Original Article

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Biochemistry Section

Role of Serum Retinol Binding Protein 4 (RBP4) Concentration in Patients with Primary Hypertension: A Case-control Study

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ABSTRACT

Introduction: Serum Retinol Binding Protein 4 (RBP4), an adipokine that transports vitamin A from the liver to other tissues, is reported to be elevated in hypertensive subjects. Positive correlation between RBP4 and cardiovascular risk factors has been noted in a few studies.

Aim: To evaluate serum RBP4 levels in newly diagnosed primary hypertensive cases and non hypertensive controls and to correlate serum RBP4 levels with lipid profile.

Materials and Methods: This case-control study was conducted at the Department of Biochemistry in collaboration with the Department of Medicine, from August 2019 to June 2020 in MKCG Medical College and Hospital, Berhampur, Odisha, India. A total of 51 newly diagnosed primary hypertensive patients and 51 healthy age and sex-matched individuals between the ages of 18-50 years were enrolled in the study. Systolic and Diastolic Blood Pressure (SBP and DBP) were measured in both cases and controls. Serum RBP4 was measured by Enzyme-Linked Immunosorbent Assay (ELISA) and other biochemical parameters were measured by TBA120FR autoanalyser. Statistical analysis of the data was done by using Statistical Package for Social Sciences (SPSS) software, version 22.0.

Results: The mean age for controls was 36.98±8.7 years and that in cases was 39.00±8.4 years. Proportion of males was higher (56.8%) than females (43.2%). The mean SBP in the case group was significantly higher as compared to the control group. The cases had a higher level of serum RBP4 (31.82 mg/L) compared to the control groups (15.5 mg/L) RBP4 ($p<0.001$). The cases had a higher level of mean serum triglyceride (196.20±67.81 mg/dL) and lower level of mean serum High-Density Lipoprotein cholesterol (HDLc) (37.53±11.90 mg/dL) as compared to the control groups which had a mean triglyceride of 104.25±39.89 mg/dL and HDLc of 57.18±10.73 mg/dL ($p<0.001$). The serum RBP4 level positively correlated with SBP ($r=0.644$, $p<0.001$), DBP ($r=0.444$, $p<0.001$), serum triglycerides ($r=0.649$, $p<0.001$), and negatively correlated with HDLc ($r=-0.313$, $p=0.025$)

Conclusion: A high serum RBP4 level was found in the newly diagnosed hypertensive cases as compared to normotensive controls. A significant positive correlation was observed between RBP4 with SBP and DBP along with triglyceride levels. A significant negative correlation was observed between RBP4 with HDLc.

Keywords: Adipokine, Cardiovascular risk, Lipid profile

INTRODUCTION

Hypertension, per se, is asymptomatic but when not treated in time causes permanent organ damage in the future [1]. Being asymptomatic, it remains undiagnosed for a longer period and eventually leads to lethal complications like Cardiovascular Diseases (CVDs) and damage to vital organs of the body. Various researches are going on to diagnose the disease as early as possible and so is the quest for detecting its early markers to halt the deleterious effects of uncontrolled or poorly controlled hypertension in the long run.

There are various factors contributing to the pathogenesis of hypertension. Altered immunity and inflammation are important in the genesis of hypertension and also contribute to its complications [2]. Hypertension is an inflammatory process [3]. Two main mechanisms oxidative stress and endothelial dysfunction are crucial for the development of hypertension. Biomarkers, immune cell subtypes, cytokines, Toll-like Receptors (TLRs) etc. are found to be associated with both inflammation and hypertension [2]. Further, inflammatory cytokines, vascular adhesion molecules and the formation of an atherosclerotic plaque which when destabilises leads to cardiovascular accidents [4]. Oxidative stress attenuates Nitric Oxide (NO) production by the vessel wall and also contributes to the development of atherosclerosis [5]. The severity of the inflammatory state is measured with the help of various biomarkers. Serum high sensitive C-Reactive Protein (hsCRP) and serum RBP4 are one of the markers of inflammation. RBP4 is believed to cause inflammation by decreasing the endothelial tone [5].

Retinol binding protein-4 (RBP4) is a new adipokine belonging to the lipocalin family of proteins [5]. RBP4 is synthesised mainly by the liver. Adipose tissue produces only 20-40% of that secreted by the liver [6]. The role of RBP4 in the serum is to carry retinol from the liver to the tissues. Novel biomarker RBP4 has a role in decreasing vascular tone in patients with primary hypertension. It is very recently discovered as a new biomarker linking both inflammation and hypertension [7]. It is believed to act via the TLRs to increase blood pressure and it acts by decreasing the NO release by the endothelial wall [8,9]. Besides its well-established function in the induction of insulin resistance, it has also been found in recent years to be closely associated with CVDs and other risk factors, such as hypertension, coronary heart disease, heart failure, obesity and hyperlipidaemia [10]. No relevant documentation about the role of RBP4 in newly diagnosed primary hypertension was found in Southern Odisha. Thus, the present study was conducted with the primary aim to evaluate serum RBP4 concentration and its correlation with hypertension and lipid profile in newly diagnosed cases of primary hypertension.

