Original Research Article

Platelet Indices and Antiphospholipid Syndrome in Patients with Recurrent Pregnancy Loss

ABSTRACT

•**Introduction:** Spontaneous pregnancy loss is a common occurrence. Recurrent pregnancy loss (RPL) is defined as two or more failed clinical pregnancies as documented by ultrasonography or histopathologic examination before 20 weeks gestation, ectopic, molar, and biochemical pregnancies are not included.

Comment [MMS1]: New sentence. ... not included in the definition.

Aim: To examine the relationship between platelet indices and the presence of antiphospholipid syndrome (APS) in RPL patients.

Methodology: This study was conducted on fifty first-trimester pregnant females with a history of RPL. Control group included fifty first-trimester pregnant females without history of RPL with at least one live birth. Lupus anticoagulant (LA) testing with simplified dilute Russell's Viper venom test (DRVVT) and anticardiolipin (aCL) antibodies detection with Human Anti-Cardiolipin IgG/IgM ELISA. CBC for mean platelet volume (MPV), Platelet distribution width (PDW), and plateletcrit (PCT) was done for all patients.

Results: The age and the gravida number of the patients were significantly higher than of the control. All platelet indices were significantly higher among RPL group compared to control. According to the positivity of LA and aCL antibodies, RPL patients were classified into 2 groups, 25 patients each, positive and negative for APS respectively. Comparing platelet indices between both subgroups, PCT and MPV were significantly higher among APS positive patients, while PDW did not attain any significance. Receiver operating characteristic (ROC) curve analysis was applied to assess the best cut off value for predicting RPL in patients with APS who may benefit from early treatment.

Conclusion: These low-cost and easily measurable indices can be used for prediction of fetal loss and may help clinicians start early management of high-risk RPL cases.

- **Keywords:** *Platelecrit, MPV, PDW, antiphospholipid, recurrent pregnancy loss*

Key Messages

Platelet indices are significantly higher in patients with recurrent pregnancy loss and can be used for prediction of pregnancy loss among high risk cases and allow early intervention and prevention.

INTRODUCTION

Spontaneous pregnancy loss is a shockingly common occurrence. Only 30% of all pregnancies result in a live birth [1]. Spontaneous pregnancy loss can be physically and emotionally exhausting for couples, particularly when associated with recurrent losses. Recurrent pregnancy loss (RPL) is defined as two or more failed clinical pregnancies as documented by ultrasonography or histopathologic examination before 20 weeks gestation, ectopic, molar, and biochemical pregnancies are not included [2].

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At present, there exist few accepted etiologies for RPL. These include parental chromosomal abnormalities, hypothyroidism, uncontrolled diabetes mellitus, some uterine anatomic abnormalities, and antiphospholipid antibody syndrome (APS) [3]. Other possible etiologies include other endocrine disorders, heritable and/or acquired thrombophilias, immunologic abnormalities, infections and environmental factors. After assessment for these causes, around half of all cases will remain unexplained [4].

One specific autoimmune disorder, APS, requires particular attention as it has been clearly linked with many poor obstetric outcomes, including RPL. Besides, it is the most frequently acquired risk factor for thrombophilia, with a prevalence of 3% to 5% in the general population. The laboratory diagnosis of APS depends on tests documenting the presence of antibodies such as, anti-β2 glycoprotein-I (anti-β2GPI) antibodies, anticardiolipin (aCL) antibodies or lupus anticoagulant (LA) on two or more occasions at least 12 weeks apart [5].

The natural inclination towards thrombophilia in pregnancy is due to the rise in the levels of several clotting factors, including factor I, factor VII, factor VIII and von Willebrand [6]. Micro-emboli within the uteroplacental circulation,

that cause placental insufficiency and inflammation, are know[8]n to cause recurrent miscarriage in pregnant women with thrombophilia [7].

