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SERUM LDH, ALP AND URIC ACID IN HYPERTENSIVE DISORDERS OF PREGNANCY

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ABSTRACT

Background: *Gestational hypertension (BP>140/90 mm of Hg without proteinuria) and Preeclampsia (BP>140/90 mm of Hg with proteinuria) are classified under hypertensive disorders of pregnancy (HDP). There is widespread endothelial dysfunction leading to hypertension and damage to vital organs such as liver, kidney, brain etc. Damage to such organs may lead to elevation in serum levels of specific enzymes and metabolites which can be used for predicting severity of the disease.* ***Objectives:*** *This study was done to compare serum levels of lactate dehydrogenase(LDH), alkaline phosphatase(ALP) and uric acid(UA) among women with HDP, normal pregnancy and non-pregnant healthy controls and to evaluate role of their estimation in HDP.* ***Materials and methods:*** *Case control study was done taking 30 women with preeclampsia (group I), 30 with gestational hypertension (group II), 30 with normal pregnancy (group III) and 30 age matched healthy non-pregnant women (controls). Serum levels of LDH, ALP and UA were measured using commercially available kits. Statistical analysis was done using SPSS 17.0.* ***Results:*** *Serum levels of LDH, ALP and UA were significantly increased in women with HDP compared with controls. LDH & ALP were significantly high in group I compared with group II, and LDH, ALP & UA were high in group II compared with group III. Their levels significantly positively correlated with systolic and diastolic BP.* ***Conclusion:*** *Serum LDH, ALP and UA levels gradually increase as the disease severity increases. Regular monitoring of their serum levels in women with HDP may give a clue of disease severity and associated organ damage.*

KEYWORDS

Alkaline phosphatase, gestational hypertension, lactate dehydrogenase, preeclampsia, uric acid

INTRODUCTION

Pregnancy is a physiological state associated with many alterations in metabolic, biochemical, physiological, hematological and immunological processes. If there are no complications, all these changes are reversible following a few days to a few months after delivery [1].

Globally, an estimated 287,000 women died during pregnancy and childbirth in 2010 of which India accounted for approximately 19% (56,000) deaths [2]. Hypertensive disorders of pregnancy and their complications rank as one of the major cause of maternal mortality and morbidity in the world after obstetric hemorrhage, preexisting medical disorders, sepsis and abortions[3]. HDP occurs in approximately 6-8% of all pregnancies, 10% of first pregnancies, and 20-25% of women with a history of chronic hypertension [4]. It accounts for approximately a quarter of all antenatal admissions [5]. In addition, as it is strongly associated with fetal growth retardation and prematurity, it also contributes largely to perinatal mortality and morbidity [6].

Several studies have been carried out till date to understand the pathophysiological basis of this disease. But still the exact pathophysiology of this disease is not known. Impaired trophoblast invasion leading to atherosclerotic lesions in placenta is implicated as a causal factor in pathogenesis of the disease [7]. Many other theories such as immunological intolerance between maternal and fetal tissue, genetic

predisposition, nutritional imbalance, oxidative stress etc are also postulated [8, 9].

These lesions in placenta cause narrowing of uterine spiral arterioles leading to placental ischemia. Placental ischemia leads to alteration in expression of various factors which affect the endothelial function. It leads to reduced expression of vascular endothelial growth factor (VEGF), placental growth factor (PlGF), prostacyclin (PGI_2), nitric oxide (NO) etc from vascular endothelium. On the other side, there is increased expression of antiangiogenic factors such as tumour necrosis factor-α (TNFα), interleukins (ILs), soluble Fms like tyrosine kinase 1 (sFlt 1), Angiotensin 1 receptor autoantibody (AT1-AA), endothelin-1 (ET-1), thromboxane A_2 (TxA_2) etc. This angiogenic imbalance leads to wide spread endothelial dysfunction all over the body [10, 11].

Lactate Dehydrogenase (LDH) is mainly an intracellular enzyme. It is responsible for interconversion of pyruvate and lactate in the cells. Its levels are several times greater inside the cells than in the plasma. So its levels are increased in the scenario of increased cell leakiness, hemolysis and cell death [12].

Alkaline phosphatase (ALP) is a nonspecific enzyme which hydrolyses aliphatic, aromatic or heterocyclic compounds. It has several isoenzyme forms, of which α_2 heat stable ALP is of placental origin. Normal serum level of placental isoform is only 1% of total ALP but in pregnancy and pregnancy associated disorders its level rises enormously [12].

