it is one of the families of cysteine-rich secretory proteins.[2] RETN plays important regulatory roles and also roles in IR, T2DM, atherosclerosis, cardiovascular disease (CVD), hypertension and autoimmune disease.[3] High serum RETN levels were founded in T2DM, increasing weight, hypertension and the acute coronary syndrome (ACS).[3] One possible mechanism underlying the correlation between the RETN and hypertension might be mediated via the toll-like receptor4 (TLR4).[4] The local secretion of RETN causes an increase in the release of endothelin 1, intercellular adhesion molecule 1 (ICAM1), vascular cell adhesion molecule 1 (VCAM1) and monocyte chemotactic protein-1 (MCP-1).[5] This affects the vascular endothelial cells when the level of it increases and also affects vascular smooth muscle cell migration, the endothelial function, and the development of hypertension.[6] TLR4, a member of the toll-like receptors family (TLRs), is a receptor belonging to the transmembrane lipopolysaccharide (LPS) receptor that once activated it induces the release of inflammatory cytokines, chemokines and antimicrobial peptides, also have an important role in initiating the innate immune system.[7] Kim et al. (2007) found that human TLR4 have an important role that may affect the onset of T2DM by increasing the IR.[8]

The summary of previous studies' findings shows that TLR4 has a fundamental role in IR, lowering the prevalence of DM, hypertension, atherosclerosis, and cardiovascular disease. Some researchers studied the role of brain TLR-4 in Angiotensin II promoted hypertension and assess whether central TLR-4 blockade has a protective role in myocardial inflammation and cardiac function incited by Angiotensin II.[4] Another study by Dange et al., in 2004, investigated the role of brain TLR-4 in modulating vasodilatory components and vasoconstrictory components of the cardiac renin-angiotensin-aldosterone (RAAS).[4]

The IR is a pathological condition in which cells fail to respond normally to the normal levels of exogenous or endogenous insulin hormone in cells, tissue or the whole body. IR is well-known as one of the risk factors for T2DM, cardiovascular disease, hypertension, polycystic ovary syndrome and metabolic syndrome that are leading causes of mortality and morbidity worldwide.[9] The signs and symptoms may include lethargy, difficulty in concentrating, and hunger.[10] In addition, other signs that appear in populations with IR are obesity especially around the middle, high total cholesterol levels and hypertension. IR can be determined through several ways, these ways depend on fasting blood concentrations for glucose and insulin with the use of various calculations.[11]

One of important methods to calculate IR is homeostatic model assessment (HOMA), the HOMA (HOMA1) has been used firstly by Matthews in 1985.[12] The equation is greatly used and simplify to:

$$HOMA - IR = (FPI \times FPG)/22.5$$

Where FPI is fasting plasma insulin concentration (μIU/ml) and FPG is fasting plasma glucose (mmol/L).

An updated method was also used to assess the IR called HOMA-2 which was reported in 1998. HOMA-2 was designed to calculate the IR in addition to insulin sensitivity (%S) and beta-cell function percentage (%B). HOMA-2 has been used to evaluate the IR and metabolic syndrome in Brazilians.[13] Only a very few studies measured serum TLR4 and RETN at the same time in hypertensive subjects with T2DM and their correlation with IR and arrived to different suggestion according to their experimental.[14,15] The information on serum TLR4 and RETN in human are rare, especially in the cases of hypertensive subjects with T2DM. This study was clarified the missing points of the previous studies and finding the association between serum TLR4 and RETN with IR in hypertensive subjects and T2DM subjects. Moreover, we finding the correlation between TLR4 and RETN hormone in all study subject's groups.

MATERIALS AND METHODS

Study design and subjects

This cross-sectional study was conducted on 120 men classified into four different groups; each group consists of 30 patients. These groups consist of: 30 apparently healthy subjects as controls, 30 patients with hypertension, 30 patients with T2DM and 30 hypertensive patients with T2DM.

