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Research Article

ApoB/ApoA-1 ratio and nitric oxide levels in pregnancy induced hypertensive women

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ABSTRACT

Background: Pregnancy induced hypertension is considered as the major cause of maternal and perinatal mortality. Even though occurrence of PIH is due to abnormal placentation, endothelial dysfunction plays a pivotal role in the genesis of the multisystem disorder that develops in pre eclampsia and eclampsia. Various studies have proved that hyperlipidemia is one of the major causes of endothelial dysfunction. Since ApoB/apoA-I ratio is a dyslipidemic indicator, the study was designed to determine ApoB/ApoA-I in PIH women and to analyse whether this ratio can be correlated with ED in PIH women.
Methods: A cross-sectional analytical study involved normotensive, preeclamptic and eclamptic pregnant women with hundred subjects in each group. They were investigated for serum lipid profile, ApoA, ApoB, NO, MDA, FRAP in the 3rd trimester of pregnancy.
Results: The SBP & DBP were significantly high between 3 groups. The mean plasma TC, TGL, VLDL, LDL, MDA, ApoB levels, ApoB/ApoA-I were significantly high & HDL, ApoA-I, NO, FRAP levels were significantly low between 3 groups. The ApoB/ApoA-I was positively correlated with TC, TGL, VLDL, LDL, malondialdehyde and negatively correlated with HDL, FRAP & NO.
Conclusions: Our results indicate that women with PE & E exhibit markedly elevated concentrations of TGL-rich lipoproteins. The negative correlation between ApoB/ApoA-I with NO indicates that the hyperlipidemia is directly related with severity of ED in PIH. So, careful monitoring of ApoB/ApoA-I along with NO might be helpful to predict the onset and progression of the disease.

Keywords: Pregnancy induced hypertension, ApoB/ApoA-I ratio, Nitric oxide

INTRODUCTION

Hypertensive pregnancy disorders complicate 10% of all pregnancies.[1] WHO estimates that at least one woman dies every seven minutes from complications of hypertensive disorders during pregnancy.[2] The incidence of PIH is high among the women who have conceived for the 1st time.[3] Preeclampsia (PE) is currently classified as a pregnancy-specific syndrome characterized by the presence of new-onset hypertension in a previously normotensive woman after 20 weeks of gestation with proteinuria. Eclampsia is classified as presence of seizures, non-attributable to other causes, in women diagnosed with PE.[4] If undetected, preeclampsia can lead to eclampsia which is one of the top five causes of maternal illness, infant illness and death. The risk of developing pre-eclampsia appears to be greater in women who have family histories of essential hypertension.[5]

Maternal endothelial dysfunction is considered as a classic hallmark of preeclampsia.[6] Previous studies had stated that preeclamptic women had presented arterial

lesions at the uteroplacental implantation site which are characterized by areas with fibrinoid necrosis surrounded by lipid-laden macrophages.[7] Pregnancy is known to affect biochemical metabolic processes involved in carbohydrate, protein, lipid and lipoprotein metabolism. These metabolic changes have evolved to meet the metabolic demands of the growing fetus. However, in some cases, such metabolic changes particularly lipid and lipoprotein are found to be exaggerated which may lead to ED.[8]

The Apolipoproteins have important roles in lipid transport and metabolism. They have specific structural domains that are recognized by cell receptors. They often diffuse from one lipoprotein and bind to another.[9] ApoB and ApoA-I are the two major apo lipoproteins involved in lipid transport and is reported to influence the process of atherosclerosis. ApoB is a large protein that exists in the plasma in two isoforms, apoB48 and apoB100. ApoB48 is exclusively expressed in the intestine and is present on chylomicrons and chylomicron remnants. ApoB100 is mainly expressed in the liver and is present on all atherogenic lipoprotein particles, including VLDL, IDL, LDL and Lp (a). In clinical practice, apoB can be used as a marker to estimate the total number of atherogenic lipoprotein particles.[10] ApoA-I is the major protein in HDL particles. It reflects the anti-atherogenic potential.[11] ApoB/apoA-I ratio is regarded as atherogenic index and it is also an index of dyslipidaemia which has a direct effect on the alteration of endothelial function. Endothelial dysfunction has been attributed to a reduction in nitric oxide (NO) bioactivity through the formation of peroxy nitrite.[12] Therefore this clinical study was designed to determine ApoB/apoA-I ratio in PIH women and to analyze whether this ratio can be correlated with ED in PIH women.

