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Original Research Article

Significance of maternal hemogram parameters as new inflammatory markers for prediction of threatened preterm labor and preterm premature rupture of membranes

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ABSTRACT

Background: Non-invasive, inexpensive and easily available simple markers are needed for timely prediction of preterm labor and preterm premature rupture of membranes (PPROM) in women at risk. Aims and objectives of current study was to study the significance of maternal hemogram parameters i.e., NLR, PLR, MPV, PDW and plateletcrit in TPL, PPROM.
Methods: A total number of 150 pregnant women, 50 with PPROM (group 1), 50 with TPL (group 2) and 50 gestation matched healthy controls (group 3) attending antenatal clinic and labor room in SVBP Hospital and associated LLRM Medical College, Meerut were recruited in study. Complete blood count was done from blood sample collected in EDTA vials using 5-part automated cell analyzer device. Hematological parameters like NLR, PLR, MPV, PDW and plateletcrit were measured from CBC.
Results: Patients with PPROM had increased NLR, PLR, MPV, PWD and plateletcrit than patients with threatened preterm labor group and healthy control group.
Conclusions: NLR, PLR, MPV, PDW, and Plateletcrit were significantly increased in both PPROM group and TPL group compared to healthy control group and have a predictive value in PPROM and TPL. Monitoring of these parameters can be promising and cost-effective methods in prediction of TPL and PPROM.

Keywords: Neutrophil/lymphocyte ratio, PLR, MPV, Platelet distribution width, Threatened preterm labor, Plateletcrit, Complete blood count, Ethylenediamine tetra-acetic acid

INTRODUCTION

Preterm birth may be defined as birth between the age of viability and before 37 completed weeks of gestation and complicates 5-8% of pregnancies in most developed and developing countries and incidence is still increasing worldwide.[1] The term "premature rupture of membranes" (PROM) refers to the amniotic fluid leaking before the onset of labour. Preterm premature rupture of the membranes is what is meant if this happens before 37 weeks of pregnancy (PPROM). PPROM complicates upto 3% of pregnancies and contribute to 30-40% of preterm birth. It causes significant neonatal morbidity and mortality primarily from prematurity, sepsis, cord prolapse and pulmonary hypoplasia.[2] Although many etiological factors may be considered with spontaneous preterm labor and preterm premature rupture of membrane (PPROM) (such as previous preterm birth, urinary tract infections and sexually transmitted infections, previous or current cervical surgical procedures, low socioeconomic status and low maternal body mass index (BMI), amniocentesis etc.), the actual cause of membrane weakening and rupture is not known. Regardless of causes, inflammation is the only pathologic process for a strong causal relation with preterm labor and PPROM.[3] Priming and activation of maternal leucocytes in peripheral blood is a key

component of parturition and this inappropriate preterm priming of leucocytes might initiate preterm labor and delivery.[4] Cytokines released from inflammed areas in choriodecidua during early stage of inflammation may result in change in number of leucocytes subset. Platelets also play a role in inflammatory process. Platelet/Lymphocyte ratio (PLR) has been proposed as a predictive and prognostic parameter for many kind of diseases such as cardiovascular diseases, acute appendicitis and malignancies. It is well known that pregnancy itself and labor cause platelet activation.[6] Therefore, platelet indices are also raised in preterm labor and PPROM. We hypothesize that PPROM and threatened preterm labor may be associated with alteration of these hematological parameters in the maternal serum. So, the aim of this study was to assess and quantify peripheral platelets, neutrophils and lymphocytes count in women with PPROM and TPL and to use various hematological parameters like NLR, PLR, plateletcrit and MPV in their prediction.

METHODS

A total of 150 antenatal women of reproductive age group attending antenatal OPD and emergency in the department of Obstetrics and Gynaecology of SVBP Hospital associated with LLRM Medical College, Meerut were recruited for study as per the inclusion and exclusion criteria during a period of 18 months. Pregnant females having gestational diabetes mellitus, pregnancy induced hypertension, infection of urinary, respiratory or gastro-intestinal tract and any acute or chronic infectious conditions, multiple gestation, previous history of hematopoietic system disorder, malignancies, hepatic disorders, history of auto immune disease were excluded from the study.