Study subject's ages were matched in all four groups as well as sex (men only). The age range was 40-48 years. Each participant, in this study, was thoroughly interviewed according to a well-structured questioner. Men included were collected from Al-Imamain Al-Kadhimain Medical City in Baghdad. The practical work was conducted at the Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University. The Institutional Review Board (IRB) of the College of Medicine, Al-Nahrain University approved this study; before participation, all men were given an idea about the research study, and their written informed consent was taken. Physical examination was done and their weight, height, body mass indexes (BMI), systolic and diastolic blood pressure were recorded. The T2DM was diagnosed according to standard American Diabetes Mellitus Association criteria.[16] Any participant with the following diseases was excluded: Thyroid disease, liver disease, renal disease, alcoholic and smoker subjects, as well as those with inflammatory disorders. About seven milliliters of blood samples were obtained from each fasting (8 hours fast) participant in this study.

Biochemical analyses

Fasting blood samples were taken from all participants during their visit. Each blood samples divided into two parts: the first part of two milliliters was collected on EDTA

tube for the determination of glycated hemoglobin (HbA_1c), and the second part of five milliliters was left for 20 minutes in the gel tube at room temperature (25-28^oC). After coagulation sera were separated by centrifugation at 2000*xg* for 10 min. Glucose (FSG), lipid profile, renal function tests and liver function tests were then evaluated using serum samples and by applying appropriate enzymatic colorimetric method, with commercially available kits.

Residual sera stored at -20^o C for the subsequent assay of serum RETN, TLR4 and fast insulin hormone. They were determined by using ELISA. IR was assessed by HOMA, in two formulas HOMA1-IR (which mentioned in the introduction) and HOMA2-IR (computerized). HOMA2 Calculator v2.2.3 from Diabetes Trials Unit, University of Oxford was used in this research.[17]

Statistical analysis

All statistical analyses were performed using SPSS statistical software, version 29 (IBM Corporation, USA) and medcalc. version 19. All Tables and figures were made using Microsoft excel and Word softwares. Normally distributed data were presented as mean±standard deviation and groups were compared by analysis of variance (ANOVA). Non-parametric Kruskal-Wallis test was applied for testing the variance among the groups for those with non-normally distributed, which were expressed as median and interquartile range and post-hoc analysis (Conover) was conducted to indicate a pairwise difference between each two group whenever Kruskal-Wallis test was significant. Two-tailed Spearman's correlations were performed between the study biomarkers and other variables for testing the association between them. A p value of <0.05 was considered statistically significant.

RESULTS

Both age and BMI, of all groups, were matched and there were no significant differences among groups. Table 1 shows the mean age in healthy control group and all patient groups. Among all study groups, no significant difference (p<0.05) was found in the age. Also, the BMIs of the control group and patients were not significantly different (p<0.05).

Diagnostic parameters of study subjects are displayed in Table 2. It shows the clinical and laboratory characteristics of all groups. High significant differences (p<0.001) were noted among all groups in fasting blood sugar (FBS), HbA_1 *c*, diastolic blood pressure and systolic blood pressure.

The serum TLR4 in ng/ml of the control group and patient's groups (T2DM patients, HT patients and hypertensive patients with T2DM.) were 0.34±0.12, 3.55±0.49, 4.10±0.97 and 3.26±0.35 ng/ml, respectively. Statistically, significant differences (p<0.001) were estimated among the study groups as shown in Table 3.

Table 1 **The comparison of age and body mass index (BMI) between the control group and patients.**

Variable	Group	Mean±SD	SEM	Min	Max	*P*
Age	Control	44.43±2.74	0.50	40.00	49.00	0.835
	DM	44.03±2.58	0.47	40.00	49.00	
	DMHT	44.13±2.68	0.49	41.00	49.00	
	HT	43.90±2.47	0.45	41.00	49.00	
BMI (Kg/m^2)	Control	29.42±3.11	0.57	24.97	37.11	0.06
	DM	28.26±4.73	0.86	19.26	37.22	
	DMHT	30.69±4.03	0.74	23.45	39.79	
	HT	30.31±5.21	0.95	18.73	42.94	

BMI, body mass index; **DM,** diabetes mellitus patients group; **HT,** hypertension patients group; **DMHT,** diabetes mellitus with hypertension patients group; **SEM,** standard error mean.

