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Serum Retinol Binding Protein-4 and Insulin Resistance in Post-menopausal Women with Cardiovascular Disease

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Abstract

Background: Retinol binding protein-4 (RBP-4) is a newly discovered adipokine, which is mainly secreted by liver and originally known to be the only specific transport protein for vitamin A in the circulation. Recently, RBP-4 is found to be expressed in adipose tissue and correlated with obesity, insulin resistance (IR) and type 2 diabetes mellitus (T2DM). It is well known that there is a higher prevalence of cardiovascular risk factors and metabolic syndrome (MS) in postmenopausal women. Postmenopausal status is believed to be a risk factor for IR in women. IR has a causal role in the development of T2DM. Even in the absence of hyperglycemia or diabetes, IR contributes to an increased risk of cardiovascular disease (CVD).

Objective: The aim of the present work was to evaluate the influence of menopausal status on RBP-4 concentration and to investigate the association of plasma RBP-4 with IR in post-menopausal women with and without CVD.

Subjects and Methods: The present study included 25 apparently healthy premenopausal women, 25 apparently healthy postmenopausal women and 25 postmenopausal women With CVD. Blood samples were collected from all subjects and the level of plasma RBP-4 and plasma insulin were measured by enzyme linked immunossorbent assay (ELISA).

Results: RBP-4 concentrations in postmenopausal women were higher than those in premenopausal women. Postmenopausal women with CVD have higher plasma RBP-4 concentrations than in healthy postmenopausal women and were positively correlated with age, TC, TG, TG/HDL-C, fasting plasma glucose, postprandial glucose, plasma insulin, HOMA-IR and ALT.

Conclusions: Postmenopausal women with CVD showed significantly higher plasma RBP-4, blood pressure (systolic and diastolic), lipid profile (TC, LDL-C, TG and TG/HDL-C ratio), insulin and HOMA-IR than those in postmenopausal women. In addition, RBP-4 significantly positively correlated with FG, postprandial glucose, insulin, HOMA-IR, TC, TG, TG/HDL-C ratio and ALT.

Keywords: Retinol binding protein-4, insulin resistance, cardiovascular disease, Postmenopausal women.

1. Introduction

Cardiovascular diseases (CVD) are a principal cause of death worldwide and are linked to obesity and metabolic syndrome. Several adipokines secreted by the increased adipose tissue mass, together with the infiltrating macrophages, have been identified as key components of the 'adipo-cardiovascular axis' and are main contributors to the pathogenesis of atherosclerosis and other cardiovascular diseases. Among these, RBP4 has been identified as adipokines associated with obesity, type 2 diabetes (T2DM) and metabolic syndrome[1].

Retinol binding protein-4 (RBP-4), is the sole carrier of retinol (vitamin A) in blood and serves to transport it from liver stores to the peripheral tissues. Circulating RBP4 levels have been shown to rise and positively correlated with body mass index (BMI) [2,3] and to be associated with insulin resistance (IR) [2-5]. Growing evidence suggests that RBP4 plays a role in lipid metabolism to an even greater extent than insulin resistance. In fact, many human studies have found a strong relationship between RBP4 and triglycerides, some finding an association with insulin resistance [3,6] and others failing to do so [7-9].

Menopause is defined as the permanent cessation of menses as a consequence of the loss of ovarian follicular function or of surgical removal of ovaries. During this period, many psychological, physiological, and pathological modifications occur; in particular cardiovascular disease (CVD). It is well known that there is a higher prevalence of cardiovascular risk factors and metabolic syndrome (MS) in postmenopausal women. Postmenopausal status is believed to be a risk factor for IR in women. IR has a causal role in the development of T2DM. Even in the absence of hyperglycemia or diabetes, IR contributes to an increased risk of CVD [1,10].

The aim of the present work was to evaluate the influence of menopausal status on RBP-4 concentration and to investigate the association of plasma RBP-4 with IR in post-menopausal women with and without CVD.

The present study included 25 apparently healthy premenopausal women, 25 apparently healthy postmenopausal women (Both were chosen from the staff members of MRI) and 25 postmenopausal women With CVD (were recruited from the Internal Medicine Department, Cardiology unit MRI).

