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Original Article

Biochemistry Section

Evaluation of Serum ApoA1 and ApoB Levels in the First-degree Relatives of Patients with Essential Hypertension at a Tertiary Care Centre in Eastern India: A Cross-sectional Study

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ABSTRACT

Introduction: An altered serum lipid profile and lipoprotein levels are major modifiable risk factors for hypertension. Apolipoprotein A1 and B100 (ApoA1 and ApoB100) are the chief structural proteins of High-density Lipoprotein Cholesterol (HDL-C) and Low-density Lipoprotein Cholesterol (LDL-C), respectively. The level of ApoB in the serum represents overall atherogenicity, whereas the level of ApoA1 can indicate total antiatherogenicity.

Aim: To evaluate the serum levels of ApoA1 and ApoB in the first-degree relatives of individuals with essential hypertension.

Materials and Methods: This cross-sectional observational study was conducted in the Clinical Biochemistry laboratory in association with the Department of General Medicine at SCB Medical College and Hospital, Cuttack, Odisha, India, from February 2018 to March 2019. It consisted of 165 participants: group I included hypertensive patients (n=55); group II included first-degree relatives of the above hypertensive patients (n=55); and group III included non hypertensive healthy age-matched controls (n=55). The waist-hip ratio, Blood Pressure (BP), fasting plasma glucose, serum cholesterol, triglycerides, HDL-C, LDL-C, ApoA1, ApoB, and the ApoB/ApoA1 ratio were all measured. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software version 25.0.

Results: The mean age of subjects in groups I, II, and III was 49±8.3, 38±5.2, and 37±5.4 years, respectively, with males constituting 56% and females 44% of the total participants. The ApoA1 levels were lowest in group I (104.9±16.3 mg/dL) and highest in group III (117.4±7.3 mg/dL). The serum ApoB levels and the ratio of ApoB/ApoA1 were highest in group I (104.2±14.1; 1.1±0.64 mg/dL) and lowest in group III (60.9±18.2; 0.5±0.15 mg/dL). A highly significant negative correlation (r=-0.40, p<0.01) was found between Systolic Blood Pressure (SBP) and ApoA1. A significant correlation (r=0.26, p=0.04) was observed between SBP and the ApoB/ApoA1 ratio in group III.

Conclusion: Serum ApoB levels and the ratio of ApoB/ApoA1 were significantly elevated in the first-degree relatives of hypertensive patients, thus emphasising the importance of screening individuals with a positive family history of hypertension.

Keywords: Apolipoproteins, Atherogenicity, Diastolic blood pressure, Dyslipidemia, Family history, Siblings

INTRODUCTION

Hypertension is a leading global non communicable disease, affecting approximately 1.3 billion people and resulting in about 10 million annual deaths [1]. Essential hypertension is defined as a Systolic Blood Pressure (SBP) of ≥140 mm Hg and a Diastolic Blood Pressure (DBP) of ≥90 mm Hg, irrespective of medication [2]. In India, the prevalence of hypertension is nearly 33% in urban areas and 25% in rural areas [3]. Family history plays a vital role in the development of hypertension [4]. The risk of essential hypertension in childhood increases considerably if, both parents are hypertensive. The genetic heritability of hypertension can be accounted for at 15-40%. Screening healthy individuals with a positive family history of hypertension helps to develop insights into the familial nature of hypertension [5-6].

Altered serum lipid profiles and lipoprotein levels are significant modifiable risk factors for essential hypertension. Hypertension, dyslipidemia, and other metabolic disruptions in an individual can act synergistically to accelerate atherosclerosis and the occurrence of cardiovascular diseases [7].

Apolipoprotein B 100 (ApoB) is the major apoprotein of atherogenic LDL-C. LDL-C contains variable amounts of cholesterol but has only one ApoB. Therefore, ApoB reflects the number of LDL particles better than LDL-C [8]. ApoB is a superior predictor of Cardiovascular Disease (CVD) compared to total blood cholesterol and triglyceride levels [4]. Apolipoprotein A1 (ApoA1) is the chief structural protein of antiatherogenic HDL-C. It plays an essential role in reverse cholesterol transport and cellular cholesterol balance. It is isolated as a prostacyclin-stabilising factor and thus may have anticlotting actions [9].