Table 2 **The diagnostic parameters by groups using F-tests (ANOVA)**

Variable	Group	Mean±SD	SEM	Min	Max	*P*
FBS (mg/dl)	Control	100.43±7.15	1.31	85.00	114.00	<0.001
	DM	163.07±52.43	9.57	106.00	260.00	
	DMHT	173.03±67.55	12.33	123.00	365.00	
	HT	100.43±6.40	1.17	91.00	113.00	
HbA$_1$*c* (%)	Control	5.17± 0.30	0.05	4.59	5.80	<0.001
	DM	7.46± 1.80	0.33	5.32	11.30	
	DMHT	7.82± 1.68	0.31	5.60	11.60	
	HT	5.13± 0.22	0.04	4.80	5.56	
Systolic BP (mmHg)	Control	115.83±4.86	0.89	110.00	122.00	<0.001
	DM	132.13±3.86	0.70	123.00	135.00	
	DMHT	165.15±28.54	5.21	16.50	180.00	
	HT	159.33±7.28	1.33	145.00	170.00	
Diastolic BP (mmHg)	Control	83.57±3.42	0.63	77.00	89.00	<0.001
	DM	86.70±1.88	0.34	85.00	89.00	
	DMHT	94.50±3.47	0.63	90.00	100.00	
	HT	92.27±3.27	0.60	89.00	100.00	

FBS, fasting blood sugar; **HbA$_1$*c*,** hemoglobin A$_1$ *c*; **DM,** diabetes mellitus patients group; **HT,** hypertension patients group; **DMHT,** diabetes mellitus with hypertension patients group; **BP,** blood pressure.

However, serum (RETN) in ng/ml of the controls and patients (T2DM patients, HT patients and hypertensive patients with T2DM.) were 2.68±2.11, 7.39±1.45, 8.00±1.19 and 6.24±1.17ng/ml, respectively (Figure 1). Significant differences (p<0.001) were recorded among all study groups.

Moreover, serum insulin in μIU/ml of the controls and other groups also shows highly significant differences (p<0.001) among all groups. Notable differences (p<0.001) in HOMA1-IR, HOMA2-IR, β-cell function (%B) and insulin sensitivity (%S) were also found among the study groups between all study groups as shown in Table 3.

According to post-hoc analysis (Conover), pairwise comparisons are significantly different (p<0.05) for serum levels of RETN Figure 1. The box plot showed an increase in the

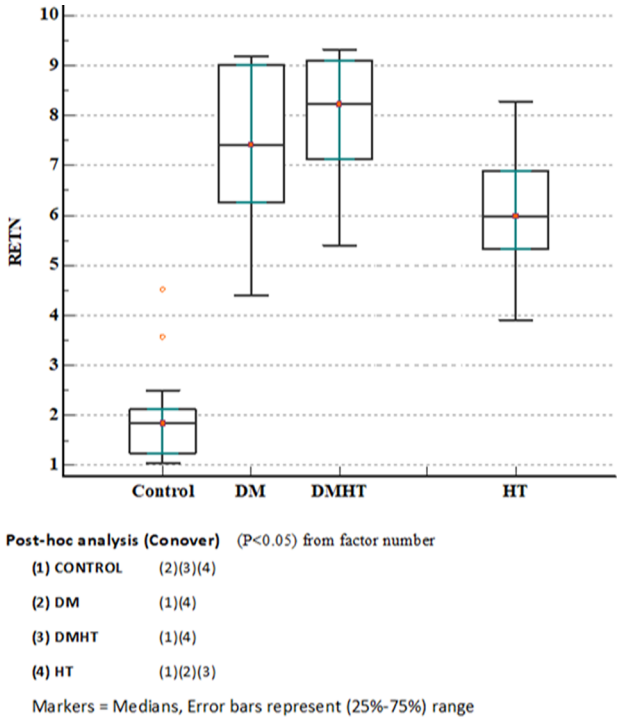

Figure 1 **Box plot of serum RETN comparison among the studied groups presenting post-hoc analysis (Conover) pairwise comparison.** ***DM,*** *diabetes mellitus patients group;* ***HT,*** *hypertension patients group;* ***DMHT,*** *diabetics with hypertension group.*

