Assessment of Laboratory Derangements in Preeclampsia: Revisiting Traditional Biomarkers

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Abstract

Objectives: To assess the routine laboratory analytes, serum Thyroid Stimulating Hormone (TSH), Lactate Dehydrogenase (LDH) and Uric acid in women with preeclampsia compared to normotensive healthy pregnant women and to determine the concentration of liver enzymes in women with preeclampsia. Materials and Methods: The study was carried out in the Department of Biochemistry of MVJ Medical College and Research Hospital. Thirty women with preeclampsia and thirty normotensive pregnant women admitted to the Department of OBG were recruited for the study. Blood samples were collected and analysed for serum TSH, LDH, uric acid and liver enzymes. Mann Whitney U test was employed to evaluate the significance of the differences obtained between the groups. Results: All three analytes were significantly higher in the case group when compared to controls. LDH and ALT (Alanine transaminase) showed significant difference even within the mild and severe preeclampsia groups. A moderate positive statistically significant correlation (r=0.348, p=0.006) was obtained between serum TSH and LDH. Serum TSH level showed a sensitivity of 66.64%, specificity of 73.33% and a positive predictive value of 71.4%. A De Ritis ratio of 1.82 was observed in preeclampsia. Conclusion: TSH, LDH and Uric acid showed significant elevation in preeclampsia. TSH and LDH levels showed a positive correlation. Hence, TSH, LDH and Uric acid could serve as markers of preeclampsia. A hypothyroid status in early pregnancy might be seen as a risk factor for preeclampsia. An elevated ALT and a high De Ritis might indicate an underlying liver and cardiac compromise in preeclampsia.

Keywords: Preeclampsia, TSH, LDH, Uric Acid, De Ritis ratio.

List of abbreviations

TSH: Thyroid Stimulating hormone
LDH: Lactate Dehydrogenase
ALT: Alanine Transaminase
HDP: Hypertensive disorders of pregnancy
ACOG: American College of Obstetricians and Gynecologists
NICE: National Institute for Health and Care Excellence
PCR: Protein – creatinine ratio
ACR: Albumin – creatinine ratio
BP: Blood pressure
HELLP syndrome: Haemolysis (H), elevated liver enzymes (EL), and low platelet count (LP) occurring in pregnancy
IUD: Intra uterine death
RDS: Respiratory distress syndrome
AST: Aspartate transaminase
SGOT: Serum Glutamate Oxaloacetate Transferase
SGPT: Serum Glutamate Pyruvate Transferase

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INTRODUCTION

Hypertensive disorders of pregnancy (HDP), encompassing preeclampsia and eclampsia are the prime contributors of maternal and perinatal morbidity and mortality [1]. Preeclampsia is defined as a pregnancy specific rapidly progressive multi systemic syndrome characterised by denovo onset hypertension developing after 20 weeks of pregnancy [2, 3]. Traditionally defined and diagnosed by the classical clinical triad of increased blood pressure (140/90 mm Hg), fluid retention and proteinuria, the ACOG and the NICE revised diagnostic guidelines defines preeclampsia as an abrupt onset persistent hypertension with the coexistence of proteinuria (PCR≥30 mg/mmol or ACR 8≥ mg/mmol or [2+] on dipstick) or renal insufficiency (creatinine>1.1 mg/dL) or liver involvement (doubling of serum transaminases), haematological complications such as thrombocytopenia (platelet count<100,000/microlitre), disseminated intravascular coagulation or haemolysis or neurological manifestations (seizures, altered mental status, visual disturbances, stroke, clonus, severe headaches or persistent visual scotomata) non attributable to other explanatory neurological causes, or uteroplacental dysfunction such as IUGR, abnormal umbilical artery doppler waveform analysis. It emphasizes that in absence of proteinuria, diagnosis can be made with the presence of hypertension coexisting with any one or more cardinal manifestations [2-4].

