RESEARCH

Effect of metformin on reducing platelet dysfunction in gestational diabetes mellitus: a randomized controlled trial

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Abstract

Objectives: To evaluate the effect of metformin in improving platelet dysfunction in women with gestational diabetes mellitus (GDM).
Patients and methods: A randomized controlled trial was conducted on pregnant women diagnosed with GDM. Singleton low-risk pregnancies meeting the inclusion criteria were randomly allocated at 27–31 weeks to receive metformin and placebo through the rest of pregnancy. Thirty-seven and 39 cases were recruited into the metformin group and the placebo group, respectively. MPVs, P-selectin, and 8-isoprostane levels were determined at the time of allocation and 6 weeks after treatment. Obstetric and neonatal outcomes were also assessed.
Results: Most baseline characteristics of the two groups were comparable. The levels of P-selectin after 6 weeks of treatment were significantly higher in the metformin group (68.9 ± 14.4 vs 60.6 ± 11.3; *P*-value = 0.006), indicating more platelet activation. All of the obstetric and neonatal outcomes were comparable except that birth weight was significantly lower in the metformin group (3018 ± 364 g vs 3204 ± 393 g; *P*-value = 0.037).
Conclusion: Metformin, in addition to diet and lifestyle modifications, does not improve or worsen oxidative stress and platelet dysfunction in women with GDM. Nevertheless, metformin significantly reduces fetal weight in women with GDM, theoretically preventing macrosomia.

Key Words

- gestational diabetes mellitus
- metformin
- oxidative stress
- platelet dysfunction
- P-selectin
- 8-isoprostane

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Highlight

- Metformin significantly reduces fetal weight in women with GDM, theoretically preventing macrosomia.
- Metformin does not improve oxidative stress and platelet dysfunction in women with GDM.

Introduction

Gestational diabetes mellitus (GDM), one of the most common complications in pregnancy, is defined as glucose intolerance of variable severity with onset or first recognition during pregnancy (1). GDM usually disappears shortly after birth but up to 50% will develop type 2 diabetes mellitus 10 years after GDM (2, 3, 4, 5). Women with GDM typically have different levels of insulin resistance and possible chronic low-grade inflammation, which trigger vascular injury and dysfunction and subsequent platelet activation (6, 7). GDM may probably be related to oxidative stress in the insulin resistance pathway, as documented in DM type 2 (8). Nevertheless, the relationship between GDM and oxidative stress is unclear. The levels of oxidative stress

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in GDM such as 8-isoprostane (8IsoP), TNF-α, and IL-10 have been studied in a very limited number of reports, in which the results are contradictory, though some showed higher levels of oxidative stress markers in pregnancies with GDM (9, 10).

P-selectin is an adhesion molecule found in the α-granules of platelets and the Weibel-Palade bodies of endothelial cells. Circulating degranulated platelets rapidly shed surface P-selectin, producing the circulating plasma protein soluble P-selectin. The anti-P-selectin antibody has been shown to inhibit thrombus formation (11), thus providing evidence that P-selectin plays a role in thrombosis, closely associated with platelet activation. Also, increased P-selectin levels are associated with a higher risk of DVT and subsequent vascular events in healthy women (12, 13). P-selectin is critical in the progressive change of atherosclerosis and leukocyte recruitment in the plaque. Also, a soluble form of P-selectin has pro-atherogenic and pro-thrombotic effects. It is involved in platelet dysfunction and facilitating thromboembolism and it can also be reflective of platelet dysfunction in GDM. Therefore, P-selectin is a potential clinical biomarker for innovation of therapy in subclinical inflammatory diseases or metabolic syndrome (14).

Mean platelet volume (MPV) is generally used to evaluate platelet morphology and can be used as an indicator of platelet activity (15). Increased MPV is associated with increased platelet activation and is commonly seen in diabetic patients (9, 16, 17). Platelet hyperactivity is accompanied by increased synthesis of thromboxane (18), reflecting a relationship between platelet function and micro-and macrovascular complications of diabetes mellitus (16, 17, 18, 19). MPV was significantly increased in pregnant women with GDM compared with healthy pregnant women (20). The pathogenesis for increased MPV in GDM has been suggested that insulin resistance is a major determinant of platelet activation which can be measured by MPV. In that case, MPV may serve as an indicator of the chronic inflammatory status of GDM (20).

