The role of serum retinol binding protein 4 in hypertensive male patients and its relationship with metabolic syndrome and associated cardiovascular risk

Bothaina I. Saleh[1], Salwa R. Ali[2], Ghada F. El-Mahaseb[3], Mostafa I. Saleh[4], Amani M. Tawfik[5]

Departments of Clinical Pathology[1], Internal Medicine[2,3], Cadiology[4], Microbiology[5], Faculty of Medicine, Al-Azhar University, Cairo, Egypt
bothina_i@yahoo.com

Abstract: Background: Retinol binding protein 4 (RBP4) is an adipokine that had been reported to induce insulin resistance and to play a role in the pathogenesis and severity of essential hypertension (EH). **Aim:** was to evaluate RBP4 levels in patients with EH and to investigate the relationship between raised serum RBP4 and insulin resistance in hypertensive (HTN) male patients and its relation with the severity of hypertension and risk of left ventricular hypertrophy (LVH). **Subjects and Methods:** serum RBP4, , insulin, , blood glucose, uric acid, creatinine, high sensitivity C-reactive protein (hs-CRP) and lipid profile were investigated in HTN male patients, together with measurement of homeostasis model assessment (HOMA) index for insulin resistance, body mass index (BMI) and waist circumference. The patients were divided into two groups; hypertensive patients with metabolic syndrome (MS) and hypertensive patients without MS and compared with age and sex matched apparently healthy individuals as control. **Results:** HTN patients with MS were found to have significant higher values of RBP4, BMI, waist circumference, HOMA index, uric acids and triglycerides with high prevalence of LVH compared to patient group without MS. Serum RBP4 was found to be positively correlated with high levels of systolic and diastolic blood pressure and with high values of HOMA index, and negatively correlated with HDL. **Conclusion:** serum RBP4 can be used as a predictive marker for the severity of hypertension and associated risk of LVH in HTN patients with MS.

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Introduction

Essential hypertension (EH) is a major public health problem of worldwide distribution. It is responsible for one half of coronary heart disease and about two thirds of cerebrovascular accidents **(Jaddou *et al.*, 2011)**. Accumulating evidence suggested that insulin resistance plays an important role in the development of essential hypertension **(Anan *et al.*, 2003)**.

Metabolic syndrome (MS) is the clustering of abnormalities in glucose metabolism, lipid metabolism, and blood pressure (**Park *et al.*, 2011)**. Abdominal obesity and insulin resistance appear to be the predominant underlying risk factors for its pathogenesis **(Lin *et al.*, 2009)**. MS had been found to be associated with an increased prevalence of subclinical damage in a variety of organs and increase the risk of organ damage in hypertensive patients (**Park *et al.*, 2011)**.

It was well established that, adipose tissue is the main site of inflammatory and proatherogenic mechanisms that play an important role in the pathogenesis of diffuse vascular damage **(Fantuzzi *et al.*, 2007)**. Additionally, excess secretion of various adipocyte-derived molecules had been linked with insulin resistance, obesity, type 2 diabetes and cardiovascular disease **(Chiba *et al.*, 2010)**.

Retinol-binding protein 4 (RBP4) is a transport protein for retinol (vitamin A) in the circulation. It transports retinol from the liver to the peripheral tissues **(Kotnik *et al.*, 2011)**. It is synthesized mainly by the hepatocyte and secreted into the circulation bound to vitamin A and transthyretin. Although hepatocytes are regarded as the principal source of circulating RBP 4 under normal conditions, adipose tissue has the second highest expression level **(Christou *et al.*, 2012)**.

Various studies had demonstrated the possible importance of RBP4 as a mediator for insulin resistance (**Yao-Borengasser *et al.*, 2007)**. The glucose-transporter 4 (GLUT4) is the major glucose transporter protein that mediates glucose uptake in adipose tissue and skeletal muscle, and it is thus a key regulator of glucose homeostasis **(Matsuzawa, 2006)**. In insulin resistant states, the expression of GLUT4 is down-regulated selectively in adipocytes but not in skeletal muscle; this results in impaired insulin-stimulated glucose transport in adipocytes, which precedes glucose intolerance **(Smith, 2002)**. Down regulation of adipocyte GLUT4 in insulin

resistance states and type 2 diabetes is therefore thought to represent the mechanism for up-regulation of RBP4 secretion **(Mostafaie *et al.*, 2011)**.