Globally, preeclampsia is a leading cause of maternal and infant morbidity and mortality accounting for 30,000 maternal deaths, 230,000 HDP related near-miss events, about half a million to a million infant deaths and ten to twenty times greater incidence of neonatal morbidities per year [1]. In India, the incidence of preeclampsia is 8-10% among pregnant women [6].

The major symptoms of preeclampsia include rise in blood pressure (BP), severe headaches, nausea, vomiting, blurring of vision, light sensitivity all of which can be easily ignored. The risk factors which predispose women to preeclampsia are varied and include nulliparity, multifetal gestations, obesity, diabetes mellitus, maternal age above 35 years etc [7]. The serious effects of undiagnosed or delayed diagnosis or risk assessment of preeclampsia include severe maternal consequences of placental abruption, HELLP syndrome, eclampsia, maternal coma and death [1]. The other end of the spectrum highlights adverse perinatal outcomes due to the underlying fetal, maternal distress and the resultant iatrogenic prematurity, encompassing IUGR, IUD, RDS, neonatal hypoglycemia, perinatal asphyxia, hypoxic-ischaemic encephalopathy and neonatal death [1]. The severe adverse outcomes hence warrant the need for markers which could help in early and cost-effective risk assessment and diagnosis which could lead to its effective management and ensure desirable outcomes.

Current research on “the disease of theories” aims to evaluate the role of laboratory-based biomarkers in early prediction and risk stratification of preeclampsia. Though emerging novel and promising signalling markers, angiogenic and growth factors have been proposed, yet none has been implemented into routine and widespread clinical practice for early risk assessment and diagnosis of preeclampsia. This could be due to shortcomings in translation of these markers into the routine laboratory analytical platform and the associated high expenses, especially relevant in the resource constrained environment of developing countries. Thus, we aim to investigate derangements and hence the efficacy and the predictive potential of relatively simple, cost effective laboratory based routinely analysed biomarkers within easy access of the Indian rural population, we cater to.

Thyroid hormone being a key regulatory hormone of foetal growth, development and pregnancy being a state of physiological hyperthyroxinemia, thyroid hormone perturbations could be assessed as a candidate marker and risk predictor of impending preeclampsia [8-11]. Preeclampsia pathogenesis focuses placental dysregulations with an altered angiogenic development, thus markers of supposed hypoxic origin might be effective risk assessors of the underlying placental dysfunction [12]. Lactate dehydrogenase (LDH), an intracellular enzyme of anaerobic glycolysis, usually released during cellular death, may be robust predictor of chronic anoxemia and placental ischemia in preeclampsia [13, 14]. Uric acid, a routinely assayed metabolite, is believed to increase in preeclampsia due to multiple hypothesised reasons like oxidative stress, tissue ischemia, altered renal function etc [15]. The present study aims to investigate the association between thyroid stimulating hormone (TSH) and preeclampsia in the rural South Indian population, if any, which will aid in early detection and targeted management of preeclampsia in hypothyroid patients. It also aims to evaluate whether serum LDH and uric acid can be used as cost effective markers of preeclampsia and can be used to grade the severity of preeclampsia. In addition to these parameters, we also aim to assess the liver enzymes in patients to understand the frequency of liver compromise in women presenting with preeclampsia.

MATERIALS AND METHODS

Subjects

This cross-sectional observational study enrolled thirty women (34.7± 5.62 weeks POG) with preeclampsia admitted to the Department of Obstetrics and Gynaecology in MVJ Medical College and Research Hospital, Bangalore (rural), India between the periods of April to September 2018. Patients diagnosed with preeclampsia [BP (≥140/90 mmHg) on at least 2 occasions, six hours apart and/or proteinuria (≥300mg/24 hours or ≥1+ dipstick) after 20 weeks of gestation], voluntarily consenting to participate in the
study were recruited as cases. Patients with known history of chronic hypertension, any renal disease, hypothyroidism, hyperthyroidism, any metabolic disorder prior to pregnancy or patients with multifetal gestations or having undergone a recent trauma were excluded from the study. Thirty normotensive gestational age matched (34.9 ± 6.34 weeks POG) pregnant women attending antenatal clinic in OBG Department of MVJMC & RH were recruited as controls. The study was approved by the Institutional Ethical Committee and informed consent was obtained from all the participants.

