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Platelets and White Blood Corpuscles' Indices for the Prediction of Premature Rupture of Membranes

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ABSTRACT

Introduction and aim: Premature rupture of membranes (PROM) is a common condition that is usually associated with adverse maternal and fetal outcome. Early identification is crucial to institute proper management strategies. The current study aimed to evaluate the role of platelets and white blood cells (lymphocytes and neutrophil) in prediction of PROM

Methodology: The study included 100 pregnant females. All were subjects to full clinical evaluation. Then vaginal examinations were completed to inspect for the active flowing of amniotic fluid from the cervix. A transabdominal ultrasound was done to confirm gestational age and estimate amount of amniotic fluid and turbidity. Complete blood cell count and differential count of leucocytes was performed and specific ratios were calculated.

Results: We have two groups, the study (PPROM) and females without PROM (control group). White blood cell, platelets, and neutrophils were significantly increased, while lymphocytes and monocytes were significantly decreased in the study than the control group. Mean platelet volume (MPV) and amniotic fluid index (AFI) were significantly reduced in the study than the control group (8.37±0.49, 4.39±0.65 vs. 10.04±0.68 and 11.20±1.18 respectively). However, platelet lymphocyte and neutrophil lymphocyte ratio were significantly increased in the study than control group (139.88±9.34, 5.58±0.70 vs. 127.50±4.70 and 4.06±0.54 respectively). The predictors of PPROM were AFI (η=0.619) followed by mean platelet volume (η=0.254) and finally NLR (η=0.075).

Conclusion: Blood cell count indices are useful indicators in screening for possible development of PPROM. In addition, MPV and NLR as useful predictors of PPROM. The availability, simplicity and low cost of such tests increased its value as a potential indicators for development of PPROM.

Keywords: **Premature Rupture of Membranes; Mean Platelet Volume: Total Leucocyte Count; Neutrophil to Lymphocyte Ratio; Prediction.**

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INTRODUCTION

Preterm premature rupture of membranes (PPROM) is a common clinical condition. It usually affects about 3% of all pregnancies. It is defined as a spontaneous rupture of membranes before the beginning of labour before 37 weeks of gestation [1]. It is the commonest cause of premature delivery, and it's linked to maternal and/or fetal infections. Chorioamnionitis developed in 6-10% of cases of PPROM, which increased to 40% if the condition lasts > 24 hours [2]. Females with chorioamnionitis have a two-fold increased risk of neonatal infection that increased with the presence of PPROM. Neonatal jaundice and hypoxia are also increased in association with PPROM [3]. The specific patho-physiologic mechanism (s) of PPROM is (are) not well known. It seems to be a complex and multifactorial in nature. Inflammation has a key role in the rupture of membranes [4].

In daily clinical practice, a complete blood count is a routine tests used to check and follow up different disease conditions. It had the advantages of being a simple, inexpensive, and readily available test. Previous studies linked the increased platelet counts to different conditions (e.g., infection, inflammation, and cancer) [5]. In addition, markers originated from complete blood count could be helpful in the diagnosis or prediction of severe chronic inflammatory conditions. This lies on the severe apoptosis and accelerated proliferation in precursor megakaryocytes [6].

The platelet-to-lymphocyte ratio (PLR) is a simple, readily available, cheap and accurate indicator. It's been suggested as a predictive and prognostic biomarker for different conditions (e.g., cardiovascular diseases and cancers including gynecologic cancers) [7-9]. It has been also linked to gestation-associated clinical conditions (e.g., gestational diabetes, recurrent pregnancy loss, preeclampsia and preterm labor) [10]. Another unique indicator is the neutrophil-to-lymphocyte ratio (NLR). It was recognized as an inflammatory biomarker, which associated with bad outcome in a variety of pathologic conditions (e.g., preeclampsia) [11].

Considering the simplicity, cost and availability of resources to do complete blood and differential cell count is advantageous to use indicators originated of such analysis in diagnosis, prognosis and prediction of disease conditions. However, the role of such indicator in did not studied well in premature rupture of membranes.

THE AIM OF THE WORK

The aim of this study was the evaluation of platelets and white blood cells (lymphocytes and neutrophil) in prediction of premature rupture of membrane (PROM).