Insulin resistance (IR) is typically defined as decreased sensitivity and/or responsiveness to metabolic actions of insulin that promote glucose disposal. This important feature of diabetes, obesity, glucose intolerance, and dyslipidemia is also a prominent component of cardiovascular disorders, including hypertension, coronary artery disease, and atherosclerosis, which are characterized by endothelial dysfunction **(Kim *et al.*, 2006)**.

Even in the absence of hyperglycemia or diabetes, IR contributes to an increased risk of cardiovascular disease and constitutes a pathophysiologic link between obesity and atherosclerosis **(Suh *et al.*, 2010)**. A major risk factor for developing insulin resistance is obesity (**Mostafaie *et al.*, 2011)**.

Left ventricular hypertrophy (LVH), one of the complications of EH is characterized by an increase in chamber mass produced largely by an increase in the size of cardiomyocytes **(Mitsuhashi *et al.*, 2007)** and it is, in turn an important risk factor for myocardial infarction, heart failure, stroke, and sudden cardiac death **(Guerra *et al.*, 2011)**.The determinants of LVH include age, elevated blood pressure (BP), obesity, and insulin resistance **(Mitsuhashi *et al.*, 2007)**.

The aim of this work was to evaluate RBP4 levels in patients with EH and to investigate the relationship between raised RBP4 serum levels and insulin resistance in hypertensive (HTN) male patients and its relation with the severity of hypertension and risk of LVH.

2. Subjects and methods

A cross sectional study was carried out in the period between January 2011 and May 2011. Twenty five HTN male patients, (age range between 45 and 59 years), admitted to the Internal Medicine Department of Al – Zahraa University Hospital, Cairo, Egypt, were included in this study. Fifteen - age and sex matched- apparently healthy individuals were taken as a control group. Consent was obtained from all subjects before inclusion in the study.

2.1) Exclusion criteria

Patients with the following conditions were excluded from the study: those with secondary causes of hypertension, diabetes, liver failure, renal failure (serum creatinine levels greater than 1.4 mg/dL), congestive heart failure, valvular heart disease, previous myocardial infarction, atrial fibrillation, previous stroke, systemic inflammatory or neoplastic disease and those taken lipid lowering agents within the last 6 months to rule out the effect of these drugs.

All participants were subjected to the following:

2.2) Full history and complete clinical examination

According to **Joint National Committee (JNC 7) guidelines, 2004**, EH was defined as blood pressure ≥140/90 mmHg or use of antihypertensive treatment at the time of enrollment in the study with absence of clinical signs suggestive of secondary hypertension. Also, HTN patients were classified into 2 stages; stage I (140-159/90-99 mmHg) and stage II (≥160/100 mmHg) hypertension.**(Chobanian *et al.*, 2003)**

MS was diagnosed based on the Third Report of the National Cholesterol Education Program's Adult Treatment Panel **(NECP ATP III, 2002)**, if 3 or more of the following conditions were present: high blood pressure (systolic and/or diastolic blood pressures ≥130/85 mmHg or patients receiving anti-hypertensive drugs), hyperglycemia (fasting plasma glucose ≥110 mg/dL or patients receiving oral hypoglycemic agents), hypertriglyceridemia (fasting plasma triglycerides ≥150 mg/dL), low high-density lipoprotein cholesterol (HDL-C) (fasting HDL-C < 40 mg/dL for men), or central obesity (waist circumference of ≥90 cm for men).

Body mass index (BMI) was calculated as the ratio of the weight to the height squared (kg/m^2). Waist circumference was taken with a tape measure horizontally at the umbilicus, midpoint between the lower rib margin and the iliac crest while subjects were in the standing position after normal expiration.

2.3) Imaging study

All HTN patients had subjected to transthoracic echocardiography for diagnosis of LVH. Left ventricular dimensions were measured by echocardiography (Powervision 6000, Toshiba**,** probe frequency 2.5 MHz) following the American Society of Echocardiography recommendations **(Sahn *et al.*, 1978)**. For each patient the following measurements were taken: end-diastolic and end-systolic interventricular septum thickness (IVSD and IVSS, respectively), posterior wall thickness (PWD and PWS, respectively), and left ventricular diameters (LVDD and LVDS, respectively); left atrial diameter (LAD). LVM was calculated (M-mode tracings under two-dimensional control, left parasternal short axis view, mean of three cardiac cycles) by using the Devereux's formula and indexed by $height^{2.7}$ ($LVM/h^{2.7}$) **(De Simone *et al.*, 1995)**. LVH was defined on the basis of the $LVH/h^{2.7}$, using ≥ 49.2 $g/m^{2.7}$in men as partition values **(Gerdts *et al.*, 2008)**.

