How to Cite:
Kassem, A. A., El-Khyat, A. M., El-Dakhakhni, R. A., Soliman, G. A., & Hamoda, M. S. (2022). Evaluation of platelet indices in cases of spontaneous abortion and recurrent pregnancy loss. *International Journal of Health Sciences*, *6*(S4), 9567–9580. https://doi.org/10.53730/ijhs.v6nS4.11197

Evaluation of platelet indices in cases of spontaneous abortion and recurrent pregnancy loss

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***Abstract*---**Background: Spontaneous abortion is defined as a clinically recognized pregnancy loss before the 20th week of gestation, or an expulsion of an embryo weighing 500 g or less. .Pregnancy is a condition of hypercoagulation. study aimed to evaluate the change of platelet indices namely platelet count, platelet crit , platelet distribution width and mean platelet volume among patients . Methods: This study is observational case control study . participants were 135 and divided into three groups Group1: 45 women with spontaneous abortion. Group2: 45 women with recurrent pregnancy loss.Group3: 45 normally pregnant women Results At cut off 14.12, platelet distribution width diagnose abortion (P=0.008) with sensitivity, specificity, PPV, NPV and accuracy of 74.4%, 53.3%, 76.1%, 51.1%, 67.4% respectively.At cut off 6.98, Mean platelet volume diagnose abortion (P=0.002) with sensitivity, specificity, PPV, NPV and accuracy of 76.7%, 44.4%, 75.0%, 57.1%, 70.4% respectively. At cut off 0.31, Platelet crit diagnose abortion with sensitivity, specificity, PPV, NPV and accuracy of 31.1%, 97.8%, 96.6%, 41.5%, 53.3% respectively. There were univariate regression for value of platelet indices in prediction of abortion in the studied

Manuscript submitted: 27 April 2022, Manuscript revised: 18 June 2022, Accepted for publication: 9 July 2022

sample. Conclusions: Platelet indices are markers for the prediction of spontaneous abortion and recurrent pregnancy loss.

***Keywords*---**platelet indices, spontaneous, abortion, pregnancy loss.

Introduction

Spontaneous abortion is defined as a clinically recognized pregnancy loss before the 20th week of gestation, or an expulsion of an embryo weighing 500 g or less. [1] It is the most common pregnancy complication and affects approximately 15% of all pregnancies. [2] Many factors have been associated with spontaneous abortions, such as genetic disorders, chromosomal abnormalities, endocrinology imbalances, and immunologic dysfunctions. [3]

According to guidelines from European Society of Human Reproduction and Embryology (ESHRE), recurrent pregnancy loss (RPL) is three or more consecutive pregnancy losses before 22 weeks of gestation. It is classically defined in the literature as three or more spontaneous fetal losses before the 20th week of pregnancy, but in some studies, two or more losses were considered Sufficient to diagnose RPL. There have been many studies showing that oxidative-inflammation caused by immune mechanisms and thrombosis is responsible for pathogenesis of RPL. [4]

In thrombophiles, the risk of thrombosis increases. [5] Some studies have shown that mean platelet volume (MPV) was significantly increased in subjects with arterial thrombotic events, i.e., acute myocardial infarction (MI). Platelet Distribution Width (PDW) and plateletcrit (PCT) values were found high in MI patients. [6] Plateletcrit (PCT) was reported recently by Aynioglu et al as a low-cost, widely available marker for prediction of RPL in patients with a history of at least 1 abortus. [7]

Mean platelet volume (MPV) , platelet crit (PCT), and platelet distribution width (PDW) were significantly higher in studies that investigated platelet parameters in cases with recurrent pregnancy loss. [7-12] . The aim of this study is To evaluate the change of platelet indices namely platelet count (PLC), platelet crit (PCT), platelet distribution width (PDW) and mean platelet volume (MPV) among patients with recurrent pregnancy loss (RPL) and spontaneous abortion.

Patients and Methods

This study is observational case control study was conducted at outpatient clinic and/ or inpatient department of Obstetrics& Gynaecology at Tanta University Hospitals and the study was conducted directly after approval From January 2020 till the October 2021.