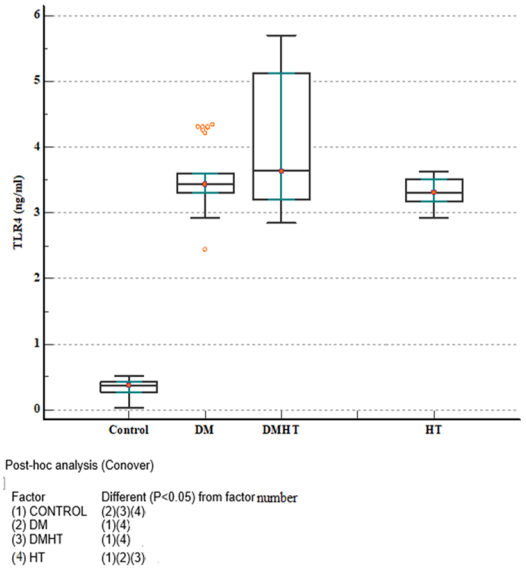

Figure 2 **Box plot of serum TLR4 comparison among the studied groups presenting post-hoc analysis (Conover) pairwise comparison.** ***DM,*** *diabetes mellitus patients group;* ***HT,*** *hypertension patients group;* ***DMHT,*** *diabetics with hypertension group.*

Table 3 **The mean differences of the TLR4, RETN, Insulin, HOMA1-IR, HOMA2-IR, % Beta cell, and %S by groups using Kruskal-Wallis test.**

Variable	Group	Mean±SD	Median	SEM	Min	Max	*P* -value
TLR4 (ng/ml)	Control	0.34±0.12	0.37	0.02	0.04	0.52	< 0.001
	DM	3.55±0.49	3.44	0.09	2.44	4.34	
	DMHT	4.10±0.97	3.64	0.18	2.85	5.70	
	HT	3.26±0.35	3.31	0.06	1.79	3.62	
RETN (ng/ml)	Control	2.68±2.11	1.93	0.39	1.04	8.28	< 0.001
	DM	7.39±1.45	7.41	0.27	4.39	9.17	
	DMHT	8.00±1.19	8.22	0.22	5.40	9.32	
	HT	6.24±1.17	5.98	0.21	3.91	8.28	
Insulin (μIU/ml)	Control	8.03±1.05	7.90	0.19	6.50	11.00	<0.001
	DM	12.79±1.67	12.10	0.30	10.32	15.78	
	DMHT	15.24±1.33	15.00	0.24	13.00	19.00	
	HT	9.49±2.04	9.30	0.37	7.00	13.00	
HOMA1-IR	Control	2.00±0.34	1.97	0.06	1.47	2.93	< 0.001
	DM	5.19±1.92	4.45	0.35	2.96	8.88	
	DMHT	6.59±2.95	5.20	0.54	3.95	15.32	
	HT	2.36±0.54	2.28	0.10	1.59	3.31	
HOMA2-IR	Control	1.08±0.14	1.05	0.03	0.85	1.49	< 0.001
	DM	1.88±0.31	1.82	0.06	1.45	2.51	
	DMHT	2.42±0.81	2.21	0.15	1.80	5.1	
	HT	1.26±0.27	1.24	0.05	0.92	1.70	
%Beta cell	Control	78.11±9.36	77.75	1.71	61.10	100.10	< 0.001
	DM	52.88±21.95	58.10	4.01	17.30	89.50	
	DMHT	56.37±23.06	62.00	4.21	15.30	89.50	
	HT	88.66±16.32	85.95	2.98	66.30	128.60	
%S	Control	94.54±11.79	95.45	2.15	67.30	117.50	< 0.001
	DM	56.35±11.86	57.30	2.17	39.90	99.90	
	DMHT	38.50±11.83	42.90	2.16	11.80	55.50	
	HT	82.70±16.98	80.95	3.10	59.00	109.20	

levels of serum RETN in HT group, DM patients group and DMHT group compared with the control group. The serum RETN concentration is higher in DMHT patients in comparison with HT group. In addition, serum RETN concentration is higher in DM group when compared with the HT group.

Post-hoc analysis (Conover) for TLR4 shows significantly different levels between the control group and all the other three patient groups Figure 2. Similarly, post-hoc analysis has also shown statistically significant differences (p<0.05) between the control group and all the other three groups in HOMA2-IR as illustrated in Figure 3.

Correlation matrix (Spearman) for patient groups (DM patients, HT patients, and DMHT patients) were statistically calculated and presented inTable 4, Table 5 and Table 6, respectively.