2.4) laboratory investigations

After 12 hrs overnight fasting, venous blood samples were obtained from the subjects by venipuncture under aseptic condition. The samples were transferred into clean, plain tubes and centrifuged within 30 minutes of collection for 10 minutes; part of the serum was stored at – 20 °C until it was assayed for RBP4. The remaining part was used for measurement of the following parameters:

- Lipid profile was measured photometrically using Cobas C-311 autoanalyzer. Reagents were supplied by Roche diagnostics (F. Hoffmann-La Roch Ltd., Basel, Switzerland). Low density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula (**Friedewald *et al.*, 1972)**.
- Serum creatinine, uric acid and blood glucose levels were measured by enzymatic colorimetric assay, using Cobas C-311 autoanalyzer. Reagents were supplied by Roche diagnostics.
- hs-CRP was done by ELISA using a complete set of ELISA reader model SLT Spectra 216687. The Kits was supplied by Monobind Inc. CA, USA. (Product code 3125-300).
- Insulin level was measured by chemiluminescent immunoassay using an Immulite 2000 analyzer (Siemens Healthcare Diagnostics Inc., West Sacramento, Calif., USA). Insulin resistance, defined by the homeostasis model assessment IR (HOMA-IR), was calculated using the following equation: HOMA-IR = fasting insulin (µU/L) X fasting glucose (mmol/L)/22.5 (**Suh *et al.*, 2010)**. IR was defined if HOMA index ≥ 2.5 according to the Japanese guideline for the treatment of diabetes **(Matthews *et al.*, 1985, Hara *et al.*, 2006)**.
- RBP4 serum level was measured by ELISA using a complete set of ELISA reader model SLT Spectra 216687 with Quantikine® Human RBP4 ELISA Kit supplied by R&D Systems, Inc. Minneapolis, MN, USA (Cat. no. DRB400, Lot no.293307).

Statistics

All statistical calculations were performed using a commercially available statistical package for social science (SPSS) version 17. Qualitative data were presented as number and percentages while quantitative data were presented as mean, standard deviations and ranges. The comparisons between two groups with quantitative data was done by using Independent t-test for parametric data and Mann-Whitney test for non parametric while comparison between two groups with qualitative data was done by using Chi-square test. The Pearson correlation coefficient was used to correlate and assess the relation between two parameters in the same group. A value of $p \leq 0.05$ was considered statistically significant (Sokal and James,1995).

3. Results

Our study included 35 male subjects: 15 healthy individuals and 25 HTN (15 patients with stage I HTN and 10 with stage II HTN). Thirteen hypertensive patients were met the NCEP criteria for MS and the rest 12 patients were categorized as HTN without MS.

Table 1: comparison of clinical and biochemical parameters between hypertensive patients and control group

Groups / Variables	Patients (n=25)		Control (n=15)		Independent t-test		
	Mean	±SD	Mean	±SD	T	*p*-value	
Age (years)	49.32	3.26	48.30	3.97	1.748	0.098	NS
BMI (kg/ m^2)	27.79	1.03	24.73	1.48	7.000	0.01	S
Waist C. (cm)	83.36	5.72	75.90	5.36	3.547	0.001	HS
SBP (mmHg)	159.60	12.41	113.00	6.75	11.165	0.000	HS
DBP (mmHg)	98.00	7.64	74.00	5.16	9.099	0.000	HS
T.cholesterol (mg/dl)	205.00	43.49	134.20	13.54	5.011	0.000	HS
HDL-C (mg/dl)	42.60	11.28	49.70	5.74	-1.884	0.068	NS
LDL-C (mg/dl)	133.00	45.16	66.10	13.62	4.565	0.000	HS
TG (mg/dl)	147.00	50.56	92.00	21.37	3.300	0.002	HS
S. creatinine (mg/dl)	0.95	0.18	0.93	0.13	0.344	0.733	NS
FBG (mg/dl)	100.16	13.89	95.10	13.34	0.984	0.332	NS
Insulin level (µU/ml)	16.16	9.83	6.90	2.47	2.919	0.006	HS
HOMA-IR	4.04	2.60	1.63	0.64	2.876	0.007	HS
RBP4 (ng/ml)	105.34	54.38	27.71	13.52	4.423	0.000	HS
hs-CRP (µg/mL)	1.12	0.87	0.83	0.28	1.039	0.306	NS
Uric acid (mg/dl)	6.10	1.27	4.80	0.81	2.977	0.005	HS

BMI: body mass index, Waist C: waist circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure, T. cholesterol: total cholesterol, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol,TG: triglycerides, FBG: fasting blood glucose, HOMA: homeostatic model assessment of IR, RBP4: retinol binding protein 4, hs-CRP: highly sensitive C reactive protein, LVH: left ventricular hypertrophy, S: significant, NS: non significant, HS: highly significant.

