RESEARCH ARTICLE

The Effect of Angiotensin Converting Enzyme activity on Resistin and TLR-4 levels in Hypertensive Subjects with or without Type 2 Diabetes Mellitus

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ABSTRACT

Background: Hypertension (HT) and type 2 diabetes mellitus (T2DM) are the most prevalent chronic disease in the Iraqi population, leading to increasing heart disease, kidney problem, and coronary artery disease or atherosclerosis. One of the famous cause of the chronic diseases like hypertension and T2DM is obesity. Resistin (RETN) is a principle hormone link between obesity and Type2 Diabetes Mellitus (T2DM) because it induces insulin resistance. It is one member of cysteine-rich secretory proteins family. A putative receptor for RETN is TLR-4, it is conceder immunomodulators of the immune response. Immune responses participate in arterial hypertension. A case-control study was conducted to estimate the relation between serum Toll-like receptor 4 (TLR4) level and serum resistin (RETN) levels with angiotensin converting enzyme (ACE) activity in patients with hypertensive subjects and hypertensive patients with T2DM. We also studied the effect of angiotensin converting enzyme inhibitor (captopril 50 mg) on Resistin and TLR-4 levels in hypertensive subjects with type 2 diabetes mellitus.

Objectives: Study the effect of ACE activity on serum level of RETN and TLR-4 and their association between the Resistin hormone and its receptor in hypertensive subjects with or without T2DM subjects, and study the effect of Angiotensin Converting Enzyme Inhibitory on serum level of RETN and Toll-like receptor4 (TLR4) in hypertensive subjects with T2DM using captopril 50 mg treatment.

Materials and Method: A case-control study was conducted from September 2019 to July 2020 at the Department of Chemistry and Biochemistry, College of Medicine in Al-Nahrain University on 90 subjects classified into three groups; each group consists of 30 patients. Study subjects ages will be matched in all three groups and their gender (Men) Age range was between (40–48) years old. This group consists of 30 patients with hypertension, 30 hypertensive patients with T2DM and 30 hypertensive patients with T2DM using captopril drug. For all the subjects in the research serum RETN and TLR-4 were estimated by using enzyme-linked immunoassay (ELISA) technique. Also ACE were estimation by using a spectrophotometric assay.

Results: Serum resistin and its receptor TLR-4 were higher in hypertensive patients with T2DM subject than in hypertensive patients. In addition, serum Angiotensin Converting Enzyme (ACE) activity was higher in hypertensive patients with T2DM subject than in hypertensive patients. Furthermore, a positive correlation was found between TLR4 and resistance in hypertensive patients with T2DM subject group.

Conclusion: The Results indicated that ACE inhibitor (captopril) decreases the serum levels of Resistin and TLR-4 in hypertensive subjects with T2DM. Moreover, evaluation the serum level of ACE activity, RETN, and TLR4 in all studies groups.

Keywords: Angiotensin Converting Enzyme (ACE), Captopril, Hypertension (HT), Resistin (RETN), Toll-like receptor-4 (TLR-4), Type 2 Diabetes Mellitus (T2DM).

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INTRODUCTION

Hypertension is the most silent killer of chronic disease prevalence worldwide. It is an essential contributor to heart problems and stroke.[1] High blood pressure is one of the famous causes of death worldwide. In addition, hypertension was the top risk factor for the development of cardiovascular disease. There are two types of high blood pressure, either essential or secondary.[2] In essential hypertension, the etiology

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is unclear reasons but in secondary hypertension, a known pathological disorder is the cause.[3] The controlled risk factors that elevate your chances of developing hypertension are smoking, obesity, diabetes, high blood cholesterol and an unhealthy diet. The renin-angiotensin-aldosterone system (RAAS) is one of the major essential hormonal mechanisms in controlling hemodynamic stability by regulating sodium-potassium balance, blood pressure, and fluid volume. RAAS is composed of three essential compounds: renin, angiotensin II, and aldosterone. These three compounds elevate arterial pressure to decrease renal blood pressure and lowered salt delivery to the distal convoluted tubule. ACE-I is a nonspecific dipeptidyl carboxypeptidase associated with the RAAS. The main role of ACE is to convert Angiotensin I to Angiotensin II by degradation of bradykinin therefor the increasing activity of ACE causes hypertension, so the inhibition of Angiotensin I-converting enzyme activity can be contribute to the regulation and decrease of blood pressure and elimination of related pathogeneses.[4] Since 1981, ACE inhibitors have been prescribed as antihypertensive drugs. One of the most famous ACE inhibitor is captopril that has two primary functions. First function decreases the amount of sodium retained in the kidneys and the Second function is to stop the production of angiotensin II, reducing arterial pressure, venous pressure, and blood volume.[5] The other benefits of using ACE-I e drug are reducing the risk of heart attack and delaying the onset of T2DM. ACE-I enhances glucose uptake in peripheral tissues by increasing the translocation of glucose transporter-4 (GLUT4) and improving insulin resistance. The mechanism by which Angiotensin-converting enzyme inhibition lessens the advancement of T2DM in patients with essential hypertension is unclear. Patients with hypertension frequently have impaired glucose tolerance related to change in the skeletal muscle tissue. There is also a rise in fat interspersed between skeletal muscle tissues that strongly correlate with insulin resistance.[6] Finally, there become clear to post-receptor insulin signaling abnormalities. Angiotensin-II effects on post-receptor insulin signaling, so Angiotensin II (AngII) inhibits phosphatidyl inositol-3 kinase (PI3-K), therefore causing insulin resistance, Figure 1.[7]