METHODS

Patients and controls

This study was approved by the Institutional Ethics Committee of Annapoorana medical college & hospital and informed consent was obtained from all participants. Hospitalized patients with pre eclampsia and eclampsia in the department of obstetrics and gynecology were randomly selected for this study. PIH patients were defined according to the NHBPEP (National high blood pressure education programme) guidelines.[13] The details of Socio demographic characters, maternal age, marriage age, gestational age at screening, parity & gravidity, H/o diabetes, hypertension, kidney disease, dyslipidemia, cardiovascular diseases, previous history & family history of PIH/HTN in first degree relatives were collected. Individuals with past history of cardiac, renal, hepatic illness, diabetes, and hypertensions were excluded. Age matched normotensive pregnant ladies were randomly selected as controls.

Based upon the criteria, the study subgroups are divided into following groups:

Group 1: Normotensive pregnant women (n=100)
Group 2: Preeclamptic women (n=100)
Group 3: Eclamptic women (n=100)

Fasting venous blood samples were obtained from both patients and controls. Samples were biochemically analysed for TC, TGL, HDL, ApoA-I, and ApoB using commercially available standard kits (Agappe diagnostics) in a semi auto analyser. VLDL & LDL values were computed with the friedewald formula. Nitric oxide was done using Griess method. MDA was done using TBARS. Total antioxidant capacity was estimated using FRAP method. The data was processed on computer software package SPSS version 20. The numerical data was presented as mean+SD. One way ANOVA was used to evaluate mean differences in maternal serum lipid, lipoproteins & apolipoprotein concentrations between patients and controls. A value of $P<0.05$ at 95% CI was considered as statistically significant. Correlation was done using Pearsons correlation analysis.

RESULTS

The socio demographic characters between controls, PE & E groups were compared in Table 1. The maternal age, parity and gestational weeks were almost comparable between the 3 groups. The mean SBP/DBP was significantly high between the 3 groups.

Table 1: Distribution of mean values and standard deviations of socio demographic features between controls and PIH groups.

Character	Control	Pre eclampsia	Eclampsia
Maternal age (Mean±SD)	23.97± 3.30	24.62±4.07	25.71±3.71
Gestational age (weeks)	31.57± 2.67	32.42±3.12	31.05±2.90
Parity (n)	1.4±0.7	1.40±0.71	1.31±0.67
Systolic BP (mm Hg)	116±5.45	162.18± 18.26*	170± 15.52*a*
Diastolic BP (mm Hg)	75±5.99	107.5± 11.35 *	112.28± 10.59*a*

Values are expressed as mean± SD .values that are marked with a star* differ significantly from the control at P= <0.05.Values that differ significantly at P=<0.05 between PE & E groups are marked with a*

Table 2 compares mean lipid profile and lipoprotein levels between the 3 groups. The TC, TGL, VLDL, LDL, ApoB, MDA, levels were significantly high between the 3 groups. The HDL, ApoA-1, NO, FRAP levels were significantly low between the 3 groups. ApoB/apoA-I ratio was significantly high between the 3 groups.

Table 3 shows the correlation coefficient (r) of ApoB/apoA-I ratio with lipid, lipoproteins, NO, MDA & FRAP.

Table 2: Distribution of mean values and standard deviations of Lipids, lipoproteins, apolipoproteins, Nitric oxide & ApoB/apoA-I ratio between Controls and PIH groups.

Parameter	Control	Preeclampsia	Eclampsia
Total cholesterol (mg/dl)	209.7± 34.90	223.59± 39.46*	244.14± 43.56*a*
Triglycerides (mg/dl)	203.6± 37.31	246.53± 34.29 *	318.48± 78.39*a*
HDL (mg/dl)	44.02 ±7.71	35.65 ± 7.64 *	29.97 ± 4.61*a*
VLDL calculated (mg/dl)	40.72± 7.46	49.30± 6.85*	63.69± 15.67 * a*
LDL calculated (mg/dl)	124.95± 34.30	138.63± 34.66*	150.47± 46.0*a*
Apo A-1 (mg/dl)	184.9± 17.04	131.18± 23.72 *	144.65± 32.05 *a*
Apo B-100 (mg/dl)	129.22± 16.69	153.82± 26.7 *	182.14± 16.17* a*
ApoB/Apo A ratio	0.76±0.17	0.87± 0.15 *	1.33± 0.36 *a*
MDA (µmol/L)	1.08±0.86	4.49± 1.75*	5.50± 1.97* a*
FRAP (µmol/L)	2.21±0.89	0.67± 0.42*	0.407± 0.38 *a*
Nitric oxide (µmol/L)	117.37± 14.77	43.87± 6.13*	38.6± 9.94* a*

Values are expressed as mean± SD .values that are marked with a star* differ significantly from the control at P= <0.05.Values that differ significantly at P=<0.05 between PE & E groups are marked with a*

Table 3: Correlation coefficient (r) of ApoB/apoA-I ratio with lipid, lipoproteins and Nitric oxide levels.

