

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.

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. CBC for MPV, PDW, PCT was done for all patients. Lupus anticoagulant testing with simplified dilute Russell's Viper venom test (DRVVT) and anticardiolipin antibodies detection with Human Anti-Cardiolipin IgG/IgM ELISA.

Results: The age and the gravida number of the patients were significantly higher than of the control. All PLT indices were significantly higher among RPL group compared to control. According to the positivity of LAC and anticardiolipin 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.

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

28 • **Keywords:** *Platelecrit, MPV, PDW, antiphospholipid, recurrent pregnancy loss*

29 INTRODUCTION

30 Spontaneous pregnancy loss is a shockingly common occurrence. Only 30% of all pregnancies result in a live birth
31 ^[1]. Spontaneous pregnancy loss can be physically and emotionally exhausting for couples, particularly when
32 associated with recurrent losses. Recurrent pregnancy loss (RPL) is defined as two or more failed clinical
33 pregnancies as documented by ultrasonography or histopathologic examination before 20 weeks gestation, ectopic,
34 molar, and biochemical pregnancies are not included ^[2].

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

40 One specific autoimmune disorder, APS, requires particular attention as it has been clearly linked with many poor
41 obstetric outcomes, including RPL. Besides, it is the most frequently acquired risk factor for thrombophilia, with a
42 prevalence of 3% to 5% in the general population. The laboratory diagnosis of APS depends on tests documenting
43 the presence of Abs such as, anti- β 2 glycoprotein-I (anti- β 2GPI) antibodies, anticardiolipin (aCL) antibodies or
44 lupus anticoagulant (LA) on two or more occasions at least 12 weeks apart ^[5].

45 The natural inclination towards thrombophilia in pregnancy is due to the rise in the levels of several clotting factors,
46 including factor I, factor VII, factor VIII and von Willebrand ^[6]. Micro-emboli within the uteroplacental circulation,
47 that cause placental insufficiency and inflammation, are know^[8]n to cause recurrent miscarriage in pregnant women
48 with thrombophilia ^[7].

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

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

54 **MATERIALS and METHODS**

55 This study was conducted on fifty first-trimester pregnant females with a history of RPL. Control group included
56 fifty first-trimester pregnant females without history of RPL and had at least one live birth. Patients were selected
57 from the Obstetrics and Gynecology Clinics of Ain Shams University Hospitals during the period from December
58 2017 to June 2018. Pregnant females with a history of RPL due to thyroid dysfunction, Diabetes Mellitus (DM) or
59 uterine anomalies were excluded. Patients with history of deep vein thrombosis (DVT) or those using drugs
60 affecting PLT functions such as aspirin, non-steroidal anti-inflammatory drugs, oral contraceptives, anti-PLT, or
61 anticoagulant drugs were also excluded.

62 All patients were subjected to the following after taking their informed written consent; complete history taking,
63 thorough clinical examination and laboratory investigations. Three venous blood samples were collected from each
64 patient; the first one on EDTA for complete blood count (CBC) for the following parameters; MPV, PDW and PCT
65 using Beckman Coulter LH750 hematology analyzer (Beckman Coulter Inc., USA). The other two samples were
66 used for APS evaluation; one on 3.2% tri-sodium citrate for lupus anticoagulant testing with simplified dilute
67 Russell's Viper venom test (DRVVT) (Siemens, Germany). The third sample is a plain one to provide serum sample
68 for anticardiolipin antibodies detection with Human Anti-Cardiolipin IgG/IgM ELISA kit (Creative Diagnostics,
69 USA). The results of APS testing were used to classify the study group into positive and negative for APS. The
70 negative APS group included 25 patients negative for LAC and anticardiolipin antibodies, while the positive group
71 included 25 patients who were positive for the mentioned tests.

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73 **STATISTICAL ANALYSIS**

74 The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science
 75 (SPSS 15.0.1 for windows; SPSS Inc., Chicago, IL, 2001). Mean, Standard deviation (\pm SD) and range were used for
 76 parametric numerical data, while Median and Interquartile range (IQR) were used for non-parametric data. Student
 77 T Test was used to assess the statistical significance of the difference between two study group means. Mann
 78 Whitney Test (U test) was used for the difference of a non-parametric variable between two groups. A P-value of
 79 <0.05 was considered statistically significant.

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81 RESULTS

82 All the included females were between 21 and 37 years old. A total of 50 patients with history of RPL were
 83 compared to 50 participants of the control group regarding age and obstetric history (Table 1). The age and the
 84 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 \pm SD/ Median)	Control Group (Mean \pm SD/ Median)	P Value	Significance
Age (years)	31.8 \pm 8.6	26.7 \pm 3.8	0.002*	S
Live births (n)	0	2	<0.001 *	S
Gravida (n)	3.3 \pm 1.2	2.1 \pm 0.9	0.001*	S

*Student t test; S: significant

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86 Platelet indices among the studied groups is shown in Table (2). All the studied PLT indices were significantly
 87 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

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89 According to the positivity of LAC and anticardiolipin antibodies, patients of the RPL group were classified into 2
 90 groups, 25 patients each who were positive and negative for APS respectively. LAC is strongly present if the ratio
 91 between patient's LA1 screening reagent clotting time and patient's LA2 confirmation reagent clotting time is
 92 greater than 2.0 and is moderately present if the ratio is between 1.5 and 2.0, according to the manufacturer; while
 93 positive anticardiolipin antibodies is detected in patients who have > 40 GPL or MPL. On comparing the studied
 94 platelet indices between both subgroups, PCT and MPV were significantly higher among patients who had APS
 95 while PDW did not attain any statistical significance (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

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97 Receiver operating characteristic (ROC) curve analysis was applied to assess the best cut off value of platelet indices
 98 for predicting RPL in patients with APS who may benefit from early treatment, and revealed that the best cut off
 99 level for MPV was > 8.4 fL, with a diagnostic sensitivity 91.8% and specificity 88%. The negative predictive value
 100 (NPV) was 70% and positive predictive value (PPV) was 95.5%. While the best cut off level for PDW was > 15%,

101 with a diagnostic sensitivity 78% and specificity 87%, NPV was 43% and PPV was 97%. The best cut off value of
 102 PCT for predicting RPL patients was > 0.2, with a diagnostic sensitivity 58% and specificity 100%. The NPV was
 103 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

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105 DISCUSSION

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

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

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

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

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

139 It was observed in our study that all the studied PLT indices were statistically higher among RPL patients as
140 compared to control. We observed that increased MPV, PCT and PDW was associated with recurrent pregnancy
141 loss. Same results regarding MPV were detected by Yilmaz et al.; that would suggest that increased MPV is a risk
142 factor in the vascular pathogenesis of RPL since MPV correlates with platelet function and activation, whether
143 measured as aggregation, thromboxane synthesis, β -thromboglobulin release, procoagulant function, or adhesion
144 molecule expression ^[7]. This result is also similar to that reported by Abdul-Rahman Al-Aghbary et al., Aynioglu et
145 al., Dundar et al. and Avcio lu et al. ^[18, 9, 20, 21].

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

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

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

160 Furthermore, we used the ROC curve for each index alone and the results were significant area under the curve for
161 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
162 similar results, PCT was reported recently by Aynioglu et al. as a cheap marker for prediction of RPL in patients
163 with a history of at least 1 abortus ^[9]. Similar finding was reported by Dundar et al. where increased PDW was found
164 to be associated with RPL among their patients ^[20].

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

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171 **COMPLIANCE with ETHICAL STANDARDS**

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

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UNDER PEER REVIEW