The study participants were divided into 2 groups: normotensive pregnant women (Group A) and preeclamptic women (Group B). Group B patients were further divided into mild and severe group. Severity of preeclampsia was based on systolic and diastolic blood pressure. Mild preeclampsia was defined as systolic BP 140 mm Hg -159 mm Hg or diastolic BP 90 mm Hg -109 mm Hg and proteinuria of ≥300 mg/day. Severe preeclampsia was defined as systolic BP ≥160 mm Hg or diastolic BP ≥110 mm Hg, with severe proteinuria and/ or signs and symptoms of target organ damage [16].

Materials/ Blood sampling
Routine venepuncture (under aseptic conditions) was carried out, blood samples were collected in anticoagulant-free (Plain red) tubes, following which the samples were left undisturbed 15 - 20 minutes to ensure clotting and prevent haemolysis and then centrifuged at 3000 rpm for 8 minutes.

Methods
Freshly obtained serum samples were assayed for TSH by Chemiluminescence method (cobas e411, Roche Diagnostics), LDH by Deutsche Gesellschaft für klinische Chemie (DGKC) (EM 360, Transasia Biomedicalcs Pvt Ltd) method and uric acid was by Uricase method (EM 360, Transasia Biomedicals Pvt Ltd) in the Clinical Biochemistry Department, MVJ Medical College and Research Hospital. Serum samples from the preeclampsia patients were further assayed for liver enzymes (by IFCC kinetic method, EM 360, Transasia Biomedicalcs Pvt Ltd).

STATISTICAL ANALYSIS
Quantitative characteristics are tabulated and presented using descriptive statistics. Categorical variables were expressed as counts and percentages. Group differences are evaluated using Mann Whitney U test, p value less than 0.05 is considered statistically significant. The relationship between serum LDH and TSH is quantified by Spearman’s Rank correlation. ROC analysis for serum TSH is performed using MedCalC software (https://www.medcalc.org).

RESULTS
Our study enrolled 60 women in their third trimester of pregnancy, out of which 30 belonged to normotensive group (Healthy subjects) and 30 belonged to our study group (Preeclampsia Group). The mean maternal age, gestational age and blood pressure observations along with the target analytes (TSH, LDH and uric acid) are presented in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Units</th>
<th>Healthy Subjects (n = 30)</th>
<th>Preeclampsia Group (n = 30)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Age</td>
<td>Years</td>
<td>23.63 ± 2.65</td>
<td>24.4 ± 4.47</td>
<td>0.453</td>
</tr>
<tr>
<td>Gestational Age</td>
<td>Weeks</td>
<td>34.7 ± 5.62</td>
<td>34.9 ± 6.34</td>
<td>0.8885</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>mm Hg</td>
<td>113.07 ± 7.71</td>
<td>158.5 ± 19.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>mm Hg</td>
<td>70.87 ± 6.80</td>
<td>100 ± 9.83</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TSH</td>
<td>µIU/mL</td>
<td>2.065 (1.41, 4.10)</td>
<td>3.965 (1.97, 4.97)</td>
<td>0.02</td>
</tr>
<tr>
<td>LDH</td>
<td>U/L</td>
<td>371.0 (278, 505)</td>
<td>527 (445, 853)</td>
<td>0.004</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>mg/dL</td>
<td>4.55 (3.75, 5.12)</td>
<td>5.0 (4.4, 7.3)</td>
<td>0.021</td>
</tr>
</tbody>
</table>

TSH, LDH and uric acid showed statistically significant elevations in the case group when compared to healthy subjects. 67% participants showed elevated TSH (above 3.00 µIU/mL), 46% showed elevated LDH (525 U/L) and 50% participants showed elevated uric acid in preeclampsia group compared to only 26% TSH elevations, 20% LDH elevation and 23% uric acid elevation in the healthy group.

