Original Article

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Raised neutrophil lymphocyte ratio and serum beta hCG level in early second trimester of pregnancy as predictors for development and severity of preeclampsia

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Summary **Early detection and prediction of preeclampsia (PE) may avert serious materno-fetal complications. This prospective nested study was conducted to evaluate the role of serum beta human chorionic gonadotropin (hCG) and the neutrophil-lymphocyte ratio (NLR) in predicting the development and severity of PE. Four hundred and forty primigravidas, between 16 to 18 weeks of gestation, were recruited in the study. Serum beta-hCG and NLR were measured at the time of recruitment and they were followed and monitored for the development of PE and severe PE. Out of these 440 women, 64 (14%) developed PE; of which 25 (39%) developed severe PE. The mean values of NLR and serum beta hCG were significantly higher in patients developing PE and severe PE. NLR, with a cutoff value of 5.6, predicted the development of PE with 73.4% sensitivity and 88.6% specificity and severe PE with sensitivity 93.3% and specificity 86.6% respectively. The sensitivity and specificity of serum beta hCG in predicting the development of PE was 75% each for a cutoff value of 25,415 IU/mL whereas these values were 86.7%, and 79.1% respectively, for a cut-off value of 29,654 IU/mL for predicting the development of severe PE. These findings suggest that NLR and serum beta hCG can be used as excellent biomarkers in predicting both the development of PE and its severity. Multicentric studies involving subjects of multiple ethnicities should be done for establishing its utility as a routine screening test.**

Keywords: Preeclampsia, severe preeclampsia, neutrophil-lymphocyte ratio, NLR, beta hCG

1. Introduction

Preeclampsia (PE) is a common condition characterized by the development of hypertension after 20 weeks of gestation with or without proteinuria and/or multi-organ involvement. Studies suggest that the pathology for the development of PE is initiated in the second trimester itself, well before the actual development of signs and symptoms of the disease (*1*). Early detection and prediction of PE/severe PE can be instrumental as it can save the mother and the newborn from the detrimental complications of PE by close monitoring and timely intervention.

A number of clinical, biochemical and biophysical markers have been investigated as potential predictors in the development of PE (*2*). However, none of them has proven to be suitable for routine clinical practice (*3*). Serum beta-human chorionic gonadotropin (hCG) and neutrophil-lymphocyte ratio (NLR) are low-cost investigations that are readily available and have recently attracted the interest of researchers. Beta hCG is secreted by the syncytiotrophoblasts of the placenta and has been incriminated to have an initial role in the endothelial dysfunction of PE (*3*). Increase in the level of serum beta hCG reflects the placental reaction to PE and can be used for both the prediction of development and severity of PE. Furthermore, the placental changes in PE may also be attributed to the hyperactive

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inflammatory system. The NLR has been proposed as a new indicator of systemic inflammation and shown to have prognostic value in cancers and cardiac diseases (*4*). It has also been studied as a novel marker of prognosis in patients with PE (*2,5*). However, its role as a predictor of the development and severity of PE hasn't been studied elaborately. Hence, we planned this study to evaluate the role of both serum beta hCG and NLR in predicting the development and severity of PE.

2. Materials and Methods

This prospective nested case-control study was conducted in the Department of Obstetrics and Gynecology of a tertiary care teaching hospital of India. Primigravida women, between 16 to 18 weeks of gestation, attending antenatal care clinic and willing to follow up in the same hospital were recruited for the study. Women with the history of chronic medical illnesses, obesity (BMI > 30), smoking, ongoing infection or systemic inflammatory infections or any autoimmune disorders were excluded from the study. These women were followed as per hospital protocol and were monitored for the development of PE.

The venous samples of these women were taken for measuring beta hCG level and NLR between 16-18 weeks of gestation. Serum beta hCG was measured by the ELISA technique and NLR by automated coulter counters. NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count.

