Original article

Correlation of maternal platelet to lymphocyte ratio, neutrophil to lymphocyte ratio, C-reactive protein with gestational age at delivery and fetal outcome - A prospective observational study from tertiary care centre

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ABSTRACT

Background: Economic burden of preterm births can be reduced by screening and treating them at the early stage. The mechanism behind preterm birth is not clear and therefore there is no effective globally accepted treatment. Screening methods were mainly based on the previous obstetric history of the pregnant woman. Evidences indicate inflammatory markers such as neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) might be potential predictors of preterm deliveries. This study was aimed to determine the relationship between preterm births and NLR, PLR and CRP levels in a singleton pregnant woman.

Material and methods: This prospective observational study was conducted in the Department of Obstetrics and Gynaecology in a tertiary care hospital located at Chengalpattu, Kanchipuram, Tamil Nadu with an objective to determine the relationship between inflammatory markers and the foetal outcome among singleton pregnant women. The Study period was from August 2022 to July 2023 for a period of 1 year. This study enrolled pregnant women between 28 and 32 weeks of gestation attending regular antenatal clinic and those willing to give informed consent.

Results: There was mean difference between both the groups in terms of WBC count (white blood cell) [11.87 (±3.7) in pre term vs. 10.71(±2.76) in term], platelet count [262.7(±59.1) in pre term vs. 234.5(±73.6) in term], Absolute lymphocyte count [262.7(±59.1) in pre term vs. 234.5(±73.6) in term], Neutrophil lymphocyte ratio (NLR) [6.3(±2.8) in preterm vs. 3.7(±0.8) in term] and platelet lymphocyte ratio (PLR) [165.1(±87.9) in pre term vs. 120.7(±39.1) term] and all these difference were found to be statistically significant (p-value < 0.05*).

Conclusion: This prospective observational study deduced the association between the inflammatory markers and preterm delivery in a singleton pregnant woman. Rise in inflammatory parameters like neutrophil lymphocyte ratio, platelet lymphocyte ratio and C-reactive protein proved to be useful in predicting spontaneous preterm labour (before 37 weeks of gestation).

1. Introduction

Normal human parturition and labour are multi-factorial physiologic events involving an integrated set of changes within the maternal tissues and foetal membrane. Subclinical inflammation is tissue infiltration by neutrophils, macrophages, and lymphocytes. There is mounting evidence that labour is an inflammatory process, and both clinical and subclinical infection and inflammation play an essential role in the aetiology of preterm birth.1,2 It has been reported that most histopathological inflammation and chorioamnionitis cases are subclinical in preterm and term delivery.1

Preterm delivery, defined as delivery before 37 weeks of gestation, is a significant hurdle in obstetrics and children’s health. It has been estimated that 5–18 % of all pregnancies end up in preterm delivery, which poses an extensive healthcare burden mainly due to neonatal morbidity and mortality.3,4 Accordingly, one million neonates die out of 15 million neonates born preterm.5 Along with the risk of mortality, it has been shown that children born preterm suffer from various short and long-term morbidities and adverse outcomes such as neurological deficits, learning disabilities, and respiratory problems.5,6
Pregnancy is accompanied by increased expression of cell adhesion molecules, chemotactic agents such as interleukin (IL)-8, and proinflammatory cytokines, WBC activation. The neutrophil-lymphocyte ratio is the ratio between the absolute neutrophil count and the absolute lymphocyte count. It often indicates the body’s immune response to harmful agents. It is also viewed as a rapid and simple parameter indicative of systemic inflammation and stress. The platelet lymphocyte ratio was defined as the absolute platelet count divided by the total lymphocyte count. It is another parameter known to increase during thrombosis and inflammation.

The economic burden of preterm births can be reduced by screening and treating them at the early stage. The mechanism behind preterm birth needs to be clarified; therefore, there is no effective globally accepted treatment. Screening methods were mainly based on the previous obstetric history of the pregnant woman. Evidences indicate that measurement of neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) might have prognostic significance for diseases related to chronic low-grade inflammation and inflammatory markers have been suggested previously to be potential predictors of preterm deliveries. CRP is an acute phase reactant and a marker of inflammation. Maternal CRP can predict preterm birth with reasonable accuracy. Therefore, in this study, we aimed to study the correlation between preterm birth and NLR, PLR, and CRP levels in singleton pregnant women.