The ApoB level in serum represents gross atherogenicity, whereas the ApoA1 level can represent total antiatherogenicity [10]. Frank PG and Marcel YL, in a review, stated that the diffusional and translocational efflux of cholesterol from cells is better explained by the ApoA1 structure, and hence, its measurement is a better predictor of atherosclerotic potential than HDL [9]. Lima LM et al., suggested that measuring ApoA has a methodological advantage over quantifying LDL-C and non-HDL-C, as these parameters are usually calculated using different formulas, while ApoA is analysed using internationally standardised methods [10]. Numerous studies have revealed that ApoB is a more potent indicator of cardiovascular risk than LDL-C and non-HDL-C [11]. In a discordance analysis for cardiovascular risk assessment, Yun YM concluded that ApoB is superior to LDL-C levels [12].

A literature search revealed that the evaluation of serum ApoA1 and ApoB levels in the first-degree relatives of patients with essential hypertension has not yet been studied in India. Therefore, this study aimed to assess the serum ApoA1 and ApoB levels in the first-degree relatives of individuals with essential hypertension.

MATERIALS AND METHODS

The present cross-sectional observational study was conducted in the Clinical Biochemistry labouratory in association with the Department of General Medicine at SCB Medical College and Hospital, Cuttack, Odisha, India, from February 2018 to March 2019, after obtaining approval from the Institutional Ethical Committee (IEC 149/2018). The subjects were recruited for the study after obtaining their written informed consent.

Inclusion criteria: Patients aged 25-55 years diagnosed with essential hypertension according to Joint National Committee (JNC) 8 criteria [2], as well as first-degree relatives (parent, child, or sibling) of the aforementioned hypertensive patients who are not known cases of essential hypertension, were included in the study.

Exclusion criteria: Patients diagnosed with diabetes mellitus, renal disease, or endocrinopathy were excluded. Pregnant females, patients on treatment with immunosuppressants, and those taking drugs that induce the development of hypertension were also excluded. In the control group, subjects with a family history of hypertension, renal diseases, chronic metabolic diseases, and patients unwilling to participate in the study were excluded.

Sample size calculation: A convenient sampling method was adopted as present study was initiated as a small-scale investigation. Hypertensive patients visiting the Medicine Outpatient Department (OPD) were asked to include their first-degree relatives in the study. The final sample consisted of 165 participants, divided into three groups as follows:

Group I (n=55): Hypertensive patients;

Group II (n=55): First-degree relatives of the above hypertensive patients;

Group III (n=55): Non hypertensive healthy age-matched controls;

Study Procedure

Waist and hip circumferences were measured using the criteria established by the World Health Organisation, and the waist-hip ratio was calculated [13]. Blood pressure was measured after allowing the subjects to relax for five minutes in a sitting position, with the BP apparatus kept at heart level. Two readings were taken one minute apart; if the readings differed significantly, a third reading was taken. The lower of the two readings was used to indicate BP (SBP and DBP).

Analysis of biochemical parameters: A 5 mL sample of venous blood was collected under aseptic precautions for biochemical analysis. Serum cholesterol, triglycerides, HDL-C, and fasting plasma glucose were analysed using the Toshiba 120 FR fully automated chemistry analyser. The LDL-C level was calculated using Friedewald's formula. Serum levels of ApoA1 and ApoB100 were analysed using the Automated Clinical analyser Biolis 24i Premium (Tokyo Boeki Machinery Ltd.). Internal quality control for the above parameters, procured from Agappe Diagnostics, was performed daily, and the monthly Coefficient of Variation (CV%) was satisfactory [Table/Fig-1] [14,15].

Parameters	Biological reference interval	Method of estimation
Fasting plasma glucose	75-100 mg/dL	Glucose oxidase peroxidase
Serum cholesterol	<200 mg/dL	Cholesterol oxidase peroxidase
Serum triglyceride	50-150 mg/dL	Lipase/GPO-PAP
Serum HDL-C	40-60 mg/dL	Cholesterol esterase oxidase method
Serum LDL-C	<100 mg/dL	Friedwald's formula
Serum ApoA1	110-230 mg/dL	Turbidimetry
Serum ApoB	60-140 mg/dL	Turbidimetry

[Table/Fig-1]: Biological reference interval and method of estimation of the analytes studied [14,15].
GPO-PAP: Glycerol-3-phosphate oxidase-phenol-aminophenazone

