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ORIGINAL PAPER

Maternal β-hCG and Neutrophil Lymphocyte Ratio during Pregnancy to Predict High-Risk Neonates: an Observational Study

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ABSTRACT

Background: *Maternal serum biomarkers assist in identifying various maternal and foetal complications. In this manner, the present study was conducted to assess the birth of high-risk infants using β-hCG level and neutrophil lymphocyte ratio and their correlation with the development of low birth weight and poor APGAR score.*

Methods: *A tertiary hospital-based prospective observation study was conducted among primi gravida attending the Department of Obstetrics & Gynaecology of Vardhaman Mahavir Medical College and Safdarjung Hospital, New Delhi, India. Written informed consent was obtained from prim gravida who met the eligibility criteria. Basic details on socio-demographics and selective blood investigations,* i.e., *β-hCG and neutrophil-to-lymphocyte ratio (NLR), were examined and followed-up until postdelivery to assess the neonatal outcome. Data was analysed using SPSS version 21.0 with appropriate statistical methods.*

Results: *The mean (±SD) age of participants (N=440) was 23.7 (±1.6). Overall, the mean (±SD) of birth weight and APGAR score at five minutes were 2.6 (±0.6), and 8.8 (±1.2), respectively, within the normal limits. Maternal values of NLR and β-hCG (IU/mL) were negatively correlated to neonatal outcomes,* i.e., *low birth weight and poor APGAR score. The mean values of NLR were significantly high in neonates with poor outcomes (LBW, poor APGAR). The sensitivity and specificity of β-hCG as a predictor for poor APGAR score was 83% and 66% at 16-18 weeks (AUC -0.82, cut-off 22721) and 83%, and 90%, respectively at 32-34 weeks (AUC-0.79, cut-off 14825). The sensitivity and specificity of NLR as a predictor for poor APGAR score were 78% and 61% at 16-18 weeks (AUC-0.76, cut-off 4.5), and 89% and 53%, respectively at 32-34 weeks (AUC-0.74, cut-off 4.5).*

Conclusion: *High levels of maternal NLR and β-hCG resulted in low birth weight neonates and poor APGAR score. The negative impact of these biomarkers should be further explored on a larger scale basis. Ascertaining this would lead to reduction in poor fetal outcomes.*

Keywords: neutrophil lymphocyte ratio, antenatal care, high-risk neonate, beta-HCG, prediction, low birth weight.

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INTRODUCTION

The production of human chorionic gonadotrophin (β-hCG) hormone by placenta in early pregnancy is fundamental for implantation and safeguarding of blastocyst. human chorionic gonadotrophin consists of alpha and beta subunits and is largely made by the placental syncytiotrophoblast (1). Throughout a normal pregnancy, its concentration increases rapidly, reaching a peak by 10 weeks of gestation and achieving a significant level by 20 weeks (2). Therefore, β-hCG levels can be used to assess placental function in the early stage of pregnancy.

Neutrophil-to-lymphocyte ratio (NLR) has been projected as a novel marker of systemic inflammation. Recently, NLR has been of much interest in their prognostic values in several different topics such as cancers and cardiac diseases. Neutrophil-to-lymphocyte ratio is devised from complete blood count (CBC) and has been studied as an innovative indicator of prognosis in patients with pre-eclampsia. It is ordinarily used as a biomarker of systemic inflammatory status, *i.e.*, chronic low grade reliable inflammation (3, 4). In addition, it is an evolving marker for both cardiac (5) and non-cardiac disorders. Recent studies demonstrated its prognostic role in stable coronary artery disease, acute coronary syndromes, heart failure as well as patients having vascular surgeries (6, 7), ulcerative colitis (8), or repeat cerclage in women with prolapsed membranes (9).

Although β-hCG levels and NLR were identified as predictors for the development and severity of pre-eclampsia, they were not correlated with the neonatal outcome. Hence, this study was undertaken to appraise the role of mother's β-hCG and NLR during the antenatal period in predicting the birth of high-risk neonates and correlated with poor APGAR score. ❑