Uric acid (UA) is an end product of purine metabolism. It is filtrated through glomeruli and almost completely reabsorbed in proximal convoluted tubules (PCT) by both active and passive carrier mediated process. It is also actively secreted into the tubules. 85% of total excreted UA is derived by tubular secretion [13]. Hyperuricemia is found to be one of the earliest laboratory manifestations of preeclampsia. It is likely to be resulted from reduced UA clearance from reduced glomerular filtration rate (GFR) and reduced tubular secretion. Its increased levels suggest serious impending damage to kidney functions [8].

This study was undertaken to evaluate the effect of hypertensive disorders of pregnancy on serum concentrations of LDH, ALP and UA and to correlate their levels with severity of the disease.

MATERIALS AND METHODS

A cross sectional study was conducted taking women with hypertensive pregnancy & healthy pregnant women as cases and healthy non-pregnant women as controls. The study cases were selected from Bapuji Hospital and Chigateri Hospital, Davangere (both these hospitals are attached to teaching institute, J.J.M. Medical College, Davangere). The controls were selected from surrounding community. Each participant gave an informed consent and this study was approved by the ethical and research committee of J.J.M. Medical College, Davangere to use human subjects in the research study.

A) Selection of study subjects

Based on inclusion and exclusion criteria a total number of 120 subjects (90 cases and 30 controls) were selected for the present study.

Inclusion Criteria used to select the study subjects:

Women with hypertensive disorders of pregnancy were selected on the basis of definitions given by National high blood pressure education program (NHBPEP 2000) [8]. Case subjects were subdivided into three groups.

Case group I- It included 30 diagnosed cases of preeclampsia in age group of 20-45 years.

Pregnant female of ≥20 weeks of gestation with blood pressure ≥140/90 mm of Hg noted first time during pregnancy on ≥2 occasions at least

6 hours apart with proteinuria of ≥1+ (≥30mg/dl) by dipstick method in a random urine sample was considered as having preeclampsia.

Case group II- It included 30 diagnosed cases of gestational hypertension in age group of 20-45 years.

Pregnant female of ≥20 weeks of gestation with blood pressure ≥140/90 mm of Hg noted first time during pregnancy on ≥2 occasions at least 6 hours apart without proteinuria was considered as having gestational hypertension.

Case group III- It included 30 age matched healthy normotensive pregnant women of ≥20 weeks of gestation without any major illness and who are not on any medication.

Controls- It included 30 age matched healthy normotensive non-pregnant women without any major illness and who are not on any medication.

Exclusion Criteria:

The women with history of chronic hypertension, diabetes mellitus, drugs intake, smoking, alcoholism, liver, cardiac or renal diseases or any other major illness were excluded from the study.

Based on the inclusion and exclusion criteria, age matched cases and controls were included in the present study after obtaining informed consent. A proforma was used to record relevant information and patient's data.

B) Collection of blood samples:

About 3 ml of blood was drawn under aseptic precautions from selected subjects in a plain vial for serum. Serum was separated by centrifugation and used for estimation of serum levels of LDH, ALP and UA.

Concentration of serum LDH, ALP and UA were analyzed by using analytical kits from ERBA Diagnostics Mannheim GmbH in semi-autoanaylzer (CHEM-5 Plus V_2, Erba Mannheim).

Values were calculated as mean±SD and the statistical analysis was done using SPSS 17.0 software. Student's unpaired t-test was used for comparison between two groups and one way ANOVA test was used to compare all the groups simultaneously. Pearson's correlation coefficient was used to see the correlation between blood pressure and the parameters. The p-value of less than 0.05 was considered as statistically significant.

RESULTS

Table-1 shows that the mean level of systolic BP, serum ALP and serum UA was significantly higher in Group I, II and III when compared with controls. The mean levels of diastolic BP and serum LDH were significantly higher in Group I and II when compared with healthy non-pregnant controls. There was no significant difference in diastolic BP and serum LDH in Group III when compared with controls. No significant difference was found in POG and age of mother in all the study groups. One way ANOVA test showed significant difference in levels of all the parameters among study groups except POG and age of mother which was non-significant.

Table-2 and figure 1, 2, 3 shows that there is highly significant positive correlation of systolic & diastolic blood pressure with serum LDH, ALP and UA concentrations.

Table 1: comparison of parameters among study groups.

