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Study of Platelet Volume and Platelet Count Changes during Pregnancy as a Marker for Prediction of Preterm Premature Rupture of Membrane

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Abstract

Aim: To investigate the level of platelet volume and number precede Preterm Premature Rupture of Membrane (PPROM) development and to determine the predictive value of these markers for prediction of PPROM.

Methods: A total of 979 pregnant women who received regular antenatal care until delivery were included. Participants were divided into 2 groups: Pregnant women with PPROM (n=140), and women without PPROM (n=839). Blood samples were collected during antenatal visits and/or during the period of inpatient hospital stay and changes in Mean Platelet Volume (MPV) and Platelet Count (PC) were compared between the two groups.

Results: Compared with controls, women with PPROM had significantly increased levels of PC and significantly decreased levels of MPV in the first trimester (p<0.001). The area under the receiver operator curve was 0.9 for MPV and 0.67 for PC. The cut-off values of MPV ≤7.9fL and PC ≥270 X 10^3/µl predicted PPROM with a sensitivity of 69% and 70% and specificity of 58% and 50%, respectively.

Conclusion: MPV can be used as a more efficient predictor for an early diagnosis of PPROM in the first trimester between 12 and 14 weeks' gestation than PC. However, further research combining other markers is needed to increase the efficiency of prediction.

Key Words: *Blood platelet count – Mean platelet volume – Preterm premature rupture of the membranes – Serum markers.*

Introduction

PREMATURE rupture of the membranes (PROM) is rupture of fetal membranes before onset of labor. If it occurs before 37 weeks' gestation, it is called preterm premature rupture of membrane (PPROM). It occurs in approximately 2-3% of all pregnancies and is the leading identifiable cause of preterm delivery [1]. Some of the possible causes of PPROM are an intra-amniotic infection, smoking, multiple pregnancies, nutritional deficiencies, antepartum hemorrhage, polyhydramnios and cervical incompetence [2,3]. Chronic infection of fetal membranes has a clear role in the initiation and propagation of molecular events leading to PPROM [4].

Previous studies have identified that intrauterine infection triggers an increase of several cytokines in maternal serum as well as amniotic fluid [5-7]. As inflammatory markers are in close relation with cytokines, we hypothesize that PPROM may be associated with alteration of these markers in maternal serum in the first trimester. Both C-Reactive Protein (CRP) and leukocyte levels were shown to be increased in serum of patients preceding PPROM [8,9]. Platelet Count (PC) and Mean Platelet Volume (MPV) constitute part of the data detectable by Complete Blood Count (CBC) test. The applicability of these indices for the clinical and pathophysiological understanding of several diseases, as in PPROM has not been investigated. Platelet activation has been noticed in the pathophysiology of infection, inflammation, and malignancy [10]. The MPV is a reliable indicator of platelet size that reflects platelet function and activation. Previous studies reported the association of MPV with both pro-thrombosis and pro-inflammation [10]. The aim of this observational study was to investigate the diagnostic performance of platelet volume and number changes during pregnancy for prediction of PPROM.

Subjects and Methods

Study design:

This was an observational longitudinal study of women Attending Antenatal Clinic (ANC) and/or admitted to the maternity ward at our hospital during period from June 2016 to June 2017. The

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study protocol was approved by Zagazig University Ethical Committee. A clear explanation of the study protocol was given for participating women, and a written informed consent was then obtained from all of them.

Inclusion criteria: Healthy pregnant women at ≥6 weeks' gestation with a live singleton fetus were eligible for recruitment.

Exclusion criteria: Women with the following conditions were ineligible and excluded: Multiple pregnancy, morbid obesity with Body Mass Index (BMI) ≥40Kg/m^2, poor past obstetric history (PTL (Pre-Term Labor), IUGR (Intrauterine Growth Restriction) [estimated fetal weight <1 0 th percentile for gestational age], invasive prenatal diagnostic procedures, cervical surgery, prior preterm labor, PPROM or currently suffering from a systemic disease (hypertension, gestational or insulin-dependent diabetes, heart disease, renal or hepatic dysfunction).