MATERIALS AND METHODS

This case-control study was carried out from August 2019 to June 2020 in the Department of Biochemistry in collaboration with the Department of Medicine, MKCG Medical College and Hospital, Berhampur, Odisha, India. The Institutional Ethics Committee had approved the study (IEC no. 674/2018).

Sample size calculation: Zhu YY et al., reported that raised RBP4 levels are associated with an increased risk of the poor functional outcome by 144% Odds Ratio (OR): 2.44; 95% confidence interval (CI): 1.22–5.03) [11]. Taking into account the odds ratio of 3, and assuming raised RBP4 levels in 50% of the normal controls (as the proportion of raised RBP4 in normotensive healthy individuals is unknown.); the sample size at 80% power and 95% CI was 110 as calculated by Epitools (Auvset) [12]. A total of 55 in the case group and 55 in the control group were enroled in the study. Four samples were discarded due to faulty or erroneous results. The corresponding samples were also excluded resulting in the total exclusion of eight samples. Hence, a total of 102 samples were included in the final study.

Inclusion criteria: Total of 51 newly diagnosed primary hypertensive patients (18-50 years), and 51 age and sex-matched normotensive healthy controls were considered. The diagnosis of hypertension was done as per the recommendations of the World Health Organisation and Joint National Committee (JNC) VII [13,14].

Exclusion criteria: Patients with secondary hypertension and conditions like diabetes mellitus, chronic inflammatory diseases, tuberculosis, autoimmune diseases, stroke, hepatic diseases, renal disease, acute infection and sepsis, gout, critically ill individuals, recent history of trauma, drug treatment as lipid-lowering drugs, any cancers, smokers and alcoholics.

Study Procedure

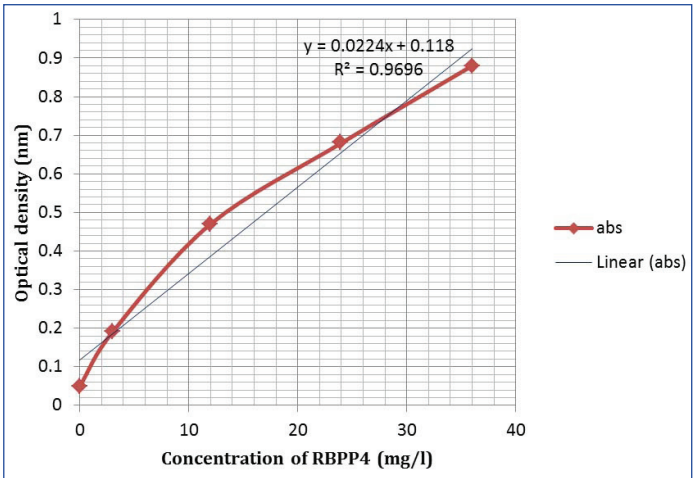
A total of 5 mL of whole blood was collected after obtaining the written consent form in both english and the local language duly signed by the participants. A 1 mL of blood for plasma was taken for estimation of fasting plasma glucose and 4 mL of blood for serum was taken for the rest of the biochemical parameters and RBP4 estimation. On the same day of collection of the sample the plasma glucose and serum urea, creatinine lipid profile and electrolyte were analysed by the TOSHIBA TBA120FR autoanalyser maintaining internal and external quality control. In two aliquots 1 mL each of serum was stored at -20° for estimation of serum RBP4 by ELISA kit, 96T Enzyme-linked Immunosorbent Assay (ELISA) for quantitative detection serum RBP4 [15].

ELISA Method for Estimation of RBP4

Principle of the assay: The human RBP4 ELISA kit has microtiter plate wells coated with Purified Human RBP-4 antibody (solid phase antibody). To the wells, test samples (containing human RBP-4 antigen) are added which combine with the Human RBP-4 antibody. Then the wells are washed to remove any unbound substance. After washing a Horseradish Peroxidase (HRP)-conjugated antibody specific for RBP4 is added to the wells and washed again. A Tetra Methyl Benzidine (TMB) substrate solution is added and the solution becomes blue colour. The addition of sulphuric acid solution terminates the HRP enzyme-catalysed reaction and the colour change to yellow. The yellow colour is measured spectrophotometrically at a wavelength of 450 nm. The concentration of human RBP-4 in the samples was then determined by comparing the Optical Density (O.D.) of the samples to the standard curve [Table/Fig-1,2].