PATIENTS AND METHODS

This Prospective study included 100 pregnant women, selected from the Department of Obstetrics and Gynecology, Al-Azhar University Hospital (New Damietta). We included females in their reproductive age, with singleton pregnancy and gestational age at 24- 37 weeks of gestation. On the other side, exclusion criteria were hematologic disorders, malignancies, hepatic disease; any acute or chronic infectious or inflammatory diseases; pregnancies with fetal chromosomal anomalies, intrauterine growth restriction, or any fetal infection; women who underwent any invasive procedures such as amniocentesis; and pregnant women with urinary tract infection and genital infection.

All females were subjected to full medical history taking (e.g., maternal age, gravidity, parity, maternal weight, last menstrual period (LMP), as well as presence of any disease). All patients were asked for risk factors and any fluid leakage before 37 weeks' gestation and regular uterine contractions. In addition, all underwent abdominal examination to assess fundal level and gestational age. The vaginal examination by sterile Cusco's speculum was performed to verify the active flowing of amniotic fluid from the cervix, under complete aseptic condition. All females submitted to trans-abdominal ultrasound to assess gestational age by fetal biometry, amount of amniotic fluid and turbidity

A venous sample, 10 ml was drawn from antecubital vein under complete aseptic condition for complete blood count to determine platelets count and white blood cells (count and types). The reference platelet range is 150,000 to 400,000 per ml. The normal number of WBCs in the blood is 4,500 to 11,000 WBCs per microliter (4.5 to 11.0 × 10^9/L). A differential blood count gives the relative percentage of each type of white blood cell and also helps to reveal abnormal white blood cell populations (eg, blasts, immature granulocytes, and circulating lymphoma cells in the peripheral blood). The test was performed by SYSMEX XN-3000 automatic five classifications hematology analyzer (SYSMEX, Kobe, Japan). Reference ranges for differential white blood cell counts are as follows: Neutrophils 2500-8000 per mm^3 (55-70%); Lymphocytes 1000-4000 per mm^3 (20–40%); Monocytes 100-700 per mm^3 (2–8%); Eosinophils 50-500 per mm^3 (1–4%); Basophils 25-100 per mm^3 (0.5-1%) [12].

Ethical consideration:

The study protocol had been provided and accepted by the research and ethics institutional review board, Damietta faculty of Medicine, Al-Azhar University. All participating females singed an informed consent. Data were anonymized before distribution and only used for the porpoise of research. Full rights of all females were assured and no harm was provided for participating females. All data used in statistical analysis are available on request.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp, USA). Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation. Significance of the obtained results was judged at the 5% level. The Chi square test, Mann-Whitney and student "t" test were used for comparison when appropriate.

RESULTS

This Prospective study include 100 pregnant women (A convenient sample). They were selected from the Department of Obstetrics and Gynecology, Al-Azhar University Hospital (Damietta). They were divided into two equal groups. The first (study group) included patients with PROM and the second group for normal females (without PROM) as a control group. The maternal age ranged between 19 and 33 years, while body mass index (BMI) ranged between 25 and 32 kg/m2. There was no significant difference between study and control groups. In addition, no significant difference was registered between study and control groups regarding parity or gestational age. In addition, comorbidities were increased in study than control group (30.0% vs 20.0%). However, the difference was non-significant. The commonest in both groups were hypertension and diabetes mellitus (Table 1).

In the current work, the white blood cell count was significantly increase in the study than control group (10.41±1.23 Vs. 7.72±1.00 x 10^3, respectively). However, platelet count was significantly increased in the study than the control group (267.96 ± 14.45 vs 249.40 ± 8.90 x 10^3 respectively), although both groups were in the normal range of platelet count (the range extended between 234 and 287). On the other side, no significant difference was observed regarding hemoglobin, hematocrit or red cell distribution width (Table 2).

In the current work, neutrophils showed statistically significant increase, while lymphocytes and monocytes revealed significant decrease in the study than control group (Table 3).

In the current work, there was significant decrease in mean platelet volume and significant increase of PLR, NLR and amniotic fluid index in the study than control group (Table 4).

Regarding sensitivity of different biomarkers, the most sensitive for diagnosis of PROM was AFI followed by mean platelet volume (100.0%). WBCs, platelets, neutrophils, PLR and NLR had the same sensitivity (86.0%). However, the most specific biomarkers were WBCs, PLR and AFI (100.0%), followed by NLR and lymphocytes (90.0%) (Table 5).