Study protocol:

During the enrolment of ANC visit at ≥6 weeks' gestation, past, medical, surgical and obstetric history was reviewed. General and abdominal examinations were carried out, fetal heart rate was checked and findings were recorded. Routine investigations including CBC were arranged. In women with prior regular periods, gestational age was estimated from the date of last menstrual period. Otherwise, gestational age was estimated from early ultrasound scan (USS) at 7 weeks' gestation. Gestational age was verified by the 13 th week USS. Fetal anomaly USS was arranged at around 20 weeks' gestation. Women were then reviewed as per the routine antenatal clinic protocol where they attended every 4 weeks until 28 weeks' gestation, every 2 weeks until 36 weeks' gestation then weekly thereafter until delivery. Those who developed PPROM were admitted, a blood sample for CBC was taken at each clinic visit, PPROM diagnosis is confirmed by the presence of pooling of amniotic fluid in the vagina by sterile speculum examination. Using EDTA samples tubes (5ml); venous blood samples were drawn from all participants. Samples were processed within two hours after venipuncture. Automated blood counter Cell-Dyn 4000 (Abbott Diagnostics, Santa Clara, CA, USA) was used after calibration to assess CBC parameters including platelet indices (PC and MPV). The primary outcome was to determine the correlation if any, between changes in selected platelet indices during pregnancy on one hand and occurrence of PPROM on the other hand.

Ethical approval: Informed consent was taken from each patient. The research protocol was approved by the Ethical Committee of Zagazig University Hospitals.

Statistical analysis:

The data are presented as the mean ± Standard Deviation (SD) and were processed and analysed using SPSS version 20 (Statistics for Windows, IBM Corp, Armonk, NY, USA). We determined the statistical significance between the two groups using one-way ANOVA (analysis of variance) supplemented with the post hoc LSD test. We compared paired quantitative continuous data using a paired *t*-test. Receiver Operating Characteristic (ROC) curve analysis was used to reveal the cut-off values, Areas Under the Curve (AUCs) and 95% Confidence Intervals (CIs) for PC and MPV. A p-value of ≤0.05 was deemed to indicate statistical significance.

Results

We summarized the demographic and baseline characteristics for all participants in (Table 1). Both groups were parallel in all features registered except for gestational age at delivery, which is expected finding in PPROM.

Table (2) showed comparisons between changes in PC, MPV and in both study groups. With the exception of few patients in the PPROM group, participants in all groups had a normal PC, but showed a continuous increase throughout pregnancy starting from 12-14 weeks' gestation onwards. Compared with the baseline values, MPV in PPROM group showed a continuous decrease throughout pregnancy starting from 12-14 weeks' gestation onwards. These changes preceded the occurrence of PPROM by 8-20 weeks despite significantly higher MPV and lower PC in the first trimester than the control group (p<0.001).

ROC analysis revealed an AUC of 0.09 (95% CI 0.12-0.05, p<0.001) for MPV and an AUC of 0.67 (95%CI 0.6-0.7, p<0.001) for PC. The best cut-off value with optimal sensitivity and specificity for MPV was ≤7.9fL. The application of this cut-off value predicted PPROM with a sensitivity of 69%, a specificity of 58%, a Positive Predictive Value (PPV) of 62.5%, a Negative Predictive Value (NPV) of 65.6%, and an accuracy of 64%. The best cut-off value with optimal sensitivity and specificity for PC was ≥270 X 10^3 / µL. The application of this cut-off value predicted PPROM with a sensitivity of 70, a specificity of 50%, a PPV of 64.9%, a NPV of 67.4%, and an accuracy of 66% (Table 3).

Table (1): Socio-demographic characteristics of both groups.

Variable	Group I PPROM (n=140)	Group II without PPROM (n=839)	*t*-test	*p*
Maternal age (years):				
Mean ± SD	26.1±4.3	26.4±4.2	0.3	0.7
Range	20-34	19-34		
GA at delivery (weeks):				
Mean ± SD	28.4±0.7	38.4±0.7	0.3	0.001*
Range	17-35	37-39		
BMI:				
Mean ± SD	26.2±2.5	25.9±2.2	0.4	0.7
Range	20.6-30	21-30		
History of previous				
Abortions:				
Mean ± SD	43.1±9.4	38.4±7.5	0.4	0.7
Range	25-60	25-60		
Gravidity:				
Mean ± SD	2.5±1.1	2.4±1.2	0.2	0.8
Range	1-5	1-5		
Parity:				
Mean ± SD	1.3±0.7	1.2±0.5	0.6	0.5
Range	0-2	0-3		

PPROM : Preterm Premature Rupture of Membrane.
SD : Standard Deviation.
BMI : Body Mass Index.
GA : Gestational Age.
* : Statistically significant difference ($p \leq 0.05$).
** : Statistically high significant difference ($p \leq 0.001$).

