Original Research Article

TheRole of Neutrophil-LymphocyteRatio,Platelet-LymphocyteRatio,andABOBloodGroupsinEarly Pregnancy Loss

ABSTRACT

Background: Intrauterine pregnancy loss referred to natural pregnancy loss before the twentieth weeks of gestation. Women who show signs of stress are three times more likely to lose their pregnancy during the first three weeks. Blood group system has been linked with development of mental stress, anxiety or disease. Medical literature has also increasingly supported neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) as strong indicators of inflammation and oxidative stress.
Place and Duration of Study: Department of Haematology, Gynaecology andObstetrics unit both of Enugu State University of Science and Technology Teaching Hospital, between August and December 2023.
Methodology: A total of 100 participants were enrolled comprising individuals who had experienced between 1-2 miscarriages. Fifty healthy normal pregnant women who had no history of miscarriage were used as control. Demographic information such as age and number of miscarriages was obtained through questionnaire. Neutrophil, lymphocyte, monocyte, eosinophil, packed cell volume (PCV) and platelet (PLT) were analyzed by automated haematology analyzer, while ABO and Rhesus blood group were done by tile method. The data were analyzed using SPSS version 25. Analysis of variance (ANOVA), Chi-square test and pie chartwere used. P-Value < 0.05 was considered as statistically significant.
Results: Out of the 100 women, blood group A was 27, B was 24, AB was 1 and O was 48. Platelet count and NLR values were higher in miscarriage group compared to healthy normal pregnant women (p=0.039 and <0.001 respectively). Lymphocyte count were found to be lower in miscarriage group compared to healthy normal pregnant women (p=0.042). There was no significant relationship between NLR, PLR and ABO blood group (p=0.199).
Conclusion: Increased platelet count and NLR were observed in women who had early pregnancy loss. Measurement of low cost test like platelet count and NLR before twentieth weeks should be recommended as one of the ways to predict pregnancy leading to spontaneous abortion.

Keywords: ABO, NLR, pregnancy, loss, ratio, miscarriage, platelet

1. Introduction

“Miscarriage is the loss of a pregnancy before twentieth weeks. A spontaneous abortion or miscarriage is a process that can be grouped into 4 stages—threatened, inevitable, incomplete, and complete” [1]. “Miscarriage is an incident that usually leads to various maternal complications such as anemia, injuries to the uterus and endometrium, and infection. The combination of oxidative stress and defective placentation can cause increased serum ischemia-modified albumin levels, which in turn can contribute to the pathophysiology of early pregnancy loss” [2, 3].“Albumin effect alters under ischemic attacks linked with oxidative stress, generation of reactive oxygen species, and acidosis. Under these conditions, ischemia-modified albumin (IMA) produced has a lower metal-binding capacity, especially for transition metals, such as copper, nickel, and cobalt” [4, 5]

“In general, miscarriage rate is reported as 15-20%, meaning that 15-20% of recognized pregnancies result in miscarriage. Maternal age increases the occurrence of spontaneous miscarriage. Around 80% of miscarriages occur within the first trimester. And the rate of miscarriage reduces with increasing gestational age” [6]. “The immune response remodel leads to abnormal placental function, with ischemia of epithelial covering of the highly vascular embryonic placental villi and the presence of products are the crucial reasons for endothelial integrity damage. The incidence of endothelial dysfunction activates an inflammatory response within blood vessels that requires intravascular leukocytes, as well as the clotting and complement systems” [7]. “It is assumed that the increase in systemic inflammatory response markers like neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), mean platelet volume (MPV), and plateletcrit are associated to the pathology of missed abortion and preeclampsia. It can be assumed that these markers would have notable changes in spontaneous abortion” [8]. Several studies have been done on neutrophil-lymphocyte ratio and platelet-lymphocyte ratio focusing on pathologic conditions such as gestational diabetes [9], endometriosis [10] and gynecological cancers [11]. In addition, the ability of prognosticating early pregnancy loss and missed miscarriage of NLR, PLR, and MPV values has been studied [12]. Complete blood count (CBC), ABO and Rhesus blood group are simple, inexpensive, practical, and most sensitive test, are among the normal screening tests during pregnancy. Several studies had reported relationship between ABO blood group and spontaneous abortion [13, 14, and 15]. In 1972, study on ABO incompatibility as a cause of spontaneous abortion was done by Takno and Miller [16]. “Neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) can be extracted from the complete blood count test results” [17]. Bearing in mind the foremost importance of finding out the diagnostic and prognostic factors connected with miscarriage, the results of this study will be of great help in reducing the incidence of miscarriage. This study aims to determine the NLR and PLR in blood of women presenting miscarriage at first trimester.

2. Materials and Methods

2.1.1StudyArea

A case-control cross sectional study was conducted at the Enugu State University Teaching Hospital (ESUTH), gynaecology and obstetricsunit from August to December, 2023.

