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Assessment of the Predictive Value of Platelet Parameters in Gestational Diabetes Mellitus: A Comparative Institutional Study

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Abstract

Introduction: Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. Women with GDM alone or with type 2 DM are at increased risk of cardiovascular disease (CVD) in future. Literature has shown that platelets play a significant role in the pathogenesis of GDM.There are very few studies relating GDM and platelet parameters emphasized on platelet count (PLT), Mean platelet volume (MPV) and Platelet distribution width (PDW) but not about plateletcrit (PCT) and P-LCR (platelet large cell ratio). The objective of this present study is to compare the platelet parameters including platelet count (PLT), MPV, PDW, PCT and P-LCR in gestational diabetic subjects with healthy pregnant women and to evaluate the effect of GDM on platelet parameters. *Materials and Methods*: Prospective cross sectional study was conducted for 6 months duration. Ninety GDM patients diagnosed by Oral glucose challenge test (OCT) and Oral glucose tolerance test (OGT) along with ninety normal pregnant females as control were taken up for study. Investigation panel including platelet parameters were done. *Results*: Statistically significant increase was noted in MPV ($p<0.002$) and platelet distribution width ($p<0.004$)and significant decrease was noted in platelet count ($p<0.01$) in gestational diabetics compared with control group. PCT is decreased in test group but it is only weekly significant ($p<0.09$), there is no significant difference in P-LCR ($p<0.25$) among the groups. *Conclusion:* Determination of these platelet parameters are simple, inexpensive, routinely available and can be used as a predictive markers to identify GDM patients who are at increased risk of CVD in later life.

Keywords: Gestational Diabetes Mellitus; Platelet Parameters; Predictive Value; Screening Methods.

Introduction

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. Perinatal outcome is highly dependent on early recognition and adequate treatment of this entity. Literature has shown that platelets play a significant role in the pathogenesis of gestational diabetes mellitus [1].

GDM is a frequently encountered complication affecting 5-6% to 15–20% of pregnant females worldwide. Incidence mainly depends on the type of screening methods and diagnostic criteria we apply and also depends on maternal life style [2]. There is 20 to 60% increased risk of developing into type 2 diabetes mellitus and cardio vascular disease in future, which depends on severity as well as grade of insulin resistance and hyperglycemic levels in pregnancy period. Glycemic deregulation occurs due to incomplete insulin secretion by beta cells of pancreas during increased insulin resistance in pregnancy.[3] GDM patients are at high risk of acquiring atherogenic abnormalities such as dyslipidemia, hypertensive disorders, vascular endothelial dysfunction and elevated homocysteine levels [4,5].

In diabetes mellitus altered platelet morphology and functions were already reported. There can be decreased activity of the enzyme nitric oxide synthase

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and increased production of peroxynitrate. When platelet synthesis increases the volume also increases proportionately, thus acts as a direct indicator of synthesis and activity [6]. Its well known fact that during normal pregnancy there is activation of coagulation and it is due to increase in platelet synthesis thereby definite increase in MPV (Mean platelet volume) and also changes in other parameters [7]. These parameters are also increased in ischemic stroke, acute myocardial infarction, renal artery stenosis as well as in early eclampsia, hence these alterations can be related to increased incidence of venous thromboembolism and vascular disease [8]. These issues have been widely investigated in above mentioned conditions but not in GDM. Women with GDM alone or when diagnosed with type 2 DM are at increased risk of cardiovascular disease (CVD)in future [9].

Larger platelets are found to be metabolically and enzymatically more active.Mean Platelet volume and platelet distribution width (PDW) are a marker of platelet activation. Increase in the MPV and alteration of other platelet parameters have been demonstrated in conditions such as metabolic syndrome, obesity and hypertension. Platelet-related indices and their determination by automated cell counters are cost effective and routinely ordered markers, the importance of these easily available markers are mostly underutilized or ignored [10,11].

There are very few studies relating GDM and platelet parameters emphasized on platelet count, MPV and PDW but not about plateletcrit and P-LCR (platelet large cell ratio), moreover most of the studies have not done a comparative study with the healthy pregnant controls. The objective of this present study is to compare the platelet parameters including platelet count, MPV, PDW, plateletcrit and P-LCR in gestational diabetic subjects with healthy pregnant women and to evaluate the effect of GDM on platelet parameters.