The preeclampsia group had sixteen cases of mild preeclampsia and fourteen cases of severe preeclampsia. Table 2 presents the differences in biomarker concentrations between these two subgroups. Serum LDH and ALT were found statistically different between the groups. Further, the De Ritis ratio (AST/ALT) was found to be 1.82 (1.55, 2.20) for the preeclampsia group.
Table 2: Comparison of target analytes between healthy subjects and Preeclampsia Group

<table>
<thead>
<tr>
<th>Target analytes</th>
<th>Units</th>
<th>Mild preeclampsia (n = 16)</th>
<th>Severe preeclampsia (n = 14)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>µIU/mL</td>
<td>4.085 (2.08, 4.78)</td>
<td>3.90 (1.85, 6.74)</td>
<td>0.9</td>
</tr>
<tr>
<td>LDH</td>
<td>U/L</td>
<td>472.5 (417, 697)</td>
<td>704 (502, 1306)</td>
<td>0.013</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>mg/dL</td>
<td>4.8 (4.1, 6.17)</td>
<td>5.4 (4.67, 7.92)</td>
<td>0.163</td>
</tr>
<tr>
<td>AST/SGOT</td>
<td>U/L</td>
<td>24 (23, 30)</td>
<td>25 (23, 57)</td>
<td>0.5</td>
</tr>
<tr>
<td>ALT/SGPT</td>
<td>U/L</td>
<td>13 (11, 15)</td>
<td>18.5 (12, 26)</td>
<td>0.02</td>
</tr>
<tr>
<td>ALP</td>
<td>U/L</td>
<td>159 (132, 176)</td>
<td>175 (115, 235)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

A ROC for serum TSH levels showed an AUC of 0.675 (95% CI: 0.538 – 0.811, p value 0.01), sensitivity of 66.64%, specificity of 73.33%, positive predictive value of 71.43% and a negative predictive value of 68.75% (Cut off: 3.00 µIU/mL) for predicting preeclampsia. Further, a moderate positive statistically significant correlation was observed between serum TSH and LDH levels (Figure 1).

![Fig-1: Spearman correlation between serum TSH and LDH](image)

**DISCUSSION**

Hypertensive disorders of pregnancy, characterized by heterogenous clinical and laboratory manifestations and varied pathogenesis, are one of the leading causes of maternal and neonatal morbidity and mortality [17, 18]. In the era of laboratory guided diagnosis, laboratory assessment is gaining momentum in evaluation of HDPs. Irrespective of the associated cost of such heightened surveillance, laboratory generated cues become prime and indispensable since it facilitates risk stratification for adverse outcomes, increased interventions, observational hospitalisations and the clinical decision of the appropriate time of preterm delivery [19]. Hence, we aimed to estimate the frequency of derangements observed in routinely assessed and key laboratory analytes in preeclampsia and observed significant derangements in a few parameters.

The most plausible explanations of the pathogenesis of preeclampsia focus on the placenta, its alteration in growth, angiogenesis and related factors, hence abnormal circulatory levels of proteins, enzymes and factors produced by the placenta which may inhibit angiogenesis, trigger vessel constriction, oxidative stress and mitochondrial pathology etc is at the heart of laboratory assessment of preeclampsia [12].

Thyroid hormone plays a significant role in the development and metabolic functioning of the foetus [8, 9]. Prior to 18th – 20th week of gestation, the foetus solely depends on thyroid hormones of maternal origin [10]. Pregnancy being a state of mild hyperthyroxinemia, it is believed that preeclamptic women have a high incidence of hypothyroidism that might correlate with the severity of preeclampsia [11].