The following were done for all participants:

1- Clinical examination

- Through history taking: with special stress on family history of diabetes mellitus and any drug intake.
- Complete physical examination. .

2- Anthropometric measurements

- Body mass index (BMI).
- Waist to hip ratio (WHR).
- Blood pressure.

3- Laboratory measurements

- Lipid profile including Total cholesterol (TC) by enzymatic method [11], high density lipoprotein cholesterol (HDL-C) by enzymatic method [12,13], low density lipoprotein cholesterol (LDL-C= [TC-HDL-C-(TG/5)]) [14], Triglyceride (TG) by enzymatic method [15] and TG/HDL-C ratio.
- Fasting and postprandial plasma glucose level by enzymatic method [16].
- Determination of plasma insulin by ELISA method [17].
- Assessment of IR by homeostasis model assessment HOMA score (HOMA-IR = Fasting glucose (mg/dl) × Fasting Insulin (µIU/ml) / 405) [18].
- Serum creatinine by kinetic method [19].
- The estimated glomerular filtration rate (eGGFR) was calculated using Cockcroft Gault equation: eGFR = (140-age) × body weight (kg) × 0.85 if female / 72 × serum creatinine (mg/dl) [20].
- Liver enzymes including serum aspartate aminotransferase (AST) by kinetic method [21], serum alanine aminotransferase (ALT) by kinetic method [21].
- Determination of plasma RBP-4 by ELISA method [22].

2. Results

2.1 Results of age (year), body mass index (kg/m^2), waist circumference(cm), waist/hip ratio and blood pressure [systolic and diastolic (mmHg)] (table 1)

Table (1) showed the statistical analyses of age (year), body mass index (kg/m2), waist circumference (cm), waist/hip ratio and blood pressure [systolic and diastolic (mmHg)] of the three studied groups. Data showed a significant difference in age and waist circumference ($p1 < 0.05$) and insignificant difference in BMI, WHR and blood pressure (systolic

and diastolic) ($p_1 > 0.05$) between premenopausal and postmenopausal women. Compared with the postmenopausal women, the postmenopausal women with CVD had significant higher age and blood pressure (systolic and diastolic) ($p_2 < 0.05$) while there was no significant difference between the two groups in BMI, waist circumference and WHR ($p_2 > 0.05$).

Table 1: The statistical analyses of age (year), body mass index (kg/m2), waist circumference (cm), waist/hip ratio and blood pressure [systolic and diastolic (mmHg)] of the three studied groups

	Premenopausal women (n = 25)	**Postmenopausal women (n = 25)**	**Postmenopausal women with cardiovascular diseases (n = 25)**
Age (year)			
Range	27.0 – 52.0	47.0 – 59.0	52.0 – 66.0
Mean ± SD	37.60 ± 7.65	52.56 ± 3.28	59.52 ± 4.72
p_1		$p_1 < 0.001^*$	
p_2			$p_2 < 0.001^\#$
BMI (kg/m^2)			
Range	20.69 – 36.79	25.30 – 35.38	24.03 – 40.31
Mean ± SD	29.13 ± 4.48	30.03 ± 2.79	32.48 ± 3.44
p_1		$p_1 = 0.684$	
p_2			$p_2 = 0.064$
Waist circumference(cm)			
Range	78.0 – 113.0	90.0 – 116.0	89.0 – 118.0
Mean ± SD	95.88 ± 10.19	103.88 ± 7.59	106.56 ± 9.18
p_1		$p_1 = 0.010^*$	
p_2			$p_2 = 0.580$
WHR			
Range	0.68 – 0.95	0.72 – 1.03	0.77 – 1.06
Mean ± SD	0.84 ± 0.06	0.88 ± 0.06	0.90 ± 0.08
p_1		$p_1 = 0.102$	
p_2			$p_2 = 0.742$
Systolic (mmHg)			
Range	90.0 – 130.0	100.0 – 140.0	130.0 – 180.0
Mean ± SD	113.20 ± 11.89	120.20 ± 9.52	146.40 ± 14.69
p_1		$p_1 = 0.136$	
p_2			$p_2 < 0.001^\#$
Diastolic (mmHg)			
Range	60.0 – 90.0	70.0 – 90.0	80.0 – 100.0
Mean ± SD	75.60 ± 6.97	79.20 ± 7.73	90.0 ± 5.40
p_1		$^{MW}p_1 = 0.149$	
p_2			$^{MW}p_2 < 0.001^\#$

p_1 :p value compared to premenopausal women. **p_2** :p value compared to postmenopausal women.
* :Significantly different from premenopausal group. # :Significantly different from postmenopausal group.
Significance was considered at the level of $p < 0.05$. MW : Mann-Whitney test.