2.1.2 Methodology

One hundred women presenting miscarriage in their first trimester at gynaecology and obstetrics units in the age range of 16-45 years were recruited for the study. The approval for this study was given by the Research Ethics Committee of Enugu State University of Science and Technology Teaching Hospital (ESUTH) Enugu. Written informed consent was obtained from each woman. Those who were unable to sign inform consent form was excluded. Fifty ages matched pregnant women with no history of miscariage were recruited as controls.

2.1.3 Sample collection

Three milliliters of blood samples were collected via venepuncture. Complete blood count (CBC) was analyzed by automated haematology analyzer (Mindray/BC-5150). ABO and Rhesus blood group were done by tile method.

2.1.4Statistical analysis: This was done using the SPSS version 25; means of variables are reported as mean ± standard deviation. Test of significant difference between means of variables was determined using the student "T" test, one way ANOVA. Chi-square test of independence was used to determine association between ABO blood group and inflammatory response markers. A value of ≤ 0.05 was considered significant.

3. Results:

Table1 showed mean +/-SD ofsome haematological parameters. There were statistically significant increase in the values of platelet (249.93 +/- 142.23 x 10^9/l) and NLR (2.11 +/- 1.54) in the subjects that had experienced miscarriage, compared to control subjects (PLT 212.27+/-100.02 x 10^9/l, p = 0.039; MCHC 1.61+/-0.03, p = 0.012). The mean values of lymphocytes and PCV were significantly decreased in the subjects that had experienced miscarriage, (34.95+/-16.74 %; 28.30+/-5.18 %), compared to control subjects (39.18 +/-13.68 %, p = 0.042; 33.80+/-2.24 %, p = 0.001)

Table 1: Mean ± SD of some haematological parameters

Parameters	test (N=100)	control (N=50)	p-value
Neutrophils (%)	54.81 ± 18.11	51.82 ± 14.49	0.227
Lymphocytes (%)	34.95 ±16.74	39.18 ± 13.68	0.042*
Monocytes (%)	6.86 ± 2.25	5.82 ± 1.60	0.368
Eosinophils (%)	2.58 ±1.89	3.86 ± 2.44	0.182
PCV (%)	28.30 ± 5.18	33.80 ±2.24	<0.001*
Platelet count ($10^{9/L}$)	249.93 ± 142.23	212.27 ± 100.02	0.039*
NLR	2.11 ±1.54	1.61 ± 0.03	0.012*
PLR	107.52±85.39	92.79 ±41.39	0.125

Table.2 compared mean +/- SD of NLR of ABO blood group in women who had experienced loss of 1-2 pregnancies with pregnant women who had not experience pregnancy loss. There was statistically significant increase in the values of NLR of blood group B rhesus D positive subjects (1.96 +/-1.14), compared to control subjects (0.89 ± 0.35, p=0.001).

Table.2: mean +/- SD of NLR in ABO blood group

	A +ve	B +ve	O +ve
Test (N=94)	1.79 ± 1.22	1.96 ± 1.14	2.91 ± 1.61
control (47)	1.53 ± 0.82	0.89 ± 0.35	1.83 ± 1.10
p value	0.233	<0.001	0.183

Abbreviation: +ve = positive

Table 3: compared mean+/-SD of PLR of ABO blood group in women who had experienced loss of 1-2 pregnancies with women who had not experience pregnancy loss. The result showed no significant difference between test and control.

Table 3: mean+/-SD of PLR in ABO blood group

	A +ve	B +ve	O +ve

Test (N=94)	87.16 ± 45.96	82.82 ± 47.10	92.82 ± 47.13
control (47)	86.72 ± 50.98	92.89 ± 18..66	99.07 ± 34.92
p value	0.965	0.171	0.462

Table 4 compared mean+/-SD of NLR and PLR among A B O Rhesus D positive women that had experienced loss of 1-2 pregnancies. However, the result showed no statistically significant changes.

.Table 4: Mean+/- SD of NLR and PLR among A B O Rhesus D postive blood groups

	NLR	PLR
A +ve	1.795 ± 1.224	87.159 ± 45.939
B +ve	1.939 ± 1.139	82.818 ± 47.103
O +ve	2.190 ± 1.610	92.818 ± 47.127
F (p) value	0.958 (0.386)	0.507 (0.604)
A vs B	0.839	0.900
A vs C	0.410	0.836
B vs C	0.683	0.582

Table 5 showed relationship between NLR, PLR and ABO blood group of women who had experienced loss of 1-2 pregnancies using Chi-square test. The result showed no significant relationship between NLR, PLR and ABO blood groups (p = 0.199)

Table.5: Relationship between NLR, PLR and ABO blood group using Chi-square test

	Value	df	Asymp. Sig (2-sided)
Pearson Chi-square	6.000 [a]	4	0.199
Likelihood Ratio	6.592	4	0.159
Linear-by-Linear association	0.859	1	0.354
N of valid cases	3		