Our study shows that women with preeclampsia have a significantly elevated serum TSH level when compared to normotensive gestational age matched pregnant women and a fairly appreciable predictive potential for preeclampsia. Our findings are consistent with many other studies. It has been reported that preeclampsia is observed in 16.7% of subclinical hypothyroidism cases and 43.7% of cases of overt hypothyroidism [11]. Study done by Divya et al, has shown that there is a state of hypothyroxinemia in preeclampsia [20]. Sangita Nangia Ajmani et al. also show the high prevalence of thyroid disorders...
associated with adverse maternal and foetal outcomes and a significant correlation of preeclampsia and subclinical hypothyroidism [21]. Mustafa Bashug et al. reported a significant increase in serum TSH in preeclamptic-eclamptic group when compared to normotensive pregnant women concluding that moderate decreases in thyroid hormone and the associated TSH increase correlates with severity of preeclampsia [22]. Study by Wilson Karen et al. also showed high incidence of HDP in women with subclinical hypothyroidism [23].

Placental and endothelial dysfunction, deranged estrogen production and reduced peripheral conversion of T4 to T3, play a significant role in the development of hypothyroidism and the pathogenesis of preeclampsia [24]. Reports indicate that patients with subclinical hypothyroidism have impaired endothelial derived vasodilation attributed to decreased nitric oxide secretion [23]. A large retrospective observational cohort of 1981 pregnant women by Hernández et al reports a higher median first trimester TSH of group of pregnancies with miscarriage as well as pregnancies leading to pre-eclampsia with TSH showing a negative correlation with adverse birth outcomes [25]. Modest decreases in thyroid hormones with complementary increase in TSH levels are known to correlate with the severity of preeclampsia and high levels of endothelin [10]. Hypothyroidism induced vascular smooth muscle contraction (systemic and renal vessels) leads to increased diastolic hypertension, peripheral vascular resistance and decreased tissue perfusion. Thus, there lies a strong connection between hypothyroidism and preeclampsia [26].

LDH, a predominantly intracellular cytoplasmic enzyme of anaerobic glycolysis catalyses the inter conversion of pyruvate to lactate [13]. It is usually released from its tissue of origin to the general circulation during cellular death or dysfunction [13]. Glycolysis being the principle source of energy for the placenta, chronic anoxemia due placental ischemia/ under perfusion is believed to augment the induction of trophoblastic LDH activity leading to vigorous glycolysis [13].

The present study shows that the control group of normotensive pregnant women had serum LDH well within the recommended cut off of 524 U/L, while women with preeclampsia showed statistically significant elevations in serum LDH [27]. The difference of LDH between mild and severe preeclampsia groups was also found to be statistically significant. This is in agreement with many of the previous studies. Jaiswar S et al. shows high serum LDH levels correlate well with the severity of the disease and outcomes in patients of preeclampsia [28]. He S et al. shows serum LDH levels were significantly increased in preeclamptic women than those in normal pregnancy. Further, preeclamptic women with low birth weight infants had significantly higher LDH levels than preeclamptic women with infants of birth weight appropriate of gestational age [29]. Study done by Vinitha Padmini Mary et al. also suggests increased serum levels of LDH, uric acid and liver enzymes in severe preeclampsia and infers that LDH values greater than 800U/L correlates with increased risk of perinatal mortality [30]. Further, a study by Purnima and Sonal and another study by Munde et al. also concludes that LDH and GGT can be effectively used as biochemical markers as it reflects the severity of preeclampsia and maybe helpful in its management [31, 32]. Neha V Bhave further claims that LDH effectively predicts maternal and perinatal outcomes along with the severity of the disease [17].

Uric Acid, the end product of purine metabolism, gradually decreases until 16 weeks of gestation, remains stable during the second trimester and increases in the 3rd trimester [15, 33]. Preeclampsia has been long associated with elevations in uric acid levels while studies have shown contrasting evidences. Our study showed significantly elevated serum uric acid levels in the preeclampsia group compared to the control group. Further, the elevation of uric acid was higher in women with severe preeclampsia than the mild preeclamptic group, though the difference is not statistically significant, which might be due to the limited sample size of our study.