Metformin, the first-line therapy for type 2 diabetes mellitus, had favorable action on platelet function. Previous studies showed that metformin improved oxidative stress, preserved anti-oxidant function, and limited platelet activation (21, 22). Furthermore, metformin can decrease MPV (23), in contrast to increased MPV, which is strongly related to vascular complications (24, 25). Second- and third-trimester metformin treatment of GDM appears to be safe in the short term and is effective in glucose control and reducing the rate of macrosomia, and accepted for the treatment of GDM (26), though some studies suggest that it is not completely safe for pregnancy (27). To the best of our knowledge, no study has been published using metformin to reduce platelet dysfunction in GDM. We hypothesize that women with GDM who take metformin during pregnancy have lower platelet dysfunction in maternal blood and cord blood, also less estimated blood loss after delivery, and lower incidence of adverse pregnancy outcomes, when compared with those who do not take metformin. Therefore, we conducted this study to compare the levels of soluble P-selectin and oxidative stress biomarker (8IsoP levels) between the intervention group (taking metformin) and placebo group (taking placebo) at 27–31 weeks' gestation and before delivery (after taking drugs 6 weeks), as primary objectives and to compare the MPV, the blood clotting time of pregnant woman and cord blood, the estimated blood loss after delivery, postpartum complications and neonatal outcomes as the secondary objectives. To the best of our knowledge, this is the first trial to study the effect of metformin on platelet function in pregnant women with GDM.

Patients and methods

A parallel-group double-blind randomized controlled trial was conducted on pregnant women, attending antenatal care at Maharaj Nakorn Chiang Mai Hospital (Chiang Mai University, Thailand), between July 2020 and June 2021. The study received ethics approval from the Institute Review Board (study code: OBG-2562-06696) and was also registered on thaiclinicaltrials.org (TCTR20200517003). All participants were enrolled with written informed consent.

Study population

All recruited participants met the following inclusion criteria: (i) singleton pregnant women attending our antenatal clinic; (ii) maternal age of 20–45 years; (iii) diagnosed with GDM using the criteria recommended by the National Diabetes Data Group (screened with 50 g glucose challenge test, using cut off at 140 mg/dL and diagnosed using the standard 100 g oral glucose tolerance test (OGTT), diagnosed if two or more of the following thresholds were met (fasting 105 mg/dL, 1h 190 mg/dL, 2h 165 mg/dL, and 3h 145 mg/dL), before 32 weeks of gestation; (iv) having good glycemic control, defined as fasting blood glucose of <95 mg/dL, 2-h postprandial glucose of <120 mg/dL; (v) all women with GDM had normal medical histories before pregnancy. Exclusion criteria were as follows: (i) women with GDM whose blood

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glucose was poorly controlled, who needed insulin therapy before 28 weeks of gestation; (ii) women with systemic diseases such as chronic hypertension, heart disease, renal disease, connective tissue disease, hepatic disease, etc.; (iii) pregnancies with fetal major structural anomaly; (iv) obstetric complications: pregnancy-induced hypertension and preterm labor before 28 weeks of gestation in the current pregnancy; (v) poor obstetric history in previous pregnancy, for example recurrent pregnancy loss, previous occurrence of pre-eclampsia, and intrauterine growth restriction.

Randomization

During antenatal care, the participants were randomly assigned by the study coordinator into one of two study groups according to a computer-generated random allocation sequence with blocks of size of four, with a ratio of 1:1. Sequentially numbered sealed opaque envelopes would be employed to provide allocation concealment. The two groups included intervention (taking metformin) and control (taking placebo).

Study procedures

After informed consent was obtained from the women meeting the inclusion criteria, each woman in the intervention group received metformin (500 mg) 1 tablet oral once a day from day one of joining the study (27–31 weeks of gestation) throughout the entire study period. For the placebo group, each woman received one placebo tablet orally once a day. Both groups received the same standard antenatal care, intrapartum and postpartum management. The treatment was blinded to the participants and caretakers. Both groups received metformin or placebo from 27 to 31 weeks of gestation until the day they were admitted to the hospital for giving birth. For medication adherence measures, the study coordinator would use the pill count method which is an objective measurement. Both groups were counseled and monitored for lifestyle modification and diet control as our standard protocol in the management of GDM. The fasting and 2-h postprandial glucose levels were routinely assessed by the attending physician on every antenatal care visit and also self-home monitoring after training. The diet control was based on our nutritionists (Typically, the daily caloric intake of 30–35 kcal/kg including carbohydrate intake limited to 40% of total calories and the remaining calories apportioned to give 20% as protein and 40% as fat). Regular physical activity that incorporates aerobic and strength-conditioning exercise and avoiding smoking, as well as alcohol, were also advised. The target of treatment followed the recommendation of The American College of Obstetricians and Gynecologists (ACOG) (26).