The correlation between serum RETN and BMI in the HT group was positive and significant at ($p\leq$0.05), also there was a positive correlation but non-significant in the other

Table 4 **Spearman's correlation analysis in DM group.**

Variables	TLR-4 (ng/ml)	RETN (ng/ml)	Insulin (uIU/ml)	HOMA1-IR	HOMA2-IR	%Beta-cell	%S
Age (years)	0.33	0.07	-0.08	0.23	0.19	**-0.41**	-0.29
BMI (kg/m$^{2)}$	**0.49**	0.20	-0.05	0.06	**0.42**	0.15	**0.45**
FBS (mg/dl)	0.21	0.13	0.14	**0.78**	**0.48**	**-0.88**	-0.34
HbA$_1$*c* (%)	0.31	0.12	0.10	**0.67**	**0.39**	**-0.75**	-0.35
Systolic BP (mmHg)	**0.55**	**0.44**	**0.43**	**0.47**	**0.52**	-0.20	**-0.43**
Diastolic BP (mmHg)	**0.37**	**0.85**	**0.73**	**0.46**	**0.64**	0.08	-0.33
Urea (mg/dl)	-0.04	-0.20	-0.05	0.19	-0.01	-0.23	-0.07
Creatinine (mg/dl)	0.16	0.22	0.34	0.01	0.01	0.25	**0.40**
ALT (U/l)	-0.14	-0.34	-0.17	-0.14	-0.30	0.16	**0.41**
AST (U/l)	-0.18	0.13	0.06	0.29	0.12	-0.17	-0.32
Total cholesterol (mg/dl)	0.31	0.02	-0.04	-0.11	-0.15	0.16	0.27
Triglycerides (mg/dl)	0.15	0.03	-0.02	0.11	-0.02	-0.15	0.17
HDL (mg/dl)	-0.04	-0.15	-0.13	-0.22	-0.12	0.14	-0.09
LDL (mg/dl)	0.34	0.09	0.02	-0.09	-0.09	0.19	0.17
VLDL (mg/dl)	0.15	0.03	-0.02	0.11	-0.02	-0.15	0.17
TLR-4 (ng/ml)	**1**	0.13	0.08	0.27	0.32	0.22	**-0.45**
RETN (ng/ml)		**1**	**0.85**	**0.58**	**0.69**	0.06	-0.27
Insulin (uIU/ml)			**1**	**0.68**	**0.60**	0.16	-0.21
HOMA1-IR				**1**	**0.72**	**-0.54**	**-0.41**
HOMA2-IR					**1**	**-0.45**	**-0.61**
%Beta-cell						**1**	0.34
%S							**1**

Values in bold are different from zero with a significance level alpha equal to 0.05.

BMI, *Body Mass Index;* ***BP,*** *blood pressure;* ***FBS,*** *fasting blood sugar;* ***ALT,*** *alanine aminotransferase;* ***AST,*** *aspartate aminotransferase;* ***HDL,*** *high-density lipoprotein;* ***LDL,*** *low-density lipoprotein;* ***VLDL,*** *very low-density lipoprotein;* ***%S,*** *insulin sensitivity percentage.*