Procedure

Participants were categorized into, Group 1- 50 women with Preterm premature rupture of Membranes (PPROM), Group 2- 50 women with threatened preterm labor (TPL), Group 3- 50 Gestation matched healthy controls. 2-3 ml venous blood was drawn from antecubital vein on admission under full aseptic condition using a dry sterile disposable syringe and needle and the sample was transferred to standardized tubes containing Ethylene-diamine tetraacetic acid (EDTA) vials for complete blood count using 5-part automated cell analyzer device. Blood was mixed promptly with EDTA by rolling the container between palms and gentle inversion several times to avoid sample clotting. Blood sample was sent to laboratory for testing as soon as possible. In case of delay, sample can be stored for 24-48 hours at ambient temperature of 40°C. All blood samples were taken before steroid and antibiotic administration. All the haemogram parameters including total and differential leucocyte count, platelet indices Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), Plateletcrit and CRP were measured. NLR and PLR were calculated. The Neutrophil Lymphocyte Ratio (NLR) was calculated by dividing the number of neutrophil by the number of lymphocyte count.

$$\text{Neutrophil Lymphocyte Ratio } (NLR) = \frac{Number\ of\ Neutrophil}{Number\ of\ Lymphocyte}$$

The platelet lymphocyte ratio (PLR) was calculated by dividing the number of platelets by the number of lymphocyte count.

$$\text{Platelet Lymphocyte Ratio } (PLR) = \frac{Number\ of\ Platelet}{Number\ of\ Lymphocyte}$$

All women with threatened preterm labor & PPROM were managed according to standard protocol. Data was collected and labour outcome was evaluated. Data were collected and analyzed.

RESULTS

Demographic and clinical characteristics of the patients are shown in (Table 1). There was no difference between the three study groups in the terms of age, gravida, gestational age.Total leucocyte count,platelet count , MPV, NLR, PLR, plateletcrit all were significantly higher in the PPROM group and TPL group as compared to gestation matched healthy control. Group 1 had significantly higher value of hematological inflammatory markers compared to healthy control. Group 2 participants had higher value of inflammatory markers as compared to group 3 but the difference was not statistically significant. In cases of PPROM (group 1) CRP is a very important marker of inflammation and was found raised significantly in group 1 as compared to group 3. The neonatal outcomes of pregnancies are represented in (Table 2). The PPROM and TPL groups had significantly lower birth weight and low APGAR score compared to healthy control. Poor neonatal outcome was noted among group 1 and group 2 compared to group 3 as more neonate required NICU admission for sepsis and RDS.

DISCUSSION

Although various mechanisms and factors, including inflammation, infection, defective placentation, malfunctions in genetic, immunologiocal, hormonal and angiogenic mechanism and increased oxidative stress have been implicated in preterm births but precise pathophysiology of the condition is still unknown.[10] Pro-inflammatory cytokines and various leucocyte subtypes have been extensively explored on the link between inflammation and premature birth. High levels of C-reactive protein (CRP), cytokines and ferritin are only a few of the sample characteristics that have been studied because of their potential value in predicting preterm birth and PPROM.

Table 1: Demographic and clinical characteristics of patients.

Parameters	Group 1 (PPROM) (N=50)	Group 2 (TPL) (N=50)	Group 3 (Healthy control) (N=50)	P value
Age (years)	25.78±3.29	27.24±4.45	25.90±4.05	0.127
Gestational age (weeks)	32.19±3.9	33.3±1.7	33.53±2.8	0.08
Total leucocyte count (/mm3)	14039±3759	10137±2086	8548±1713	0.001
Platelet count (X 1000 cells/mm)	254±80	213±55	199±40	0.001
Plateletcrit (%)	0.27±0.08	0.22±0.06	0.20±0 .04	0.001
Mean platelet volume (fl)	10.66±1.44	10.01±1.55	9.66±1.43	0.003
NLR	6.71±2.53	4.75±2.1	3.57±1.20	0.001
PLR	14.49±5.44	12.95±6.31	11.31±4.89	0.019
CRP (mg/dl)	3.69±3.77	2.66±0.85	1.06±0.37	0.002

Table 2: Neonatal outcomes of pregnancies.