2. Material and methods

This prospective observational study was recently conducted by the Department of Obstetrics and Gynaecology in a tertiary care hospital in Chengalpattu, Kanchipuram, Tamil Nadu. The study aimed to investigate the relationship between inflammatory markers and foetal outcomes among women with singleton pregnancies. The study period ran from August 2022 to July 2023, spanning one year. This study enrolled only the pregnant women in their 28–32 weeks of gestation attending regular antenatal clinics and those willing to give informed consent. The study exclusion criteria were those with multiple pregnancies, foetal anomalies, previous history of preterm birth, haematological disease, and other comorbidities complicating the present pregnancy. Based on the results of previously available literature with similar objectives, the sample size for the data collection was calculated using the formula for the difference between two means ± SD. With the level of significance at 5 % and setting power at 80 %, the required sample size for the study was calculated to be 151. The study was conducted after obtaining ethical clearance from the institutional ethical committee.

In this study, preterm births were defined as delivery before 37 weeks of gestation, and any delivery ≥ 37 weeks will be considered a term birth. After the informed consent by the patients, detailed obstetric and antenatal history was taken, followed by clinical examination and lab investigation for inflammatory parameters (such as WBC count, platelet count, and CRP), and they were followed up till their delivery. The collected data were first entered into the Excel sheet and then imported to SPSS (statistical package for social sciences (SPSS) v21 software) for data analysis. Chi-square (χ²) was used to adjudicate the association between two categorical variables. Similarly, the mean difference between two independent samples (between preterm and term delivery) was determined using the independent sample t-test, and the difference between two independent proportions was calculated using the z-test. A scatter plot was done to determine the linear relationship between inflammation markers (NLR and PLR) and gestational age at delivery, and correlation coefficient (r) was calculated, and the value of <0.05* was considered statistically significant.

3. Results

This prospective observational study included one hundred fifty-one pregnant women attending the regular antenatal clinic of a tertiary care hospital in Chengalpattu. Out of 151, 61 (40.4 %) had preterm labour (<37 weeks) and 90 (59.6 %) had term labour (≥37 weeks). The mean age of the participant delivered preterm was 28.1 years (SD ± 3.3 years) vs. term delivery 27.2 (SD ± 3.8 years), and the difference between these two groups was not statistically significant (p-value = 0.29). Among the study participants, 35 (57.4 %) were multigravida women who delivered preterm vs. 45 (50 %) in term. There were no significant differences in other delivery-related characteristics, such as mode of delivery and sex of the baby. However, in terms of neonatal characteristics such as NICU admission [21(34.4 %) in preterm vs. 69 (76.7 %) in term, p < 0.01] and birth weight [2.6 ± 0.4 in preterm vs. 2.9 ± 0.3 in term, p-value = 0.03], there was the difference in proportion and mean between both groups and this difference was statistically significant. The mean gestation age during the time of lab investigation was 29.6 weeks ±1.3 in the preterm labour vs. 30.1 weeks ±1.2 in term labour, and the mean difference was not statistically significant (p-value = 0.08). The details of Baseline and delivery-related characteristics of both groups are shown in Table 1.

A comparison of blood cell parameters between the two groups was depicted in Table 2, and the values are presented in mean±SD. There was a mean difference between both groups in terms of WBC count (white blood cell) [11.87±(3.7) in preterm vs. 10.71±(2.76) in term], platelet count [262.7±(59.1) in preterm vs. 234.5±(73.6) in term], Absolute lymphocyte count [194.0±(93.6) in preterm vs. 203.8±(63.3) in term], Neutrophil lymphocyte ratio (NLR) [6.3±(2.8) in preterm vs. 3.7±(0.8) in term] and platelet lymphocyte ratio (PLR) [165.1±(87.9) in preterm vs. 120.7±(39.1) term] and all these differences were found to be statistically significant (p-value <0.05*). However, regarding Neutrophil and Lymphocyte count, there was no significant difference in the mean between both groups.

The univariate analyses of associations between C-reactive protein and fetal outcome were shown in Table 3. Those who had CRP positive during pregnancy had a higher risk of undergoing preterm labour by 7.6 times (95 % CI: 2.1–28.3), and this association was found to be

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison of baseline characteristics between subjects delivered preterm and term baby.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age in years (mean±SD)</strong></td>
<td>Pre term (&lt;37 weeks), n = 61</td>
</tr>
<tr>
<td>28.10±3.26</td>
<td>27.18±3.77</td>
</tr>
<tr>
<td><strong>Gravidity (weeks), n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Primigravida</td>
<td>26(42.6 %)</td>
</tr>
<tr>
<td>Multigravida</td>
<td>35(57.4 %)</td>
</tr>
<tr>
<td><strong>Mode of delivery, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Normal vaginal delivery</td>
<td>29(47.5 %)</td>
</tr>
<tr>
<td>Emergency LSCS</td>
<td>12(19.7 %)</td>
</tr>
<tr>
<td>elective LSCS</td>
<td>20(32.8 %)</td>
</tr>
<tr>
<td><strong>Birth weight of the baby (mean±SD)</strong></td>
<td>2.63±0.48</td>
</tr>
<tr>
<td><strong>Sex of the baby, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25(41.0 %)</td>
</tr>
<tr>
<td>Female</td>
<td>36(59.0 %)</td>
</tr>
<tr>
<td><strong>NICU admission, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>21(34.4 %)</td>
</tr>
<tr>
<td>No</td>
<td>40(65.6 %)</td>
</tr>
<tr>
<td><strong>Gestational age of the mother during lab investigation (mean±SD)</strong></td>
<td>29.69±1.35</td>
</tr>
<tr>
<td><strong>Gestational age of mother at delivery (mean±SD)</strong></td>
<td>35.74±1.51</td>
</tr>
</tbody>
</table>