Standard solutions	Concentration of RBP4 in mg/l	Absorbance in nm
SD1	0	0.058
SD2	03	0.191
SD3	12	0.471
SD4	24	0.681
SD5	36	0.883

[Table/Fig-1]: Absorbance of RBP4 standard solutions and their concentration.



[Table/Fig-2]: Standard curve of RBP4.

STATISTICAL ANALYSIS

Data was entered in Microsoft excel version 21, and was analysed. Statistical analysis of the data was done by using Statistical Package for Social Sciences software, version 22.0 (SPSS Inc., Chicago, IL, USA). Comparisons between the continuous variables were done by Students' tests and Pearson's correlation coefficient. A p-value of less than 0.05 was considered significant.

RESULTS

The mean age for controls was 36.98±8.7 years and for cases was 39.00±8.4 years. The proportion of males was higher (56.8%) than females (43.2%) [Table/Fig-3]. However, these differences were not significant statistically as they were selected after matching their age and sex.

Parameters	Cases Mean±SD	Controls Mean±SD	p-value
Age (years)	39.00±8.4	36.98±8.7	0.241
Systolic BP (in mmHg)	144.16±4.78	112.67±10.58	<0.01
Diastolic BP (in mmHg)	92.90±5.48	78.08±5.57	<0.01
Fasting blood sugar (mg/dL)	92.41±10.4	91.69±6.70	0.677
Urea (mg/dL)	24.24±6.03	21.55±8.14	0.061
Creatinine (mg/dL)	0.83±0.21	0.82±0.18	0.35
Total cholesterol (mg/dL)	184.33±50.68	160.02±49.28	0.016
Triglyceride (TG) (mg/dL)	196.20±67.81	104.25±39.89	0.001
HDL cholesterol (mg/dL)	37.53±11.90	57.18±10.73	0.001
LDL cholesterol (mg/dL)	103.22±43.34	90.31±27.78	0.077

[Table/Fig-3]: Demographic and biochemical data of cases and controls.
p-value ≤0.05 is significant; n=51 in each group; BP: Blood pressure; HDL: High density lipoprotein; LDL: Low density lipoprotein

The mean SBP in the case group was 144.16±4.78 mmHg and DBP was 92.90±5.48 mmHg, as compared to the controls in which the SBP was 112.67±10.58 mm of Hg and DBP, was 78.08±5.57 mmHg. The difference was statistically significant.

The case group had a higher level of serum triglyceride and a lower level of serum HDLc as compared to the control groups. The difference was highly significant [Table/Fig-3]. The cases had a higher level of serum RBP4 as compared to the control groups and the difference is statistically significant [Table/Fig-4].

RBP4 (mg/L)	Range	Mean±SD	t-value	p-value
Cases	18.37-52.8	31.82±8.7	11.678	0.001
Controls	7.7-25.5	15.5±4.7		

[Table/Fig-4]: Serum RBP4 levels in cases and controls.
p-value=0.05 is significant

The [Table/Fig-5] shows that the serum RBP4 level positively correlated with SBP (r=0.644, p<0.001), DBP (r=0.444, p<0.001),

Parameters		RBP4	SBP	DBP	TG	HDL
RBP4	Pearson correlation	1	0.644**	0.444**	0.649**	-0.313**
	p-value (2-tailed)		0.001	0.001	0.001	0.025
SBP	Pearson correlation	0.644**	1	0.302*	0.191	-0.330*
	p-value (2-tailed)	0.001		0.031	0.180	.018
DBP	Pearson correlation	0.444**	0.302*	1	0.088	-0.287*
	p-value (2-tailed)	0.001	0.031		0.537	0.041
TG	Pearson correlation	0.649**	0.191	0.088	1	-0.267
	p-value (2-tailed)	0.001	0.180	0.537		0.059
HDL	Pearson correlation	-0.313**	0.330*	0.287*	-0.267	1
	p-value (2-tailed)	0.025	0.018	0.041	0.059	

[Table/Fig-5]: Correlation matrix of serum RBP4, SBP, DBP, TG and HDLc.
**. Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed); n=51 in each group

serum Triglycerides (r=0.649, p<0.001), and negatively correlated with HDLc (r=-0.313, p=0.025).