The mean platelet volume (MPV), plateletcrit (PCT), and platelet distribution width (PDW) have been investigated as the markers of platelet activation and predictors of thrombophilic disorders. Moreover, the combination of MPV and PDW may predict activation of coagulation with more efficiency

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The objective of this study was to examine the relationship between platelet indices namely plateletcrit, MPV, PDW and the presence of APS in patients with RPL.

MATERIALS and METHODS

This study was conducted on fifty first-trimester pregnant females with a history of RPL. Control group included fifty first-trimester pregnant females without history of RPL and had at least one live birth. Patients were selected from the Obstetrics and Gynecology Clinics of Ain Shams University Hospitals during the period from December 2017 to June 2018. Pregnant females with a history of RPL due to thyroid dysfunction, Diabetes Mellitus (DM) or uterine anomalies were excluded. Patients with history of deep vein thrombosis (DVT) or those using drugs affecting PLT functions such as aspirin, non-steroidal anti-inflammatory drugs, oral contraceptives, anti-PLT, or anticoagulant drugs were also excluded.

All patients were subjected to the following after taking their informed written consent; complete history taking, thorough clinical examination and laboratory investigations. All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Three venous blood samples were collected from each patient; the first one on EDTA vacutainer for complete blood count (CBC) for the following parameters; MPV, PDW and PCT using Beckman Coulter LH750 hematology analyzer (Beckman Coulter Inc., USA). The other two samples were used for APS evaluation; one on 3.2% tri-sodium citrate for lupus anticoagulant testing with simplified dilute Russell's Viper venom test (DRVVT) (Siemens, Germany). The third sample is a plain one to provide serum sample for anticardiolipin antibodies detection with Human Anti-Cardiolipin IgG/IgM ELISA kit (Creative Diagnostics, USA). Testing for both LA and aCL antibodies was repeated after 3 months according to ISTH guidelines. The results of APS testing were used to classify the study group into positive and negative for APS. The negative APS

group included 25 patients negative for LAC and anticardiolipin antibodies, while the positive group included 25 patients who were positive for the mentioned tests.

STATISTICAL ANALYSIS

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 15.0.1 for windows; SPSS Inc., Chicago, IL, 2001). Mean, Standard deviation (±SD) and range were used for parametric numerical data, while Median and Interquartile range (IQR) were used for non-parametric data. Student T Test was used to assess the statistical significance of the difference between two study group means. Mann Whitney Test (U test) was used for the difference of a non-parametric variable between two groups. A P-value of <0.05 was considered statistically significant.

RESULTS

All the included females were between 21 and 37 years old. A total of 50 patients with history of RPL were compared to 50 participants of the control group regarding age and obstetric history (Table 1). The age and the gravida number of the patients were significantly higher than of the control group.

Table (1): Age and obstetric history of RPL an control groups

Parameter	**RPL Group (Mean ± SD/ Median)**	**Control Group (Mean ± SD/ Median)**	**P Value**	**Significance**
Age (years)	31.8 ± 8.6	26.7 ± 3.8	0.002*	S
Live births (n)	0	2	<0.001*	S
Gravida (n)	3.3 ± 1.2	2.1 ± 0.9	0.001*	S

*Student t test; S: significant

Platelet indices among the studied groups is shown in Table (2). All the studied PLT indices were significantly higher among RPL group when compared to the control group.

Table (2): Platelet indices of studied patients

Parameter	RPL Group (Mean ± SD/ Median)	Control Group (Mean ± SD/ Median)	P Value	Significance
MPV (fl)	10.2 ± 2.1	9.1 ±1.3	0.001*	S
PDW (%)	16.9 ± 2.4	16.4 ± 2	0.001*	S
PCT (%)	0.26 ± 0.1	0.18 ± 0.04	0.032**	S

* Student t test; ** Mann-Whitney test; S: significant

According to the positivity of LAC and anticadiolipin antibodies, patients of the RPL group were classified into 2 groups, 25 patients each who were positive and negative for APS respectively. LAC is strongly present if the ratio between patient's LA1 screening reagent clotting time and patient's LA2 confirmation reagent clotting time is greater than 2.0 and is moderately present if the ratio is between 1.5 and 2.0, according to the manufacturer; while positive anticardiolipin antibodies is detected in patients who have > 40 GPL (IgG phospholipid units) or MPL (IgM phospholipid units). On comparing the studied platelet indices between both subgroups, PCT and MPV were significantly higher among patients who had APS while PDW did not attain any statistically significant difference (Table 3).