LSCS – Lower segment caesarean section; Z-test is used to determine the difference between two proportions and independent sample t-test is used to determine the difference between two group means; * p-value <0.05 is considered statistically significant.
considered statistically significant. The p-value is used to determine the association between categorical variables; *p-value <0.05 is considered statistically significant.

**Table 2**

<table>
<thead>
<tr>
<th>parameters</th>
<th>Pre term (mean ± SD)</th>
<th>Term (mean ± SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (x10³/mm³)</td>
<td>11.87 ± 3.70</td>
<td>10.71 ± 2.76</td>
<td>0.03*</td>
</tr>
<tr>
<td>Platelet (x10³/mm³)</td>
<td>262.75 ± 59.07</td>
<td>234.45 ± 73.61</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Neutrophil (10³/mm³)</td>
<td>8.10 ± 0.64</td>
<td>7.25 ± 0.59</td>
<td>0.68</td>
</tr>
<tr>
<td>Lymphocyte (10³/mm³)</td>
<td>1.46 ± 0.47</td>
<td>2.03 ± 0.43</td>
<td>0.18</td>
</tr>
<tr>
<td>Absolute lymphocyte count (x10³/mm³)</td>
<td>194.03 ± 93.67</td>
<td>203.81 ± 63.32</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>NLR</td>
<td>6.31 ± 2.89</td>
<td>3.72 ± 0.87</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>PLR</td>
<td>165.07 ± 87.96</td>
<td>120.74 ± 39.12</td>
<td>&lt;0.01*</td>
</tr>
</tbody>
</table>

Independent sample t-test is used to determine the difference between two group means; * p-value <0.05 is considered statistically significant.

**Table 3**

<table>
<thead>
<tr>
<th>CRP</th>
<th>Preterm n (%)</th>
<th>Term n (%)</th>
<th>$\chi^2$ value</th>
<th>cOR (95 % CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>13 (21.3 %)</td>
<td>3 (3.3 %)</td>
<td>12.12</td>
<td>6.69 (2.09-28.32)</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Negative</td>
<td>48 (78.7 %)</td>
<td>87(96.7 %)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CRP – C-reactive protein; cOR – Crude odd ratio; Chi-square test is used to determine the association between categorical variables; * p-value <0.05 is considered statistically significant.

statistically significant (p-value <0.01*)

Figs. 1 and 2 depict a scatter plot that shows the correlation between gestational age at delivery and blood cell parameters such as platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR). Both PLR (r = −0.326) and NLR (r = −0.405) are negatively correlated with gestational age at delivery. This correlation is statistically significant (P < 0.01*), which means that the relationship between these factors is not due to chance.

**4. Discussion**

Preterm births contribute to long-term morbidity and mortality. Many mechanisms have been linked with the pathophysiology of preterm delivery, like inflammation and infection, placental defects, hormonal, immunological, and genetic defects, and increased oxidative stress. Still, the exact pathophysiology remains challenging to understand. There is little evidence showing labour as an inflammatory process. So, this study aimed to establish the association between preterm birth and various inflammatory changes like NLR, PLR, and CRP.

This study included one hundred fifty-one pregnant women attending the regular antenatal clinic of a tertiary care hospital located in Chengalpattu. Out of 151, 61(40.4 %) had preterm labour (<37 weeks) and 90(59.6 %) had term labour (≥37 weeks). Among the study participants, 35 (57.4 %) were multigravida women who delivered preterm vs. 45(50 %) in term. Similarly, dudhrejia et al. did a study to determine factors associated with preterm births and stated that 58 % of preterm births were associated with multigravida.11 Regarding neonatal birth weight [2.6 ± 0.4 in preterm vs. 2.9 ± 0.3 in the term, value = 0.03], there was a difference in proportion and mean between both groups, which was statistically significant. This is expected as term babies stay longer inside the mother’s uterus and gain weight compared to preterm births.