The study participants were 135 participants and were divided into three groups as following:

- Group1: 45 women as study group who came with spontaneous abortion.

- Group2: 45 women as study group who came with recurrent pregnancy loss.
- Group3**:** 45 normally pregnant women with no history of pregnancy loss as control group.

Inclusion Criteria

Age range between 18 to 38 years, Gestational age (6-20) weeks, BMI (≥18-≤30) kg/m^2 and Recurrent pregnancy loss

Exclusion Criteria

Smoking or alcohol use, Women with chronic disease, rheumatological disease , uterine anomaly , who are on anticoagulant and who take any drugs affecting platelets as Aspirin. After taking informed written consent from all pregnant women, all selected participants were subjected to the following: Personal history, History of present illness ,Menstrual ,Obstetric , present pregnancy history, Past history of any medical disease and family history. Alslo vital signs measuring**,** Abdominal examination , pelvic examination and per vaginal (PV) examination.

Ultra-sonographic scanning

For estimation of gestational age:
Gestational age was determined using the last menstrual cycle (LMC), if not known it is measured by U/S as the following:

1- In early pregnancy (till 12^{th} week i.e first trimester) : gestational age is measured by transvaginal U/S by measuring crown rumb length CRL.
2- After 12^{th} week (in second trimester) : gestational age is measured by U/S by many biometric variables as biparietal diameter (BPD) , head circumference (HC), abdominal circumference (AC) and femur length(FL).

Laboratory testing as blood group and RH, RBS, urine analysis, thyroid function test, TORCH infection *and* CBC for :

- Heamoglobin and anemia
- Platelet count and their indices which are
- mean platelet volume (MPV)
- platelet crit(PCT)
- platelet distribution width (PDW)

Blood samples were obtained from antecubital vein. Ethylene diamine tetra acetic acid (EDTA) tube were used, and samples were examined within 2 hours, Abortion and recurrent pregnancy loss group blood samples were withdrawn immediately after diagnosis. the tests were performed with fully automatic blood cell counter (PCE – 210N – ERMA INC.)

Figure 1 PCE – 210N

Statistical analysis

SPSS statistics for windows (Statistical Package for the Social Sciences) version 26 (IBM, Armonk, NY, USA) was used for statistical analysis of the collected data. Shapiro-Wilk test was used to check the normality of the data distribution. All tests were conducted with 95% confidence interval. P (probability) value < 0.05 was considered statistically significant. Quantitative variables were expressed as mean and standard deviation while categorical variables were expressed as frequency and percentage. One-way ANOVA with Bonferroni post hoc analysis and Kruskal Wallis with Dunn's post hoc analysis tests were used for inter-group comparison of parametric and non-parametric continuous data respectively. Categorical Group differences with Fisher exact and Chi square tests were used for inter-group comparison of nominal data. Bivariate Correlations were assessed using Pearson's or Spearman's correlation coefficient depending on the nature of data. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated using the receiver operating characteristic (ROC) curve .A binary logistic regression model was conducted to determine the value of platelet indices in prediction of abortion (R2).

Results

Table (1) demonstrates demographic characteristics of the studied groups. There were no statistically significant differences among the three studied groups concerning all demographic features (age, BMI, occupation, and residency) (P>0.005).

Table 1 Demographic characteristics of the studied groups

		Group I (n= 45)	Group II (n= 45)	Group III (n= 45)	P	P1	P2	P3
Age (years)	Range	18.00, 37 .00	19.00, 38 .00	18.00, 37 .00	0.5 42	0.42 5	0.79 0	0.28 8
	Mean	24.80 ± 4. 181	25.53 ± 4. 551	24.56 ± 4. 309				
BMI (kg/m2)	Range	19.92, 27 .99	19.65, 29 .02	19.65, 29 .02	0.9 03	0.67 4	0.94 7	0.72 4

	Mean	23.96 ± 2.153	24.16 ± 2.281	23.99 ± 2.288				
Occupation	House wife	64.4% (29)	53.3% (24)	64.4% (29)	0.460	> 0.05	> 0.05	> 0.05
	Worker	35.6% (16)	46.7% (21)	35.6% (16)				
Residency	Urban	33.3% (15)	24.4% (11)	28.9% (13)	0.649	> 0.05	> 0.05	> 0.05
	Rural	66.7% (30)	75.6% (34)	71.1% (32)				
Data is expressed as mean and standard deviation or as percentage and frequency. P is significant when < 0.05. P1: Group I & Group II. P2: Group I & Group III. P3: Group II & Group III.								