Materials and Methods

Prospective cross-sectional study was conducted in SRM Medical College Hospital and Research centre, Kattankulathur, Tamil Nadu, India. Study was done for a period of 6 months from June 2016 to November 2016, between 90 GDM subjects as a study population and 90 normal pregnant females as a control group. Institutional Ethical Committee permission and approval obtained. Written informed consent was taken from all the study subjects. The socioeconomic status, age, weight and duration of gestation were comparable between the study and the control groups.

Detailed history related to personal habits, treatment history, similar issues in family, any prolonged and significant past illness using a pretested questionnaire were taken. Subjects with history of coagulation disorders, diabetes, hypertension, preeclampsia, hypothyroidism, peripheral vascular disease, hyperlipidemia, chronic renal disease, hepatic disease, previous history of intrauterine growth retardation, intrauterine death and any infectious illness and those who are on any drugs influencing coagulation were excluded from this study. All subjects were not using tobacco or alcohol.

The following tests were performed on subjects: blood pressure recording and lipid profile to rule out hypertension and hyperlipidemia respectively. All subjects were screened with a 75-gram (g), 2-hours (h) oral glucose challenge test (OCT) between 24 and 28 weeks of pregnancy. According to the American diabetes association recommendations, subjects with a plasma glucose value more than or equal to 140 mg/dl after glucose load in nonfasting conditions were taken up as tests for the present study. While the plasma glucose level reached ≥140 mg/dl, 50g oral glucose tolerance test (OGT) was administered. The patients who had high values from both tests were considered to have GD. The patients were advised regarding further investigations, diet and required physical activity. HbA1c and Body mass index of the patients were calculated at the time of diagnosis. Urine routine including sugar and ketone bodies were performed.

2ml peripheral venous blood sample was collected from all the subjects in ethylene diamine tetra acetate (EDTA) coated vacutainer and analyzed using automated 5 part cell counter Sysmex (XT 1800i) to measure complete blood count (CBC) and platelet parameters such as platelet count (PLT), plateletcrit (PCT), MPV, PDW, and platelet-large cell ratio (P-LCR). Slides were also stained by Leishman stain according to standard operative protocol and manual microscopic examination of peripheral smears was done for confirmation of the parameters

MPV was calculated as follows: MPV (fL) = [(plateletcrit (%)/platelet count (109 /l)] 105 . PCT was analyzed as the ratio of the platelet volume to the whole blood volume. PDW and P-LCR were calculated from a histogram of platelet size distribution. PDW was defined as the platelet distribution width at the level of 20% and the P-LCR was defined as percentage of platelets with a size of more than 12 fL [12].

All the results of laboratory investigations were

entered and calculated in computerized SPSS 16.0 programmer, and statistical significance were carried out by applying nonparametric tests such as unpaired Student's ''t'' test and ANOVA. Results were finally expressed in mean ± standard deviation (SD) values. The P value of 0.05 or less has been considered as significant.

Results

The age, parity and body mass index between gestational diabetic patients and controls were compared and displayed in Table 1. Though there is increase in these parameters in diabetic patients the difference is not statistically significant. Total leukocyte count (TLC) and HbA1C levels also compared, among these TLC is not significantly increased (p<0.11), but there is significant increase in HbA1C levels (p<0.004)in test group.

The comparison of platelet parameters between test and control groups were shown in Table 2. Statistically significant increase was noted in MPV (p<0.002) and platelet distribution width (p<0.004) and significant decrease was noted in platelet count (p<0.01) in gestational diabetics compared with control group. PCT is decreased in test group but it is only weekly significant (p<0.09), there is no significant difference in P-LCR (p<0.25) among the groups. Graphical depiction of comparison and significance of differences among platelet parameters are shown in Figure1.

Platelet histogram in GDM patient taken in 5 consecutive samples is shown in Figure 2, there is thrombocytopenia and extension of graph towards right without touching baseline indicates increased MPV and PDW.