Table (2): PC and MPV in both studied groups.

Variable	Gestational age (weeks)	Group I PPROM (n=140)	Group II without PPROM (n=839)	*t*-test	*p*
• PC ($X 10^3/mm^3$)	• 12-14 (onset of change)	262±55	240.6±43	7.8	0.001
	• 24-35 (onset of diagnosis)	270±65	245.2±45	4.9	0.001
• MPV (fL)	• 12-14 (onset of change)	9.9±1.7	8.7±1.5	5.3	0.001
	• 24-35 (onset of diagnosis)	8.8±1.3	10.2±1.1	9.5	0.001

Data are represented as mean ± SD.
PPROM : Preterm Premature Rupture of Membrane.
PC : Platelet Count.
MPV : Mean Platelet Volume.
SD : Standard Deviation.

Table (3): Diagnostic performance of PC and MPV for prediction of PPROM.

	PC (X 103/mm3)	MPV (fL)
Accuracy (%)	66	64
Sensitivity (%)	70	69
Specificity (%)	50	58
PPV (%)	64.9	62.5
NPV (%)	67.4	65.6
AUC (95% CI)	0.67 (0.6-0.7)	0.09 (0.12-0.05)
Cut-off	270	7.9
p-value	0.001	0.001

PPROM : Preterm Premature Rupture of Membrane.
PC : Platelet Count.
MPV : Mean Platelet Volume.
PPV : Positive Predictive Value.
NPN : Negative Predictive Value.
AUC : Area Under Curve.
CI : Confidence Interval.

Discussion

Our study indicated that PC was significantly increased and MPV was significantly decreased from 12 to 14 weeks' gestation onwards, and these changes preceded the occurrence of PPROM by 8 to 20 weeks. Early prediction of a patient who may be prone to PPROM, could provide prophylactic measures for these patients.

The risk factors for PPROM were mostly based on maternal characteristics and past obstetric history, such as history of PTL or PPROM are the most suggestive risk factor [11]. However, no risk factors can be identified in most patients. Reactive thrombocytosis that occurs early in cases of PPROM is a form of thrombocytosis that occurs secondary to medical or surgical conditions [10], can explain early MPV decrement with a high PC in the prediction of PPROM. During normal pregnancy, it was suggested that platelet function is evaluated more by changes in MPV rather than PC [12]. Decreased MPV may be related to several chronic inflammations such as inflammatory bowel disease. It plays a role as a marker not only for inflammation but also for prognosis and follows up of anti-inflammatory treatment [10]. The most common cause of PPROM among the involved multiple mechanisms is intrauterine inflammation [4], which is mostly subclinical in nature [13]. In cases of intra-amniotic infection, cytokines are produced within the uterine cavity and then they reach the maternal circulation. Overproduction of cytokines, such as interleukin IL-4, IL-6 and tumor necrosis factor, influence platelet characteristics by interfering with megakaryopoiesis and subsequent release of predominantly small platelets from the bone marrow [10]. It is important to note that infection or inflammation at sites distant from female genital tract may also contribute to the occurrence of PPROM due to increase level of circulating pro-inflammatory cytokines and potentially small platelet [14]. Ekin et al., [15] have found that patients who develop PPROM showed significantly lower MPV and higher PC in the first trimester than control group.