There was a significant increase in RBP4 in HTN patients compared to control groups (Table 1, Fig.1). Also, there were significant difference in BMI, waist circumference, systolic and diastolic BP, total and LDL-C, TG, insulin, HOMA index, and uric acid between HTN patients and control groups. Serum creatinine, HDL-C, fasting blood glucose and hs-CRP showed no significant difference.

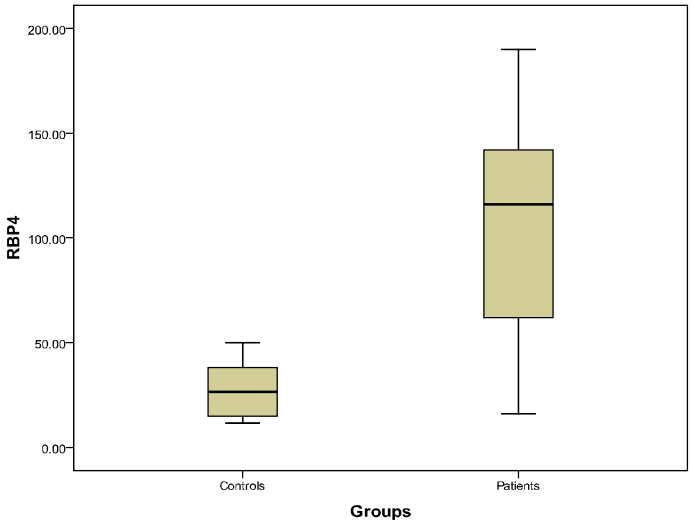

Figure 1: comparison between hypertensive patients and control group as regard serum level of RBP4

Table 2: Comparison of Clinical characteristics and biochemical parameters of hypertensive patients

Groups / Variables	HTN with MS (n=13)		HTN without MS (n=12)		Independent t-test		
	Mean	±SD	Mean	±SD	T	*P*-value	
Age (years)	51.23	3.32	47.25	1.49	3.811	0.001	HS
BMI (kg/ m^2)	28.40	0.74	25.12	0.88	3.920	0.01	S
Waist C.(cm)	92.38	2.93	78.67	1.83	6.515	0.001	HS
SBP (mmHg)	166.92	9.47	146.67	4.92	6.620	0.001	HS
DBP (mmHg)	103.08	6.30	90.00	0.00	7.174	0.001	HS
T. cholesterol (mg/dl)	242.15	20.51	164.75	16.43	10.357	0.001	HS
HDL-C (mg/dl)	32.92	5.33	53.08	3.99	-10.634	0.001	NS
LDL-C (mg/dl)	171.54	19.59	91.25	19.35	10.298	0.001	HS
TG (mg/dl)	188.46	31.38	102.08	16.44	8.508	0.001	HS
Insulin level (µU/ml)	24.08	6.38	7.58	3.45	7.937	0.001	NS
HOMA-IR	6.21	1.59	1.70	0.67	9.078	0.001	NS
RBP4 (ng/ml)	144.62	26.65	62.79	43.35	5.737	0.001	HS
hs-CRP (µg/dl)	1.56	1.01	0.65	0.28	3.017	0.006	HS
Uric acid (mg/dl)	6.98	1.05	5.14	0.65	5.218	0.001	HS
Smoking n(%)	10 (83.3%)		5 (41.7%)		1.930	0.164	NS
LVH n(%)	11 (84.6%)		3 (25.0%)		6.744	0.009	HS

MS: metabolic syndrome, BMI: body mass index, Waist C: waist circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure, T. cholesterol: total cholesterol, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, TG: triglycerides, FBG: fasting blood glucose, HOMA: homeostatic model assessment of IR, RBP4: retinol binding protein 4, hs-CRP: highly sensitive C reactive protein, LVH: left ventricular hypertrophy, NS: non significant, S: significant, HS: highly significant.

The hypertensive patients with MS had significantly higher values of body mass index, waist circumference, systolic and diastolic BP, total and LDL-C, triglycerides, insulin, HOMA index, uric acid, RBP4 and hs-CRP with significantly lower values of HDL-C in comparison to the hypertensive patients without MS. Also, there was significant increase in the percentage of LVH in patients with MS.