METHODS

A hospital-based prospective observational study was conducted from October 2015 to January 2016 in the Department of Obstetrics & Gynaecology (OBG), Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India. A cohort of pregnant women were followed-up from their antenatal period until discharge following delivery at this tertiary care centre. The minimum estimated number of samples was estimated by the standard formula, *i.e.*, 4pq/d2, where p was the proportion of prim gravidae attending the Safdarjung Hospital, which was about 50%, considering a relative precision of 10%. An additional, 10% of subjects were added in view of non-response. Therefore, we obtained a final sample size comprised of 440 subjects. Primi gravidae women aged 20-35 years who were attending the antenatal clinic (ANC) between 16th-18th weeks of gestation, agreed to follow-up and were planning to deliver in the study hospital were considered for recruitment using the consecutive sampling technique. The following exclusion criteria were used: patients with a history of heart disease, chronic hypertension, diabetes, overt liver disease, obesity (BMI >30), cancer, chronic renal disease, alcohol, smoking and drug abuse, thyroid disorders, haematological disease, any ongoing infection, or systemic inflammatory diseases or any autoimmune disorders. Four hundred and forty study participants who met the eligibility criteria were enrolled after obtaining their written informed consent. Venous samples (5 mL) were taken on enrolment at 16-18 weeks of gestation. The values of β-hCG and NLR were measured. Blood investigations were repeated at around 32-34 weeks. Participants were followed according to hospital protocol until delivery and discharge. Positive findings were recorded at each visit. Maternal and fetal outcomes were also noted. Those diagnosed with pre-eclampsia and PIH were followed-up and managed as *per* our institutional protocol.

Serum β-hCG was measured by using ELISA technique. Solid phase enzyme-linked immunosorbent assay (ELISA) exploits a goat anti α-hCG antibody as solid phase immobilization and another mouse monoclonal anti β-hCG antibody in the antibody enzyme (horseradish peroxide) conjugate solution. The test serum is added to the coated micro titre wells and intubated with the zero buffer at room temperature (30 min). If β-hCG is present, it will coalesce with the antibody on the well. Then, the well is washed to take away any lingering test specimen and β-hCG antibody labelled with horseradish peroxidase (conjugate) is added. The conjugate will immunologically bind to the β-hCG, resulting in the β-hCG molecules being

sandwiched between the solid phase and enzyme linked antibodies. After incubation (15 min) at room temperature, the wells are rinsed with water to remove unbound-labelled antibodies. A solution of TMB reagent is then added for incubation at room temperature (20 min) and a blue colour appears. Colour formation is stopped by adding 1 N HCL; the resulting solution turns to yellow and is measured spectrophotometrically at 450 nm (10). The concentration of β-hCG is directly proportional to the intensity of colour in the test sample. Neutrophil and lymphocyte count was estimated using automated CBC machines.

Outcome variables

The period of gestation (in weeks) during delivery, type of delivery, birth weight, APGAR score, NICU admission and neonatal mortality were measured.

Operational definition

The birth weight was considered low if the weight was < 2.5 kg (LBW).

APGAR score was based on five signs, including breathing effort, heart rate, muscle tone, grimace reflex, and baby color. It was measured at one minute and five minutes, and reading at five minutes was used for analysis. The total score was interpreted as normal (> 8), moderate (7-8), and severe (< 7) (11).

Statistical analysis

Data were entered in Microsoft (MS) EXCEL spreadsheet and analysed using the Statistical Package for Social Sciences (SPSS) version 21.0 software. Categorical variables were presented as number and percentage (%) and continuous variables as mean (± SD) and median, appropriately. Data normality was tested by Kolmogorov-Smirnov test. A non-parametric test was used for non-normal patterns. P value less than 0.05 was considered statistically significant.

Ethical clearance

The Institute Ethics Committee approved the study for the purpose of research. Informed consent was obtained from each study participant and confidentiality was ensured. Appropriate medical care was given to all subjects who experienced a negative outcome. ❑

RESULTS

A total of 440 antenatal women with a mean (±SD) age of 23.7 (±1.6) years participated in the study. Antenatal women had a minimum and maximum age of 21 and 28 years, respectively. The average (±SD) APGAR score at five minutes was 8.8 (±1.2).

There was one extremely preterm neonate (< 28 weeks), two very preterm (28 to 32 weeks), and 47 moderate to later preterm (32 to 37 weeks) neonates. The majority of them (94%) were in moderate to later preterm period. The one who was born extremely preterm (24 weeks) died soon after delivery. The mean (±SD) birth weight in kilograms was 2.6 (±0.6), which was in the normal range (Table 1).

The overall mean (±SD) of NLR at 16-18 weeks and 32-34 weeks was 4.7 (±0.8) and 5.0 (±0.9), respectively. The overall median value of β-hCG (IU/mL) at 16-18 weeks and 32-34 weeks was 17,123 and 8,517, respectively.

APGAR score was negatively correlated with NLR at 16-18 weeks and was found significant. Similarly, β-hCG had a significant negative correlation with APGAR score (Table 2).