Parameter	r value	P value
Total cholesterol	0.290**	0.000
Triglycerides	0.517**	0.000
HDL	-0.323**	0.000
VLDL	0.517**	0.000
LDL	0.183**	0.000
MDA	0.406**	0.000
FRAP	-0.406**	0.000
Nitric oxide	-0.368**	0.000

DISCUSSION

PIH, being the leading cause of maternal death and perinatal morbidity, the need for a reliable marker in order to identify the disease in early stages is increasing steadily. Endothelial dysfunction is considered as a centre to the multiple-organ pathophysiology of preeclampsia-eclampsia.[14] The clinical feature of PE is new onset of maternal hypertension and proteinuria that resolves after delivery. The maternal hypertension results from diffuse ED and the proteinuria is ascribed to glomerular endotheliosis.[15] The release of seizure-provoking factors or other deleterious proteins or pro-inflammatory cytokines due to maternal ED in PE may lead to BBB disruption and increased permeability which may cause seizure onset.[16] The hallmark of endothelial dysfunction is impaired NO bioavailability.[17] NO is an endothelium derived factor responsible for vasodilatation and platelet activation inhibition. It is involved in various stages of pregnancy including implantation, maintenance of uterine acquiescence during pregnancy, control of uterine contractions and relaxation, basic physiological adaption for successful gestation and regulation of blood pressure.[18] Our analysis had shown a significantly lower NO levels between the 3 groups indicating that the ED plays an important role in the progression and complication of the disease. ED can be caused by several conditions like diabetes, smoking, and physical inactivity.[19] One of the factor for ED is hyperlipidemia.[20]

Healthy pregnancy is characterized by a progressive increase in the serum concentrations of total cholesterol, triglycerides and hence increases in the concentration of lipoproteins like LDL and very-low-density lipoproteins (VLDL), to satisfy the demands of the developing fetus. During early pregnancy the increase in hepatic production of TG and enhancement in the removal of TG from circulation occurs. During late pregnancy due to stimulation of lipase enhanced release of FFA's from adipocytes occurs. These are important metabolic changes that must occur and allow the gravid female to meet the energy requirements of late gestation.[21]

Several studies have reported a dramatic abnormal rise of lipid profile in PIH women.[10] This observation holds true even in our study also. The TGL levels were significantly high among the PE & E women when compared to the control women. The levels of TGL were significantly high in eclamptic women when compared to preeclamptic women correlating with the severity of disease which is in agreement with a study by Musa, et al.[8] Pregnancy is associated with insulin resistance. The observed hypertriglyceridemia might be due to the insulin resistant visceral fat which results in increased flux of fatty acids to the liver via portal vein with two immediate consequences: hepatic steatosis due to an increase in TGL synthesis and increase in the blood VLDL.[22] Observed Hypertriglyceridemia could also be due to low activity of LPL, an insulin-dependent endothelial enzyme. Because of the decrease in the activity of LPL, the removal of chylomicrons and VLDL from circulation is low in Insulin resistant patients. Thus VLDL remains in the plasma for a longer time and leads to the accumulation of LDL. The increased TGL is likely to be deposited in predisposed vessels, such as the uterine spiral arteries and contributes to the activation of endothelial cells leading to

the production of placental derived factors which can be considered as probable contributors for the pathogenesis of PIH.[23,24]

Our study had displayed a significantly low HDL cholesterol & ApoA-I levels in PE & E women than the controls. The HDL & ApoA-I levels were significantly low in eclamptic women than the women with preeclampsia. A study by CN Ekator, et al had stated that increased TGL play a part in decreasing the HDL-cholesterol. Impaired transport of cholesterol from peripheral tissues to the target area of utilization may cause the decrease in HDL-cholesterol in serum. There is a direct correlation between adipose tissue lipoprotein lipase activity and plasma HDL cholesterol which might be responsible for low levels of HDL cholesterol.[25]