Table 3: Comparison between the studied patient groups as regard serum RBP4 level and the presence of LVH

	Stage I hypertensive (n=10)	Stage II hypertensive (n=15)	Hypertensive with MS (n=13)	Hypertensive without MS (n=12)
RBP4	52.00±38.43	140.90±27.07	144.62±26.65	62.79±43.35
***P* value**	<0.01 (S)		<0.01 (S)	
LVH %	4 (40.0%)	10 (66.7%)	11 (84.6%)	3 (25.0%)
***P* value**	0.097 (NS)		0.009 (HS)	

RBP4: retinol binding protein 4, MS: metabolic syndrome, LVH: left ventricular hypertrophy, S: significant, NS: non significant, HS: highly significant.

RBP4 was found to be significantly increased in HTN patients with MS and stage II hypertensive patients compared to in HTN patients without MS and stage I hypertensive patients respectively. Also, There was a highly significant increase in the incidence of LVH in HTN patients with MS compared to in HTN patients without MS with no significant increase in the percent of LVH in patient group with stage II hypertension compared to those with stage I hypertension (Figure 2).

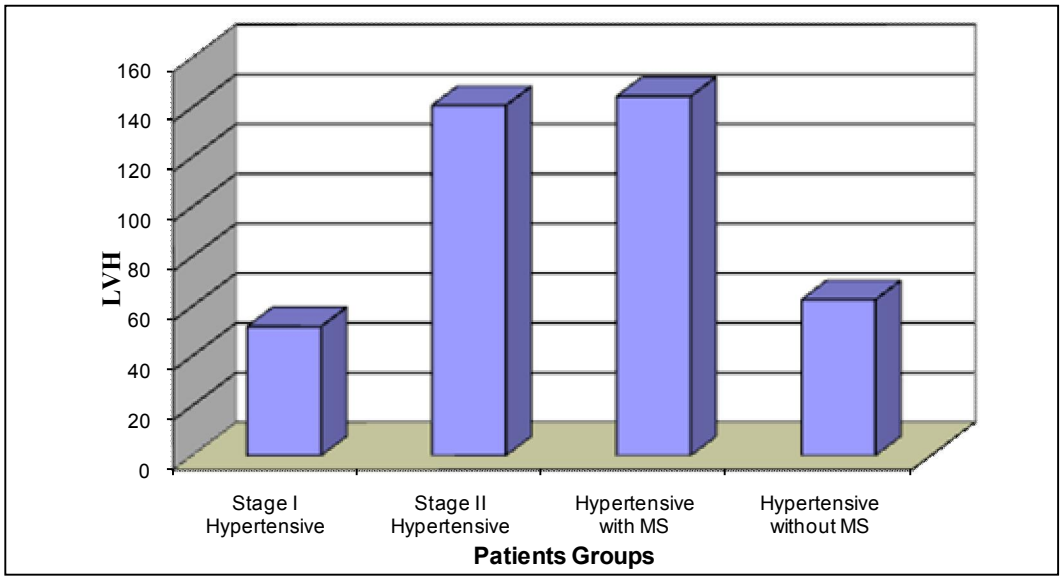

Figure 2: Comparison between the studied patient groups as regard the presence of LVH

Table 4: Comparison of serum RBP4 levels in the patient groups versus control group

Groups / Variables	Control (RBP4 level = 27.71±13.52)			
	Stage I hypertensive	Stage II hypertensive	Hypertensive with MS	Hypertensive without MS
RBP4 level	52.00±38.4	135.95±26.72	144.66±26.65	62.79±43.35
***P* value**	0.076 (NS)	0.000 (HS)	0.000 (HS)	0.023 (S)

RBP4: retinol binding protein 4, MS: metabolic syndrome, NS: non significant, S: significant, HS: highly significant.

This table showed that, although there was a non significant difference in the level of RBP4 when comparing control group with patients with stage I hypertension, but there was a highly significant increase in HTN patients with MS and patients with stage II hypertension, with only a significant difference when comparing HTN patients without MS to control group (Figure 3).

