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Original Research Article

Serum β-hCG and neutrophil lymphocyte ratio in early second trimester of pregnancy and prediction of pre-eclampsia

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ABSTRACT

Background: Hypertensive disorders, especially preeclampsia (PE), continues to challenge obstetric care, contributing substantially to maternal and perinatal morbidity and mortality. Reliable biomarkers for early prediction are needed. Biomarkers such as serum beta-human chorionic gonadotropin (β-hCG) and neutrophil-to-lymphocyte ratio (NLR) are emerging as potential tools for early risk stratification.
Methods: This prospective observational study included 86 pregnant women attending antenatal care at a tertiary care hospital. Serum β-hCG and NLR were measured between 13-20 weeks of gestation, and participants were followed until delivery. The development of PE was recorded, and associations with biochemical and clinical parameters were analysed using t tests, chi-square tests, and ROC curves.
Results: Of the 86 participants, 17.4% developed PE. Mean serum β-hCG was significantly higher in the PE group (60719 mIU/ml) vs. normotensive group (24952 mIU/ml, p=0.002)). MeanNLR was also elevated in PE (4.409) compared to normotensive women (3.010, p=0.002). A β-hCG cut-off of 57213 mIU/ml had a specificity of 93% for predicting PE. Higher BMI and multigravidity were significantly associated with PE (p=0.049 and p=0.029, respectively). PE was linked with earlier gestational age at delivery (35.13 vs. 37.56 weeks, p=0.013) and lower birth weight (2.53 kg vs. 2.93 kg, p=0.019).
Conclusions: Elevated second-trimester β-hCG and NLR, are significantly associated with an increased risk of PE. These accessible and cost-effective markers can aid in the early identification of high-risk pregnancies, allowing for timely interventions to improve maternal and foetal outcomes.

Keywords: Serum β-hCG, NLR, Preeclampsia, Biomarkers, Early prediction

INTRODUCTION

Hypertensive disorders of pregnancy (HDP) remain a leading cause of maternal and perinatal morbidity and mortality globally. PE, a distinct multisystem hypertensive disorder, is characterized by new-onset hypertension after 20 weeks of gestation accompanied by proteinuria or end-organ dysfunction. Globally, it affects approximately 3-8% of pregnancies and is responsible for an estimated 70,000 maternal deaths and 500,000 perinatal deaths annually.[1] India bears a disproportionately high burden, with prevalence estimates ranging from 5 to 10%.[2]

Despite advances in obstetric care, the unpredictable nature of PE, combined with its rapid progression and potential for severe complications, highlights the need for early prediction. The pathophysiology involves abnormal trophoblastic invasion, incomplete spiral artery remodelling, placental ischemia, oxidative stress, endothelial dysfunction, and systemic inflammatory response.[3] With significant placental involvement, biochemical markers originating from the placenta have emerged as potential predictors.

One such marker is serum β-hCG. It is produced by syncytiotrophoblasts. Serum β-hCG levels are altered in

pregnancies complicated by placental dysfunction. Elevated levels in the second trimester have been associated with increased risk of PE, preterm birth, foetal growth restriction, and other adverse outcomes.[4-5]

Similarly, the NLR is emerging as a simple and cost-effective marker reflecting systemic inflammation. PE is increasingly recognized as an inflammatory disorder with heightened neutrophil activation, oxidative stress, and lymphocyte suppression.[6] Studies demonstrate significantly higher NLR values in women who develop PE.[7,8] Given the burden of PE in India and limited access to advanced screening modalities, low-cost biomarkers such as β-hCG and NLR could be of substantial clinical utility.

This study aims to evaluate the predictive role of mid-trimester serum β-hCG levels and NLR in identifying women at risk for PE and to determine diagnostic cut-off values for early prediction.

Aims and objectives

Aim and objectives were to estimate serum β-hCG levels between 13 to 20 weeks of pregnancy and to correlate with the onset of PE and to evaluate the NLR in predicting the development and severity of PE.

METHODS

A prospective observational study was conducted in the Department of Obstetrics and Gynaecology, M. S. Ramaiah Medical College and Hospitals, Bangalore, from May 2023 to December 2024.

Normotensive, non-proteinuric pregnant women between 13 to 20 weeks period of gestation, attending the antenatal clinic were included in the study. Women with multiple pregnancy, essential hypertension, diabetes mellitus, molar pregnancy, connective tissue disorders, or antiphospholipid antibody (APLA) syndrome were excluded.

Inclusion criteria

Patients with singleton pregnancy, gestational age 13-20 weeks and willingness to participate and provide informed consent were included.

Exclusion criteria

Patients with multiple gestation, chronic hypertension, renal disease, autoimmune disorders, diabetes mellitus, thyroid disorders and smokers or known inflammatory disease were excluded.