Table (3): Comparison between platelet indices among RPL group

Parameter	APS positive (Mean ± SD/ Median)	APS negative (Mean ± SD/ Median)	P Value	Significance
MPV (fl)	10.8 ± 1.4	8.2 ±2.9	0.008*	S
PDW (%)	16.3 ± 2.0	16.1 ± 2.3	0.443*	NS
PCT (%)	0.25 ± 0.01	0.22 ± 0.0	0.003**	S

* Student t test; ** Mann-Whitney test; S: significant, NS: non-significant

Receiver operating characteristic (ROC) curve analysis was applied to assess the best cut off value of platelet indices for predicting RPL in patients with APS who may benefit from early treatment, and revealed that the best cut off level for MPV was > 8.4 fL, with a diagnostic sensitivity 91.8% and specificity 88%. The negative predictive value

(NPV) was 70% and positive predictive value (PPV) was 95.5%. While the best cut off level for PDW was > 15%, with a diagnostic sensitivity 78% and specificity 87%, NPV was 43% and PPV was 97%. The best cut off value of PCT for predicting RPL patients was > 0.2, with a diagnostic sensitivity 58% and specificity 100%. The NPV was 30% and PPV was 100% (Table 4).

Table (4): ROC curve analysis for platelet indices

Cut off Level	AUC (CI)	Sensitivity	Specificity	PPV	NPV	P (sig)
MPV > 8.4	0.847 (0.719 to 0.940)	91.8	88	95.5	70	0.001
PDW > 15	0.872 (0.755 to 0.959)	78	87	97	43	0.001
PCT > 0.2	0.855 (0.737 to 0.959)	58	100	100	30	0.001

DISCUSSION

Pregnancy is a hypercoagulable state within which the levels of coagulation factors, such as factors II, VII, VIII, X, increase and those of natural anticoagulants, like protein C, protein S, and antithrombin III, decrease [9]. The existence of thrombotic tendency and coagulation stimulators induced by pregnancy lead to numerous pregnancy complications, like RPL. In the pathogenesis of RPL, inflammation and coagulation disorders are proposed to possess a necessary role, since fibrin deposition and fibrinoid necrosis within the decidual bed as well as thrombi in intervillous spaces occur in RPL, leading to fetal hypoperfusion and resultant fetal loss [10].

The most important thrombophilia associated with recurrent miscarriage is APS [11]. Antiphospholipid antibodies are related to a range of medical problems, including arterial and venous thrombosis, recurrent miscarriage, and severe pregnancy with early onset, intrauterine growth retardation and fetal loss. Antiphospholipid antibodies employed in the diagnosis are lupus anticoagulant, anticardiolipin antibodies, and anti-β2-glycoprotein I [12]. Antiphospholipid syndrome is a treatable cause of recurrent miscarriage. The standard treatment for APS is low-dose aspirin and heparin. While live birth rates in untreated patients were about 10%, it was reported as 71% in treated patients [13].

It is associated with changes in platelets functions which return to normal after 12 weeks [14]. In the study of Van Dreden et al., they observed an increasing level of platelet activating factors in serum samples from females who have suffered two or more RPL and they attributed its implications to placental function and fetal growth [15]. There are many studies on platelets indices among RPL. In the study of Rai et al., they reported that a relationship between platelet indices and the increased risk of thrombosis [16]. Increased MPV has been associated clinically with cardiovascular and cerebrovascular morbidity and known as an independent risk factor for myocardial infarction in patients with coronary heart disease. In addition, MPV was found to be elevated in some conditions with increased risk of cardiovascular morbidity, including diabetes mellitus, hypercholesterolemia, obesity, hypertension, and smoking [17].