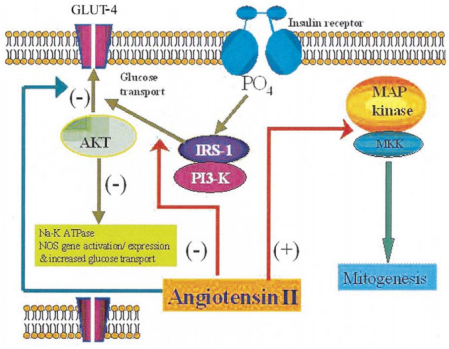

Figure 1: The effects of angiotensin-II on post-receptor insulin signaling, that causing insulin resistance. GLUT 4 = glucose transporter 4; MAP = mitogen activated protein; IRS_1 = insulin receptor substrate-1; NOS = nitric oxide MKK = MAP kinase kinases; PO_4 = phosphate

Many studies suggested that adipose tissue hormones are involved in the mechanism of action of ACE inhibitors. Very little is known as to whether the action on RETN participates in the clinical effectiveness relationship with the use of these factors. RETN was specified as a possible link between insulin resistance and obesity. Insulin resistance (IR) is a major part of the etiology of T2DM and is also linked to other pathophysiologic sequels, including atherosclerosis, hypertension, and hyperlipidemia. Several studies have supported the principle of inflammatory cytokine mediation of RETN. The mechanism underlying the correlation between the resistin and hypertension might be mediated via the TLR-4. The local secretion of RETN causes an increased release of endothelin1, intercellular adhesion molecule 1 (ICAM1), vascular cell adhesion molecule 1 (VCAM1) and MCP-1. This affects vascular endothelial cells when its level increases, affecting the endothelial function and vascular smooth muscle cell migration and development of hypertension.[8] RETN probably binds on the membrane with TLR-4 and then activates the intracellular signaling pathway. This binding activated the translocation of NFκB into the nucleus that activated the transcription of pro-inflammatory cytokine genes, contributing to the proliferation of vascular smooth muscle cell (VSMCs) and endothelial dysfunction. TLR-4 is one member of the toll-like receptor family (TLR) is a receptor belonging to trans-membrane lipopolysaccharide receptor (LPS) that activation of it induced the release of inflammatory cytokines, chemokines and antimicrobial peptides, also have a fundamental role in initiate the innate immune system. RETN-TLR4-induced inflammation and insulin resistance. In addition to induced insulin resistance and diabetes mellitus, RETN and TLR4 play a role in hypertension. TLR4 in modulating the inflammatory responses following various cardiovascular diseases such as myocardial ischemia as shown below.[9]

Finally, the recent study found that the antihypertensive and anti-inflammatory action of ACE inhibition affected TLR4 expression in a high-dose model like captopril drug. also findings TLR4 was important role in insulin resistance, a lower prevalence of DM, hypertension, atherosclerosis, cardiovascular disease and cardo-arterial disease.[10] the

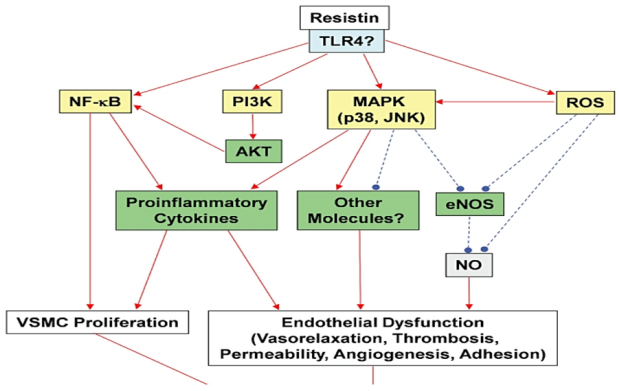

Figure 2: The signaling pathway of RETN- TLR4 binding that mediate hypertension and cardiovascular dysfunction

summary of study assess the serum Resistin and TLR-4 in hypertensive subjects with or without T2DM and their relationship with the activity of ACE. In addition, this study shows the correlation between serum Resistin and TLR-4 with insulin resistance. Finally, study the effect of ACE inhibitor (captopril 50 mg) on serum level of RETN and TLR-4 and their association between the Resistin hormone its receptor in hypertensive subjects with T2DM using captopril treatment.