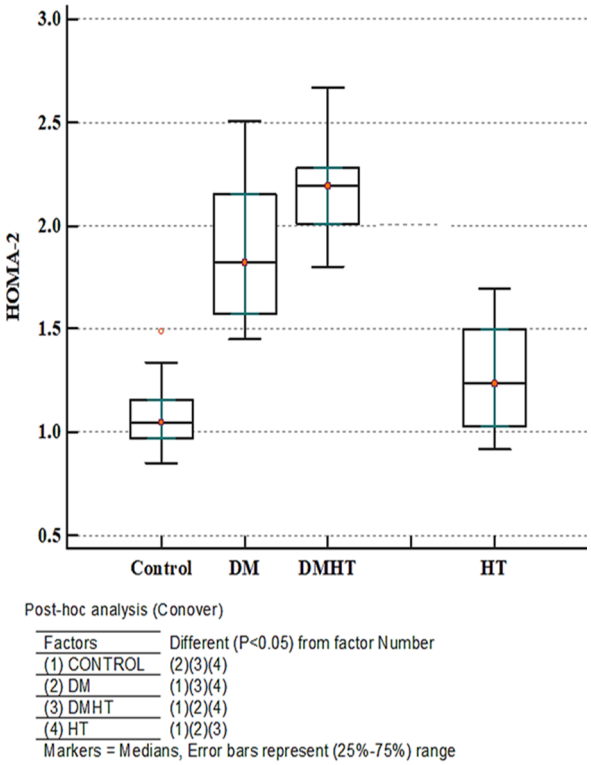

Figure 3 Box plot of HOMA2-IR comparison among the studied groups presenting post-hoc analysis (Conover) pairwise comparison. ***DM,*** *diabetes mellitus patients group;* ***HT,*** *hypertension patients group;* ***DMHT,*** *diabetics with hypertension group.*

two groups. However, serum TLR4 and RENT were not correlated with age, FBS, HbA_1 *c,* urea, creatinine, AST, ALT, triglycerides, VLDL and LDL) in all patient groups.

The association between RETN and IR (HOMA1 and HOMA2), in DM group and DMHT groups, was positive and significant at ($p \leq 0.05$). The value of positive correlation coefficient between RETN and IR (HOMA1-IR and HOMA2-IR) in DM group were 0.58 and 0.69, respectively. In addition, positive association between RETN and IR (HOMA1-IR and HOMA2-IR) in HT group at ($p \leq 0.05$) and the correlation coefficient values were 0.5 and 0.59, respectively. Furthermore, the correlation coefficient values of DMHT group between RETN and IR (HOMA1-IR and HOMA2-IR) were 0.41 and 0.49, respectively with a significant correlation. The RETN and percentage of insulin sensitivity (%S) in all patient groups were negatively associated. Finally, it was significantly and positively correlated with TLR4 in DMHT group.

DISCUSSION

In the present study, serum RETN was higher in all patient groups when compared with the control group, such findings were also seen by Zhang et.al[18] who observed increased

Table 5 **Sperman's correlation analysis in HT group.**

Variables	TLR-4 (ng/ml)	RETN (ng/ml)	Insulin (uIU/ml	HOMA1 IR	HOMA2 IR	%Beta-cell	%S
Age	-0.17	0.00	0.10	0.03	0.08	0.33	-0.08
BMI (Kg/m^2)	0.10	**0.44**	0.16	0.21	0.17	0.12	-0.17
FBS (mg/dl)	0.10	0.11	0.11	**0.41**	0.21	**-0.59**	-0.21
HbA$_1$*c*	0.10	0.11	0.11	**0.41**	0.21	**-0.59**	-0.21
Systolic BP (mmHg)	-0.02	**0.50**	0.24	0.16	0.23	0.29	-0.23
Diastolic BP (mmHg)	0.13	**0.76**	**0.70**	**0.57**	**0.67**	**0.65**	**-0.67**
Urea (mg/dl)	0.04	0.31	0.18	0.10	0.16	0.31	-0.16
Creatinine (mg/dl)	-0.08	0.00	0.20	0.14	0.22	0.26	-0.22
ALT (U/l)	-0.31	-0.10	-0.04	0.03	-0.04	-0.13	0.04
AST (U/l)	-0.14	0.17	0.13	0.03	0.14	0.23	-0.14
Total cholesterol (mg/dl)	0.15	0.09	0.13	-0.09	-0.12	0.05	0.12
Triglycerides (mg/dl)	0.12	0.16	0.24	-0.19	-0.21	-0.04	0.21
HDL (mg/dl)	-0.16	-0.24	**0.41**	0.33	0.36	0.28	-0.36
LDL (mg/dl)	0.18	0.07	-0.04	-0.01	-0.04	0.10	0.04
VLDL (mg/dl)	0.12	0.16	-0.24	-0.19	-0.21	-0.04	0.21
TLR-4 (ng/ml)	**1**	0.19	0.32	0.29	0.32	0.09	-0.32
RETN (ng/ml)	0.19	**1**	**0.60**	**0.54**	**0.59**	**0.61**	**-0.59**
Insulin (uIU/ml)	0.32	**0.60**	**1**	**0.93**	**0.99**	**0.68**	**-0.99**
HOMA1-IR	0.29	**0.54**	**0.93**	**1**	**0.96**	**0.45**	**-0.96**
HOMA2-IR	0.32	**0.59**	**0.99**	**0.96**	**1**	**0.62**	**-1.00**
%Beta-cell	0.09	**0.61**	**0.68**	**0.45**	**0.62**	**1**	**-0.62**
%S	-0.32	**-0.59**	**-0.99**	**-0.96**	**-1.00**	**-0.62**	**1**