In our study TC, VLDL, LDL & apoB-100 levels were significantly high in PE, E women than controls. The eclamptic women had displayed a significantly high level of TC, VLDL, LDL & ApoB-100 than the women with PE in our study. In most conditions more than 90% of all ApoB in blood is found in LDL.[10] In cases where LDL C is in the normal/low range, high ApoB levels indicate an increased number of small dense LDL (sd-LDL) particles, which are the most atherogenic particles.[11]

In our study ApoB/apoA-I ratio was found to be significantly high in PE &E women compared with the controls. The ApoB/apoA-1 ratio was found to be significantly high (P=0.000) in eclamptic women than the preeclamptic women correlating with the severity of disease. A study by wallidus et al stated that the lipid status determined as the ApoB/apoA-I ratio is a better marker than conventional lipid profile and lipid ratios.[11] A study by sachu, et al had reported that ApoB/apoA-I ratio can be considered as the accurate marker for hyperlipidemia and as it covers both atherogenic and antiatherogenic lipids, this ratio can be used as better predictor of Coronary artery disease than conventional factors.[26] A study by Jian Wang, et al had stated that the decreased level of apo A-I and increased level of ApoB in fetal blood may contribute to the vascular disease in fetal umbilical placental circulation during placental insufficiency. This condition could also cause 'acute atherosis' in umbilical placental circulation similar to atherosclerosis in adult life.[27] Apo A-I not only initiates the reverse cholesterol transport by activating the LCAT but also manifests antioxidant and anti-inflammatory effects. ApoA-1 can stimulate both endothelial production of nitric oxide as well as release of prostacyclin from the endothelium.[26] Level of apolipoproteins overwhelms the lipids because ApoA1 is under more genetic control than lipid components and hence depicts the number of lipoprotein particles more accurately.[11]

Apolipoprotein measurements present some methodological advantages over the measurement of lipoproteins. The LDLc quantified by the Friedewald equation cannot be extended to samples presenting triglyceride levels higher than 400 mg/dl but the same does not interfere significantly with apolipoprotein measurement. The plasma apolipoprotein levels can be measured directly by accurate and precise internationally standardized methods, using a common reference material for apo A-I and apo B and the same is not available for HDLc and LDLc measurements. Plasma apolipoprotein levels are slightly influenced by biological variables, whereas plasma lipid levels fluctuate in response to various metabolic control stimulus. Therefore preanalytical variables have less influence in the measurements of apolipoproteins A-I and B, which can be dosed without the need of prior fasting.[28]

Our correlation analysis had shown a negative correlation between ApoB/apoA-I ratio and NO. This indicates that hyperlipidemia contributes to ED by increasing the production of ROS & RNS.[29] Moreover our study had displayed a positive correlation between ApoB/apoA-I ratio and MDA besides negative correlation between ApoB/apoA-I & FRAP. In our study the mean MDA levels were significantly high and the mean FRAP levels were significantly low in PE & E women than controls. The mean MDA levels were significantly high & the mean FRAP levels were significantly low in eclamptic women than in pre eclamptic women in our study. This might be due to increased oxidative stress which is believed to result from increased formation of lipid peroxides, ROS and superoxide anion radicals, leading to an imbalance in production between prooxidant and antioxidant defences.[30] The increased serum levels of LDL in PE women undergoes oxidation which results in the generation of oxidized LDL. Ox-LDL can bind to the lectin-like oxidized LDL receptor-1 (LOX-1) on endothelial cells which is responsible for the binding, uptake, and degradation of oxLDL. The binding of oxLDL activates the NADPH oxidase enzyme system, resulting in the excessive generation of superoxide which scavenges the NO and reduces its bioavailability.[31]

Hyperlipidemia contributes to ED through the oxidative stress. For the reason that the ApoB/apoA-I ratio is most dependable proven marker for dyslipidemia and moreover it was also negatively correlated with NO which is a marker for ED, early careful monitoring of ApoB/apoA-I ratio along with NO might be helpful to predict the onset and progression of the disease.

CONCLUSION

Our results indicate that the women with PE & E exhibit markedly elevated concentrations of TGL-rich lipoproteins. The negative correlation between ApoB/apoA-I ratios with NO indicates that the hyperlipidemia is directly related with the severity of ED in PIH women. So, early careful monitoring of ApoB/apoA-I ratio along with NO might be helpful to predict the commencement and advancement of the

disease. It may help in emerging approaches for diagnosis and prevention of maternal and fetal complications.

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