METHODS

A case-control study was conducted on 90 subjects classified into three different groups; each group consisted of 30 patients. This group consists of 30 patients with hypertension, 30 hypertensive patients with T2DM and 30 hypertensive patients with T2DM using captopril 50 mg treatment. Study subjects age will be matched in all three groups and sex (Men only) Age range was between 40 to 48 years old. Each participant in this study was thoroughly interviewed according to a well-structured questioner.Men included in this study were collected from Al-Imamain Alkadhimain Medical city in Baghdad. The practical work was coordinated at the Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University. This research was approved by the Institutional Review Board (IRB) of College of Medicine, Al-Nahrain University; before participation, all men were given an idea about the research study, and their written informed consent was taken.[11] Physical examination was done, and their weight, height, BMI, and blood pressure were estimated and asked the patient about all the treatments he used. The diagnostic of T2DM was diagnosed according to standard American Diabetes Mellitus Association Criteria.[12] Fasting blood samples were taken from all participants during their visit. Any subjects with the following diseases will be excluded: Renal disease, Alcoholic and smoking subjects, Thyroid disease, Inflammatory disorders, and liver disease. Samples collection blood samples were obtained from fast men. About seven milliliters of blood samples were obtained from each fasting (8 hours fast) participant in this study. Each blood sample is divided into two parts; the first part includes five milliliters of blood samples left for 20 minutes in the gel tube at room temperature (25–28ºC), and the second part includes two milliliters collected into EDTA tube for measuring of glycated hemoglobin (HbA1c). After coagulation serum were separated by centrifugation at 2000 xg for 10 minutes. Serum was immediately measured of glucose (FSG), lipid profile, renal function tests and liver function tests using appropriate enzymatic colorimetric method, with commercially available kits.

Residual sera stored at -20ºC for the subsequent assay of serum ACE activity, RETN, TLR-4 and fast insulin hormone. They were measure by using ELISA technique. Insulin resistance (IR) was founded by homeostatic model assessment (HOMA) in two formulas HOMA1-IR as shown in the introduction and HOMA2-IR that is computer model. HOMA2 Calculator v2.2.3 from the Diabetes Trials Unit, University of Oxford was used in this research.

Statistical Analysis

All statistical analyses were performed using SPSS statistical software, version 29 (IBM Corporation, USA) and medcalc. Version 19 software, all Tables and figures were made using Microsoft excel and ward software. Normally distributed data were presented as mean ± standard deviation and groups were compared by analysis of variance (ANOVA). Non-parametric Kruskal Wallis test was employed to test the variance among the groups for those with non-normal distribution, which were expressed as median and interquartile range and Post-hoc analysis (Conover) was conducted to indicate pairwise difference between each tow group whenever Kruskal Wallis test was significant. Two-tailed Spearman's correlations were performed between the study biomarkers and other variables to test their association.[13] Significant levels were set to alpha = 0.05 with statistical significance when $p < 0.05$.

RESULTS

Body Mass Index (BMI) and age in all three groups were matched and there were no significant differences between all the three groups regarding BMI and age among all studied groups. The mean ± standard deviation (SD) of age for hypertensive subjects group (HT), hypertensive patients with T2DM group (DMHT) and hypertensive patients with T2DM using captopril 50 mg treatment (DMHTD) were 43.90 ± 2.47 years, 44.13 ± 2.68 years and 44.57 ± 2.18 years, respectively. Among all study groups, there is no significant difference ($p < 0.05$) was found in age.

The Mean ± standard deviation (SD) of Body Mass Index (BMI) in (Kg/m^2) for hypertensive subjects group (HT), hypertensive patients with T2DM group (DMHT), and hypertensive patients with T2DM using captopril 50 mg treatment (DMHTD) were 30.31 ± 5.21, 30.69 ± 4.03 and 30.88 ± 0.27, respectively. No significant difference ($p < 0.05$) was found in BMI among all study groups (Table 1).

BMI, SEM, Standard error of mean: HT, Hypertension patients group; DMHT, Diabetes Mellitus with Hypertension patients group.

The major diagnostic parameters of study subjects are displayed in Table 2.

HbA1c, hemoglobin $A1_C$, HT, Hypertension patients group; DMHT, Diabetes Mellitus with Hypertension patients group: DMHTD, Diabetes Mellitus with Hypertension patients group treatment. ALT, Alanine aminotransferase: AST, Aspartate aminotransferase.

Table 2 shows the clinical characteristics of the three study groups. Strongly significant differences ($p<0.001$) were estimated between these study groups in fasting blood sugar (FBS), HbA1c, diastolic blood pressure, systolic blood pressure and creatinine. Significant differences (p 0.128) and (p 0.48) were estimated between three ALT and AST study groups. Moreover, according to this result and the physician's follow-up, the patients were classified into three major groups.

The Mean ± SD of serum TLR-4 in ng/mL for the three subject groups (hypertensive patients, hypertensive patients

Table 1: The comparison of body mass index (BMI) and age between the three patients groups

	Variable	*Mean ± SD*	SE_M	*Min*	*Max*	*p*
BMI	HT	30.31 ± 5.21	0.95	18.73	42.94	0.06
	DMHT	30.69 ± 4.03	0.74	23.45	39.79	
	DMHTD	30.88 ± 0.27	0.05	30.24	31.23	
Age	HT	43.90 ± 2.47	0.45	41.00	49.00	0.835
	DMHT	44.13 ± 2.68	0.49	41.00	49.00	
	DMHTD	44.57 ± 2.18	0.40	41.00	49.00	

Table 2: The mean differences for fast blood sugar, HbA1c, systolic and diastolic blood pressure, by groups using F-tests (ANOVA)