STATISTICAL ANALYSIS

The statistical analysis was performed using SPSS version 25.0. The distribution of the data was assessed using the Shapiro-Wilk test. Continuous data were expressed as mean and standard deviation. An inter group comparison of the variables was conducted using Analysis of Variance (ANOVA) and Independent t-tests. Correlation analysis was performed using Pearson's correlation coefficient. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The collected data were subjected to the Shapiro-Wilk test and were found to have a continuous distribution. The mean age of subjects in groups I, II, and III was 49±8.3, 38±5.2, and 37±5.4 years, respectively. The gender distribution (male/female) in groups I, II, and III was 33/22, 39/16, and 25/30, respectively. The waist-hip ratio was higher in group I (1±0.03) compared to group II (0.9±0.04) and group III (0.9±0.04). The ApoA1 levels were lowest (104.9±16.3 mg/dL) in group I and highest (117.4±7.0 mg/dL) in group III. The serum ApoB levels and the ratio of ApoB/A1 were highest (104.2±14.1; 1.1±0.64 mg/dL) in group I and lowest (60.9±18.2; 0.5±0.15 mg/dL) in group III. An independent samples t-test was applied between groups I and II, and significant differences (p-value <0.01) in the lipid profile and serum levels of ApoA1 and ApoB were observed, as expected in the non hypertensive group. When groups II and III were compared [Table/Fig-2], significant differences were found in DBP (p=0.03), ApoB (p-value=0.01), and the ApoB/A1 ratio (p-value <0.01), thus emphasising the importance of ApoB as an indicator of dyslipidaemia, even when the difference in serum LDL-C levels is not significant [Table/Fig-2].

Parameters	Group I (n=55)	Group II (n=55)	Group III (n=55)	p-value*	p-value@ Group I vs II	p-value@ Group II vs III	p-value@ Group I vs III
Age	49±8.3	38±5.2	37±5.4	<0.01	< 0.01	0.94	<0.01
Gender distribution (Male/Female)	33/22	39/16	25/30	-	-	-	-
Waist-hip Ratio (WHR)	1±0.03	0.9±0.04	0.9±0.04	<0.01	< 0.01	1.0	<0.01
Systolic BP (SBP) (mm Hg)	154±6.6	121±9.6	118±8.7	<0.01	< 0.01	0.17	<0.01
Diastolic BP (DBP)	95±4.6	74±5.5	72±3.5	<0.01	< 0.01	0.03	<0.01
Fasting plasma glucose (mg/dL)	87±17.6	74±7.6	73±7.5	<0.01	<0.01	1.0	<0.01
Serum cholesterol (mg/dL)	197±35	144±15.8	143±15.7	<0.01	<0.01	0.85	<0.01
Serum triglyceride (mg/dL)	199±70	111±33.5	106±32.4	<0.01	0.04	0.52	<0.01
Serum HDLc (mg/dL)	45±5.7	39±8.9	38±7.9	<0.01	0.02	0.69	<0.01
Serum LDLc (mg/dL)	113±27.7	84±12.4	83±12.3	<0.01	<0.01	0.95	<0.01
Serum ApoA1 (mg/dL)	104.9±16.3	115.1±7.0	117.4±7.0	<0.01	<0.01	0.98	<0.01

Serum ApoB (mg/dL)	104.2±14.1	69.4±15.4	60.9±18.2	<0.01	<0.01	0.01	<0.01
ApoB/ApoA1	1.1±0.64	0.6±0.15	0.5±0.15	<0.01	<0.01	<0.01	<0.01

[Table/Fig-2]: Descriptive statistics and analysis of variance between the groups.
*- p-value obtained after comparing three groups using ANOVA; @- p-value obtained after two groups were compared using independent t-test

There was a negative and non significant correlation (r-value=-0.12, p-value=0.4) between the ratio of ApoB/ApoA1 and SBP in group I. A similar negative correlation (r-value=-0.03, p-value=0.8) was observed between the ratio of ApoB/ApoA1 and DBP in group I [Table/Fig-3].

Parameters	SBP (r, p-value)	DBP (r, p-value)
ApoB	0.05, 0.7	0.13, 0.4
ApoA1	-0.11, 0.4	0.04, 0.7
ApoB/ApoA1	-0.12, 0.4	-0.03, 0.8

[Table/Fig-3]: Pearson's correlation between blood pressure and ApoB, ApoA1, and ApoB/ApoA1 in Group I.

In contrast, a highly significant negative correlation (r-value=-0.40, p-value <0.01) was found between SBP and ApoA1. Additionally, a significant positive correlation (r-value=0.26, p-value=0.04) was observed between SBP and the ratio of ApoB/ApoA1 in group II [Table/Fig-4]. A significant negative correlation (r-value=-0.32, p-value=0.02) was also noted between SBP and ApoA1. However, the correlation between the ratio of ApoB/ApoA1 and SBP and DBP was not significant [Table/Fig-5].