Sample size determination

The sample size was determined by Power analysis (R-statistical software). Asper the record of cases admitted to the Department, hypothetically presumed the total population size (n=110) with margin of error was 5%. Standard z value has extracted from the table value at 1% level of significance (z=1.96). The proportion of p=q=0.5 equated.

The required sample size of the present study was (n)=86 n=population size (110) e=margin of error (percentage in decimal form) (5%) z=z-score (1.96), p=q=0.50. The z-score is the number of standard deviations a given proportion is away from the mean.

After obtaining informed consent, patient details were recorded as per a structured proforma. Gestational age was confirmed by crown-rump length measurement on first-trimester ultrasonography. All participants underwent serum beta-hCG estimation using the chemiluminescent immunoassay (CLIA) method and NLR estimation using automated Coulter counters. NLR was calculated as the ratio of absolute neutrophil count to absolute lymphocyte count.

Participants were followed up until the delivery, with regular blood pressure and urine protein assessment. PE was diagnosed according to ACOG (2013) criteria: systolic BP ≥140 mmHg or diastolic BP ≥90 mmHg on two occasions at least 6 hours apart after 20 weeks of gestation with proteinuria in a previously normotensive woman.

Statistical analysis

Data analysis was performed using SPSS version 25.0. Statistical tests including Chi-square test, t-test, logistic regression, and multivariate analysis were used. Descriptive data were presented as mean±SD, frequency, and percentage. Correlation between serum β-hCG, NLR, and PE development and severity were assessed. ROC curve used to determine diagnostic accuracy. $p<0.05$ was considered statistically significant.

RESULTS

A total of 86 antenatal women between 13 and 20 weeks of gestation were recruited for the study and all were followed prospectively until delivery. The baseline characteristics, biochemical parameters, and pregnancy outcomes were analysed to assess their association with the development of PE. The mean age of the study population was 28.4±4.7 years. Most women belonged to the 25-29-year age group, forming the largest proportion of participants, as shown in Table 1. The distribution of parity showed that 69.8% of the women were multigravida, while 30.2% were primigravida (Table 2). The mean BMI of the study group was 25.7±4.3 kg/m^2. Among these, 29% of participants were classified as overweight and 14% as obese.

During follow-up, 15 out of the 86 women (17.4%) developed PE (Table 3). Comparison of baseline characteristics between the PE group and the normotensive

group showed that BMI had a statistically significant association with PE. Women who developed PE had a higher mean BMI of 28.6±1.56 kg/m^2, whereas the mean BMI among normotensive women was 25.1±3.9 kg/m^2 (p=0.049). Parity also showed a significant difference between the groups. In the PE group, 93.3% of women were multigravida and only one woman (6.7%) was primigravida, while in the normotensive group, the distribution was broader. This difference in parity distribution was statistically significant (p=0.029).

The study analysed second-trimester β-hCG levels to assess their association with the subsequent development of PE. The mean β-hCG level in women who developed PE was 60,719 mIU/mL, which was higher than the value noted among normotensive women (24,952 mIU/mL). This difference was statistically significant (p=0.002) (Table 4). A receiver operating characteristic (ROC) curve was plotted for β-hCG to determine its predictive accuracy. The area under curve (AUC) was 0.804, which indicated good diagnostic performance. Based on the ROC curve, an optimal cut-off value of 57,213 mIU/mL was identified. At this threshold, the sensitivity was 53.3% and the specificity was 93%. Thus, a value above this cut-off correctly identified most women who did not develop PE, while identifying a portion of women who did.

The NLR was also evaluated as a possible marker for predicting PE. The mean NLR in the PE group was 4.409±1.38, whereas the mean NLR in the normotensive group was 3.010±1.40. This difference was statistically significant (p=0.002) (Table 5). ROC curve analysis for NLR showed an AUC of 0.799, indicating good predictive value. A cut-off value of 4.74 was obtained from the ROC curve analysis. At this level, the specificity was 91.5%, showing that most women below this threshold did not develop PE.

Pregnancy outcomes were compared between the PE and normotensive groups. Women who developed PE delivered at an earlier gestational age. Mean gestational age at delivery for the PE group was 35.1±3.3 weeks, whereas for normotensive women, the mean gestational age was 37.6±1.4 weeks. This difference was statistically significant (p=0.013) (Table 6). Preterm deliveries were more frequent among the women who developed PE.

Birth weight was also assessed as part of neonatal outcomes. The mean birth weight among neonates born to women with PE was 2.53±0.99 kg, which was lower than the mean birth weight of infants born to normotensive mothers (2.93±0.46 kg). This difference was statistically significant (p=0.019) (Table 7). Low birth weight was more commonly observed in the PE group.