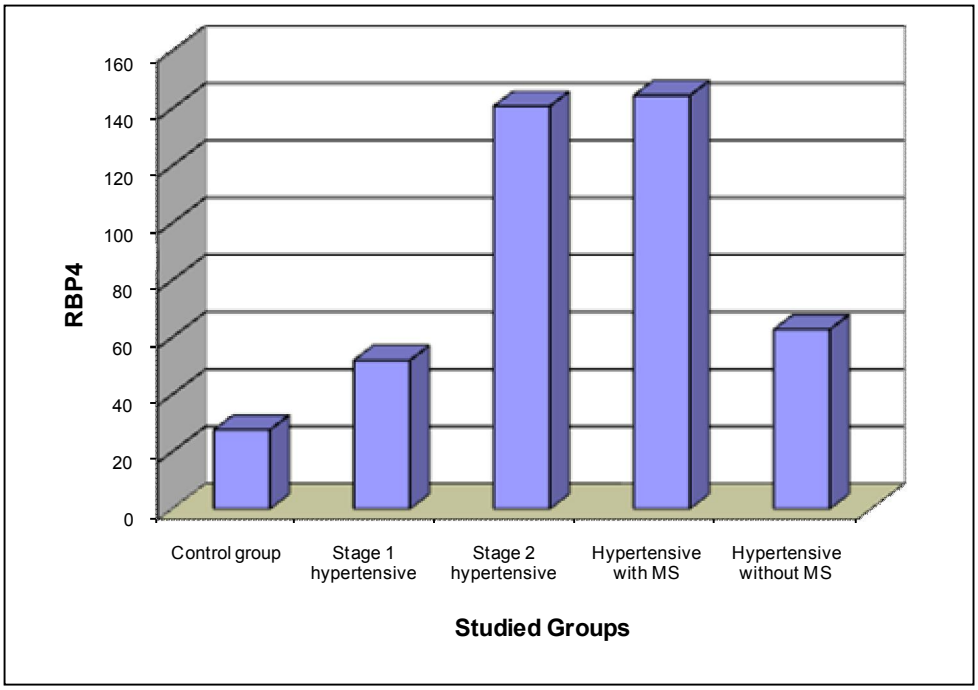

Figure 3: Comparison of serum RBP4 levels in the patient groups versus control group

Table 5: correlations between RBP4, hs-CRP and other clinical and biochemical characteristics in hypertensive patients group

	RBP4		hs-CRP	
	r	*P*	R	*P*
Age	0.915**	0.001	0.565*	0.044
BMI	0.820**	0.001	0.497	0.084
Waist C.	0.982**	0.001	0.567*	0.044
SBP	0.929**	0.001	0.579*	0.038
DBP	0.662*	0.014	0.206	0.500
T.cholesterol	0.778**	0.002	0.407	0.168
HDL-C	-0.847**	0.001	-0.372	0.210
LDL-C	0.764**	0.002	0.356	0.232
TG	0.877**	0.001	0.533	0.061
Creatinine	0.779**	0.002	0.509	0.075
FBG	0.315	0.295	0.005	0.986
Insulin	0.627*	0.022	0.393	0.184
HOMA	0.861**	0.001	0.429	0.144
uric acid	0.788**	0.001	0.380	0.200

RBP4: retinol binding protein 4, hs-CRP: highly sensitive C reactive protein, BMI: body mass index, Waist C: waist circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure, T. cholesterol: total cholesterol, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, TG: triglycerides, FBG: fasting blood glucose, HOMA: homeostatic model assessment of IR, ** Highly significant, * significant.

There were highly significant positive correlations between RBP4 and age, BMI, waist circumference, systolic and diastolic BP, total and LDL-C, triglyceride, insulin and HOMA index together, with significant negative correlation of RBP4 and HDL-C. Hs-CRP was found to have a significant positive correlation with age, waist circumference, and SBP and negative correlation with HDL-C with no significant correlations with other parameters.

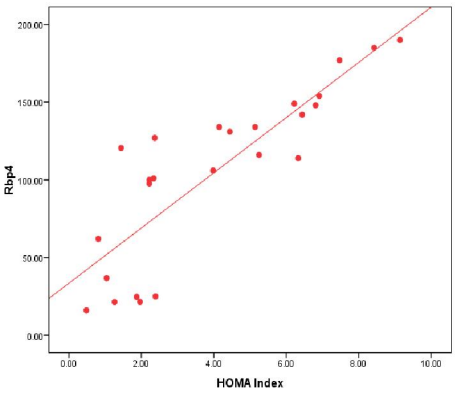

Fig. (4.1)

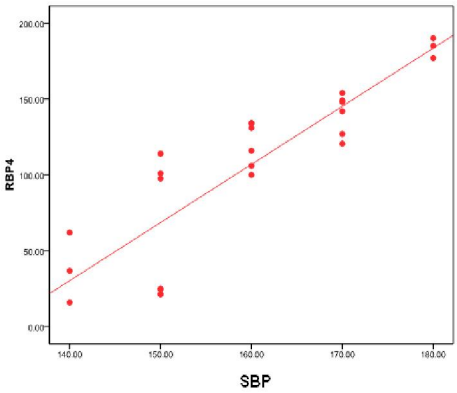

Fig. (4.2)