	Variable	*Mean ± SD*	SE_M	*Min*	*Max*	*p*
Fasting Blood Sugar	HT	100.43 ± 6.40	1.17	91.00	113.00	< 0.001
	DMHT	173.03 ± 67.55	12.33	123.00	365.00	
	DMHTD	159.97 ± 49.27	9.00	123.00	365.00	
HbA_{1C}	HT	5.13 ± 0.22	0.04	4.80	5.56	< 0.001
	DMHT	7.82 ± 1.68	0.31	5.60	11.60	
	DMHTD	7.41 ± 1.15	0.21	6.10	11.20	
Systolic Blood pressure	HT	159.33 ± 7.28	1.33	145.00	170.00	< 0.001
	DMHT	165.15 ± 28.54	5.21	16.50	180.00	
	DMHTD	140.83 ± 4.37	0.80	135.00	145.00	
diastolic Blood pressure	HT	92.27 ± 3.27	0.60	89.00	100.00	< 0.001
	DMHT	94.50 ± 3.47	0.63	90.00	100.00	
	DMHTD	84.17 ± 3.00	0.55	80.00	89.00	
creatinine	HT	1.03 ± 0.14	0.03	0.70	1.50	< 0.001
	DMHT	1.00 ± 0.28	0.05	0.70	1.90	
	DMHTD	1.04 ± 0.34	0.06	0.80	1.90	
ALT	HT	30.07 ± 4.96	0.91	21.00	40.00	0.128
	DMHT	27.23 ± 4.64	0.85	19.00	37.00	
	DMHTD	29.87 ± 5.37	0.98	22.00	40.00	
AST	HT	26.40 ± 3.74	0.68	21.00	34.00	0.48
	DMHT	25.60 ± 4.40	0.80	19.00	34.00	
	DMHTD	24.97 ± 4.90	0.90	19.00	34.00	

with T_2DM and hypertensive patients with T_2DM using captopril) were 3.26 ± 0.35, 4.10 ± 0.97, and 0.76 ± 0.158.00 ± 1.19, respectively. The high significant differences ($p<0.001$) were estimated between these three study groups.

The Mean ± SD of serum resistin (RETN) in ng/ml for the three subject groups (hypertensive patients, hypertensive patients with T2DM and hypertensive patients with T2DM using captopril) were 6.24± 1.17, 8.00± 1.19 and 3.76± 0.46, respectively. Therefore high significant differences ($p<0.001$) were estimated between these three study groups.

The Mean ± SD of serum ACE activity in U/mL for the three subject groups (hypertensive patients, hypertensive patients with T2DM and hypertensive patients with T_2DM using captopril) were 58.48 ± 4.63, 62.17 ± 5.95, and 31.08±9.62, respectively. Therefore high significant differences ($p<0.001$) were estimated between these three study groups.

The Mean ± SD of serum insulin in µIU/ml for the hypertensive patients, hypertensive patients with T2DM group and hypertensive patients with T2DM using captopril were 9.49±2.04, 15.24 ± 1.33, and 13.71 ± 0.97, respectively, so high significant differences ($p<0.001$) were estimate between these three study groups.

The Mean ± SD of HOMA1-IR, HOMA2-IR, β-cell function (% β-cell), and insulin sensitivity (%S) of 100% were found, so high significant differences ($p<0.001$) were estimated between the three study groups as shown in Table 3.

A pairwise comparison between studies groups was found according to Post-hoc analysis (Conover). Pairwise comparisons for serum resistin level are significantly different ($p<0.05$) between hypertensive patients with T2DM using captopril group and toward hypertensive patients group, on the other hand, it is significantly different ($p<0.05$) with hypertensive patients with T2DM group. In addition, serum resistin pairwise comparison was found significantly different ($p<0.05$) between each group, as shown in Figure 3.

The box plot showed a decrease in serum resistin levels

Table 3: Results for testing the mean differences results for testing of, TLR4, RETN, Insulin, HOMA1-IR, HOMA2-IR, % Beta cell, and %S by groups using Kruskal-Wallis test

	Variable	*M ± SD*	*Median*	SE_M	*Min*	*Max*	*P*
TLR-4 ng/mL	HT	3.26 ± 0.35	3.31	0.06	1.79	3.62	< 0.001
	DMHT	4.10 ± 0.97	3.64	0.18	2.85	5.70	
RETN ng/mL	DMHTD	0.76 ± 0.15	0.76	0.03	0.55	1.10	< 0.001
	HT	6.24 ± 1.17	5.98	0.21	3.91	8.28	
	DMHT	8.00 ± 1.19	8.22	0.22	5.40	9.32	
	DMHTD	3.76 ± 0.46	3.90	0.08	2.90	4.77	
ACE activity	HT	58.48 ± 4.63	58.73	0.85	49.65	69.54	< 0.001
	DMHT	62.17 ± 5.95	60.80	1.09	51.23	73.91	
	DMHTD	31.08 ± 9.62	33.89	1.76	3.43	38.65	
Insulin µIU/mL	HT	9.49 ± 2.04	9.30	0.37	7.00	13.00	< 0.001
HOMA1- IR	DMHT	15.24 ± 1.33	15.00	0.24	13.00	19.00	
	DMHTD	13.71 ± 0.97	14.00	0.18	11.00	16.00	
	HT	2.36 ± 0.54	2.28	0.10	1.59	3.31	< 0.001
	DMHT	6.59 ± 2.95	5.20	0.54	3.95	15.32	
	DMHTD	5.43 ± 1.77	5.05	0.32	3.67	12.62	
HOMA2-IR	HT	1.26 ± 0.27	1.24	0.05	0.92	1.70	< 0.001
	DMHT	2.42 ± 0.81	2.21	0.15	1.80	5.1	
	DMHTD	2.10 ± 0.6	2.00	0.11	1.57	5.10	
%Beta cell	HT	88.66 ± 16.32	85.95	2.98	66.30	128.60	< 0.001
	DMHT	56.37 ± 23.06	62.00	4.21	15.30	89.50	
	DMHTD	53.18 ± 18.56	54.85	3.40	13.90	81.9	
%S	HT	82.70 ± 16.98	80.95	3.10	59.00	109.20	< 0.001
	DMHT	38.50 ± 11.83	42.90	2.16	11.80	55.50	
	DMHTD	53.14 ± 14.14	51.80	2.59	14.60	89.40	