Parameters	Group 1 (PPROM) (N=50)	Group 2 (TPL) (N=50)	Group 3 (Healthy control) (N=50)
Birth weight(gram)(mean±SD)	1763.6±654.7	1849.0±564.2	2578.8±597.0
NICU admission (N, %)	42 (84)	38 (76)	00 (00)
Neonatal deaths (N, %)	21 (42)	17 (34)	00 (00)
Duration of stay in NICU	3.86	2.38	00
Sepsis (+) (N, %)	20 (40)	14 (28)	00
RDS (+) (N, %)	22 (44)	13 (26)	00
APGAR<7 (1 min) (N)	19 (38)	16 (32)	02 (04)
APGAR>7 (5 min) (N)	31 (62)	34 (68)	48 (96)

Several inflammatory indicators have been investigated for their capacity to effectively detect PPROM, it is not yet clear whether there is a valid diagnostic test for predicting PPROM. In our study, there was no significant difference in mean age between PPROM (25.78±3.29 years), pre-term (27.24±4.45 years) and term controls (25.90±4.05 years). Toprak et al.[9] reported that the mean age was 28.7±5.1 and 29.4±5.0 years among PPROM and control groups respectively. In our study we found that TLC was significantly more among PPROM and TPL group compared to term controls (p value=0.001). Although WBC count was thought to be a predictor of impending preterm birth, mean WBC count in the research by Daglar et al was comparable within the PPROM, TPL and healthy term control groups.[7] To evaluate platelet function during pregnancy is a challenge because platelet function vary due to physiological changes that occur in the cardiovascular and haematological systems. Increased platelet synthesis and activation normally occurs during pregnancy. Additionally, compared to normal pregnancies, pregnancies complicated by pre-eclampsia, IUGR, and recurrent loss had higher levels of platelet activation.

In our study, platelet count (Lac Cells/ mm3), Mean Platelet Volume (MPV) and plateletcrit was significantly more among PPROM compared to pre-term and term controls. Platelet Lymphocyte Ratio (PLR) was significantly more among PPROM (14.49±5.44) compared to pre-term (12.95±6.31) which was significantly more than the term controls (11.31±4.89) with a p value 0.019. Similar to our findings, Toprak et al reported significantly higher platelet count in the PPROM group compared to preterm group (244.5±60 vs. 210.6±64.8 x1000/mm^3, p<0.001).[9] Lakshmi et al observed that pregnant women with PPROM had higher mean total leucocyte counts and platelet lymphocyte ratio than healthy gestation age-matched controls.[10-12] It has been shown that the widely-used marker PLR may predict thrombotic events, inflammatory illnesses, and cancers. Increased PLR has been linked to large negative outcomes in cardiovascular illnesses and decreased survival in cancers according to several earlier research. PLR was studied in pre-eclampsia, PPROM, acute pancreatitis, and gestational diabetes in pregnant women. Toprak et al showed a significant correlation between the frequency of PPROM and PLR levels greater than 117.14. Additionally, it was shown to be an important independent discriminator for PPROM.[9]