		Preeclampsia (Group I)	Gestational Hypertension (Group II)	Normal Pregnancy (Group III)	Healthy Non-Pregnant (Controls)
POG (weeks)	Mean±SD	34.03±3.46	34.77±3.4	34.36±1.69	-
Age (years)	Mean±SD	22.6±2.22	22.83±2.98	22.03±2.38	22.27±2.61
	t test (p)[a]	0.634	0.437	0.719	-
Systolic BP (mm of Hg)	Mean±SD	157±8.77	151.3±5.71	113.0±5.34	117.67±8.88
	t test (p)[a]	< 0.001**	< 0.001**	< 0.05*	-
Diastolic BP (mm of Hg)	Mean±SD	96.87±7.0	95.0±5.09	73.67±4.9	75.87±5.94
	t test (p)[a]	< 0.001**	< 0.001**	0.123	-
Serum LDH (IU/L)	Mean±SD	356.46±158.09	282.3±120.98	151.57±47.47	130.5±44.36
	t test (p)[a]	< 0.001**	< 0.001**	0.081	-
Serum ALP (IU/L)	Mean±SD	167.37±65.09	136.16±43.25	104.16±21.43	89.27±21.43
	t test (p)[a]	< 0.001**	< 0.001**	< 0.01*	-
Serum Uric acid (mg/dl)	Mean±SD	5.83±0.71	5.63±0.55	5.02±0.72	4.63±0.72
	t test (p)[a]	< 0.001**	< 0.001**	< 0.05*	-

[a] p-value of unpaired student's t-test between respective case groups and controls.

POG – period of gestation; BP- Blood Pressure.

* Significant ** Highly Significant

Table 2: Pearson's correlation between Systolic & Diastolic BP and parameters.

	r-value for Systolic BP	r-value for Diastolic BP
Serum LDH (IU/L)	0.504**	0.546**
Serum ALP (IU/L)	0.380**	0.418**
Serum Uric Acid (mg/dl)	0.408**	0.420**

** Highly Significant (p<0.001)

Figure 1: Correlation of systolic & diastolic blood pressure with serum Lactate Dehydrogenase levels in cases.

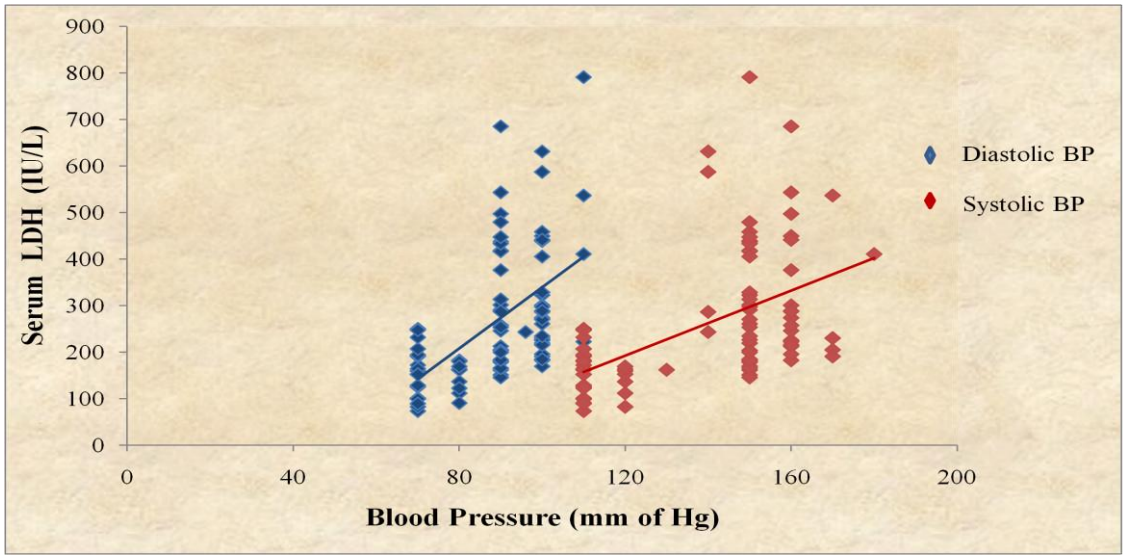


Figure 2: Correlation of systolic & diastolic blood pressure with serum Alkaline Phosphatase levels in cases.