HT, Hypertension patients group; DMHT, Diabetes Mellitus with Hypertension patients group; DMHTD, Diabetes Mellitus with Hypertension patients using captopril group; %S, insulin sensitivity percentage, ACE, TLR-4.

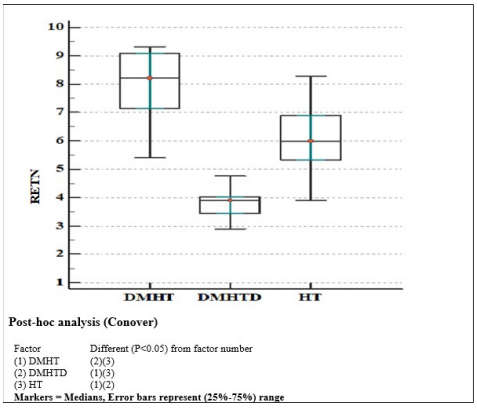

Figure 3: Box plot of serum resistin comparison among the studied groups presenting Post-hoc analysis (Conover) pairwise comparison

(RETN) in hypertensive patients with T_2DM using the captopril patients group compared with the two studies groups. The serum RETN concentration is higher in hypertensive patients with T2DM than in the hypertensive group. In addition, serum RETN concentration is higher in T2DM group compared with the hypertensive patient's group.

Pairwise comparisons for serum TLR-4 level are significantly different ($p < 0.05$) between hypertensive patients with T_2DM using captopril subjects group and hypertensive patients, also significantly different ($p<0.05$) with hypertensive patients with T_2DM group. Lowering concentration of TLR-4 was found in subjects that used captopril treatment (hypertensive patients with T2DM using captopril patients group) compared to the hypertensive patient's group. In addition, serum TLR-4 pairwise comparisons were found significantly different ($p < 0.05$) between each group, as shown in Figure 4.

Pairwise comparison for serum ACE activity are significantly different ($p < 0.05$) between hypertensive patients with T2DM using captopril subjects group and hypertensive patients, also significantly different ($p < 0.05$) with hypertensive patients with T2DM group. The lower activity of ACE was found in subjects that used captopril treatment (hypertensive patients with T2DM using captopril patients group) compared to the hypertensive patient's group.

In addition, serum Angiotensin Converting Enzyme (ACE) activity pairwise comparisons were found significant different ($p < 0.05$) between each group as shown below in Figure 5.

Insulin resistance (IR) also found according to Post-hoc analysis (Conover). It shows significant difference ($p<0.05$) between hypertensive patients with T_2DM using captopril subjects group and hypertensive patients, also significantly different ($p<0.05$) with hypertensive patients with T_2DM group in HOMA2-IR as shown in Figure 6.

The correlation matrix (Spearman) for each study group are statistically calculated to find the correlation between every parameters that we measured in the following Tables 4 to 6**.**

The result of correlation matrix (Spearman) in this study, between ACE and RETN in hypertensive patients with T2DM patients group and hypertensive patients group was a positive correlation with a significance level alpha = 0.05. The positive correlation between ACE and RETN in hypertensive patients with T2DM patients group was 0.46. There was also a positive relation between ACE and RETN in the hypertensive subject group, and the value was 0.41. In addition, correlation matrix (Spearman) for the subject that used captopril treatment was a non-significant correlation between ACE and RETN the value was 0.31.

The association between RETN and IR (HOMA1, HOMA2-IR) in hypertension group and hypertensive patients with T2DM group was a positive correlation with a significance level alpha=0.05 according to the Correlation matrix (Spearman). The value of positive correlation between RETN and IR (HOMA1, HOMA2-IR) hypertensive patients with T_2DM group were 0 were 0.41 and 0.49 respectively, also positive relation between RETN and IR (HOMA1-IR, HOMA2-IR) in hypertensive subject group and the value were 0.5 and 0.59 respectively. In addition correlation matrix (Spearman) for the subject that used captopril treatment showed a non-significant correlation between RETN and IR (HOMA1, HOMA2-IR); the values were found as 0.19 and 0.10, respectively.

The correlation between serum resistin (RETN) and serum TLR-4 in hypertensive patients with T_2DM group was positive correlation with a significance level $\alpha = 0.05$ according to Correlation matrix (Spearman), and the values were found 0.62. However, it was non-significant in hypertensive and hypertensive patients with T2DM groups using captopril group the value 0.19 and 0.35, respectively.