DISCUSSION

In the present study, the serum RBP4 level was evaluated and the mean RBP4 concentration in newly diagnosed primary hypertensive patients was higher than in the normotensive group. A positive correlation was observed between the serum RBP4 with blood pressure and serum triglyceride. A negative correlation was observed between serum RBP4 with HDLc. A large epidemiological survey in 2007 by Qi Q et al., showed that RBP4 levels were associated with blood pressure [16]. In the same year, Inoue S et al., also demonstrated that there was an elevation of RBP4 in pregnancy-induced hypertension patients [17]. A Japanese cohort study of hypertension "The Tanno and Sobetsu study" by Chiba M et al., in 2010 found that serum RBP4 levels in hypertensive men and women were similar to RBP4 levels in the present study [18]. Sun Q et al., in 2013 cohort study showed higher levels of RBP4 in coronary heart disease [19]. Deng W et al., in 2014 study on 331 subjects concluded that serum RBP4 levels were significantly increased in the hypertensive subjects than in normotensive, normal weight patients after adjusting for body mass index and waist circumference [20].

The elevation in serum levels of RBP4 in hypertensives compared to the healthy controls, indicates the role of this adipokine in the pathogenesis of hypertension. RBP4 may play a direct role in elevating BP and may lead to endothelial dysfunction, at least partly through attenuation of the NO-mediated vascular responses or by acting on the TLRs [9]. When up regulated TLR is expressed, it increases the TNF-alpha level in vascular smooth muscles contributing to inflammation. So, the TLRs modulate vascular function due to inflammation and thus contribute to hypertension [2]. New studies on RBP4 in animal models have suggested its role in inflammation mainly by decreasing the vascular tone and finally leading to hypertension [9].

This may be the mechanism by which serum RBP4 is associated with hypertension in the present study. There may be another possibility of elevated blood pressure, as RBP4 might be involved in the regulation of left ventricular diastolic function in patients with essential hypertension as stated by Li X et al., [21].

There was a positive correlation observed between serum RBP4 with blood pressure in the present study. The study by the Japanese Tanno and Sobetsu study in 2010 is in accordance with the findings of the present study [18]. Solini A et al., study in European females also had a positive correlation of RBP4 with SBP and DBP [22]. In 2017 the Zhang JX et al., multivariate study in prehypertensive cases showed similar statistical results as the present study [23]. Solini A et al., study in Italian newly diagnosed non obese hypertensive women without metabolic syndrome and with normal renal function diagnosed hypertensive females report a remarkable increase in serum RBP4 levels. They showed that serum RBP4 levels correlated positively with carotid intima-media thickness, supporting the hypothesis that RBP4 plays a role in the pathogenesis of hypertension [22].

The mean serum total cholesterol and triglycerides values in the present study group of hypertensives were significantly higher than the controls. Though, the mean LDL cholesterol in cases was higher than in controls, the difference was not statistically significant. This may be probably due to the small sample size of the study. A significant positive correlation was observed between serum RBP4 with serum triglyceride and a negative correlation observed between serum RBP4 with HDLc but no significant correlation was observed between RBP4 with total cholesterol and LDL. Kwanbunjan K et al., established a positive correlation of RBP4 with serum triglyceride [24]. Majerczyk M et al., in 2018 found associations between the levels of RBP4 and hypertriglyceridaemia, as well as low HDL cholesterol which is similar to the findings in the present study [25]. A similar study in children by Boaghi A et al., in 2020 showed an association of dyslipidaemia with RBP4 [26]. Serum RBP4 is positively correlated with the CVD risk factors such as hypertension, and deranged lipid profile as some authors hypothesise RBP4 to be a causal agent of inflammation and the correlation may be due to the ability of RBP4 to stimulate the liver for lipid synthesis [22,25,27]. The deranged lipid profile in the present study may be due to the role of RBP4 in the pathogenesis of dyslipidaemia by the synthesis of Very-low-density lipoprotein, oxidised LDL, and dysfunctional HDL and promoting hypertension and atherosclerosis indicating a lipogenic nature of RBP4 [28].

Limitation(s)

Small sample size of the study and patients receiving antihypertensive treatment and secondary hypertension were not included in the present study.

CONCLUSION(S)

In the present study, the serum RBP4 level was significantly higher in the newly diagnosed primary hypertension than in the age and sex-matched normotensive controls. A significant positive correlation was found between serum RBP4 levels with blood pressure and triglycerides and a significant negative correlation was observed between serum RBP4 and HDLc. More studies in the future will establish the role of this new serum marker as a diagnostic, prognostic, and risk stratification in primary hypertension. A study on patients receiving antihypertensive treatment and secondary hypertension will further emphasise the role of RBP4 in hypertension.

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