PE was diagnosed and classified according to the ACOG (2013) definition (*6*). A patient with systolic blood pressure of 140 mm Hg or higher or a diastolic blood pressure of 90 mm Hg or higher on two occasions at least 6 hours apart occurring after 20 weeks of gestation in a pregnant woman with previously normal blood pressure and detectable urinary protein (> 1+ by dipstick or 0.3 g/24 h and more) was diagnosed to have PE. Patients with blood pressure greater than or equal to 160/110 mm Hg with/without other evidence of severe disease like raised serum creatinine, eclampsia, pulmonary oedema, oliguria, fetal growth restriction, oligohydramnios and symptoms suggesting significant end-organ involvement were diagnosed as having severe PE. Women who met the criteria of PE but not severe PE were diagnosed as mild PE. Values of NLR and serum hCG were correlated with the development and severity of PE.

All statistical evaluation of the data was done using statistical software, STATA/SE version 14.2 (StataCorp LP, College Station, TX, USA). Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD. Normality of data was tested by QQ plot and Kolmogorov-Smirnov test. If the normality was rejected, then the corresponding non-parametric test was used. Quantitative variables were compared using the Independent *t*-test/Mann-Whitney test (when the data sets were not normally distributed) between the two groups. To find the association between qualitative variables, Chi-Square test/Fisher exact test was used. Receiver operating characteristic curve was used to find out cut off point of β-hCG and NLR for predicting PE and severe PE and accordingly sensitivity and specificity was calculated. A p value < 0.05 was considered as statistically significant.

3. Results

Out of the 440 women recruited in the study, 64 (14%) developed PE of which 25 (39%) developed severe PE. The mean values of NLR and serum beta hCG at 16-18 weeks were found to be significantly higher in patients who developed PE (Table 1) and severe PE (Table 2). On the basis of receiver operator characteristics (ROC) curve, the most discriminant NLR value at 16-18 wks for prediction of development of PE was 5.6 which gave the highest sensitivity and specificity as 76.4% and 88.6% respectively with probability 0.84 *i.e.* area under the curve (AUC) = 0.84). The best cutoff value of serum beta hCG at 16-18 weeks for prediction of development of PE was 25,415 IU/mL with AUC as 0.81, sensitivity and specificity both being 75% (Table 3). Further, for the prediction of development of PE by

Table 1. Distribution of the mean values of Serum beta hCG and NLR ratio between 16-18 weeks in normal women and women who developed PE

Variable	Normal women (n = 376), Mean ± SD	PE (n = 64), Mean ± SD	p-value
Beta hCG (IU/mL)	19165.03 ± 8044.7	29605.7 ± 8190.83	< 0.001
NLR	4.55 ± 0.66	5.55 ± 0.81	< 0.001

Table 2. Distribution of the mean values of serum beta hCG and NLR between 16-18 weeks in the women between 16-18 weeks who developed mild PE and severe PE

Variable	Mild PE (n = 49), Mean ± SD	Severe PE (n = 15), Mean ± SD	p-value
Beta hCG (IU/mL)	27,519.61 ± 7,483.04	36,420.27 ± 6,703.07	< 0.001
NLR	5.39 ± 0.84	6.08 ± 0.43	0.001

Table 3. Distribution of the sensitivity (SN) and specificity (SF) of serum beta hCG and NLR between 16-18 weeks in predicting the development of PE

Variable	AUC	95% Confidence interval	Cut off	SN	SF
Beta hCG (IU/mL)	0.81	0.76 to 0.86	> 25,415	75.0	75.0
NLR	0.84	0.77 to 0.90	≥ 5.6	73.4	88.6

Table 4. Distribution of the sensitivity (SN) and specificity (SF) of serum beta hCG and NLR between 16-18 weeks in predicting the development of severe PE

Variable	AUC	95% Confidence interval	Cut off	SN	SF
Beta hCG (IU/mL)	0.92	0.86 to 0.98	> 29,654	86.7	79.1
NLR	0.95	0.90 to 1.00	≥ 5.61	93.3	86.6

NLR and serum beta hCG together; the area under the curve (AUC) was 0.84 (95% CI 0.77 to 0.90), which is equivalent to the prediction by NLR alone.

The sensitivity, specificity and AUC of NLR ratio and beta hCG for the prediction of development of severe PE were very high as compared to that for prediction of development of PE. At the cutoff value of 5.6, the sensitivity and specificity NLR ratio (at 16-18 weeks) was 93.3% and 86.6% (AUC = 0.95). The sensitivity and specificity of beta hCG at 16-18 weeks in predicting the development of severe PE taking the cut off value as 29,654 IU/mL were 86.7% and 79.1% (AUC = 0.92) as shown in Table 4. For the prediction of development of severe PE by NLR and serum beta hCG together area under the curve (AUC) was marginally improved and was observed as 0.97 (95% CI 0.93 to 1.0).