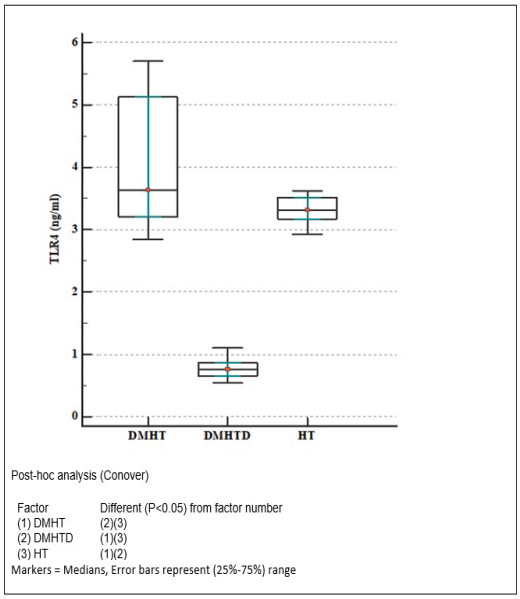

Figure 4: Box plot of serum TLR-4 comparison among the studied groups presenting Post-hoc analysis (Conover) pairwise comparison.

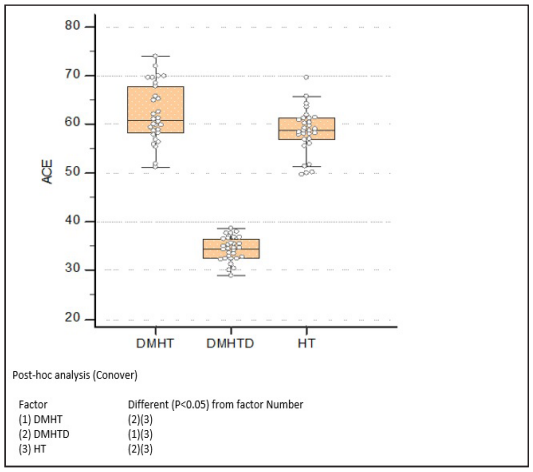

Figure 5: Box plot of serum ACE activity comparison among the studied groups presenting Post-hoc analysis (Conover) pairwise comparison

DISCUSSION

Several studies have found that adipose tissue hormones like RETN are interested in the mechanism of action of angiotensin converting enzyme inhibitors treatment. Little investigation is concerning whether the action on RETN contributes to the clinical effectiveness related to the utilization of these agents. Another study has demonstrated that increasing RETN level in T2DM and cardiovascular disease also found a positive correlation between serum resistin and vascular function.[15]

The present study showed increases in the levels of serum RETN in hypertensive patients with T2DM subjects group

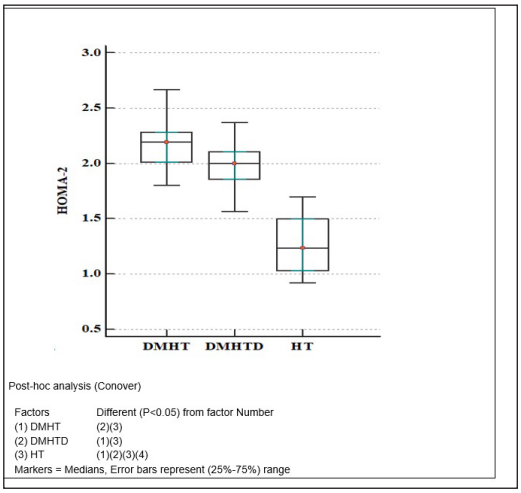

Figure 6: Box plot of HOMA2-IR comparison among the studied groups presenting post-hoc analysis (Conover) pairwise comparison

Table 4: Correlation matrix (Spearman) / Group hypertensive subjects patients with T2DM using captopril (DMHTD)

Variables	*TLR4 ng/mL*	*RETN ng/mL*	*ACE*	*Insulin uIU/mL*	*HOMA1-IR*	*HOMA2-IR*	*%Beta-cell*	*%S*
Age	0.04	-0.01	0.03	0.34	0.21	0.21	-0.23	-0.08
BMI	0.36	0.44	0.21	0.11	0.03	0.14	0.02	-0.46
FBS	0.15	0.16	0.19	0.23	0.89	0.64	-0.92	-0.56
HbA1c	0.25	0.09	0.08	-0.08	0.42	0.34	-0.51	-0.30
Systolic Blood pressure	0.19	0.31	-0.25	-0.07	-0.27	-0.33	0.34	0.25
Diastolic Blood pressure	0.01	0.20	-0.07	0.09	-0.12	-0.09	0.18	0.15
urea	0.22	0.27	0.30	-0.03	-0.03	-0.01	-0.12	-0.18
creatinine	0.20	0.11	0.31	-0.08	-0.19	-0.13	0.04	-0.08
ALT	0.00	0.01	-0.03	0.14	0.07	-0.01	0.08	0.05
AST	0.08	0.10	-0.09	0.18	0.09	0.22	-0.01	-0.02
Cholesterol	0.19	0.00	0.01	0.03	0.06	-0.04	0.00	0.15
triglycerides	0.17	-0.18	-0.06	-0.02	-0.09	-0.16	0.06	0.19
HDL	0.08	0.31	0.12	0.11	0.25	0.28	-0.16	-0.33
LDL	0.22	0.09	0.06	-0.01	0.11	0.00	-0.05	0.10
VLDL	0.17	-0.18	-0.06	-0.02	-0.09	-0.16	0.06	0.19
TLR4ng/mL	1	0.35	0.35	0.09	0.19	0.10	-0.06	0.32
RETN ng/m	0.35	1	0.31	0.15	0.22	0.20	-0.09	-0.13
ACE Activity U/mL	0.35	0.31	1	-0.03	0.10	0.10	-0.30	-0.41
Insulin uIU/mL	0.09	0.15	-0.03	1	0.57	0.79	-0.08	-0.46
HOMA1-IR	0.19	0.22	0.10	0.57	1	0.86	-0.74	-0.65
HOMA2-IR	0.10	0.20	0.10	0.79	0.86	1	-0.58	-0.72
%Beta-cell	-0.06	-0.09	-0.30	-0.08	-0.74	-0.58	1	0.56
%S	0.32	-0.13	-0.41	-0.46	-0.65	-0.72	0.56	1