Blood samples

Maternal blood samples for soluble P-selectin levels and 8IsoP were taken at 27–31 weeks gestation (the day of enrollment) and at 6 weeks after taking drugs. All of the 5 mL blood samples were drawn in a heparin tube then plasma was separated by centrifugation at 1036 g for 10 min. The separated plasma samples for measurement of soluble P-selectin and 8IsoP levels were stored at –80°C for subsequent testing in batches.

A maternal blood sample for MPV was obtained from all the participants in the third-trimester and intrapartum. To avoid the platelet swelling induced by EDTA, blood samples were analyzed within half an hour of collection (28). An automated blood counter was used to measure complete blood count (CBC) parameters including MPV.

Blood clotting time

The blood clotting time of all women was tested intrapartum (at the same time of collecting blood to measure MPV). The whole blood clotting test was performed. Two milliliters of collected venous blood were transferred directly into a clean and dry glass tube. It was maintained upright, open, undisturbed for 20 and/or 30 min at room temperature. After exactly 20 min, the tube was inverted. If a solid clot was retained, the test indicated normal coagulation. If the clot broke down quickly upon inversion of the tube or if there was no clot, the test indicated a coagulopathy (29). Additionally, an umbilical cord blood sample was obtained from all cases. The whole blood clotting test was performed using the same technique (Table 1).

Outcome assessment

The primary outcome was the measurement of the levels of soluble P-selectin and oxidative stress biomarker (8IsoP levels). The secondary outcomes were as follows: MPV of a pregnant woman with GDM, the blood clotting time of the women and cord blood, pregnancy outcomes including glucose monitoring level (fasting blood glucose and 2-h postprandial glucose), total weight gain, route of delivery, maternal anemia, antepartum hemorrhage, pregnancy-induced hypertension, and fetal weight and growth, postpartum hemorrhage, the maternal total length of stay,

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Table 1 Blood clotting grade.

	Interpretation	Visual appearance
Grade 0	Normal coagulation	A stable, solid clot that maintains its shape and clings to the glass vessel without issues after rotation of the tube
Grade 1	Abnormal, but there is some clotting activity apparent at the 20 and 30 min	There is some blood in solid form, but it fails to adhere to the glass as a single plug and either disintegrates completely (friable clot) or partially degrades ≤30 s after rotation
Grade 2	Abnormal with no clotting activity apparent at the 20 and 30 min	The entire blood sample remains in a free-flowing liquid state with no demonstrable clotting whatever upon rotation. This is immediately apparent during the rotation of the tube.

postpartum 75 g-OGTT, and neonatal outcomes including APGAR score (at 1, 5, and 10 min-of-life), birth weight, hypo- or hyperglycemia, anemia or polycythemia, the need for blood transfusion or antibiotics, respiratory distress syndrome, neonatal intensive care unit (NICU) admission, neonatal jaundice, the need for phototherapy or exchange transfusion, and neonatal length of stay.

Statistical analysis

All statistical procedures were performed using the statistical package for the social sciences (SPSS) software version 26.0 (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0: IBM Corp). The baseline data were presented as mean ± S.D. or median (interquartile range) for continuous data, as appropriate for data distribution. In comparisons of the categorical data, chi-square was used, whereas Mann–Whitney *U* test and Student's *t*-test were used for the comparisons of continuous data according to the normality of distribution which was tested using Kolmogorov–Smirnov test. A *P*-value less than 0.05 was considered statistically significant. This study needed a sample size of at least 32 participants per group for a randomized controlled study, given a power of 90% at a 95% CI with the acceptable error of