Values in bold are different from zero with a significance level alpha equal 0.05.
BMI, *Body Mass Index;* ***BP,*** *blood pressure;* ***FBS,*** *fastig blood sugar;* ***ALT,*** *alanine aminotransferase;* ***AST,*** *aspartate aminotransferase;* ***HDL,*** *high-density lipoprotein;* ***LDL,*** *low-density lipoprotein;* ***VLDL,*** *very low-density lipoprotein;* ***%S,*** *insulin sensitivity percentage.*

RETN levels in T2DM and in cardiovascular disease patients as well as the positive correlation between serum RETN and vascular function. However, Urbanovych et al. (2015) were also found significantly elevated RETN levels in patients with T2DM, when compared with individuals without T2DM.[19]

The current study also showed that the mean BMI of the participants among all study groups was ranged between overweight to obese values with non-significant difference between groups. The association between high RETN levels and BMI among all the patient groups was controversial in several studies. Heilbronn et al[20] reported no correlation between serum RETN levels and BMI or age. Another study found significant correlation between RETN and BMI as well as significant correlation with IR in obese patients and those with T2DM.[21] This study demonstrated a non-significant correlation between serum RETN and BMI in all study groups except HT group which showed a positive correlation between serum RETN and BMI.

Recently, Siddiqui et al[22] mentioned that RETN hormone is a promising biomarker of IR, in the pathogenic mechanism of endothelial dysfunction and inflammation which may

Table 6 Sperman's correlation analysis in group DMHT.

Variables	TLR4 (ng/ml)	RETN (ng/ml)	Insulin (uIU/ml	HOMA1 IR	HOMA IR	%Beta-cell	%S
Age	0.08	0.19	**-0.38**	-0.13	-0.23	-0.16	0.08
BMI (Kg/m^2)	0.15	0.21	0.01	0.20	0.18	-0.21	-0.19
FBS (mg/dl)	0.31	0.13	0.16	**0.87**	**0.63**	**-0.97**	**-0.52**
HbA$_1$*c*	0.16	0.03	0.35	**0.78**	**0.68**	**-0.72**	**-0.55**
Systolic BP (mmHg)	**0.44**	**0.86**	0.23	0.11	0.25	0.06	-0.08
Diastolic BP (mmHg)	**0.41**	**0.78**	0.16	**0.43**	**0.39**	-0.09	-0.20
Urea (mg/dl)	0.11	0.26	-0.14	-0.09	-0.13	0.07	0.03
Creatinine (mg/dl)	0.32	0.34	-0.03	-0.25	-0.23	0.29	0.15
ALT (U/l)	0.13	0.01	0.06	0.14	0.01	-0.17	-0.35
AST (U/l)	0.32	0.32	-0.14	-0.03	-0.01	-0.02	0.01
Total cholesterol (mg/dl)	0.12	**0.41**	-0.17	-0.01	-0.02	-0.01	0.06
Triglycerides (mg/dl)	0.05	-0.28	-0.21	0.08	0.07	-0.09	-0.11
HDL (mg/dl)	-0.23	**-0.39**	-0.11	0.17	0.06	-0.19	-0.04
LDL (mg/dl)	0.16	0.28	0.14	-0.24	-0.18	0.27	0.11
VLDL (mg/dl)	0.05	0.28	-0.21	0.08	0.07	-0.09	-0.11
TLR-4 (ng/ml)	**1**	**0.62**	0.05	0.30	0.17	0.37	-0.08
RETN (ng/ml)	**0.62**	**1**	**0.71**	**0.41**	**0.49**	0.11	-0.02
Insulin (uIU/ml)	0.05	**0.71**	**1**	**0.51**	**0.75**	0.001	**-0.57**
HOMA1-IR	0.30	**0.41**	**0.51**	**1**	**0.90**	**-0.79**	**-0.74**
HOMA2-IR	0.17	**0.49**	**0.75**	**0.90**	**1**	**-0.51**	**-0.73**
%Beta-cell	0.37	0.11	0.00	**-0.79**	**-0.51**	**1**	**0.42**
%S	-0.08	-0.02	**-0.57**	**-0.74**	**-0.73**	**0.42**	**1**