No cases of intrauterine foetal demise were noted in the PE group. NICU admissions were more frequent among neonates born to mothers with PE, although this difference was not statistically analysed in the present study. Mode of delivery was not significantly different between the two groups, though a higher proportion of caesarean sections was observed among women with PE due to obstetric indications.

Overall, the results showed significant differences in BMI, parity, β-hCG levels, NLR values, gestational age at delivery, and neonatal birth weight between the PE and normotensive groups. The findings indicate that both β-hCG and NLR were higher in women who later developed PE and showed good predictive accuracy on ROC analysis.

Table 1: Age distribution of patients, (n=86).

Age category (in years)	N	Percentage (%)
Less than 25	19	22.09
25 to 29	31	36.05
30 to 34	29	33.72
≥ 35	7	8.14
Total	86	100.0
Mean±SD	28.42±4.684	

Table 2: Gravida distribution of patients, (n=86).

Gravida types	N	Percentage (%)
Multi	60	69.8
Primi	26	30.2
Total	86	100.0

Table 3: BMI category PE crosstabulation.

BMI category	PE		P value
	No, (n=71)	Yes, (n=15)	
Normal	44	5	0.003 (Chi square=16.369)
Overweight	21	4	
Grade 1 obesity	5	5	
Grade 3 obesity	1	1	
Total	71	15	

Table 4: PE versus β-hCG.

PE	N	β-hCG mean	β-hCG, SD	Std. error mean	T test	P value	Mean difference
PE	15	60719.53	35779.88	9238.33	3.767	0.002	35766.67
Normotensive	71	24952.86	18436.26	2187.98			

Table 5: PE versus NLR.

PE	N	NLR mean	NLR, SD	Std. error mean	T test	P value	Mean difference
PE	15	4.409	1.38	0.356	3.564	0.002	1.399
Normotensive	71	3.010	1.40	0.166			

Table 6: PE versus GA at delivery.

PE	N	GA mean	GA, SD	Std. error mean	T test	P value	Mean difference
Yes	15	35.13	3.27	0.844	-2.821	0.013	-2.430
No	71	37.56	1.44	0.171			

Table 7: PE versus birth weight.

PE	N	Birth weight mean	Birth weight, SD	Std. error mean	T test	P value	Mean difference
PE	15	2.53	0.99	0.26	-2.384	0.019	-0.396
Normotensive	71	2.93	0.46	0.05			

DISCUSSION

PE continues to be a significant contributor to maternal and perinatal morbidity globally, especially having high burden in developing countries such as India. Early identification of risk factors contributing to PE remains a cornerstone of preventive obstetric care. This study was done to identify the association of maternal demographic characteristics like age, parity, and BMI-with the subsequent development of PE. This study is done to evaluate the predictive performance of two accessible biomarkers: mid-trimester serum β-hCG and NLR. The findings of this study help in supporting the combined clinical and biochemical approach in identifying pregnancies at higher risk and thus preventing complications.

In the present study, the mean maternal age was 28.4±4.7 years, which corresponds with national demographic trends for pregnant women in India. Historically maternal age has been widely studied as a potential risk factor for PE; the association still remains complex. Advanced maternal age (≥35 years) has been important risk factor for PE due to increasing vascular stiffness, reduced endothelial responsiveness, and increasing prevalence of metabolic comorbidities.[9] In our study, women above 35 years constituted a smaller proportion, and no statistically significant association was noted between age and PE. Similar results have been noted in studies comprising of younger study population. In contrast to our study, studies from European regions, where maternal age at first pregnancy is higher, have shown a significant correlation between advancing age and PE risk.[10] Thus, even though maternal age remains an important clinical risk factor, its impact may vary depending on study population, distribution of risk factors, and sociocultural patterns.

Parity, is another important risk factor for PE which has been consistently associated with the condition. Primigravida pregnancies are traditionally considered at higher risk for PE due to incomplete immunological adaptation to paternal antigens.[11] In our study, 93.3% of women who developed PE were multigravida in contrast to other studies. Several explanations may be considered for this deviation from the classical pattern. Multigravida women in our study may have had unreported hypertensive tendencies or vascular dysfunction from prior pregnancies.[12] Repeated pregnancies with short interpregnancy intervals may have also been associated with persistent endothelial imbalance, which predisposes it to hypertensive disorders in later pregnancies. Additionally, the larger proportion of multigravidas in the study population may have skewed the distribution. This finding highlights the possibility to evaluate PE risk not only based on parity but also with other clinical markers such as BMI and biochemical parameters.