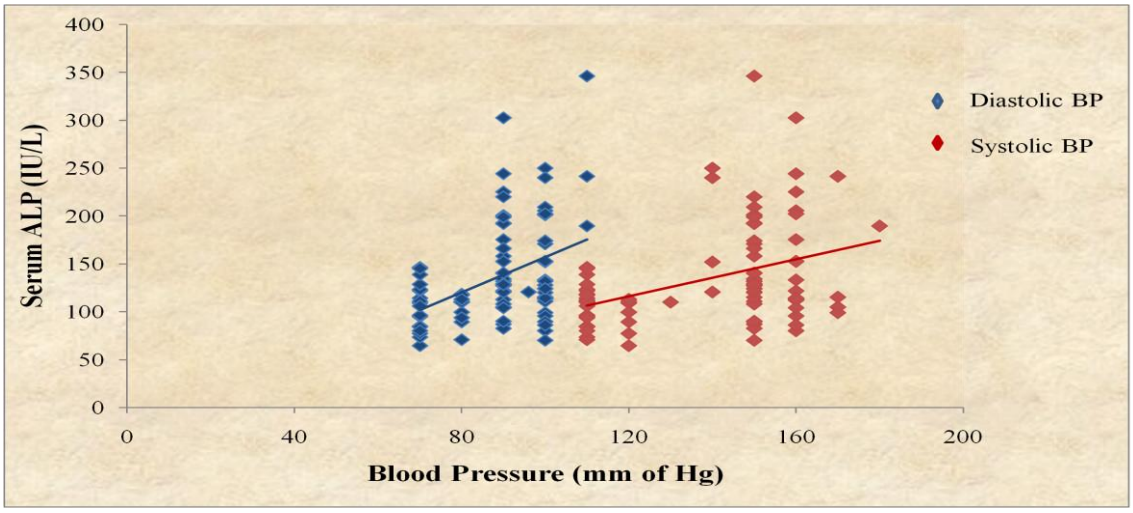
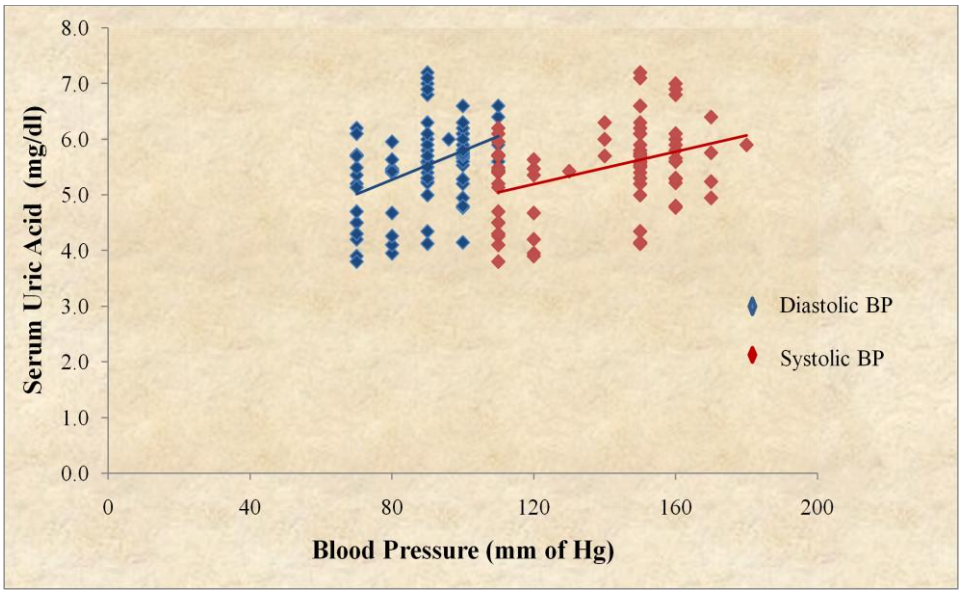


Figure 3: Correlation of systolic & diastolic blood pressure with serum Uric Acid levels in cases.



DISCUSSION

Hypertensive disorders of pregnancy are known since ancient time [14]. In spite of many recent advances, the exact pathology of this disease is not known. Many theories have suggested that endothelial dysfunction caused by factor released from ischemic placenta may be a causative factor for disease pathogenesis [15].

In our study, we found significantly elevated levels of serum LDH in women with preeclampsia and gestational hypertension compared with controls. These finding was in accordance with study done by Qublan H et al [16] and Kozic J et al [17]. They concluded that serum LDH can be a useful marker for prediction of adverse outcome of pregnancy in severe preeclampsia. Serum LDH has also found to be useful predictor for birth of small for gestational age infants in preeclamptic pregnancy [18]. A group of researchers has noted significant usefulness of LDH levels in amniotic fluid at mid-trimester for prediction of fetal growth restriction [19].

It is found that LDH-A (4) isoenzyme is immunolocalized primarily in the fetal endothelial cells while LDH-B(4) isoenzyme is predominantly present in syncytiotrophoblasts. The LDH-A(4) isoenzyme activity increased approximately by 1.6-fold in preeclampsia when compared with normal pregnancy. This may also suggest that endothelial dysfunction present at uteroplacental vessels can lead to hypoperfusion to the growing fetus & may lead to elevation of LDH isoform [20].