Variable	Number	Percentage
Period of gestation		
Preterm (< 37 weeks)	50	11.4
Term (≥ 37 weeks)	390	88.6
Type of delivery		
NVD	376	85.4
LSCS	55	12.5
Assisted vaginal delivery	9	2.1
APGAR at 5 min		
< 7	18	4.1
7 -8	87	19.8
>8	335	76.1
NICU admission		
Yes	54	12.3
No	386	87.7
Birth weight		
LBW (<2.5 kg)	147	33.4
Normal (≥ 2.5 kg)	293	66.6
Neonatal mortality		
Yes	6	1.4
No	434	98.6

TABLE 1. Characteristics of the delivery and neonatal outcomes during the postnatal period

TABLE 2. Correlation of NLR and β-hCG with APGAR score at 5 min

Variables	APGAR score	
	r	p value
NLR 16-18 weeks	-0.31	**0.000**
β-hCG 16-18 weeks	-0.24	**0.000**
NLR 32-34 weeks	-1.47	**0.002**
β-hCG 32-34 weeks	-0.19	**0.000**

TABLE 3. Correlation of NLR and β-hCG with birth weight

Variables	Birth weight	
	r	p value
NLR 16-18 weeks	-0.22	**0.000**
β-hCG 16-18 weeks	-1.97	**0.000**
NLR 32-34 weeks	-0.06	0.191
β-hCG 32-34 weeks	-0.11	**0.02**

There was a negative correlation between neonatal birth weight and maternal NLR and β-hCG during the antenatal period, with a significantly correlation with β-hCG at both 16-18 weeks and 32-34 weeks of gestation. For NLR, birth weight was significantly correlated with maternal values at 16-18 weeks of gestation (Table 3).

The difference between β-hCG at 16-18 weeks and birth weight was statistically significant (p <0.000) but its value at 32-34 weeks was insignificant. Also, the mean difference between NLR at both 16-18 weeks (p <0.000) and 32-34 weeks (p <0.007) and birth weight was statistically significant (Table 4).

Similarly, the difference between β-hCG at 16-18 weeks and 32-34 weeks was statistically significant across the groups based on APGAR score. There was a significant inter-group mean difference between NLR for both gestations and APGAR score (Table 5).

The sensitivity and specificity of β-hCG (AUC 0.82) in predicting the development of poor APGAR scores at 16-18 weeks, deciding the cut-off value as 22721, were 83% and 66% (p 0.007), respectively. Likewise, the sensitivity and specificity of β-hCG at 32-34 weeks (AUC 0.79), deciding the cut-off value as 14825, was 83% and 90% (p 0.014), respectively. The sensitivity and specificity of NLR in predicting the development of poor APGAR scores were 78% and 61% (AUC 0.76, cut-off 4.5) at 16-18 weeks, and 89% and 53% at 32-34 weeks (AUC 0.74, cut-off

Birth weight category	Normal (n=293)	Low birth weight (n=147)	p value
β-hCG (16-18 weeks)#: Median	16520	19850	**0.000**
β-hCG (16-18 weeks)#: IQR	13682 - 25678	15420 - 32436	
β-hCG (32-34 weeks)#: Median	8425	8658	0.106
β-hCG (32-34 weeks)#: IQR	7293 - 12546	7410 - 14231	
NLR (16-18 weeks)*: Mean (SD)	4.5 (0.7)	4.9 (0.9)	**0.000**
NLR (32-34 weeks)*: Mean (SD)	4.8 (0.9)	4.9 (1.0)	**0.007**

#Mann-Whitney U test

*Student t test

TABLE 4. Comparison of maternal serum biomarkers with birth weight categories

	APGAR category			
	< 7 (n=18)	7-8 (n=87)	>8 (n=335)	p value
β-hCG (16-18 weeks)#: Median	33688.5	17526	16581	**0.007**
β-hCG (16-18 weeks)#: IQR	17402 - 40152	14520-31452	14203-2665	
β-hCG (32-34 weeks)#: Median	15254.5	9631	8254	**0.001**
β-hCG (32-34 weeks)#: IQR	11156-15967	7410 - 13721	7293 - 12314	
NLR* (16-18 weeks): Mean (SD)	5.7 (0.9)	5.0 (0.8)	4.6 (0.7)	**0.000**
NLR *(32-34 weeks): Mean (SD)	5.9 (0.9)	5.0 (0.9)	4.9 (0.9)	**0.011**

#Kruskal-Wallis test

*Oneway ANOVA

TABLE 5. Comparison of maternal serum biomarkers with APGAR score (at 5 min)

	Area under the ROC curve (AUC)	Standard error	95% confidence interval	p value	Cut-off	Sensitivity	Specificity
β-hCG (16-18)	0.82	0.11	0.59-1.00	**0.007**	≥ 22721	83	66
β-hCG (32-34)	0.79	0.14	0.50-1.00	**0.014**	≥ 14825	83	90
NLR (16-18)	0.76	0.06	0.63-0.88	**0.000**	4.5	78	61
NLR (32-34)	0.74	0.05	0.64-0.84	**0.000**	4.5	89	53