2.2 Results of total cholesterol (mg/dl), high density lipoprotein-cholesterol (mg/dl), low density lipoprotein-cholesterol (mg/dl), triglyceride (mg/dl) and triglyceride/high density lipoprotein-cholesterol ratio (table 2)

Results of serum total cholesterol (TC) (mg/dl), serum high density lipoprotein-cholesterol (HDL-C) (mg/dl), serum low density lipoprotein-cholesterol (LDL-C) (mg/dl), serum triglyceride (TG) (mg/dl) and triglyceride/high density lipoprotein-cholesterol ratio were shown in table (2). Data showed that the postmenopausal group had a significantly higher TC and TG levels than those of the premenopausal group ($p_1 < 0.05$). In contrast, there was no significant difference between the two groups in HDL-C, LDL-C levels and TG/HDL-C ratio ($p_1 > 0.05$). It was also observed that there were significant differences in TC,

LDL-C, TG levels and TG/HDL-C ratio between the postmenopausal women with CVD and postmenopausal group ($p_2 > 0.05$), while there was no significant difference between two groups in HDL-C.

Table 2: The statistical analyses of total cholesterol (mg/dl), high density lipoprotein-cholesterol (mg/dl), low density lipoprotein-cholesterol (mg/dl), triglyceride (mg/dl) and triglyceride/high density lipoprotein-cholesterol ratio of the three studied groups.

	Premenopausal women (n = 25)	**Postmenopausal women (n = 25)**	**Postmenopausal women with cardiovascular diseases (n = 25)**
TC (mg/dl)			
Range	132.0 – 229.0	124.0 – 276.0	188.0 – 321.0
Mean ± SD	181.96 ± 26.36	204.20 ± 30.42	227.80 ± 28.97
p_1		$p_1 = 0.028^*$	
p_2			$p_2 = 0.018^\#$
HDL-C (mg/dl)			
Range	34.0 – 74.0	31.0 – 77.0	31.0 – 85.0
Mean ± SD	52.36 ± 9.92	57.56 ± 11.39	50.58 ± 11.74
p_1		$p_1 = 0.256$	
p_2			$p_2 = 0.089$
LDL-C (mg/dl)			
Range	66.6 – 156.8	51.0 – 192.8	97.2 – 213.6
Mean ± SD	114.36 ± 24.81	122.82 ± 30.31	143.18 ± 30.05
p_1		$p_1 = 0.579$	
p_2			$p_2 = 0.047^\#$
TG (mg/dl)			
Range	48.0 – 128.0	60.0 – 291.0	85.0 – 287.0
Mean ± SD	76.24 ± 23.77	119.12 ± 53.76	170.20 ± 46.89
p_1		$p_1 = 0.004^*$	
p_2			$p_2 < 0.001^\#$
TG/HDL-C Ratio			
Range	0.65 – 3.15	0.78 – 8.08	1.29 – 8.39
Mean ± SD	1.54 ± 0.69	2.35 ± 1.78	3.58 ± 1.46
p_1		$p_1 = 0.133$	
p_2			$p_2 = 0.010^\#$

p_1 :p value compared to premenopausal women **p_2** :p value compared to postmenopausal women
* :Significantly different from premenopausal group # :Significantly different from postmenopausal group
Significance was considered at the level of $p < 0.05$

2.3 Results of fasting glucose (FG) (mg/dl), post prandial glucose (mg/dl), plasma insulin (µIU/ml) and homeostasis model assessment insulin resistance (HOMA-IR) (table 3)

The results showed insignificant difference in FG, post prandial glucose, insulin levels and HOMA-IR ($p_1 > 0.05$) between premenopausal and postmenopausal women. On the other hand plasma insulin levels and HOMA-IR in postmenopausal women with CVD, were significantly higher than those in postmenopausal women ($p_2 < 0.05$), whereas, there was no significant difference between the two groups in both fasting and post prandial glucose levels ($p_2 > 0.05$).