Values in bold are different from 0 with a significance level alpha=0.05

Table 5: Correlation matrix (Spearman)/Group hypertensive patients with T2DM (DMHT)

Variables	*TLR4 ng/mL*	*RETN ng/mL*	*ACE (U/mL) activity*	*Insulin uIU/mL*	*HOMA1-IR*	*HOMA2-IR*	*%Beta-cell*	*%S*
Age	0.08	0.19	-0.06	-0.38	-0.13	-0.23	-0.16	0.08
BMI	0.15	0.01	0.17	0.01	0.20	0.18	-0.21	-0.19
FBS	0.31	0.13	0.22	0.16	0.87	0.63	-0.97	-0.52
HbA1c	0.16	0.03	0.33	0.35	0.78	0.68	-0.72	-0.55
Systolic Blood pressure	0.44	0.86	0.40	0.23	0.11	0.25	0.06	-0.08
Diastolic Blood pressure	0.41	0.78	0.35	0.16	0.43	0.39	-0.09	-0.20
Urea	0.11	0.26	0.00	-0.14	-0.09	-0.13	0.07	0.03
Creatinine	0.32	0.34	0.13	-0.03	-0.25	-0.23	0.29	0.15
ALT	0.13	0.01	0.22	0.06	0.14	0.01	-0.17	-0.35
AST	0.32	0.32	0.34	-0.14	-0.03	-0.01	-0.02	0.01
Cholesterol	0.12	0.41	0.50	-0.17	-0.01	-0.02	-0.01	0.06
Triglycerides	0.05	0.28	0.26	-0.21	0.08	0.07	-0.09	-0.11
HDL	-0.23	-0.39	-0.04	-0.11	0.17	0.06	-0.19	-0.04
LDL	0.16	0.28	0.23	0.14	-0.24	-0.18	0.27	0.11

Variables	*TLR4 ng/mL*	*RETN ng/mL*	*ACE(U/mL) activity*	*Insulin uIU/mL*	*HOMA1-IR*	*HOMA2-IR*	*%Beta-cell*	*%S*
VLDL	0.05	0.28	0.26	-0.21	0.08	0.07	-0.09	-0.11
TLR4 ng/mL	1	0.62	0.17	0.05	0.30	0.17	-0.37	-0.08
RETN ng/m	0.62	1	0.46	0.71	0.41	0.49	0.11	-0.22
ACE activity U/mL	0.17	0.46	1	0.41	0.50	0.38	0.18	-0.16
Insulin uIU/mL	0.05	0.71	0.41	1	0.51	0.75	0.00	-0.57
HOMA1- IR	0.30	0.41	0.50	0.51	1	0.90	-0.79	-0.74
HOMA2-IR	0.17	0.49	0.38	0.75	0.90	1	-0.51	-0.73
%Beta-cell	-0.37	0.11	0.18	0.00	-0.79	-0.51	1	0.42
%S	-0.08	-0.22	-0.16	-0.57	-0.74	-0.73	0.42	1

Values in bold are different from 0 with a significance level alpha = 0.05

Table 6: Correlation matrix (Spearman)/Group hypertensive subjects (HT)

Variables	*TLR4 ng/mL*	*RETN ng/mL*	*ACE(U/mL) activity*	*Insulin uIU/mL*	*HOMA1-IR*	*HOMA2-IR*	*%Beta-cell*	*%S*
Age	-0.17	0.00	0.07	0.10	0.03	0.08	0.33	-0.08
BMI	0.10	0.44	0.03	0.16	0.21	0.17	0.12	-0.17
FBS	0.10	0.11	-0.07	0.11	0.41	0.21	-0.59	-0.21
HbA1c	0.10	0.11	-0.07	0.11	0.41	0.21	-0.59	-0.21
Systolic Blood pressure	-0.02	0.50	0.04	0.24	0.16	0.23	0.29	-0.23
Diastolic Blood pressure	0.13	0.76	0.32	0.70	0.57	0.67	0.65	-0.67
urea	0.04	0.31	-0.16	0.18	0.10	0.16	0.31	-0.16
creatinine	-0.08	0.00	0.03	0.20	0.14	0.22	0.26	-0.22
ALT	-0.31	-0.10	0.07	-0.04	0.03	-0.04	-0.13	0.04
AST	-0.14	0.17	-0.12	0.13	0.03	0.14	0.23	-0.14
Cholesterol	0.15	0.09	0.07	-0.13	-0.09	-0.12	0.05	0.12
triglycerides	0.12	0.16	0.04	-0.24	-0.19	-0.21	-0.04	0.21
HDL	-0.16	0.24	0.02	0.41	0.33	0.36	0.28	-0.36
LDL	0.18	0.07	0.08	-0.04	-0.01	-0.04	0.10	0.04
VLDL	0.12	0.16	0.04	-0.24	-0.19	-0.21	-0.04	0.21
TLR4 ng/mL	1	0.19	0.59	0.32	0.29	0.32	0.09	-0.32
RETN ng/mL	0.19	1	0.41	0.60	0.54	0.59	0.61	-0.59
ACE activity U/mL	0.59	0.41	1	0.53	0.44	0.51	0.47	-0.51
Insulin uIU/mL	0.32	0.60	0.53	1	0.93	0.99	0.68	-0.99
HOMA1-IR	0.29	0.54	0.44	0.93	1	0.96	0.45	-0.96
HOMA1-IR	0.32	0.59	0.51	0.99	0.96	1	0.62	-1.00
%Beta-cell	0.09	0.61	0.47	0.68	0.45	0.62	1	-0.62
%S	-0.32	-0.59	-0.51	-0.99	-0.96	-1.00	-0.62	1