Peripheral smear examination of leishman stained smears (Figure 3) were performed, GDM patients showed mild to moderate thrombocytopenia (PLT) when compared with control group and also showed increased anisopoikilocytosis of platelets including giant platelets reflecting increased MPV and PDW.

Table 1: Demographic details and laboratory findings of the subjects (n=180)

Parameters	GDM Mean ± SD	Controls Mean ± SD	t value	P value
Age	30.16± 3.76	29.1± 2.31	0.55	0.59
BMI	28.2 ± 1.1	27.1 ± 2.04	1.44	0.17
Parity	2.1 ± 0.89	1.84 ± 0.75	0.9	0.35
Leukocyte count	9.31± 1.28	10.38± 0.78	-1.7	0.11
HbA1C	5.68 ± 0.61	4.42 ± 0.35	3.9	0.004*

BMI: body-mass index, HbA1c: glycosylated hemoglobin, *denotes statistically significant p value

Table 2: Comparison of platelet parameters among test and control groups (n=180)

Parameters	GDM Mean ± SD	Controls Mean ± SD	t value	P value
PLT	2.38± 0.53	2.76± 0.65	- 2.5	0.01*
MPV	11.04± 0.84	10.4± 0.78	3.01	0.002*
PDW	13.43± 2.07	12.1± 1.44	2.94	0.004*
PCT	0.245± 0.07	0.27± 0.06	1.71	0.09
P-LCR	29.7± 6.27	27.9± 6.24	1.14	0.25

PLT: Platelet count, MPV: Mean platelet volume, PDW: Platelet distribution width, PCT: Plateletcrit,P-LCR: Platelet large cell ratio, *denotes statistically significant p value

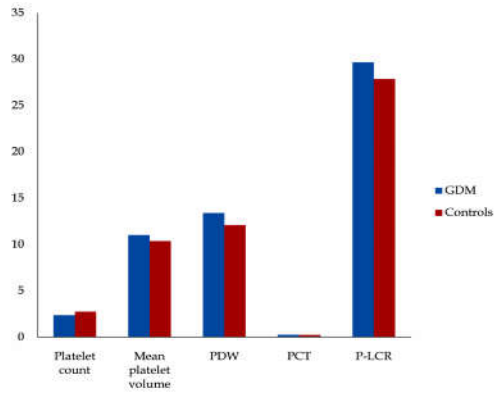

Fig. 1: Comparison of platelet parameters in GDM and non-GDM pregnant controls

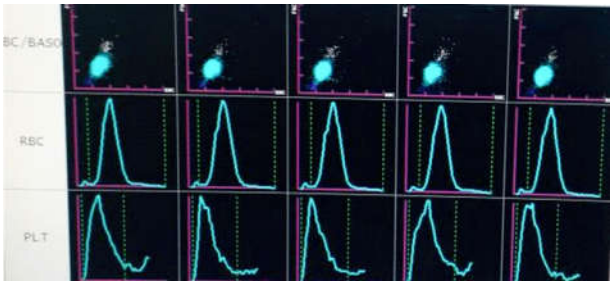

Fig. 2: WBC, RBC and Platelet histogram in GDM patient
Platelet histogram shows decreased platelet count (72x109/L), wider curve due to increased PDW (17.3FL), curve not touching baseline and rising above upper discriminator indicates giant platelets (MCV – 15.8fl) and normal p-LCR (43.7%)

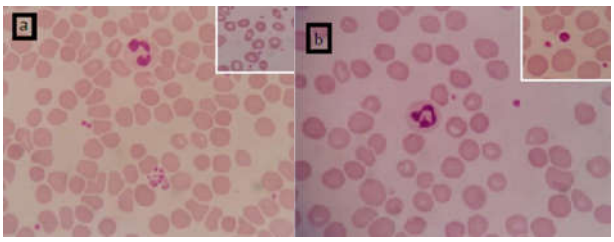

Fig. 3: Peripheral smear findings in control and GDM patient
Peripheral smear (a) from control shows normal platelet count of 2,23,000/cmm with normal morphology (inset) and clumps (b) from GDM patient shows thrombocytopenia (PLT – 72,000/cmm) and giant platelets (highlighted in inset).