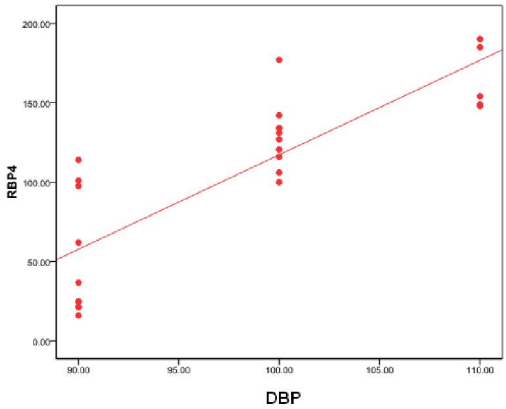

Fig. (4.3)

Figure 4: Linear relationship between the level of serum RBP4 and HOMA index (4.1); or SBP (4.2); and DBP (4.3) in hypertensive patients group

4.Discussion

Epidemiologic studies had demonstrated that hypertension is usually accompanied by major cardiovascular risk factors with less than 20% of hypertension occurring as an isolated manifestation. The risk of cardiovascular events in hypertensive patients increases in proportion to the burden of associated risk factors **(Shim *et al.*, 2010)**

Some inflammatory mediators originating from the adipose tissue can lead to local insulin resistance with an impaired inhibitory effect of insulin on the release of free fatty acids and endothelial dysfunction. Among these inflammatory mediators is retinol binding protein-4, an adipocytokine linked to insulin resistant states in humans and predicting the development of type 2 diabetes. RBP4 levels are also increased in other clinical conditions characterized by increased cardiovascular risk and a certain degree of insulin resistance, such as essential hypertension and metabolic syndrome **(Solini *et al.*, 2012)**.

RBP 4 is regarded as a novel cardiometabolic risk factor that is upregulated in insulin resistant states associated with obesity **(Christou *et al.*, 2012)**. The positive relationship between RBP4 and atherosclerosis that can lead to elevated BP may be explained by the findings that higher fat content in the vessel wall and in atherosclerotic plaques might reflect lipid-modulation activities of retinoids and retinol-binding proteins **(Solini *et al.*, 2009)**.

In the present study, comparison between HTN patients and healthy control revealed that there was a highly significant difference in systolic and diastolic BP between the two groups. Also, total and LDL-C, TG, HOMA index, RBP4 and uric acid among HTN patients were higher than control group, while serum creatinine, HDL-C, fasting blood glucose, and hs-CRP showed no significant difference.

Our results also, showed that HTN patients with MS had higher values of serum RBP4, systolic and diastolic BP, BMI, waist circumference, and serum TG, with significant decrease in HDL-C when compared with patients without MS. Also, RBP4 was found to be significantly higher in stage II HTN patients than stage I group indicating its relationship with the severity of HTN.

This was in consistent with **Park *et al.*, 2011** who found that, BMI and waist circumference were significantly higher in the HTN with MS group than in HTN patients without MS. TG levels were elevated and HDL-C levels were significantly reduced in the HTN with MS group.

In our study, RBP4 was found to be significantly correlated with SBP (r = 0.929, p = 0.001), DBP (r = 0.662, p = 0.001), waist circumference (r = 0.982, p = 0.001), HOMA index (r = 0.861, p = 0.001), and TG (r = 0.877, p = 0.001), but negatively correlated with HDL-C (r = -0.847, p = 0.001).

This in consistent with **Mostafaie *et al.*, 2011** who reported that, RBP4 concentrations were correlated strongly with TG and inversely correlated with HDL-C. The observed association between RBP4 and TG may be due to the lipid-modulating activities of retinoids and retinol-binding proteins **(Mostafaie *et al.*, 2011)**.

Another explanation by **Kim *et al.*, 2011** study, who confirmed the correlation between RBP4 concentrations and TG levels and suggested that, RBP4 metabolism appears to be closely connected with liver fat. Hypertriglyceridemia, led by hyperinsulinemia, may subsequently provoke the synthesis and secretion of RBP4 from the liver or ectopic fat.

Our results coincides with **Lin *et al.*, 2009, Chiba *et al.*, 2010 and Suh *et al.*, 2010** who reported that serum RBP4 concentrations were positively correlated with age, DBP, and HOMA-IR.

Mostafaie *et al.*, 2011 also reported that, BMI, insulin concentrations and HOMA-Index were correlated significantly with RBP4 concentrations. However, he found that there was no significant association between RBP4 and BP.