(DMHT) when compared with the hypertensive group (HT). In addition, serum RETN level is lower in hypertensive patients with T2DM using captopril group comparison with hypertensive patients with T2DM group also lower than hypertensive patients group (DMHT).[14] Several studies reported similar results that included the correlation between serum resistin level and angiotensin converting enzyme activity, especially for hypertensive subjects. The mechanism underlying the correlation between resistin and hypertension remains to be clarified. The mechanism might be mediated via the TLR-4. Moreover, many studies reported that the resistin level was significantly elevated in patients with T_2DM, when analog with individuals without T_2DM.[15-17]

Regarding TLR-4 in present results, there was a significant difference ($p < 0.001$) in means of serum TLR-4 levels in hypertensive patients group (HT), hypertensive patients with T2DM subjects group (DMHT) compared with hypertensive patients with T2DM subjects group using captopril drug (DMHTD). The serum TLR-4 concentration is higher in hypertensive patients with the T2DM group than in the hypertensive patients group. The minimum level of TLR4 in the three study groups was found in hypertensive patients with T2DM subjects group using captopril drug (DMHTD).[16] These results conform with the arrangement of serum resistin levels in all study groups that indicated TLR-4 is a putative receptor for RETN. In addition, the results confirm that TLR-4 is a receptor for RETN that has been suggested to participate in resistin-induced insulin resistance and inflammation. A recent study found Serum TLR-4 raised as a strong biomarker that gives data about the systemic status related to inflammatory conditions.[11] Recently investigation has shown that patients with hypertension showed rising TLR-4 expression on their peripheral monocytes in contrast with those with controlled hypertension.[18] This demonstrates that uncontrolled blood pressure is related to activated innate immunity, which would contribute to organ and tissue damage and thus influence disease prognosis. So, we indicated that the duration of hypertension disease might have an immune-modulating effect.[19]

Regarding serum ACE activity in present results a significant different ($p < 0.05$) between three study groups, lowering activity of ACE was found in a subject that used captopril treatment (hypertensive patients with T2DM using captopril patients group) in comparison with another group. Lowering ACE activity in the subjects that used captopril treatment ACE was the essential effect on lowering serum TLR-4 and resistin levels in this group.

Regarding correlation matrix (Spearman) in this study, the positive correlation between ACE and RETN in hypertensive patients with T_2DM patients group, also positive correlation between it in hypertensive patients group. In addition, correlation matrix (Spearman) for the subject that used captopril treatment was a non-significant correlation between ACE and RETN. The recent study, together with our study, indicates that increasing ACE could cause increase in serum RETN level and causes an inflammatory response and cardiovascular disease.[17]

Moreover, regarding correlation matrix in this study, the positive correlation between RETN and IR (HOMA1-IR, HOMA2-IR) in all groups except the group that used captopril treatment indicates its role in insulin resistance that mediated by inflammatory pathways and endothelial dysfunction also the effect of ACE on RETN.[6,20]

Regarding ACE activity in present results, many studies reported similar results that the inhibition effect of captopril increases the percentage of insulin sensitivity (%S) compared with hypertensive patients with T2DM study group and these result enhanced by the positive correlation between RETN and IR.[21,22]

CONCLUSION

In conclusion, serum levels of ACE, TLR-4 and RETN were higher in hypertensive patients with T2DM subjects group than in hypertensive patients group, minimum level of these markers was found in subject that used captopril treatment (hypertensive patients with T2DM using captopril patients group). It concludes that any increase in ACE could cause an increase in serum RETN level and causes inflammatory response and cardiovascular disease. Moreover, positive correlation between RETN and IR (HOMA1-IR, HOMA2-IR) in all groups except the group that used captopril treatment indicates its role in insulin resistance that mediated by inflammatory pathways and endothelial dysfunction also the effect of ACE on RETN. The serum TLR-4 concentration is higher ln hypertensive patients with T2DM-g. The minimum level of TLR4 in the three study groups was found in hypertensive patients with T2DM subjects group using captopril treatment. It concludes to confirm that TLR-4 is an assumed receptor for RETN that has been suggested to participate in resistin-induced insulin resistance and inflammation conditions.

Then, we suggested a close relationship between TLR4 and RETN affected by ACE activity and their positive association with insulin resistance.

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