There is significant association of BMI with PE in our study which reinforces the well-established role of maternal overweight and obesity as major factor for HDP. Women who developed PE had a significantly higher mean BMI (28.6±1.56 kg/m²) when compared to normotensive

group (25.1±3.9 kg/m², p=0.049). Obesity is known to induce chronic low-grade inflammation which increases oxidative stress, alters lipid profile, and cause endothelial dysfunction. This may contribute to the pathogenesis of PE.[13] Excessive adipose tissue increases insulin resistance and increases the secretion of pro-inflammatory cytokines, which disrupts placental vascular remodeling.[14] Given that nearly half of the study population was overweight or obese, BMI tends to be a major modifiable risk factor that must be addressed through preconception counselling and early antenatal interventions.

A crucial part of this study involved evaluating gestational age at delivery of pregnant women, which was significantly lower in PE cases (35.1±3.3 weeks) when compared with normotensive pregnancies (37.6±1.4 weeks, p=0.013). This finding suggests the typical disease trajectory of PE, which necessitates earlier delivery to reduce maternal or foetal risk. Poor placental perfusion due to uteroplacental insufficiency and rising maternal blood pressure often leads on to the clinical decision to induce labour or perform early caesarean delivery.[15] This pattern of early delivery aligns with global data showing that PE contributes for a higher proportion of medically indicated preterm births, significantly contributing to neonatal morbidity including NICU admissions, respiratory distress, and growth restriction.[16] The corresponding reduction in birth weight observed in this study in the PE group further supports the presence of utero-placental insufficiency, which depicts the severity of the condition.

Another important biomarker investigated in our study was serum β-hCG. Women who developed PE had markedly higher levels of β-hCG (60,719 mIU/mL) when compared to normotensive women (24,952 mIU/mL, p=0.002). Elevated serum β-hCG reflects syncytiotrophoblastic stress, defective trophoblastic invasion, and hypoxic placental conditions.[17] Multiple studies such as Murmu et al and Huma et al have confirmed that elevated mid-trimester β-hCG levels which strongly correlate with the development of PE.[4,18] In our study, the serum β-hCG ROC analysis yielded an AUC of 0.804, demonstrating good predictive accuracy. A cut-off value of 57,213 mIU/mL showed very high specificity (93%), making serum β-hCG an important biomarker for identifying high-risk pregnancies and prediction of PE. Although the sensitivity was moderate (53.3%), this limitation is mostly common in single-marker predictive studies and supports the need for multi-marker strategies.

The NLR demonstrated similar predictive value. Pre-eclamptic women in this study had significantly increasing levels of NLR (4.409±1.38) than normotensive women (3.010±1.40, p=0.002). NLR is a simple, cost-effective marker derived from routine complete blood counts. It reflects systemic inflammation-a central feature of PE. Elevated neutrophils indicate heightened inflammatory response and endothelial activation, whereas reduced lymphocytes reflect immune dysregulation which is seen in PE.[19] Several studies, including those by Panwar et al and Yavuzcan et al have also noticed the diagnostic potential and usage of biomarkers like NLR in distinguishing preeclamptic from normotensive pregnancies.[6-8] In the present study, ROC analysis yielded an AUC of 0.799, nearly equivalent to that of β-hCG. A higher NLR cut-off of 4.74 demonstrated specificity of 91.5%, again supporting NLR as a strong rule-in marker.

These findings reinforce that PE is a multifactorial condition influenced by demographic, hemodynamic, biochemical, and inflammatory factors. While maternal age and parity provide supportive context, modifiable factors such as BMI and measurable biomarkers such as β-hCG and NLR offer superior predictive value. Importantly, these two biochemical markers reflect distinct pathways: β-hCG indicates placental dysfunction, while NLR reflects maternal systemic inflammation. Their combined use could therefore provide a more comprehensive risk assessment profile.

The foetal morbidity associated with PE-including foetal growth restriction, preterm delivery, and neonatal complication can be reduced by early prediction, timely prophylactic measures such as aspirin initiation, calcium supplementation, enhanced surveillance, and planned delivery. However, the modest sensitivity of the biomarkers suggests that while these tests are excellent for identifying high-risk women, they may not capture all potential cases. Therefore, integration into a multi-marker risk prediction model is advisable, especially in resource-limited settings where advanced tests such as sFlt-1/PlGF may not be routinely available.

This study highlights the importance of early second-trimester biomarkers in conjunction with maternal clinical factors. Elevated β-hCG and NLR together provide a promising, accessible, and cost-effective strategy for early prediction of PE, especially in low-resource environments. Their incorporation into routine antenatal care has the potential to significantly improve pregnancy outcomes through early identification, targeted surveillance, and timely intervention.

Limitations

The number of cases were limited and the study was done for a short period.

CONCLUSION

Mid-trimester serum β-hCG and neutrophil lymphocyte ratio are significant predictors of PE. Elevated levels of both markers demonstrated strong diagnostic accuracy, with β-hCG showing high specificity and NLR showing high sensitivity. Their combined use can enhance early detection and improve maternal and neonatal outcomes, particularly in settings where advanced biochemical tests are not feasible.

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