Table (2) illustrates obstetric history of the studied groups. There were highly statistically significant differences among the three studied groups as regards parity and number of gestations being significantly increased in group II followed by group III then group I (P<0.001). However, there were no statistically significant differences among the three studied groups as regards gestational age (P>0.05).

Table 2 Obstetric history of the studied groups

		Group I (n= 45)	Group II (n= 45)	Group III (n= 45)	P	P1	P2	P3
Parity	Range	0.00, 2.00	0.00, 3.00	0.00, 3.00	< 0.001	< 0.001	0.343	0.003
	Mean	0.64 ± 0.830	1.38 ± 0.936	0.82 ± 0.886				
Number of Gestations	Range	1.00, 3.00	2.00, 7.00	1.00, 4.00	< 0.001	< 0.001	1	< 0.001
	Mean	1.64 ± 0.830	4.62 ± 1.248	1.82 ± 0.886				
Gestational age (weeks)	Range	7.00, 19.00	7.00, 20.00	6.00, 19.00	0.299	0.651	0.288	0.131
	Mean	13.27 ± 3.707	13.64 ± 4.360	12.38 ± 3.756				
Data is expressed as mean and standard deviation. P is significant when < 0.05. P1: Group I & Group II. P2: Group I & Group III. P3: Group II & Group III.								

Table (3) reveals comparison of blood parameters between the studied groups. There were no statistically significant differences among the three studied groups concerning maternal serum hemoglobin, TLC and platelets (P>0.05). However, there were statistically significant differences among the three studied groups concerning platelet distribution width , mean platelet volume and plateletcrit (P<0.05).

Table 3 Comparison of blood parameters between the studied groups

		Group I (n= 45)	Group II (n= 45)	Group III (n= 45)	P	P1	P2	P3
Maternal serum hemoglobin (gm/dl)	Range	8.32, 12.60	8.38, 13.26	8.81, 13.65	0.481	0.710	0.238	0.418
	Mean	10.72 ± 0.956	10.80 ± 1.073	10.97 ± 0.966				

TLC (*103/mm3)	Range	4.10, 13.50	1.80, 13.90	4.00, 14.00	0.176	0.063	0.363	0.338
	Mean	8.36 ± 2.186	7.51 ± 2.251	7.95 ± 1.996				
Platelet (*103/mm3)	Range	189.00, 399.00	164.00, 400.00	190.00, 378.00	0.777	0.494	0.857	0.614
	Mean	279.76 ± 49.722	272.40 ± 51.411	277.82 ± 51.285				
Platelet distribution width (fL)	Range	12.25, 19.58	12.16, 18.12	11.75, 17.59	0.029	0.840	0.028	0.017
	Mean	15.07 ± 1.569	15.14 ± 1.421	14.39 ± 1.382				
Mean platelet volume (fL)	Range	5.17, 11.12	5.53, 11.31	5.18, 9.69	0.006	0.596	0.013	0.003
	Mean	7.92 ± 1.218	8.06 ± 1.214	7.30 ± 1.108				
Plateletcrit (%)	Range	0.14, 0.39	0.15, 0.39	0.09, 0.31	0.003	0.712	0.002	0.006
	Mean	0.26 ± 0.077	0.25 ± 0.078	0.21 ± 0.066				
Data is expressed as mean and standard deviation. P is significant when < 0.05. P1: Group I & Group II. P2: Group I & Group III. P3: Group II & Group III.								