4. Discussion

We evaluated NLR and serum beta hCG as potential markers for predicting the development of PE and its severity in primigravida. Both NLR and serum beta hCG were found to be excellent biomarkers in predicting the development of PE and its severity. NLR, with a cut off value equal to or more than 5.6 at 16-18 weeks of gestation was found to predict the development of PE with 73.4% sensitivity and 88.6% specificity. The NLR was found even better in predicting the severity of PE. The sensitivity and specificity of NLR in predicting severe PE was 93.3% and 86.6% respectively, for a cut off value of 5.6. Raised NLR in PE may be attributed to the secretion of proinflammatory cytokines from the hypoxic placenta due to the defective trophoblastic invasion, which is central to the genesis of PE (*4*). These cytokines stimulate the inflammatory pathway causing free radical generation and oxidative stress, thus contributing to endothelial injury which leads to the development of PE (*3*). This activation and exaggeration of the inflammatory pathway in PE are reflected as increased leucocyte counts, mainly neutrophils and thus raised NLR (*7*).

In the recent past, only a few studies have evaluated the predictive utility of NLR in PE, with inconsistent results. In a prospective case-control study, comprising of 50 cases of PE and 51 controls, Sachan *et al*., found that NLR had sensitivity and specificity of 53% and 65% respectively, for predicting non-severe PE cases at a cut-off value of > 3.35. Moreover, they found that diagnostic accuracy of NLR was significant between non-severe PE and severe PE, at a cut-off value of 3.42, with a sensitivity of 81% and specificity of 65% (*8*). A couple of other studies have also found a statistically significant association between high NLR values and PE, however, no statistically significant relationship was found between NLR and severity of PE in these studies (*2,9*). Some other studies, however, didn't find any statistically significant association between NLR and PE (*7,10*).

In the present study, we found that women who had high values of serum beta hCG in the second trimester had a significantly higher risk of developing PE in the later part of pregnancy. Serum beta hCG, with a cutoff value equal to or more than 25,415 IU/mL at 16-18 weeks of gestation, could predict the development of PE with 75% sensitivity and 75% specificity. The predictive value of serum beta hCG for development of severe PE was even better at the cut off value of 29,654 IU/mL at 16-18 weeks with 86.7% sensitivity and 79.1% specificity. The findings of our study are in accordance with another study by Kaur *et al*., which found that the serum beta hCG estimation at mid-trimester (13-20 weeks) was a good predictor of PE and higher levels of beta hCG were associated with increased severity of PE (*11*). Increased beta hCG secretion may be attributed to the trophoblastic response to hypoxia as a result of defective placental invasion that occurs in PE. The undifferentiated cytotrophoblasts get transformed to syncytiotrophoblasts in PE, resulting in a hypersecretory state of beta hCG (*12*). This describes the linear association between the raised beta hCG and the severity of PE.

Few other studies that tried to investigate serum beta

hCG levels in the early second trimester to predict the subsequent occurrence of PE have yielded contrasting results. Karahasanovic *et al*., found that beta hCG was significantly lower in pregnancies that subsequently developed PE (*13*). Raty *et al*., concluded that maternal mid-trimester serum level of free beta-hCG is not predictive for the development of PE (*14*). A couple of other studies have found a modest or clinically insignificant increase in the risk of developing PE among women with abnormal second-trimester levels of hCG (*15,16*). However, there are studies from recent past that suggest that elevated hCG levels are associated with developing early PE, and measuring levels of beta hCG during the second trimester of pregnancy can be useful in clinical practice to identify pregnant women who will develop PE (*17,18*).

5. Conclusion

Researchers from different parts of the world are looking for biomarkers that can predict the development and severity of PE. NLR and serum beta hCG are cheap, rapid, non-invasive biochemical markers that have been studied in PE. In our study, both NLR and serum beta HCG were found to be excellent biomarkers in predicting both the development of PE and its severity. Multicentric, prospective, large-scale studies involving subjects of multiple ethnicities should be done for implementation of these markers as a routine screening test in pregnancy for predicting PE.

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