Parameters	SBP (r, p-value)	DBP (r, p-value)
ApoB	0.15, 0.25	0.21, 0.12
ApoA1	-0.40, <0.01*	0.01, 0.92
ApoB/ApoA1	0.26, 0.04*	0.13,0.31

[Table/Fig-4]: Pearson's correlation between blood pressure and ApoB, ApoA1, and ApoB/ApoA1 in Group II.
*p-value <0.05

Parameters	SBP (r, p-value)	DBP (r, p-value)
ApoB	-0.02, 0.89	-0.07, 0.6
ApoA1	-0.32, 0.02*	-0.15, 0.27
ApoB/ApoA1	0.05, 0.71	-0.05, 0.7

[Table/Fig-5]: Pearson's correlation between blood pressure and ApoB, ApoA1, and ApoB/ApoA1 in Group III.

DISCUSSION

The familial nature of hypertension has been explored in various studies, highlighting the importance of screening individuals with a positive history of essential hypertension to narrow the search for the disease. Some families may have essential hypertension spanning generations, which increases the likelihood of hypertension in their descendants [4,16]. The present study showed that BP among patients with essential hypertension (group I) was significantly higher than that of their first-degree relatives (group II). DBP was significantly higher and differed significantly between the controls and group II. This finding correlates with studies that have concluded that higher DBP is associated with a greater risk of future development of hypertension in adults under 50 years of age, and it should not be overlooked, as both DBP and SBP can independently predict cardiovascular risk [17,18].

The lipid profiles of group I were significantly higher than those of group II. Dyslipidaemia is strongly associated with the development of hypertension; both conditions share similar pathophysiological mechanisms related to endothelial dysfunction and are independently associated with an increased cardiovascular risk [19]. The difference in lipid profiles between group II and the control group was not statistically significant.

The ApoA1 levels were significantly lower in hypertensive patients compared to their first-degree relatives and the control subjects. There was a highly significant negative correlation between ApoA1 and SBP. The ApoA1 levels in serum correlate strongly with HDL-C. In addition to its antiatherogenic properties, ApoA1 exhibits endothelium-stabilising properties. In a health survey by Forvall G et al., in Uppsala, Sweden, ApoA1 strongly predicted CVD and its associated mortality. Some research groups are focused on increasing the ApoA1 fraction and HDL using ApoA1 peptides and mimetics [20]. A study in China also concluded that high serum ApoA1 could lower the likelihood of developing essential hypertension in patients with coronary artery disease [21].

The serum ApoB levels were significantly higher in hypertensive patients than in group II and the control subjects. The first-degree relatives of the patients had substantially higher levels of ApoB compared to controls, thereby predisposing them to a higher risk of developing hypertension. A longitudinal study conducted on American Japanese subjects over a period of 10 years suggested that individuals who developed hypertension during the study had elevated serum ApoB levels. The retention of lipids containing ApoB in the arterial wall can interact with subendothelial proteoglycans and initiate a series of inflammatory events, eventually leading to atherosclerosis and, subsequently, hypertension [22]. A systematic review by Nathir I et al., stated that an increase in serum ApoB concentration in hypertensive individuals exacerbates the risk of cardiovascular events [23].

Authors found that the ratio of ApoB/ApoA1 was significantly higher among the three groups for obvious reasons. This ratio was significantly elevated in the first-degree relatives compared to controls, emphasising its role as a predictor of hypertension in subjects with a positive family history. Additionally, we found a positive and significant correlation between ApoB/ApoA1 and SBP. This ratio is a better predictor of Coronary Artery Disease than conventional lipid particles and lipid ratios, as it signifies the balance between proatherogenicity and antiatherogenicity [22].

Limitation(s)

This study was limited by a small sample size. The hypertensive participants could have been stratified into groups based on their blood pressure levels. Additionally, the first-degree relatives were not monitored for the future development of hypertension.

CONCLUSION(S)

The present study concludes that ApoA1, ApoB, and the ApoB/ApoA1 ratios are significantly higher in individuals with hypertension. The first-degree relatives were found to have a higher DBP compared to control subjects, thus emphasising the need for screening individuals with a positive family history of hypertension. Serum ApoB levels and the ratio of ApoB/ApoA1 were significantly elevated in the first-degree relatives. These parameters correlated significantly with SBP compared to conventional lipid profiles. The ApoB/ApoA1 ratio may be more instrumental than the individual apolipoprotein levels in identifying hypertension within families. Future longitudinal studies with a larger sample size should be conducted to investigate the inheritance patterns of essential hypertension.

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