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Since most of the diagnostic tests for assessment of recurrent miscarriage are expensive and time-consuming, we investigated in this study the relationship between platelet indices, as a simple, non-invasive and relatively low cost test that can be simply carried out at primary health care center level, with the aim of early identification of high risk cases and in an effort to prevent early pregnancy loss through early intervention.

In the present study, age and gravida number were statistically higher among RPL patient group when compared to control group. In a study by Aynioglu et al., who compared 208 patients with a history of RPL with 95 participants in the control group who had not experienced a pregnancy loss, and revealed significantly higher median age in the control group; while the number of gravida was higher in the RPL group [9]. On the contrary, a study by Abdul-Rahman Al-Aghbary et al., showed that no significant difference between RPL patients and the control in regard to patients' age. Only parity was statistically significantly higher among the control [18]. Also no difference between both groups regarding the age was among the findings of the study by Yilmaz et al., and that by Meena et al. [7, 19] .

It was observed in our study that all the studied PLT indices were statistically higher among RPL patients as compared to control. We observed that increased MPV, PCT and PDW was associated with recurrent pregnancy loss. Same results regarding MPV were detected by Yilmaz et al.; that would suggest that increased MPV is a risk factor in the vascular pathogenesis of RPL since MPV correlates with platelet function and activation, whether measured as aggregation, thromboxane synthesis, β-thromboglobulin release, procoagulant function, or adhesion

molecule expression [7]. This result is also similar to that reported by Abdul-Rahman Al-Aghbary et al., Aynioglu et al., Dundar et al. and Avcio lu et al. [18, 9, 20, 21].

Only PDW values were higher among patients in the study conducted by Meena et al. [19]. PCT showed no statistically significant difference between both groups while MPV was not evaluated in the study.

Since the association of RPL with acquired thrombophilia has been reported, as thrombotic tendency is increased during pregnancy by changes in clotting nfactors or an allo-auto-immune response to the fetal graft, we classified patients in the RPL group as positive and negative for APS and evaluated the relationship between the studied platelet indices and APS. To our knowledge very few studies has addressed the relationship between platelet indices and APS in RPL patients. The higher MPV and PCT among RPL patients who were positive for APS in our study, came in agreement with the results by Korkmaz et al. who concluded that MPV was increased at initial thrombotic event of APS [22].

Also, MPV was significantly higher in patients with clinically and laboratory confirmed APS in comparison with the controls in the study done by Rupa-Matysek et al., who revealed that MPV significantly predicted thrombosis recurrence [23]. In contrast to our results and most previous studies demonstrating that higher MPV was related to risk of thrombosis in APS, Lood et al. found that decreased platelet size is associated with platelet activation and APS in systemic lupus erythematosus (SLE) [24].

Furthermore, we used the ROC curve for each index alone and the results were significant area under the curve for the three studied indices and the cut off values for the three that could be used as a predictor for RPL in this study. In similar results, PCT was reported recently by Aynioglu et al. as a cheap marker for prediction of RPL in patients with a history of at least 1 abortus [9]. Similar finding was reported by Dundar et al. where increased PDW was found to be associated with RPL among their patients [20].

With the increase of data about thrombotic tendency in RPL, these low-cost and easily measurable PLT indices can be used for prediction of fetal loss and may help clinicians start early management of high-risk cases for RPL.

Conclusion

We conclude from the present study that antiphospholipid syndrome has a significant role in the pathogenesis of recurrent pregnancy loss which leads to significantly higher MPV, PDW and PCT in patients than in the control

group. Complete blood count, including platelet indices, is a simple, easy and non-invasive test that can allow early identification of high risk cases so that patients can be timely managed and pregnancy loss can be prevented.

COMPLIANCE with ETHICAL STANDARDS

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

COMPETEING INTERESTS

The authors declare that he has no conflict of interest.

FINANCIAL DISCLOSURE

There are no financial conflicts of interest to disclose.

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