Table 3: The statistical analyses of fasting glucose (mg/dl), post prandial (mg/dl), insulin (µIU/ml) and homeostasis model assessment insulin resistance of the three studied groups

	Premenopausal women (n = 25)	Postmenopausal women (n = 25)	Postmenopausal women with cardiovascular diseases (n = 25)
FG (mg/dl)			
Range	61.0 – 101.0	77.0 – 99.0	78.0 – 128.0
Mean ± SD	84.92 ± 9.69	91.32 ± 6.01	98.0 ± 12.58
p_1		$p_1 = 0.076$	
p_2			$p_2 = 0.061$
Post prandial glucose (mg/dl)			
Range	70.0 – 117.0	78.0 – 139.0	77.0 – 131.0
Mean ± SD	97.72 ± 10.33	104.28 ± 16.65	105.28 ± 12.56
p_1		$p_1 = 0.232$	
p_2			$p_2 = 0.966$
Insulin (µIU/ml)			
Range	2.10 – 9.40	2.05 – 18.80	3.0 – 22.60
Mean ± SD	4.52 ± 2.21	5.54 ± 4.04	7.54 ± 4.40
p_1		$^{MW}p_1 = 0.587$	
p_2			$^{MW}p_2 = 0.012^{\#}$
HOMA-IR			
Range	0.49 – 2.02	0.42 – 4.55	0.58 – 6.42
Mean ± SD	0.94 ± 0.49	1.27 ± 0.97	1.90 ± 1.33
p_1		$^{MW}p_1 = 0.265$	
p_2			$^{MW}p_2 = 0.007^{\#}$

p1 :p value compared to premenopausal women **p**2 :p value compared to postmenopausal women
:Significantly different from postmenopausal group Significance was considered at the level of $p < 0.05$
MW: Mann-Whitney test.

2.4 Results of serum creatinine (mg/dl) and glomerular filtration rate (GFR) (ml/min.) (table 4)

Data showed that creatinine levels in premenopausal and postmenopausal women were nearly within the same range and showed insignificant difference ($p_1 > 0.05$). The same result was also noticed between postmenopausal women with CVD and that of postmenopausal women ($p_2 > 0.05$). On the other hand, GFR was significantly higher in postmenopausal women than that premenopausal woman. However, GFR did not significantly between postmenopausal women with CVD and that of postmenopausal group.

Table 4: The statistical analyses of creatinine (mg/dl) and glomerular filtration rate (ml/min.) of the three studied groups.

	Premenopausal women (n = 25)	Postmenopausal women (n = 25)	Postmenopausal women with cardiovascular diseases (n = 25)
Creatinine (mg/dl)			
Range	0.80 – 1.10	0.70 – 1.10	0.80 – 1.30
Mean ± SD	0.89 ± 0.09	0.91 ± 0.09	0.92 ± 0.12
p_1		$^{MW}p_1 = 0.313$	
p_2			$^{MW}p_2 = 0.950$
GFR (ml/min.)			
Range	71.50 – 129.20	70.77 – 135.09	63.55 – 106.60
Mean ± SD	103.42 ± 14.04	88.78 ± 14.37	85.88 ± 12.12
p_1		$p_1 = 0.001^{*}$	
p_2			$p_2 = 0.753$

p_1 :p value compared to premenopausal women p_2 :p value compared to postmenopausal women
* :Significantly different from premenopausal group Significance was considered at the level of $p < 0.05$
MW: Mann-Whitney test.

2.5 Results of serum aspartate transaminase (AST) activity (U/L) and serum alanine transaminase (ALT) activity (U/L) (table 5)

In comparison with premenopausal women, the mean value of ALT of postmenopausal women was significantly higher ($p_1 < 0.05$) whereas, AST levels showed insignificant difference ($p_1 > 0.05$). Data revealed that there was significant higher ($p_2 < 0.05$) ALT levels in postmenopausal women with CVD than that in postmenopausal group ($p_2 > 0.05$), whereas, AST showed no significant difference ($p_2 < 0.05$).