Values in bold are different from zero with a significance level alpha equal 0.05.
BMI, *Body Mass Index;* ***BP,*** *blood pressure;* ***FBS,*** *fastig blood sugar;* ***ALT,*** *alanine aminotransferase;* ***AST,*** *aspartate aminotransferase;* ***HDL,*** *high-density lipoprotein; LDL, low-density lipoprotein;* ***VLDL,*** *very low-density lipoprotein;* ***%S,*** *insulin sensitivity percentage.*

accelerate the development of IR in T2DM. Moreover, the important note that we concluded in this study was the positive correlation between RETN and IR (HOMA1, HOMA2) that indicated the role of RETN in IR that is mediated by endothelial dysfunction and inflammatory pathways. Negative correlations between RETN and the percentage of insulin sensitivity (%S) in all patient studies groups enhanced the positive correlation between RETN and IR.

Regarding RETN in our results, similar results were reported by Jiang et al. (2016) who suggested the presence of correlation between serum RETN concentration and HT. The mechanism underlying the correlation between RETN and hypertension remains to be clarified. One possible mechanism might be mediated via TLR4.[15]

The data on serum TLR4 levels in humans is rare, especially in the cases of hypertensive subjects with T2DM. In accordance with recently published studies, Li et al[23] concluded that serum TLR4 level could be potential biomarkers for determining acute aortic dissection. Another study found that serum TLR4 has been raised and suggested as a strong biomarker

that gives data about the systemic status related to inflammatory conditions.[24] The essential results of the current research show that there was a significant difference (p<0.001) in means of serum TLR4 levels in all patient groups when compared with the healthy group. The maximum levels of serum TLR4 among the studied groups were observed in the HT patients with T2DM. In addition, TLR4 concentration is higher in DM group compared with the HT patient group. The increasing TLR4 levels alongside RETN levels in all patient groups confirm that TLR4 is an assumed receptor for RETN that has been suggested to participate in RETN-induced IR and inflammation. Another significant note of the current study was that serum levels of RETN and TLR4 were positively correlated in DMHT patients, which suggests that both RETN and TLR4 would be probable biomarkers to distinguish between normal healthy subjects and hypertensive patients with T2DM patients. Moreover, the increase in serum levels of RETN and TLR4 gives an indication of the extent to which tissues and cells are affected by IR and inflammations as the result of the severity of the diabetes and the HT. However, there is lacking evidence on serum TLR4 levels in hypertensive subjects with T2DM, one available study has shown that patients with HT showed elevated TLR4 expression on their peripheral monocytes in contrast with those with controlled HT. This demonstrates that uncontrolled blood pressure is related to the activated innate immunity, which would participate in organ and tissue damage and thus influence disease prognosis.[25] Another report on obesity patients with DM found that TLR4/NF-κB pathway is upregulated in obese patients with DM.[26] Finally, our study found that the increasing RETN can be associated with IR in HT and DM.

CONCLUSIONS

In conclusion, serum TLR4 and RETN were higher in diabetic patients than in healthy subjects, also they were correlated with diabetes-induced IR. Also, the highest levels of serum RETN and TLR4 were found in DMHT patients. We finally suggest that there is a close relationship between TLR4 and RETN and their positive association with IR.

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DECLARATIONS

Authors' contributions

Conceptualization, data curation, formal analysis, methodology, project administration, resources, validation, visualization, writing-review & editing: MKA, RSB and MBH. Funding acquisition: N/A. Investigation, software, writing-original draft: MKA and RSB. Super-

vision: RSP and MBH. All the authors reviewed and approved the final version before publication.

Conflict of interest

There are no conflicts of interest to declare.

Ethical approvals

Institutional Review Board of the College of Medicine at Al-Nahrain University approved the current study (No.: 5/1/52/142, Date: January 31, 2019). In addition, all participants agreed to participate in the study, signed and given the written informed consent.

Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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