Consistently, circulating RBP4 had been found by **Qi *et al.*, 2007** and **Ingelsson *et al.*, 2009** to be positively correlated with elevated TG, and high blood pressure, body mass index, waist circumference and waist to hip ratio, indicating that RBP4 is related with abdominal obesity.

In our study, hs-CRP was found to be significantly increased in HTN patients with MS in comparison to patients without MS (P<0.006), while there was no significant increase in hs-CRP in HTN patients compared to control group (P=0.306). Hs-CRP was found to have significant positive correlations with age (r = 0.565, P = 0.044) waist circumference (r = 0.579, P = 0.038) and SBP (r = 0.567, P = 0.044) and negative correlation with HDL-C (r = -0.372, P = 0.210) with no significant correlations with other parameters.

Hyperuricemia was related to an increased incidence of high BMI, high BP and high TG **(Park *et al.*, 2011)**. It was also found to be associated with the development of end-organ damage such as increased carotid intimal media thickness **(Ishizaka *et al.*, 2005)** and it is regarded as a cardiovascular risk factor and a determinant of MS **(Coutinho *et al.*, 2007)**. Uric acid, in our study, was found to be significantly elevated in HTN with MS group which was in agree with a study conducted by **Park *et al.*, 2011** who found that the HTN with MS group had significantly higher values of serum uric acid than the HTN without MS group.

On the other side, some studies had reported contradictory findings regarding the association of RBP4 with IR and other metabolic parameters. **Shim *et al.*, 2010** study revealed that, there were no significant differences in blood pressure, total cholesterol, LDL-C, hs-CRP and metabolic parameters among non diabetic HTN patients with and without MS. Also, he found that there was no significant correlation of RBP4 with waist circumference, HDL-C, and the HOMA index, although RBP4 was positively correlated with TG and uric acid. These findings were in accordance with findings reported by **Takashima, *et al.*, 2006** which showed that serum RBP4 did not correlate with fasting blood glucose, HDL-C, waist hip ratio, BMI, and fasting insulin, HOMA-IR, but significantly correlated with TG.

Qi *et al.*, 2007 and Takebayashi *et al.*, 2007 had found that there was no correlation between RBP4 and hs-CRP. This may explained by that, although high levels of CRP and RBP4 were closely associated with an increased risk of MS, increased RBP4 per se was an independent risk factor for the MS, even within the lowest CRP. Therefore, it is possible that RBP4 may promote the MS risk through a pathway not fully overlapping with CRP

There were some other possible explanations for this controversy between our study and other studies. First, HOMA-IR may not be an ideal measurement of insulin sensitivity, especially in subjects with impaired fasting glucose or impaired glucose tolerance **(Tripathy *et al.*, 2004)**. **Stefan *et al.*, 2007** had reported that RBP4 is associated with an elevation of liver fat but not visceral fat (abdominal obesity) in humans. There may be some other factors responsible for the contradictory results. Perhaps the difference in results may be attributed to a difference in ethnicity, degree of obesity, and subset of the patients analyzed.

LVH represents an independent risk factor for cardiovascular morbidity and mortality in essential hypertension **(Sciacqua *et al.*, 2011)**. Cardiomyocyte hypertrophy can be triggered by activation of insulin signaling pathways or altered adipokine levels suggesting that metabolic alterations can play a role

in the pathophysiology of LVH. Insulin stimulates protein synthesis and inhibits protein breakdown in the heart, and clinical studies had found that elevated plasma insulin is associated with LVH **(Sharma *et al.*, 2007)**.

Circulating RBP4 had been found to be associated with some measures of subclinical cardiovascular disease. Specifically, plasma RBP4 levels had been shown to be positively correlated with the echocardiographically measured left ventricular wall thickness and carotid intima-media thickness (**Christou *et al.*, 2012)**.

In our study we found that there was an increase in the prevalence of LVH in HTN patients with MS (84.6% versus 25.0% in patients without MS) with significant increase in the percent of LVH in patients group of stage II hypertension (66.7% versus 40.0% in stage I HTN patients). Our results were in concordance with **de Simone *et al.*, 2009** who reported that MS is associated with increased prevalence of LVH. **Guerra *et al.*, 2011** reported that, HTN patients with MS had significantly higher BMI, systolic and mean BP, interventricular septum than those without MS.

Conclusion:

Serum RBP4 was found to be strongly associated with MS in HTN patients especially those with stage II hypertension with positive correlation with systolic and diastolic BP and increased prevalence of LVH. So, circulating RBP4 may be considered as a potential MS promoting molecule that can be used as a predicting marker for the severity of hypertension and the risk of LVH in HTN patients.

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