Running single univariate regression analysis revealed that, lymphocytes, MPV NLR and AFT are the predictors of PROM development. However, with multiple regression analysis, the only predictors were AFI, MPV and NLR (Table 6).

Table (1): Comparison between the two studied groups according to demographic data

		Study (n = 50)	Control (n = 50)	Test	p
Maternal age (years)	Min. – Max.	19.0 – 33.0	19.0 – 33.0	0.169	0.866
	Mean ± SD.	25.84 ± 4.23	25.70 ± 4.07		
BMI (kg/m²)	Min. – Max.	26.0 – 30.0	25.0 – 32.0	0.240	0.811
	Mean ± SD.	27.88 ± 1.38	27.80 ± 1.91		
Parity	Null para	2 4.0	5 10.0	1.860	0.401
	Primary para	25 50.0	20 40.0		
	Multi para	23 46.0	25 50.0		
	Min. – Max.	0.0 – 3.0	0.0 – 2.0	1170.0	0.547
	Mean ± SD.	1.56 ± 0.79	1.40 ± 0.67		
	Median (IQR)	1.0 (1.0 – 2.0)	1.50 (1.0 – 2.0)		
GA (weeks)	Min. – Max.	26.0 – 36.0	26.0 – 36.0	0.971	0.334
	Mean ± SD.	32.02 ± 3.09	31.40 ± 3.30		
Comorbidities	No	35 (70.0%)	40(80.0%)	1.33	0.25
	Yes	15 (30.0%	10(20.0%)		
Distribution of Comorbidities	DM	5(10.0%)	3(6.0%)	1.50	0.82
	HTN	7(14.0%)	5(10.0%)		
	Renal	2(4.0%)	1(2.0%)		
	SLE	1(2.0%)	1(2.0%)		

BMI: Body mass Index, GA: parity, IQR: Inter quartile range; SD: Standard deviation; DM: diabetes mellitus, HTN: hypertension; SLE: Systemic lupus erythematosus.

Table (2): Comparison between the two studied groups according to complete blood count

		Study (n = 50)	Control (n = 50)	t	p
WBC count(10^3/mm^3)	Min.–Max.	8.0 –12.5	5.678 –8.9	***11.934***	***<0.001****
	Mean±SD.	10.41±1.23	7.72±1.00		
Hemoglobin (g/dl)	Min.–Max.	9.0 – 12.0	9.0 – 12.0	0.702	0.485
	Mean±SD.	10.83 ± 0.83	10.71 ± 0.94		
HCT%	Min.–Max.	30.40 – 35.40	30.40 – 35.40	1.309	0.193
	Mean±SD.	32.38 ± 1.57	32.79 ± 1.54		
RDW (µm)	Min.–Max.	12.50 – 14.20	12.60 – 14.20	0.555	0.580
	Mean±SD.	13.33 ± 0.60	13.39 ± 0.55		
Platelets (10^3/mm^3)	Min.–Max.	245.0 – 287.0	234.0 – 265.0	***7.733***	***<0.001****
	Mean±SD.	267.96 ± 14.45	249.40 ± 8.90		

WBC: White blood cells; HCT: Hematocrit; RDW: Red cell distribution width.

Table (3): Comparison between the two studied groups regarding neutrophil, lymphocyte and monocyte count

		Study (n = 50)	Control (n = 50)	t	p
Neutrophils(10^3/mm^3)	Min. – Max.	6.50 – 8.90	5.60 – 7.50	9.381*	<0.001*
	Mean ± SD.	7.77 ± 0.87	6.35 ± 0.62		
Lymphocytes(10^3/mm^3)	Min. – Max.	1.30 – 1.80	1.67 – 1.90	8.866*	<0.001*
	Mean ± SD.	1.53 ± 0.18	1.78 ± 0.08		
Monocytes(10^3/mm^3)	Min. – Max.	0.45 – 0.65	0.50 – 0.65	2.721*	0.008*
	Mean ± SD.	0.55 ± 0.07	0.58 ± 0.05		

Table (4): Comparison between the two studied groups regarding mean platelet volume, platelet lymphocyte ration, neutrophil lymphocyte ration and amniotic fluid index