TABLE 6. Sensitivity and specificity of maternal serum biomarker as a predictor of poor APGAR score (at 5 min)

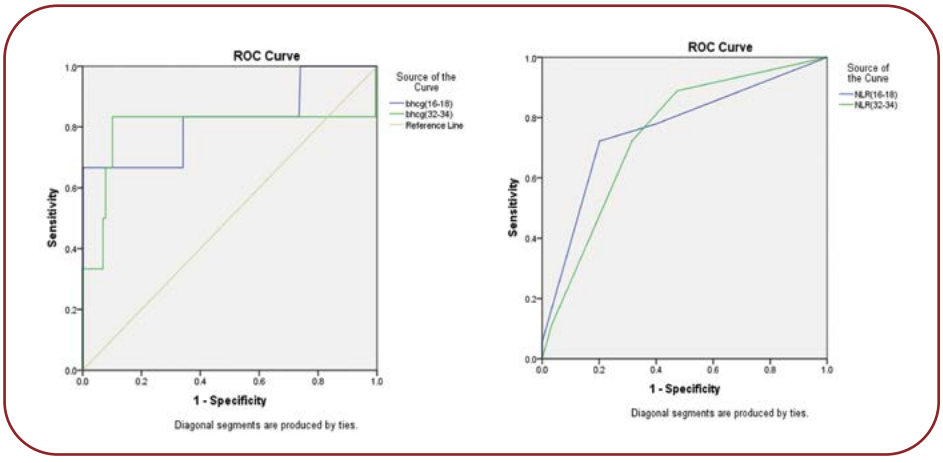

FIGURE 1. ROC curve of sensitivity and specificity of maternal serum biomarker at 16-18 and 32-34 weeks in the prediction of poor APGAR score (at 5 min)

4.5), respectively. The p value (< 0.000) was statistically significant (Table 6, Figure 1). ❑

DISCUSSION

This study explored the correlation between maternal NLR and β-hCG and neonatal outcome among primi gravida women who met the eligibility criteria. Likewise, elevated maternal NLR and β-hCG in the second trimester (16-18 weeks) of pregnancy will assist in predicting low birth weight neonates. This would help to further reduce the risk of neonatal mortality. Usually, the growth of the fetus occurs during the second and third trimesters, which is visible through maternal gestational weight gain (12). The role of maternal NLR and β-hCG during pregnancy as early predictors for the development and severity of pre-eclampsia was studied and described elsewhere (13).

In this study, there was a significant reduction in the birth weight of neonate with the rise in NLR and β-hCG, which are elevated during any acute inflammation or any form of stress in the body. The elevated maternal serum biomarkers could be suggestive of an underlying inflammatory mechanism indirectly. Early prediction of risk for low birth weight babies by diagnosis in the second trimester would lead to nutrition intervention and more close follow-up for antenatal women. Few studies reported that maternal β-hCG could not be recommended as a single predictor for delivery of low birth weight babies (14).

The present study correlated the elevated NLR with poor APGAR score at five minutes and low birth weight. The values were measured in both the second and third trimester and they were statistically significant with poor fetal outcomes. Similarly, other studies had demonstrated the poor fetal outcome in terms of prematurity and fetal loss in high-risk mothers. Balciunience G *et al* found that NLR was a good predictor of histological chorioamnionitis, which would cause preterm premature of rupture of membranes (15). Aslan MM *et al* reported that the elevated

NLR in the third trimester was associated with fetal loss in patients with severe pre-eclampsia (16). Also, NLR and β-hCG can be measured among women to predict high-risk neonates as an alternative. Overall, this would in turn avert materno-fetal complications. Future studies can further explore this aspect among antenatal women and assist in uneventful delivery and birth of healthy babies with no to minimal risk.

Study limitations

Given that our research was a tertiary care hospital-based study, results could not be generalised. The study population consisted only of primi gravida. The role of maternal serum biomarkers in terms of maternal and fetal outcomes in multi gravida and women with other comorbidities was not ascertained. Thus, larger studies need to be planned to address this issue. ❑

CONCLUSION

Antenatal women with high NLR and β-hCG had given birth to neonates with lower APGAR score and low birth weight. The risk increased with increase in the value of NLR and β-hCG during pregnancy irrespective of the gestational age. Further, the use of both NLR and β-hCG as one of the parameters for the prediction of high-risk neonates alternatively needs to be studied in the future in order to reduce neonatal mortality rate. ❑

Conflicts of interest: none declared.
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