2.6 Results of plasma retinol binding protein–4 (RBP-4) (mg/L) (table 6)

The statistical analyses of these results in table (6) showed a significant higher levels of RBP-4 between premenopausal and postmenopausal women ($p_1 < 0.05$). In addition, the mean value of RBP-4 levels of postmenopausal women with CVD was significantly higher than that of postmenopausal women ($p_2 < 0.05$).

Table 5: The statistical analyses of aspartate transaminase activity (U/L) and alanine transaminase activity (U/L) of the three studied groups

	Premenopausal women (n = 25)	**Postmenopausal women (n = 25)**	**Postmenopausal women with cardiovascular diseases (n = 25)**
AST (U/L)			
Range	13.0 – 26.0	14.0 – 25.0	11.0 – 34.0
Mean ± SD	18.72 ± 3.76	19.60 ± 2.74	20.00 ± 5.19
p_1		$p_1 = 0.742$	
p_2			$p_2 = 0.940$
ALT (U/L)			
Range	10.0 – 22.0	12.0 – 23.0	11.0 – 27.0
Mean ± SD	14.08 ± 2.99	17.08 ± 2.87	19.68 ± 4.72
p_1		$p_1 = 0.018$ *	
p_2			$p_2 = 0.046$ #

p_1 : p value compared to premenopausal women **p_2** : p value compared to postmenopausal women
* : Significantly different from premenopausal group # : Significantly different from postmenopausal group
Significance was considered at the level of $p < 0.05$

Table 6: The statistical analyses of retinol binding protein–4 (mg/L) of the three studied groups

	Premenopausal women (n = 25)	**Postmenopausal women (n = 25)**	**Postmenopausal women with cardiovascular diseases (n = 25)**
RBP-4 (mg/L)			
Range	29.61 – 68.71	53.33 – 91.79	62.05 – 120.00
Mean ± SD	54.28 ± 10.36	70.06 ± 11.81	90.09 ± 13.02
p_1		$p_1 < 0.001$ *	
p_2			$p_2 < 0.001$ #

p_1 : p value compared to premenopausal women **p_2** : p value compared to postmenopausal women
* : Significantly different from premenopausal group # : Significantly different from postmenopausal group
Significance was considered at the level of $p < 0.05$

2.7 Correlation of plasma RBP-4 levels with clinical and biochemical parameters

Correlation analysis of plasma RBP-4 levels with clinical and biochemical parameters in premenopausal women, postmenopausal women and postmenopausal women with CVD were illustrated in table (7).

Our results revealed that plasma RBP-4 was positively correlated with serum total cholesterol (TC) (r = 0.409*, p = 0.042), post prandial glucose (r = 0.412*, p = 0.041) and serum alanine transaminase (ALT) activity (r = 0.540**, p = 0.005) in

postmenopausal women and with age (r = 0.540**, p = 0.005), serum total cholesterol (TC) (r = 0.442*, p = 0.027), serum triglyceride (TG) (r = 0.654**, p < 0.001), triglyceride/high density lipoprotein-cholesterol ratio (r =0.627**, p = 0.001), fasting plasma glucose (FG) (r = 0.475*, p = 0.017), plasma post prandial glucose (r = 0.404*, p = 0.045), plasma insulin (r_s = 0.431*, p = 0.032), homeostasis model assessment insulin resistance(HOMA-IR) (r_s = 0.455*, p = 0.022) and serum alanine transaminase (ALT) activity (r = 0.441*, p = 0.027) in postmenopausal women with CVD.

Table 7: Correlation of plasma RBP-4 levels with clinical and biochemical parameters