		Group I (n = 50)	Group II (n = 50)	t	p
MPV/fl	Min. – Max.	7.80 – 9.0	8.90 – 10.90	14.126	<0.001*
	Mean ± SD.	8.37 ± 0.49	10.04 ± 0.68		
PLR	Min. – Max.	124.0 – 154.50	121.0 – 134.0	8.372	<0.001*
	Mean ± SD.	139.88 ± 9.34	127.50 ± 4.70		
NLR	Min. – Max.	4.50 – 6.70	3.40 – 5.0	12.175	<0.001*
	Mean ± SD.	5.58 ± 0.70	4.06 ± 0.54		
AFI	Min. – Max.	3.40 – 6.0	9.0 – 13.0	35.789	<0.001*
	Mean ± SD.	4.39 ± 0.65	11.20 ± 1.18		

MPV: mean platelet volume; PLR: platelet lymphocyte ratio; NLR: Neutrophil lymphocyte ration; AFI: Amniotic fluid index

Table (5): Area under the curve and cutoff values of significant parameter

	AUC	St. Error	95% CI	p	Cutoff	Sensitivity	Specificity
WBCs	0.95	0.020	0.91-0.99	<0.001*	> 9350	86.0%	**100.0%**
Platelets	0.86	0.036	0.79-0.93	<0.001*	>255.5	86.0%	70.0%
Neutrophils	0.89	0.030	0.83-0.95	<0.001*	>6.75	86.0%	70.0%
Lymphocytes	0.85	0.038	0.77-0.92	<0.001*	<1.685	64.0%	**90.0%**
Monocytes	0.60	0.057	0.49-0.71	**0.085**	<0.58	78.0%	40.0%
MPV	0.97	0.012	0.95-0.996	<0.001*	<9.40	**100.0%**	**80.0%**
PLR	0.91	0.031	0.85-0.97	<0.001*	>133	86.0%	**100.0%**
NLR	0.96	0.017	0.92-0.99	<0.001*	>4.7	86.0%	**90.0%**
AFI	1.0	0.00	1.00-1.00	<0.001*	<7.5	**100.0%**	**100.0%**

WBCs: White blood cells; MPV: mean platelet volume; PLR: platelet lymphocyte ratio; NLR: Neutrophil lymphocyte ration; AFI: Amniotic fluid index; AUC: area under the curve; CI: Confidence interval

Table (6): Single and multiple regression analysis to detect predictability of different biomarkers for PROM

	Single regression analysis				Multiple regression analysis					
Source	SoS	M.Sq.	F	p	SoS	M.Sq.	F	Sig.	η	Power
WBCs	0.002	0.002	0.19	0.658	0.002	0.002	0.198	0.658	0.002	0.072
Neutrophil	0.004	0.004	0.36	0.546	0.004	0.004	0.368	0.546	0.004	0.092
Lymphocytes	0.105	0.105	**9.27**	**0.003***	0.105	0.105	9.273	0.003	0.092	0.854
Platelets	0.003	0.003	0.26	0.608	0.003	0.003	0.265	0.608	0.003	0.080
MPV	0.352	0.352	**31.03**	**<0.001***	0.352	0.352	**31.030**	**<0.001***	0.254	1.000
PLR	0.004	0.005	0.004	0.949	0.004	0.004	0.004	0.949	0.000	0.050
NLR	0.084	0.084	**7.37**	**0.008***	0.084	0.084	**7.376**	**0.008**	0.075	0.766
AFI	1.675	1.675	**147.64**	**<0.001***	1.675	1.675	**147.641**	**<0.001***	0.619	1.000

SoS: Type III sum of squares; M.Sq.=mean square, F: Analysis of variance; η: partial Etta

DISCUSSION

Blood cell count is a routine test for the hospitalized patients. It includes about 24 important indicators, mainly white blood cell and its differential count, red blood cell count, hemoglobin concentration, hematocrit and red blood cell indices, platelet count, and platelet indices. Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR). Different blood cell count indicator has been shown to be useful in the early prediction of cancer, thrombus and diabetes [13].

In the current study we aimed to evaluate platelets and white blood cells (lymphocytes and neutrophils) in prediction of premature rupture of membrane (PROM). We did not find significant difference between study and control groups regard maternal demographics.