Table (4), Figure (2-4) demonstrate the diagnostic profile of platelet indices in diagnosis of abortion in the studied sample. At cut off 14.12, platelet distribution width (AUC=0.641) could diagnose abortion (P=0.008) with sensitivity, specificity, PPV, NPV and accuracy of 74.4%, 53.3%, 76.1%, 51.1%, 67.4% respectively. At cut off 6.98, Mean platelet volume (AUC=0.662) could diagnose abortion (P=0.002) with sensitivity, specificity, PPV, NPV and accuracy of 76.7%, 44.4%, 75.0%, 57.1%, 70.4% respectively. At cut off 0.31, Plateletcrit (AUC=0.650) could diagnose abortion (P=0.005) with sensitivity, specificity, PPV, NPV and accuracy of 31.1%, 97.8%, 96.6%, 41.5%, 53.3% respectively

Table 4 Diagnostic profile of platelet indices in diagnosis of abortion in the studied sample

	Platelet distribution width	Mean platelet volume	Plateletcrit
AUC	0.641	0.662	0.650
95% CI of AUC	0.542, 0.741	0.565, 0.759	0.558, 0.743
P	0.008	0.002	0.005
Cut off point	14.12	6.98	0.31
Youden's index	0.278	0.278	0.289
Sensitivity	74.4%	76.7%	31.1%
Specificity	53.3%	44.4%	97.8%
PPV	76.1%	75.0%	96.6%
NPV	51.1%	57.1%	41.5%
Accuracy	67.4%	70.4%	53.3%
P is significant when < 0.05.			

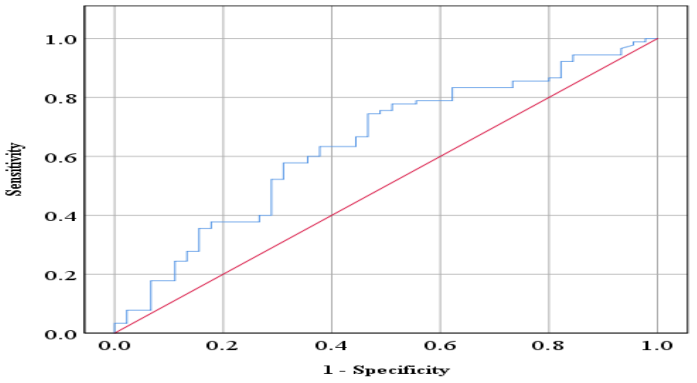

Figure 2 ROC curve of Platelet distribution width in diagnosis of abortion in the studied sample

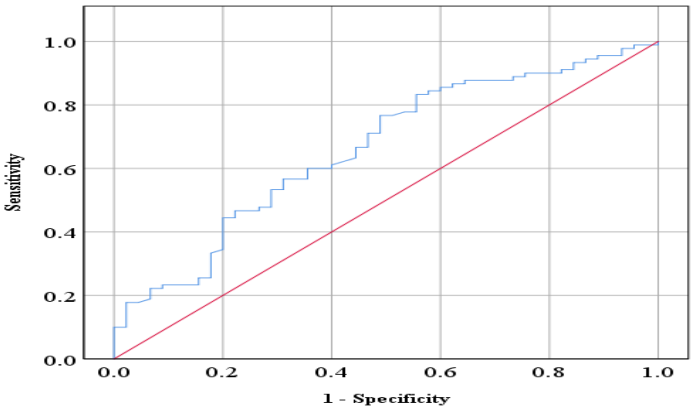

Figure 3 ROC curve of Mean platelet volume in diagnosis of abortion in the studied sample

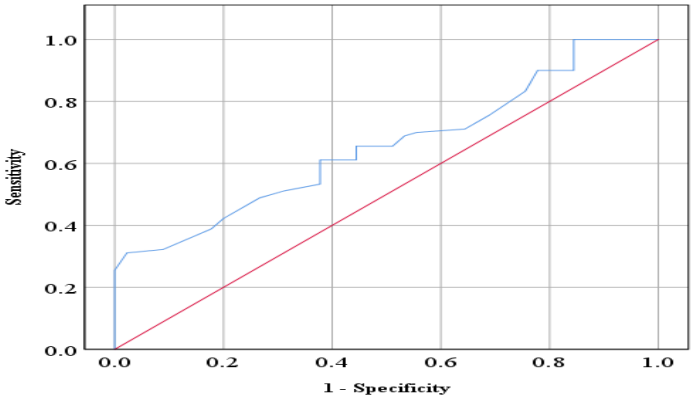

Figure 4 ROC curve of Plateletcrit in diagnosis of abortion in the studied sample

Table (5) demonstrates univariate regression for value of platelet indices in prediction of abortion in the studied sample. All platelet indices (Platelet distribution width, mean platelet volume and Plateletcrit) could be used as reliable predictors for abortion ($P<0.05$).