These hypertensive disorders are also associated with complication such as HELLP syndrome. It is one of the serious complications which may endanger the life of both mother and fetus. It is found to occur in 0.5 to 0.9% of all pregnancies and in 10–20% of cases with severe preeclampsia. HELLP syndrome is a mnemonic of the syndrome characterized by Hemolysis, Elevated Liver enzymes and Low Platelet count. According to the Tennessee Classification System, diagnostic criteria for HELLP syndrome are hemolysis with increased serum LDH (>600U/L), serum AST (≥70 U/L) and platelet count less than $100x10^9$/L [21].

In HDP, activation of the complement and coagulation cascades, increased vascular tone, platelet aggregation, and alteration of the thromboxane:prostacyclin ratio etc. events are present. They lead to systemic endothelial and microvascular injury and causing

microangiopathic hemolytic anemia, periportal hepatic necrosis, and thrombocytopenia and thus, leading to HELLP syndrome. As RBC and hepatocytes contain good amount of LDH enzyme, elevated levels of serum LDH may signify the presence of hemolysis and hepatic cell death [22].

Liver function tests are found to be abnormal in 20–30% of patients with preeclampsia. They may reflect liver dysfunction resulting from vasoconstriction of the hepatic vascular bed. Many Doppler studies have demonstrated hepatic necrosis arising from endothelial dysfunction and vasoconstriction of hepatic vessels in women with HDP. Alkaline phosphatase is often noted to be elevated in normal pregnancy. But the levels may be further increased in women with HDP [23]. In our study, we found significantly elevated levels of serum ALP in cases when compared with controls.

Acute fatty liver of pregnancy, ruptured liver hematoma, intrahepatic cholestasis of pregnancy etc. are other rare but severe complications associated with marked maternal morbidity and mortality [22]. Serum ALP is found to be significantly raised in such women alongwith other liver markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), prothrombin time, etc. So, regular workup for liver injury may prove to be an advisable step to reduce and prevent the liver complications in women with HDP [24].

Hypertensive disorders of pregnancy are commonly associated with decrease in renal function due to damage done by hypertension and wide spread endothelial dysfunction. Glomeruli undergo structural changes with pronounced endothelial cell swelling, vacuolization and hypertrophy of the cytoplasmic organelles known as “glomerular endotheliosis”. The net effects are reduced renal blood flow, reduced GFR, impaired tubular reabsorption & secretary function [6, 8].

In our study, we found that the mean serum UA levels were significantly higher in cases when compared with controls. This finding is in accordance with the study done by Punthumapol C et al [25]. It is found that estimation of serum UA is as important as proteinuria in identifying the risk of renal involvement and fetal compromise [26]. Maternal hyperuricemia is found to be a strong predictor of maternal disease progression and fetal outcome. Thus, it can be used as useful and inexpensive marker for predicting disease severity, renal function status and fetal growth retardation in women presenting with HDP [27].

In our study, the mean serum levels of LDH and ALP were significantly higher in group I when compared with group II ($p<0.05$). And levels of serum LDH, ALP & UA were significantly higher in group II when compared with group III ($p<0.05$). These findings indicate that increased levels of these parameters are seen as the disease severity increases.

In our study, it was seen that the mean serum LDH was 26% higher in group I when compared with group II & 135% higher when compared with group III. The mean serum LDH was 86% higher in group II when compared with group III. The mean serum ALP was 23% higher in group I when compared with group II and 61% higher when compared with group III. The mean serum ALP was 31% higher in group II when compared with group III. The mean serum UA was 16% higher in group I and 12% higher in group II when compared with group III. These findings also indicate that serum levels of LDH, ALP and UA rise as the disease progresses.

Pearson’s correlation analysis showed a significant positive correlation of these parameters with systolic and diastolic blood pressure in cases. It again signifies that the

serum levels of LDH, ALP and UA increases as the disease advances.

Thus, estimation of serum LDH, ALP and UA at regular interval may give insight to ongoing disease progression and organ damage. They may prove to be a useful tool to predict the maternal and fetal complications even at an earlier stage of the disease.

CONCLUSIONS

Regular estimation of LDH, ALP and UA is advisable for pregnancy diagnosed with hypertensive disorders in order to detect and prevent the morbidity and mortality in mother as well as in the fetus. It may give an idea regarding the disease severity and functioning of liver and kidney in these patients. Progressive increase in their levels should be considered as a signal for prompt intervention to improve pregnancy outcome.

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