	Group I		Group II		Group III	
	Coeff.	p	Coeff.	p	Coeff.	p
Age	r = -0.072	0.733	r = -0.143	0.496	r = 0.540**	***0.005*** #
BMI	r = -0.077	0.715	r = 0.007	0.974	r = 0.117	0.576
Waist	r = -0.072	0.734	r = 0.009	0.968	r = 0.019	0.927
WHR	r = -0.115	0.583	r = 0.130	0.534	r = -0.090	0.668
Systolic	r = -0.120	0.568	r = -0.300	0.146	r = 0.073	0.730
Diastolic	r_s = 0.214	0.303	r_s = -0.217	0.297	r_s = -0.225	0.280
TC	r = -0.052	0.806	r = 0.409*	***0.042*** #	r = 0.442*	***0.027*** #
HDL-C	r = -0.340	0.096	r = -0.107	0.610	r = -0.211	0.312
LDL-C	r = 0.043	0.839	r = 0.380	0.061	r = 0.305	0.139
TG	r = 0.200	0.338	r = 0.200	0.339	r = 0.654**	***<0.001*** #
TG/HDL-C ratio	r = 0.271	0.189	r = 0.212	0.309	r =0.627**	***0.001*** #
FG	r = -0.001	0.995	r = 0.174	0.406	r = 0.475*	***0.017*** #
Post prandial glucose	r = -0.240	0.249	r = 0.412*	***0.041*** #	r = 0.404*	***0.045*** #
Insulin	r_s = -0.144	0.588	r_s = 0.162	0.440	r_s = 0.431*	***0.032*** #
HOMA-IR	r_s = -0.127	0.544	r_s = 0.157	0.453	r_s = 0.455*	***0.022*** #
Creatinine	r_s = -0.266	0.199	r_s = 0.089	0.672	r_s = -0.163	0.435
GFR	r = 0.246	0.235	r = 0.029	0.891	r = 0.076	0.717
AST	r = -0.316	0.124	r = 0.074	0.724	r = 0.114	0.589
ALT	r = 0.002	0.993	R = 0.540**	***0.005*** #	r = 0.441*	***0.027*** #

r :Pearson coefficient rs :Spearman coefficient ** :Correlation is significant at the 0.01 level (2-tailed).
* :Correlation is significant at the 0.05 level (2-tailed). # :Statistically significant at $p \leq 0.05$.

3. Discussion

In the present study, it is evident that waist circumference of healthy postmenopausal women is significantly higher than that of healthy premenopausal women while BMI and waist/hip ratio were not significantly different. These results are in agreement with those reported by Kuk et al. (2005) [23], who stated that postmenopausal women had significantly higher mean waist circumference, but the mean BMI of pre and postmenopausal women was similar. They found a significant correlation between waist circumference and visceral fat content.

The menopause transition is associated with a dramatic change in hormonal balance, including a rapid decline of endogenous estradiol levels, leading to a period of relative androgen excess[24]. It has been suggested that this change in hormonal balance contributes to a redistribution of body fat and an increase in visceral adiposity [25] which is associated with IR in postmenopausal women [26]. It is well documented that accumulation of visceral fat is accompanied with higher risk for development of obesity related diseases such as CVD, hypertension and hyperlipidemia [27].

Hypertension is the most common disease affecting one quarter of the adult population and representing the most common major cardiovascular risk factor after the fifth decade of life in both men and women [28]. Persistent hypertension is of the risk factor for stroke, myocardial infarction, heart failure and arterial aneurysm, and is a leading cause of chronic kidney failure.

Several studies had demonstrated that postmenopausal increase in blood pressure in females is mainly caused by a loss of sex steroids, indicating that estrogen exerts protective effects against increases in blood pressure [29]. Some mechanisms of the protective effects of estrogen on cardiovascular disease were described. Estrogen inhibits the rennin angiotensin system, decreasing the expression of

angiotensin 1 (AT1) receptors in animal experiments[30,31]. Another vasoconstrictor, endothelin, also appears to be suppressed by estrogen[32]. In addition, relationships between postmenopausal hypertension and changes in insulin sensitivity [33] and salt sensitivity [31] were reported, suggesting that complex mechanisms play a role in rises in blood pressure due to the loss of estrogen in females, together with the mechanisms mentioned above.

In view of these studies we expect that blood pressure in postmenopausal women becomes higher than that in premenopausal women but the difference was insignificant. We can attributed this result to the short period of menopause and the short period of estrogen loss. On the other hand the significant elevation of systolic and diastolic blood pressure in postmenopausal women with CVD than those in healthy postmenopausal women observed in this study is logic, since high pressure is well established risk factor for CVD in postmenopausal women [33,34].

Dyslipidemia is a major cause of CVD, which in turn, is the most common cause of female morbidity and mortality [35]. The incidence of CVD increases after menopause due to changes in the plasma lipid and lipoprotein levels that occur following menopausal transition [36,37].