Discussion

GDM is a most commonly diagnosed entity related to metabolic derangements which can cause maternal and fetal adverse effects. There will be presence of low level of subclinical inflammation which also influences primary hemostasis [13]. It is well known fact that platelets play a major role in inflammation and coagulation. So evaluation of impact of GDM on platelet parameters may be helpful in understanding the pathogenesis of prethrombotic events occurring in uncontrolled GDM patients.

In the present study, all platelet parameters were compared between 90 GDM patients and 90 non-diabetic healthy pregnant controls. Table 2 shows increase in MPV, PDW and p-LCR parameters and decrease in PLT and PCT in GDM patients than healthy pregnant controls, but mean platelet count, MPV and PDW showed statistically significant difference between both groups.

Our values on platelet count and MPV values were correlating with results of Bozkurt et al [14] who showed that higher MPV values and lower platelet count in GDM patients. Decrease in platelet count may be explained by compromise in production and shortening of mean survival rate in patients with Diabetes mellitus. Study done by Muhammet Erdal Sak et al and Sahbaz A et al also showed increase in MPV values in GDM patients [13,15]. MPV is the marker of platelet activity, function and morphology which is significantly altered in case of GDM. Large platelets with high MPV values tend to be more active and secretes increased amount of mediators such as serotonin and thromboxane which are responsible for prethrombotic state. MPV values correlates well with blood glucose values also, it is found increased significantly in uncontrolled diabetics and patients presented with complications than controlled diabetics and MPV values reduced when blood values came down post treatment [1,10].

PDW value was significantly increased among GDM patients in the present study, similar result was obtained by Sahbaz A et al [13], but in their study plateletcrit had high predictive value compared with other parameters. In contrary our study failed to show significant change in plateletcrit, it showed only weak association. They also showed positive correlation between MPV and PDW. There is activation of platelets in DM which causes larger irregular platelets with pseudopodia formation. Variation of size and shape is due to enlargement and formation of pseudopodia, so there is increased anisopoikilocytosis contributes to increase PDW in GDM. These hyperactive platelets tend to release more factors which may lead to prothrombotic events in GDM patients [16].

Our study has showed one new additional finding about P-LCR which was not done in the previous studies. Though there was increase in the P-LCR value in GDM patients in comparison with controls, the difference was not statistically significant. Further studies are necessary to know the exact role of P-LCR in GDM patients.

In our study, there is a significant increase of HBA1c in GDM subjects. Due to hyperglycemia, there is decreased platelet membrane fluidity and increase in activity due to non-enzymatic glycation of the surface proteins glycoproteins Ib and IIb/IIIa which causes increased adhesion of platelets along with osmotic effect caused by glucose. All these changes might be the reason for prothrombotic events in GDM patients. Increase in HBA1c levels are also responsible for increased production of these surface proteins in turn ends up in further hyperactivity of platelet activity [17-19].

Retnakaran and Shah conducted retrospective population-based cohort study and showed that even mild hyperglycemia during pregnancy caused adverse cardiovascular outcome due to macrovascular and microvascular complications in later life [20].

Endothelial dysfunction is found to be an initiating factor in the development of atherosclerosis and CVD. Early novel potential inflammatory markers such as circulating apelin, IL-6, plasminogen activation inhibitor have been proposed recently by various studies, but these are costly, time consuming and yet to be standardized. Endothelial dysfunction results in increased platelet aggregation causes altered hemorheological properties in activated platelets [21]. Hence, platelet parameters like MPV, PDW and platelet count can be used as markers of endothelial dysfunction and markers of CVD risk assessment which are easily measured, economical and simple reliable tools available in routine laboratories. Given the rising prevalence of GDM, future larger studies should be conducted to identify specific early predictive markers of CVD in women who may develop this condition.

Conclusion

In our study, we showed statistically significant increase in platelet count, MPV and PDW in GDM patients on comparison with healthy non-diabetics pregnant subjects and no significant difference in the plateletcrit and P-LCR among the groups. Determination of these platelet parameters are simple, inexpensive and routinely available and can be used as a predictive markers to identify GDM patients who are at increased risk of CVD in later life. By using these screening parameters as an adjunct to OCT and OGT early prevention, counseling and appropriate life style advice can be given to avoid cardiovascular complications.

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