Table 5 Univariate regression for value of platelet indices in prediction of abortion in the studied sample

	R2	B	Constant	P
Platelet distribution width	7.3%	0.353	-4.515	0.010
Mean platelet volume	10.1%	0.516	-3.249	0.003
Plateletcrit	11.3%	8.598	-1.305	0.001
P is significant when < 0.05.				

There was no significant correlation between platelet indices and each other or with other studied variables except a significant correlation between Mean platelet volume and Number of Gestations as seen in table 6.

Table 6 Correlation between platelet indices and other studied parameters

	Platelet distribution width		Mean platelet volume		Plateletcrit	
	Correlation coefficient	P	Correlation coefficient	P	Correlation coefficient	P
Age (years)	-0.078	0.369	-0.041	0.634	-0.144	0.095
BMI (kg/m2)	0.187	0.030	0.049	0.571	-0.066	0.444
Worker	-0.101	0.244	0.063	0.467	0.022	0.797
Rural Residency	0.047	0.591	0.203	0.018	0.037	0.666
Parity	0.010	0.905	0.082	0.343	-0.108	0.210
Number of Gestations	0.015	0.863	0.170	0.048	0.069	0.425
Gestational age (weeks)	-0.001	0.990	0.011	0.895	0.045	0.606
Maternal serum hemoglobin (gm/dl)	0.127	0.141	-0.077	0.377	0.043	0.619
TLC (*103/mm3)	0.159	0.065	0.010	0.908	-0.059	0.493
Platelet (*103/mm3)	0.101	0.244	-0.072	0.406	-0.089	0.307
Platelet distribution width (fL)	-	-	0.157	0.069	0.052	0.551
Mean platelet volume (fL)	0.157	0.069	-	-	0.061	0.480
Plateletcrit (%)	0.052	0.551	0.061	0.480	-	-
P is significant when < 0.05.						

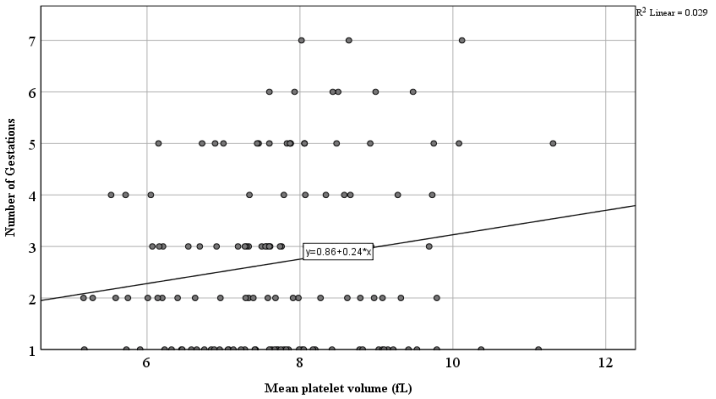

Figure 5 Correlation between Mean platelet volume and Number of Gestations

Discussion

Spontaneous abortion is defined as a clinically recognized pregnancy loss before the 20th week of gestation, or an extraction of an embryo weighing 500 g or less. RPL is identified by an ultrasound or histopathological test as two or more losses. [1] Many screening tests on frequent pregnancy loss are expensive. So, our study aimed to evaluate the change of platelet indices namely platelet count, plateletcrit (PCT), platelet distribution width(PDW) and mean platelet volume (MPV) among patients with recurrent pregnancy loss (RPL) and spontaneous abortion. In Aynıoglu et al. (2016) [7] study, in the RPL group, the number of gravida was significantly higher, although the number of live births was significantly lower than in normal pregnancy group. Also, in Amin et al. (2020) [13] study, pregnant women with RPL had significantly higher gravidity and lower parity than control pregnant women.