In this study, there was a mean difference between both groups in terms of WBC count (white blood cell) [11.87(±3.7) in preterm vs. 10.71 (±2.76) in term], platelet count [262.7(±59.1) in preterm vs. 234.5 (±73.6) in term], and this difference was found to be statistically significant (p-value <0.05*). Campbell et al. also stated that the WBC count could be used to predict upcoming preterm births.12

Neutrophil lymphocyte ratio (NLR) [6.3(±2.8) in preterm vs. 3.7 (±0.8) in term] and platelet lymphocyte ratio (PLR) [165.1(±87.9) in preterm vs. 120.7(±39.1) term] and this difference was found to be statistically significant (p-value <0.05*). Results here suggest NLR and PLR can be used as predictors of preterm as the values are significantly high in the preterm group.

In accordance with our study, Tolunay et al. concluded that the neutrophil-to-lymphocyte ratio and white blood cell count profile could guide clinicians in predicting the time of birth in threatened preterm labour cases.13 Also, Toprak et al. found in their study that the PLR might be a cost-effective, easy-to-use, and practical marker for the early diagnosis of PPROM, which can help determine the appropriate delivery waiting time and provide maternal and fetal well-being.14

In the current literature, it is well established that one highly significant risk factor for PTB is infection and inflammation. One of the most simple, economic, and routine clinical tests during pregnancy is the complete blood count (CBC). CBC and its derived parameters, including
nflammatory diseases in recent years.\(^\text{18,19,37}\), have been recognized as inflammatory markers for low-grade inflammatory diseases than in the control group, which is consistent with our study. A key difference between our study and their study was the gestational age at assessment of CBC. Their blood routine examination time was after the onset of threatened PTB (31.1 ± 1.9 W in the preterm group. In our study, CBC was routinely obtained between 28 and 32 weeks. In this study, those who had CRP positive during pregnancy had a higher risk of undergoing preterm labour by 7.6 times (95 % CI: 2.1–28.3), and this association was found to be statistically significant (p-value <0.01\(^*\)). Similar to this study, an observational study was done by Sangam et al. to use the level of C-reactive protein as a predictor for preterm deliveries. They found that the mean value of CRP has a positive association with preterm delivery. They concluded by saying that the prediction of preterm delivery by a simple biomarker like CRP could help in early intervention and subsequent prevention of preterm birth and its sequelae.

Certain precautions can be taken in patients who are suspected to have preterm labour (i.e increased NLR, PLR) to reduce prematurity related perinatal complications by offering antenatal corticosteroid prophylaxis, magnesium sulphate for neuroprotection and to rule out all other possible source of infection, if present to treat them accordingly.

5. Limitation

However, this study has some limitations. Firstly, it did not extrapolate the diagnostic ability of inflammatory parameters as individual markers for determining preterm labor. Secondly, the data was collected from a single centre, and there was an absence of data beyond 32 weeks of gestation. Additionally the study exhibited low R2 values, which indicate that the model does not explain much of variability in preterm labour occurrences based on inflammatory parameters. Furthermore, a physiological increase in white blood cells (WBC)'s is normal during pregnancy, and since there is no established cutoff to distinguish between pathological and physiological values, this also poses a limitation.

6. Conclusion

This prospective observational study deduced the association between inflammatory markers and preterm delivery in a singleton pregnant woman. The rise in inflammatory parameters like neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, and C-reactive protein proved helpful in predicting spontaneous preterm labour (before 37 weeks of gestation), however future studies are required to assess the advantage of combining these economical markers with parameters like cervical length and fibronectin values for prediction of preterm labour which would be more sensitive and specific. Also, this relationship between inflammatory parameters and preterm delivery might be due to its spurious association. In the future, further analytical studies would be required to determine its diagnostic accuracy and adjust the variable for possible confounders to establish it as a diagnostic tool.

7. Ethics clearance

Ethical approval was provided by Institutional ethics committee (REF NO: 3056/IEC/2021).Prior to the commencement of the study, informed written consent was obtained from the respondents. Before doing so, the participants were provided with a clear understanding of the study’s objectives and their right to participate or decline without any discomfort.

Source of Funding

None.

CRediT authorship contribution statement

Dr. Mrinalini Kannan: Conception and Design of the study, Acquisition of data, Analysis and/or interpretation of data, Drafting the manuscript, Approval of the version of the manuscript to be published. Dr. Sajeetha Kumari R: Conception, and design of the study, Analysis and/or interpretation of data, Drafting the manuscript, Revising the manuscript critically for important intellectual content, Approval of the version of the manuscript to be published. Dr. Vinodhini: Drafting the manuscript, Revising the manuscript critically for important intellectual content, Approval of the version of the manuscript to be published.

Declaration of competing interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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