In this study it is evident that postmenopausal women had significantly higher concentrations of total cholesterol with respect to premenopausal women ($p<0.001$). These findings are similar to other studies [38-40]. Gorodeski GI and his colleague [41] reported that TC was 19% higher in postmenopausal women compared to premenopausal women, and 1% increase in TC is associated with at least 2% increase in the incidence of CVD [42]. They attributed the elevated concentrations of TC to estrogen deficiency in postmenopausal women.

In our study, when compared to premenopausal women, postmenopausal women were having high levels of LDL, these findings are in agreement with other studies [38,43]. Circulating estrogen is a regulator of lipoprotein lipase (LPL), which catalyzes the hydrolysis of very low density lipoprotein (VLDL) to form intermediate density lipoprotein (IDL) and later LDL. After menopause due to estrogen deficiency, there will be increased plasma LPL activity causing increased level of LDL and also leads to down-regulation of LDL receptors [40,44]. The higher the small dense LDL proportion which characterizes the atherogenic shift, higher is the LDL oxidation [40], and these particles are associated with a threefold increase in CVD risk [45].

In the present study when compared to premenopausal women, postmenopausal women showed high TG and was statistically significant ($p<0.001$). These findings are in accordance with that reported by Razay et al. (1992) [42]. who stated that TG in postmenopausal women was higher by 31% when compared to premenopausal women. The elevated levels of TG observed in postmenopausal women could be interpreted in view of Razay et al. (1992) [42], they stated that in the postmenopausal women, the increased fat accumulation, resulting in increased release of free fatty acids into the circulation and excessive free fatty acids provides substrate for hepatic triglyceride and triglyceride rich lipoprotein production [46].

Furthermore, the ratio between TG and HDL-C was also estimated in post and premenopausal women. It was found that TG/HDL-C ratio (atherogenic index) in postmenopausal women was higher than in the premenopausal women. Since increased TG/HDL-C ratio is considered as independent risk factor for CVD [47,48], therefore our result may through light on the possibility of using this ratio in predicting CVD which is more common among postmenopausal women. The importance of TG/HDL-C ratio in CVD risk assessment has been established by previous study.[49].

In this study, it could be noticed that blood pressure, glucose and HOMA-IR were not influenced by menopause and that the impact of menopause is limited to adverse effect on lipids such as TC and TG and this may explain why postmenopausal women appear to be more susceptible to atherosclerotic cardiovascular events.

The present data also revealed that TC, TG, LDL-C levels and TG/HDL-C ratio in the postmenopausal women with CVD were significantly higher than those in healthy postmenopausal women; this result is expected since these parameters are among other risk factors involved in the development of CVD [47,48].

A number of different hormones produced by fat tissue have been identified in the last few years, and some of those mediate molecules have been found to be associated with the regulation of insulin sensitivity. Since visceral adiposity and insulin resistance are known to be associated with increased cardiovascular risk [50], it could be speculated that some adipocytokines such as RBP-4 mediate this relationship.

The present data revealed that RBP-4 concentrations in postmenopausal women were higher than those in premenopausal women. This result agreed with that reported by Suh et al. (2010) [51]

suggesting that menopausal status might be a major determinant of plasma RBP-4 concentrations. As women reach menopause, estrogen decrease. As such, fat amount or body fat percentage change; and visceral fat increase. As a result, lipid metabolism becomes dysregulated. This change of lipid metabolism may affect plasma RBP-4 concentrations that come from adipocytes [52].

In addition, it is important to note that some organs other than adipose tissue are important sites of RBP-4 synthesis and secretion[53]. In particular, there is compelling evidence showing that the kidneys play an important role in maintenance of whole body retinol homeostasis [54,55], which is regulated by glomerular filtration and subsequent re-absorption of RBP-4 into the proximal tubular cells [56,57]. The significant decline in the GFR observed in postmenopausal group, indicating a reduced kidney function, may be responsible for the elevated RBP-4 levels even within the limits of normal creatinine concentrations. As described earlier, the impaired catabolism of RBP-4 complex in the kidneys lead to the accumulation of RBP-4 in the plasma of postmenopausal women points to the central importance of kidney function in the regulation of plasma RBP-4 [58]. It was found in previous study [59] that a decline in estimated GFR was accompanied by gradual elevation of plasma RBP-4 level.