It is crucial to keep track of the signs of preterm labor in pregnant women to anticipate premature delivery and manage difficulties and neonatal outcomes. In our study, CRP (mg/dl) was significantly more among PPROM group (3.69±3.77), TPL group (2.66±0.85) as compared to the healthy controls (1.06±0.37) (p value=0.001) In clinical practice, the biomarkers of inflammation CRP and erythrocyte sedimentation rate (ESR) have been used to identify instances of infections that may raise the risk of premature labor. Similarly, Daglar et al also reported that CRP values are significantly elevated among threatened

pregnancy groups (both full term and preterm pregnancy) compared to control group.[7] In current study, Neutrophil Lymphocyte Ratio (NLR) was significantly more among PPROM group (6.71±2.53) compared to TPL group (4.75±2.10) which was significantly more than the term controls (3.57±1.20) (p value=0.001). Lakshmi et al observed that pregnant women with PPROM) had higher mean total counts, neutrophil counts, and neutrophil lymphocyte ratios (6.66±3.75) than were healthy gestation age-matched controls (4.22±2.09) (p value=0.0005).[12] In order to provide time for further preventive treatments, the ideal predictive test should be simple to use, repeatable, and accessible throughout pregnancy. NLR, a proinflammatory biomarker, has the benefit that its value may be determined by using a simple complete blood count test without requiring the need of extra laboratory tests. NLR has been proposed to be a measure of systemic inflammation and a prognostic marker in various illnesses, such as ovarian cancer and colorectal cancer. Increased NLR has been seen in spontaneous preterm labor. Chemokines and cytokines are what cause the decrease in lymphocytes. Recent research on the effectiveness of NLR in predicting preterm birth produced some intriguing findings. In contrast to cervical length alone or other systemic inflammatory indicators as CRP and leukocytes, Kim et al showed that a combination model made up of NLR and cervical length had a stronger diagnostic and predictive value for spontaneous preterm birth.[5] Akgun et al also linked higher NLR levels to premature births and lower birth weight babies. Some authors also proposed that the high NLR, which is a marker of maternal hyper-inflammatory state, may cause abnormal foetal development, which might result in low birth weight and early labor.[8] NLR value dramatically rises in pre-eclampsia pregnancies, particularly those with severe symptoms, and it may be a helpful biomarker for detecting pre-eclampsia early and determining its severity. Additionally, a prior study comparing the predictive accuracy of NLR and CRP for suspected late onset sepsis in preterm neonates revealed that NLR is more effective than CRP in identifying sepsis cases that have been confirmed by culture. Duration of stay in NICU was significantly more among PPROM (3.86±5.27 days) compared to Preterm (2.38±4.40 days) which was significantly more than the term controls (no hospitalization) (p value=0.001).[12] Similarly, Lakshmi et-al observed that the neonate born in PPROM had higher duration of stay in NICU than healthy gestation age-matched controls. In our study, neonatal death was significantly more among PPROM and preterm subjects than the healthy term controls. Admission in NICU were significantly more among PPROM and Preterm subjects. In contrary to our study, Toprak et al reported that regarding birth weight, RDS, APGAR score, and NICU hospitalizations, there were no significant differences between the PPROM and healthy control groups (p>0.05).[9] In the PPROM group, sepsis occurred more often (34.7% vs. 19.8%, p=0.02). The risk of RDS increases as PDW increases by about 1.33 times. The time interval between membrane rupture and delivery of fetus is a major risk for the development of maternal and neonatal complications like RDS, intraventricular hemorrhage, necrotizing enterocolitis and sepsis. An inverse relationship exits between the severity and incidence of all these complications and gestational age. Rani et-al noted that respiratory distress accounted for 77% of the newborn morbidity. In early PPROM, respiratory discomfort was prevalent. Lakshmi et-al observed that neonates delivered to PPROM women had higher NICU hospitalizations (11.4%) compared to controls (0%).[9-12]

CONCLUSION

Platelet lymphocyte ratio (PLR) and neutrophil lymphocyte ratio (NLR) values were significantly higher in PPROM group and TPL as compared to healthy controls. Both maternal PLR and NLR levels at delivery has a significant relationship with neonatal outcomes hence these ratio can be used to predict poor outcomes in preterm babies. PLR and NLR are readily available cost-effective method. We also found that platelet indices were significantly higher in PPROM group as compared to TPL group and healthy controls. Therefore, current research successfully confirmed increased PLR and NLR levels as well as other inflammatory indicators including TLC and neutrophil count for predicting PPROM and TPL. Additional trials with large sample size should be performed to support our study.

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