Ibrahim and Farag [14] reported similar results, except significant reduction of gestational age at delivery in the study than control group, which is a logic finding in PPROM. Zhan *et al*. [13] conducted a study on 70 females with PROM and 100 controls and reported significant increase of maternal age in study than control females. The older the patient, the more likely they are to develop PROM. Different sample size, inclusion and exclusion criteria could explain such contradiction. However, Ozel *et al*. [15] found that, PPROM had a higher mean maternal age than control females (p<.005). However, they reported no differences in mean gravidity, parity, or gestational age across the groups, as in the current study.

Results of the current work revealed significant increase in total leucocytic, platelet and neutrophil count in the study than control groups. Additionally, PLR and NLR were significantly increased in the study than the control group. On the other side, lymphocytes, monocytes, MPV and AFI were significantly reduced in the study than control group.

Ibrahim and Farag [14] reported significant increase of platelet count and significant reduction of MPV during 12 to 14 weeks of gestation onwards among the study than control groups. The importance of their findings are the registration of these significant variation at 8 to 20 weeks before the onset of PPROM. Thus, prophylactic measures could be applied is they are a reliable indicators (predictors) of PPROM. These results are supported by Ekin *et al*. [5] who reported significant increase of platelet count and significant decrease of MPV in the first trimester in PROM than control females. Tzur *et al*. [16] also examined the relationships between maternal leukocyte count in the first trimester of pregnancy and the risk of obstetric problems. They discovered a link between PPROM and leukocytosis throughout the first trimester. According to these studies, there is a clear association between the presence of leukocytosis and the occurrence of PPROM. Isik *et al*. [17] examined whether platelet indices are of value for predicting preterm labor and reported that, platelet indices are significantly changed in preterm deliveries**.**

In accordance with the current findings, Ozel et al. [15] found a significant increase of neutrophil and lymphocyte count in the PPROM than healthy control groups. Zhan *et al*. [13] also reported significant increase of neutrophils, and NLR in the PPROM group. However, and in contradiction to the results of the current work, Ozel *et al*. [15] reported non-significant difference between study and control groups regarding PLR. Our findings were in line with a prospective case-control research involving 121 pregnant women with PPROM and 96 age-matched pregnant women with spontaneous preterm labor. Toprak et al. [18] discovered that the PPROM group had higher NLR levels.

According to Jung *et al*. [19], a high NLR showed a significant correlation with the occurrence of spontaneous preterm labor at less than 32 weeks of pregnancy in their retrospective cohort study, which included patients at 18–24 weeks of pregnancy who underwent amniocentesis before receiving emergency cerclage for cervical insufficiency. In a recent study, Hughes et al. [20] criticized the value of ultrasound-obtained amniotic fluid index for determination of abnormal conditions (oligohydramnios or polyhydramnios) and reported that, AFI is sensitive for diagnosis of both conditions (sensitivity more than 90%). However, it was superior for identification of polyhydramnios. The serial determination of AFI is critical for assessment of at-risk gestations, including PPROM-susceptible gestations. However, due to controversy on the AFI values discriminating low from normal and high volumes, its use as a predictor for different gestational conditions is limited. The current work revealed that is the most sensitive and specific biomarker, although it is out of the scope of the study and it was used as a routine screening test. Mousavi *et al*. [21] reported that, PPROM is associated with lower AFI (<5.0). However, Günay *et al*. (22) could not identify such association (PPROM and low AFI), although they linked unfavorable outcome with lower AFI.

The current study results revealed that, AFI, MPV and NLR are the only predictors for PPROM, when all studied variables were considered (multiple regression analysis). Taking into consideration the continued controversy on the role of AFI as a predictor, the value of MPV and NLR must be considered in such condition. Ozel *et al*. [15] demonstrated that only NLR was higher in the PPROM group and could predict onset of neonatal sepsis. They suggested its use as a predictor for adverse neonatal outcome in females developed PPROM.

In short, the current study confirmed the association between blood cell count parameters and development of PPROM. Additionally, and as one of the earliest studies it pointed to the possible use of MPV and NLR as a predictors for PPROM development. However, the results must be considered cautiously due to small sample size and must be validated in future large scale studies. The importance of the current work relies on the fact that, it opened our eyes on the value of such simple indicators to predict or event to screen for potential development of a serious condition like PPROM. It will permit early anticipation and proper intervention to prevent the development or to decrease the harmful effects of PPROM.

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None to be declared.

Author contributions

All authors contributed equally

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