In Ragab et al. (2017) [14] study, RPL pregnant women had significantly higher gravidity, lower parity and lower gestational age. In contrast to Biyik et al. (2019) [1] study, in which there was no statistically significant differences between the abortion group and healthy pregnant women group regarding the gestational age, gravida, parity and currently living child derivatives. In a previous study performed by Yilmaz et al. (2015) [12], which studied patients with unexplained recurrent miscarriages and compared with data from controls. MPV increased in patients with RPL than those in the control group.

In Erdem et al. (2020) [15] study, the mean platelet volume and platelet count of recurrent pregnancy loss were higher than normal controls. And, in Aynıoglu et al. (2016) [7], the hemoglobin, haematocrit , and mean corpuscular volume (MCV) values were not significantly different between the RPL and control groups, although WBC, RDW, PLT, and PCT values in patients with RPL were significantly higher than those of the control group.In Al-Aghbary et al. (2018) [11] study, platelet count, and indices were significantly higher among RPL patients, which might suggest a role in the etiology of their RPL. Also, similar to that recently reported in India by Meena et al. (2017) [16], in Turkey by Avcıoglu et al. (2014), and Dundar et al. (2015) [4, 17].

This significantly increase in the PCT and MPV in patients with RPL is the cause of the increase in the thrombotic tendency in RPL. [7] However, these results do not agree with the study that was conducted by Kaplanoglu et al. (2015) [3], which examined the MPV to predict spontaneous miscarriage and to assess any variations in its values during pregnancy. They also showed that the MPV was statistically significantly lower in the miscarriage community than in the control group. When our study results were compared with (Uysal et al., 2013; Akdemir et al., 2013; Avcıoglu et al., 2014) [4, 10, 18]studies, it was found that no significant differences in MPV, PCT and PDW values between the women with recurrent pregnancy loss and the healthy controls.

In Bıyık et al . (2019) [1] study, it was found that in the spontaneous abortion group, Hb, Hct, MCV, and PDW were significantly higher, whereas PLT, MPV, PCT were significantly lower than in normal pregnant group. MPV value was lower in the spontaneous abortion group, PDW value was higher in spontaneous abortion group. Hb and Hct values were also found to be higher in the spontaneous abortion group. An important part of the spontaneous abortion patients consists of those with vaginal bleeding complaints, whose blood samples were taken upon hospital admission. It was suggested that higher Hb and Hct values in the spontaneous abortion group may be due to the relative hemoconcentration that occurs during the early stage of bleeding.

Other various studies investigated the platelet parameters in cases of spontaneous abortion. Kosus et al. (2011) compared MPV and PLT values between 100 spontaneous abortion cases and 100 normal pregnant women. MPV values were similar in spontaneous abortion and control groups. Eroglu et al. (2013) [19] examined MPV and PDW values of 54 threatened, 46 spontaneous abortion patients and 40 control subjects, both of which were found similar among all three groups.

These results are in contrast to Elliott and Tefferi (2003) [20] study, which found that the platelets value was reduced in recurrent pregnancy loss group compared with healthy controls. In various studies on the loss of pregnancy, MPV was found to be higher than (Akin et al., 2016) [8], lower than (Kaplanoglu et al., 2015) [3] or similar with the control group (Kara and Unluer, 2017) [9]. MPV, PCT, and PDW were significantly higher in studies that investigated platelet parameters in cases with recurrent pregnancy loss [7, 10-12].

Platelet distribution width is an indicator of volume variability in platelet size. It has been implicated as a specific marker of platelet activation. In Makled et al. (2021) [21] study, it did not find a significant difference in PDW between cases of unexplained recurrent miscarriage and controls. However, Ural et al. (2014) [22] study stated that the PDW increased with RPL. Also, Dundar et al. (2014) [17] reported that there is elevation in PDW values in women with RPL. Alaghbary et al. (2018) [11] study found that there was significant difference in PDW in women with RPL.

Plateletcrit is an indicator of circulating PLTs in a unit volume of blood. [23] In Makled et al. (2021) [21] study when compared with control women, the women with a history of recurrent miscarriage, PCT values were not significantly

different, and no cut-off value could be used to predict cases of recurrent miscarriage. This agrees with Dundar et al. (2014) and Ural et al. (2014) [17, 22] studies which reported that there was no significant difference in PCT values between the studied groups. While study by Aynıoglu et al. (2016) and Alaghbary et al. (2018) [7, 11] showed that the PCT values were highly significant different in RPL patients.