Our results are in agreement with previous studies and findings. A review by C Lam et al. reported that increased serum uric acid levels are associated with significant maternal and foetal morbidity and mortality [33]. As shown by Punthumapol C et al. hyperuricemia correlates with severe preeclampsia and the elevation may be due to several factors like altered renal function, tissue ischemia and oxidative stress [34]. Bainbridge et al. hypothesized that hyperuricemia correlates with hypertension, renal diseases and adverse foetal outcomes in pregnancy and that uric acid is not simply a marker of the severity of preeclampsia but directly contributes to the pathogenesis by promoting inflammation, oxidative stress and endothelial cell dysfunction [15]. Further, studies by Bargale et al. and Hawkins et al. indicate that increase in uric acid levels correlate with severity of preeclampsia and can identify women at increased risk of adverse maternal and fetal outcome [35, 36]. Some of the most accepted mechanisms of hyperuricemia include decreased renal clearance (due to decreased glomerular filtration, presence of circulating vasocostrictors) and increased placental production of uric acid (secondary to placental ischemia and increased trophoblastic shedding) [32]. A few studies further claim that hyperuricemia may modify the migratory and invasive phenotype of the trophoblast and may be a contributing factor to inadequate trophoblastic invasion in preeclampsia [15].
We show the statistically elevated concentration of serum ALT in women with severe preeclampsia which could indicate a higher risk of the development of HELLP syndrome in these patients [37]. An elevated De Ritis ratio (implicated in predicting and prognosis of future MACE and preeclampsia, now being considered as the first cardiac event in a woman) of 1.82 beyond the normal recommended range of 0.8 – 1.0 also emphasises the central role of laboratory assessments in understanding the underlying pathology of multi organ compromise and thus ensuring early intervention, improved immediate and long term outcomes for pregnant women as well as their neonates [38 – 40].

Thus, our study clearly shows the association of elevated TSH, LDH and uric acid with preeclampsia. TSH shows a high positive and negative predictive value for preeclampsia. LDH is also shown to significantly correlate with the severity of the disease. Further, a statistically significant moderately positive correlation was found between serum the association of LDH and TSH. This is the unique finding of our study and could provide clue that hypothyroidism in early pregnancy might indicate improper placentation, development of hypoxia and may signify the risk of preeclampsia and correlate to its severity, though a definite conclusion needs validation of this finding with further studies. Further an appreciable increase in De Ritis ratio in preeclampsia might indicate the risk of future cardiac events in such women and might warrant the need of appropriate preventive interventions and lifestyle modifications. A cross sectional study design, a small sample size and the absence of liver enzyme values in our control population can be seen as the limitations of our present study. The study needs to be replicated with a larger sample size and a prospective study should be taken up to obtain a better picture of association of these markers with the development and severity of preeclampsia.

CONCLUSION

Our study showed the significant elevation in serum concentration of all the markers in preeclampsia when compared to normotensive healthy pregnant women. Women of the severe preeclamptic group had LDH levels significantly higher than women with mild preeclampsia. Further serum TSH and LDH showed moderate positive correlation and serum TSH showed a sensitivity of 66.64%, specificity of 73.33% and a positive predictive value of 71.43%. Further, an elevated De Ritis could indicate cardiac involvement and increased incidence of future MACE in these patients, though the hypothesis should be validated by prospective follow up studies. Thus, these routine and accessible laboratory parameters when combined with classical markers, USG findings and risk assessors of preeclampsia can act as strong predictive markers to predict the risk as well as grade the severity of preeclampsia. Identification of vulnerable patients with elevated levels of these markers might aid in close monitoring, early/ prophylactic interventions and prompt management of the disease by ensuring an increased lead time or the mean time until iatrogenic prematurity and improve maternal and neonatal outcomes, if not totally circumvent or impede the occurrence of preeclampsia.

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CONFLICTS OF INTEREST STATEMENT

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