Our study showed that MPV is superior to PC as predictive factor in patients with PPROM with a cut-off value for MPV was ≤7.9fL and for PC was ≥270 X 10^3 / µL. The sensitivity, specificity, PPV and NPV of MPV were 68%, 58%, 62.5% and 65.5%, respectively, with AUC (0.09), 95% CI (0.05-0.12). The sensitivity, specificity, PPV and NPV of PC were 70%, 50%, 64.9% and 67.4%, respectively, with AUC (0.67), 95% CI (0.6-0.7). Ekin et al., [15] revealed that an AUC (0.642), 95%

CI (0.601-0.683) and $p<0.001$ for PC and AUC (0.579), 95% CI (0.536-0.622) and $p<0.001$ for MPV, indicating that MPV was superior to PC as a predictor for PPROM with a cut-off value for MPV was ≤ 8.6fL and for PC $\geq 216 \times 10^3$/gl with a sensitivity of 62% & 44%, PPV of 56% & 49% and NPV of 64% & 60%, however this study was retrospective. Also, Myatt et al., **[16]** stated that the association of MPV identified susceptible women with subclinical vascular dysfunction. In another study, Gioia et al., reported that MPV ≥ 10fL may be associated with adverse neonatal outcomes in women affected by abnormal uterine artery Doppler findings **[17]**.

The results of the present study are similar to the studies regarding other markers of inflammation. A number of studies found that pregnant women with high CRP levels during the first half of pregnancy are at risk of later development of PPROM **[8,18]**.

Tzur et al., **[9]** investigated maternal leukocyte count in the first trimester of pregnancy and the risk for development of obstetric complications. They found a significant association between leukocytosis during the first trimester and PPROM. According to these reports, it is evident that there is a strong relevance between levels of inflammation markers and occurrence of PPROM because of regulated secretion by cytokines.

The strength of this study was that all blood samples for CBC assessment were processed within two hours after venipuncture. The assessments were performed using the same anticoagulant and the same automated counter. A large number of recruited participants, as well as enrollment of women early and throughout pregnancy, give further credit to our conclusion.

Conclusion:

We found that MPV could be used as a more efficient indicator for an early diagnosis of PPROM than platelet count. Thus, our study can serve as a reference data for clinical practice in detecting those asymptomatic women with subclinical intra-amniotic infection at increased risk for PPROM and subsequent preterm delivery. The MPV is a cheap, rapid and easily applicable test for determining the patients at risk for PPROM. Due to the multifactorial origin of PPROM, early detection of all patients with a single test is rather difficult in order to develop an accurate and efficient method.

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دراسة حجم الصفائح الد موية والتغيرات فى عددها أثناء الحمل كعلامة للتنبؤ بتمزق غشاء السلوى المبكر

الهدف: دراسة التغير فى مستوى حجم الصفائح الدموية وعددها الذى يسبق حالات (PPROM) التمزق المبكر لغشاء السلوى وتحديد القيمة التنبؤية لهذه العلامات للمساعدة فى التنبؤ بـPPROM.

طرق الدراسة: تم إدراج عدد ٩٧٩ سيدة حامل تلقين رعاية طبية منتظمة من الحجم حتي الولادة. تم تقسيم المشاركين إلى مجموعتين: النساء الحوامل مع PPROM، عددهم ١٤٠، والنساء بدون PPROM وعددهم ٨٣٩ سيدة. تم جمع عينات الدم خلال الزيارات السابقة للمتابعة بالعيادات أو خلال فترة تواجدهم بالقسم الداخلى فى المستشفى وتمت مقارنة التغيرات فى متوسط حجم الصفائح الدمو ية (MPV) وعدد الصفائح الدمو ية (PC) بين المجموعتين.

النتائج: بالمقارنة مع الضوابط، زاد فى النساء ذوات PPROM بشكل كبير عدد الصفائح الدموية وانخفض متوسط الحجم بشكل كبير فى الأشهر الثلاثة الأولى من الحمل ($p<0.001$) وكانت (AUC) ٠.٩ لمتوسط حجم الصفائح و ٠.٦٧ لعدد الصفائح الدمو ية. القيمة القطعية لمتوسط الحجم ≥٧.٩ فيمتولتر والعدد≥10x270^3/ميكرولتر وقد توقع ذلك PPROM بحساسية ٦٩٪ و ٠٧٪ وخصوصية ٥٨٪ و ٥٠٪ على التوالى.

الاستنتاج: يمكن استخدام متوسط حجم الصفائح الدموية كمؤشر أكثر كفاءة للتشخيص المبكر لـPPROM (فى الأشهر الثلاثة الأولى بين ١٢ و ١٤ أسبو عاً من الحمل) من عدد الصفائح الدموية. ومع ذلك فهناك حاجة إلى مزيد من الابحاث التى تجمع بين دلالات أخرى لزيادة كفاءة التنبؤ.