This was in contrast to Al-Aghbary et al. (2018) [11], in which the higher area in the ROC curve was for plateletcrit followed by PDW and then MPV. In Bıyık et al. (2019) [1] study, the sensitivity and specificity of MPV <9.05 in predicting miscarriage was 61% and 48%. The same values for PCT <0.205 were 77.2% and 55.3%. In Amin et al. (2020) [13] study, the cutoff point for MPV to differentiate between cases and controls was found to be greater than 9.5 fl, with 91.67% sensitivity and 60.0% specificity, with an area under curve of 77.5%. Plateletcrit was reported recently by Aynioglu et al. (2016) [7] as a low-cost, widely available marker for prediction of RPL in patients with a history of at least 1 abortus.

PLTs play an important part in the pathogenesis of vascular diseases. [24] The MPV test is an indicator of PLT size. [25] It has been reported that MPV is a reflection of both proinflammatory and prothrombotic conditions like RPL. [26] Increased MPV indicates that PLT diameters are greater. An increase in MPV shows that new PLT synthesis in the bone marrow has increased. Thus bigger, younger and more functional PLTs are produced as MPV increases. Young and big PLTs produce more prothrombotic factors and groups more easily. [25] Several prospective studies and a meta-analysis have suggested a correlation between an increase in MPV and the risk of thrombosis. [26]

PDW and PCT are other and often omitted indices of PLT. Clinicians generally pay less attention to these than to PLT count and MPV. PCT is a measurement derived from the PLT count and the MPV. It was accepted as an indicator of circulating PLTs in a unit volume of blood. [27] PDW is a direct flow cytometric measurement of PLT cell volume. [28] As PDW does not increase with PLT distension, it is considered as a more specific marker of PLT activation than MPV. It has been suggested that MPV and PDW together are more meaningful for coagulation activation. [29]

Similar to Al-Aghbary et al. (2018) [11] study, when the multiple logistic regression analysis for all platelets indices was conducted among the studied pregnant women, it revealed that the PDW was the significant predictor for RPL. Similar finding was reported by Dundar et al. (2015) [17], where the elevation in PDW and red blood cell distribution width values was found to be associated with RPL among their patients. PDW is a platelet index and accepted as a platelet activation marker. [29] Platelet activation causes morphologic changes of platelets by pseudopodoia formation. Increased PDW have been shown in the vaso-occlusive crisis in sickle cell disease, ischemic heart disease. Platelet activation should be a risk factor for abortion. [30, 31]

MPV is a machine-calculated measurement of platelet size from the blood that is usually reported in the blood tests as part of the CBC. Furthermore, it has been shown that MPV is the most accurate measure of the size of platelets in stable

conditions and inversely associated with platelet count. In comparison to smaller platelets, larger platelets contain more granules and produce greater amounts of prothrombotic factors, such as thromboxane A2 and serotonin, also they aggregate rapidly under a stimulus and express a greater number of adhesion molecules, such as P-selectin and glycoprotein IIb/IIIa. [18] In Akdemir et al. (2013) [18] study, it was founded that platelet parameters such as MPV, PDW, Platecrits, wasn't accepted as an etiopathologic risk factor for early recurrent miscarriage.

Conclusion

Platelet indices are cost-effective, easily measurable, and practical markers for the prediction of spontaneous abortion and recurrent pregnancy loss complicated by thrombophilic disorder. As, platelet indices support the importance of thromboembolic events in the etiology of RPL .Our study findings revealed that, there were statistically significant differences among the three studied groups concerning platelet distribution width, mean platelet volume (being significantly increased in recurrent pregnancy loss group) and plateletcrit (being significantly increased in spontaneous abortion group) .So, all platelet indices (Platelet distribution width, mean platelet volume and Plateletcrit) could be used as reliable predictors for abortion especially Plateletcrit can be considered to be used as a screening tool due to its high specificity.

Financial support and sponsorship: Nil
Conflict of Interest: Nil

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