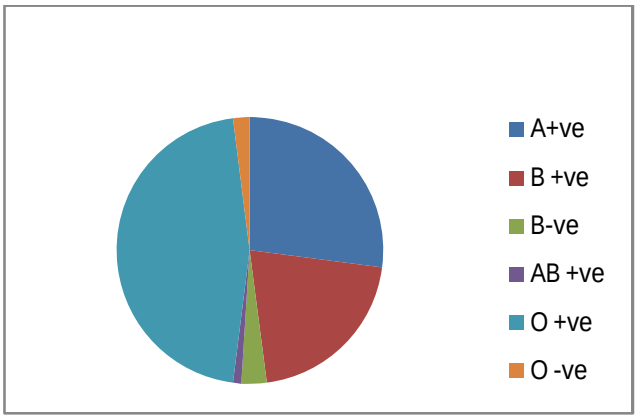

Fig 1: Blood group distribution of women with miscarriage

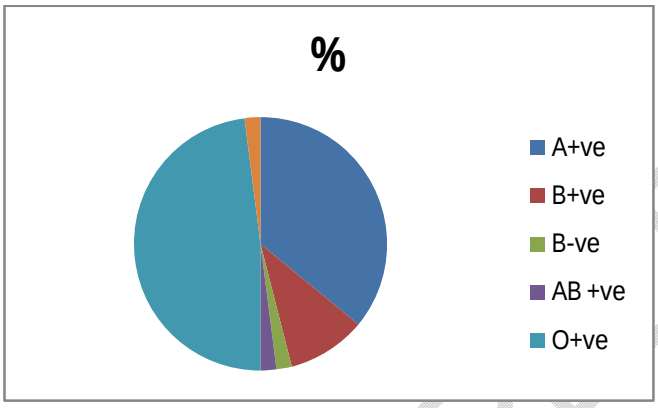

Fig 2: Blood group distribution of normal pregnant women

Discussion

Neutrophil-to-lymphocyte ratio is simply calculated from the differential cell count. It is immediately available upon admission in all the patients. It is also a reflection of physiologic stress [18]. Study had shown that in both early pregnancy failures and preeclampsia, that inflammatory markers and oxidative biomarkers are significantly high in maternal blood. The high level of markers is investigated in preeclampsia, but there are still limited studies investigating them in the early pregnancy loss [19]. In this study, NLR significantly increased in women with miscarriage compared to normal pregnancies. The finding is consistent with the results of another study, which reported increased NLR in miscarriage compared to normal pregnancy [20]. Increased platelet counts were observed in women with miscarriage compared to women with normal pregnancy. The finding is consistent with recent report on risk of early foetal demise [21]. However, PLR in this study showed no significant changes. The finding in this study is in line with previous study done in a cohort of 120 lupus patients with and without concurrent infections, where NLR had high sensitivity (75%) and specificity (90%) for predicting infections, whereas individual blood cell counts and PLR were less informative [22,23,24].

Blood group antigens are markers on surface-uncovered red cell proteins. Blood groups study also showed that an incompatibility of the blood groups can have an effect on reproduction. Miscarriage could occur in couples with incompatible blood groups [25]. In this study blood group 'O' was the dominant group occurring in 48% of the women with miscarriage, followed by groups A (27%) and group B (24%).

Only 1% of the women with miscarriage were of blood group 'AB'. Although the distribution of ABO blood groups differ from one population to the other, in most studies blood group 'O' has been reported to be the upper most group and blood group 'AB' as the lowest which is in line with this study and previous studies in Enugu State and Nigeria in general [26, 27]. The ABO prevalence in Nigeria differs slightly in different regions, but blood group 'O' has been reported as the most dominant in all reported areas of studies. In this study, blood group 'O' was found to be the most dominant blood group among the normal pregnant women without history of miscarriage. This observation is consistent with previous studies in Nigeria [28, 29]. In this study NLR was found to be significantly increased in blood group B subjects with miscarriage compared to blood group B women with normal pregnancy. The finding is consistent with previous study on ABO blood groups [30]. Many studies have reported NLR as inflammatory indicator and were closely linked to the prognosis of various diseases [31]. As a marker of inflammation, NLR has been found to be high in various diseases and complications that affect pregnancy outcome [32]. Pregnancy complications, such as gestational diabetes and preeclampsia have been associated with elevated NLR, but little is known on the direct relationship with different blood groups.

Conclusion

In conclusion, increased platelet count and NLR were observed in women who had early pregnancy loss. Measurement of low cost test including platelet count and NLR before twentieth weeks should be recommended as one of the ways to predict pregnancy leading to spontaneous abortion.

Conflict of interests

Author declared that no competing interests exist

Ethical Approval:

As per international standards or university standards written ethical approval has been collected and preserved by the author(s).

Consent

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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