It was also noticed that, plasma RBP-4 concentrations in postmenopausal women with CVD was significantly higher than that in postmenopausal women and were positively correlated with age, TC, TG, TG/HDL-C, fasting plasma glucose, postprandial glucose, plasma insulin, HOMA-IR and ALT.

Age is an independent risk factor for IR [60,61]. Many factors such as overall increased adipose tissue and decreased physical activity predispose older people to develop glucose intolerance and IR. In the present study, plasma RBP-4 concentrations positively correlated with age and HOMA-IR in postmenopausal women with CVD. We could not clearly determine why RBP-4 concentrations increase with age but the data are consistent with the suggestion that age induced IR may be reflected by plasma RBP-4 concentrations.

In the present study, RBP-4 correlate with TC, TG and TG/HDL-C ratio in postmenopausal women with CVD. Our results were in line with previous findings [62-64]. It was suggested that RBP-4 in postmenopausal women with CVD might have a direct role in the progression of lipogenesis by increasing the expression of the gene encoding fatty acid synthesase (FASN) in adipose tissue [65].

Also fasting glucose positively correlated with serum RBP-4 concentrations in postmenopausal women with CVD. The association between plasma RBP-4 concentrations and fasting glucose may be explained by the mechanism through which RBP-4 develops IR in liver. Retinol binding protein-4 induces the expression of the gluconeogenic enzyme PEPCK in the liver [66]. The present results could be due to this mechanism.

In addition, RBP-4 correlate positively with postprandial glucose in the postmenopausal women with CVD. This could be explained by findings of Broch et al. (2007) [67] who concluded that RBP-4 may impair β cell function in human subjects. As RBP-4 circulates in plasma form a complex with transthyretin, which constitutes a functional component in pancreatic β cell stimulus secretion coupling. Thus it is possible that increased serum RBP-4 prevents transthyretin from exerting its β cell stimulus secretion effects.

Results from the present study also found positive association between RBP-4 and HOMA-IR. A mechanism whereby RBP-4 modulates insulin sensitivity in muscle and liver has been suggested. In skeletal muscle, RBP-4 reduces insulin sensitivity by inhibiting both insulin receptor substrate-1 phosphorylation and phosphatidylinositol 3-kinase activation, while increasing hepatic glucose production by increasing PEPCK expression [66]. Another contributing factor for IR is that RBP-4 down-regulates GLUT4 [66,68], the insulin activated glucose transporter responsible for translocation of glucose into both muscle and fat cells [68], and has also recently been shown to induce expression and secretion of pro-inflammatory cytokines in primary human macrophages known to induce IR [69].

Insulin resistance is well established as one of the risk factors for CVD suggesting that RBP-4 might serve as an alternative biomarker of CVD [70]. This need confirmation of some future prospective studies.

Lastly, the present data also revealed a positive association between plasma levels of ALT and RBP-4 in postmenopausal women with CVD. This result is supported by other study which demonstrated that hepatic fat accumulation may lead to elevation of plasma RBP-4 [71].

4. Conclusion

- In this study it was noticed that blood pressure, glucose, insulin and HOMA-IR were not influenced by menopause and the impact of menopause in limited to adverse effect on lipid such as TC, LDL-C and TG

and this may explain why postmenopausal women appear to be more susceptible to atherosclerotic cardiovascular events.

- RBP-4 in postmenopausal women was significantly higher than that in premenopausal women, and positively correlated with postprandial glucose, total cholesterol and ALT and showed no significant correlation with fasting glucose, fasting insulin and HOMA-IR.
- Postmenopausal women with CVD showed significantly higher plasma RBP-4, blood pressure (systolic and diastolic), lipid profile (TC, LDL-C, TG and TG/HDL-C ratio), insulin and HOMA-IR than those in postmenopausal women. In addition, RBP-4 significantly positively correlated with FG, postprandial glucose, insulin, HOMA-IR, TC, TG, TG/HDL-C ratio and ALT.
- Menopausal status affect RBP-4 levels. Moreover, RBP-4 which is significantly elevated in postmenopausal women with CVD as compared to postmenopausal women, affect glucose and lipid homeostasis and contribute to the onset of IR which may play a role in the development of CVD.

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