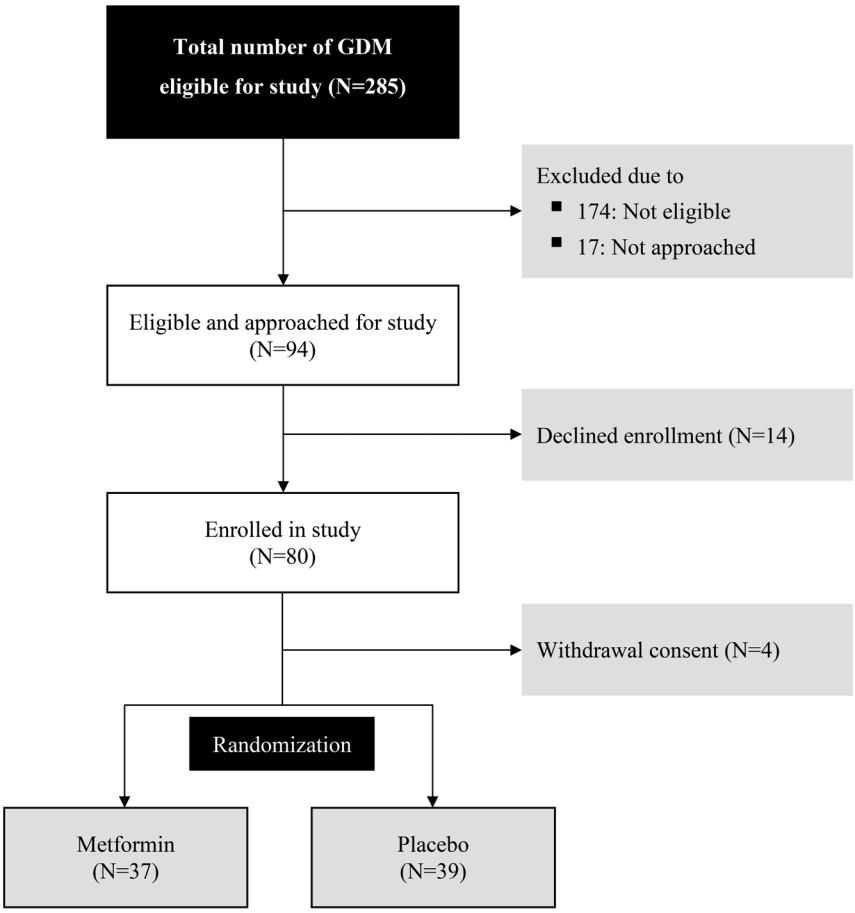

Figure 1
Flowchart of patient recruitment for randomization.

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0.1. This estimation is based on a study reported by Holmes *et al*. (30), who showed the mean P-selectin 55.4 ± 17.1 ng/mL vs 40.7 ± 16.9 ng/mL in the cases and controls.

Results

During the study period, 285 pregnant women were diagnosed with GDM. Of them, 174 were not eligible for recruitment and 17 were not approached. Ninety-four pregnant women met the eligibility criteria and were approached for enrollment, 14 declined to participate in the study. Finally, 80 were randomized into the two treatment groups (Fig. 1).

Maternal and labor characteristics by randomized treatment group are described in Table 2, the randomization achieved balanced groups. The baseline characteristics of the two groups were comparable. The median pre-pregnancy weight of both groups was around 57–60 kg, and the median maternal BMI was 23–24 kg/m^2. The maternal comorbidities were similar in both groups, such as migraine, history of thyroid disorders, or thalassemia carrier. The median gestational age at the time of diagnosis with GDM of both groups was approximately 20^{+5} weeks of gestation. Moreover, the median HbA1C was similar in both groups (HbA1C = 5.1%).

Primary outcomes

The primary outcomes are presented in Table 3. The baseline levels of 8IsoP at 27–31 weeks were not significantly different (*P*-value = 0.379) and the baseline levels of P-selectin was higher in the metformin group but not significantly different (*P*-value = 0.055) (Fig. 2).

Interestingly, while 8IsoP levels at early labor were comparable in both groups, P-selectin levels were significantly higher in the metformin group (*P*-value = 0.006) (Fig. 2). When using P-selectin levels of the placebo group as a baseline, the mean Z-score (±S.D.) of P-selectin levels at early labor is 0.734 + 1.271 (*P*-value = 0.006).

In each group, the levels of P-selectin at early labor were significantly higher than those determined at 27–31 weeks of gestation (*P*-value = 0.025). Also, the levels of 8IsoP were significantly increased (*P*-value < 0.032) in early labor, when compared to those at 27–31 weeks (Fig. 2).

Secondary outcomes

The secondary outcomes are presented in Table 4. Most of the various maternal and neonatal outcomes were not significantly different between the two groups. Nevertheless, maternal weight at delivery and total maternal weight gain were significantly lower in the

Table 2 Maternal and labor characteristics by randomized treatment group.

Characteristics	Placebo (*n* = 39)	Metformin (*n* = 37)	*P*-value
Maternal age (years)	32.2 ± 4.4	33.1 ± 5.1	0.428
Parity			0.262
Nulliparous	16 (40.0%)	21 (52.5%)	
Multiparous	24 (60.0%)	19 (47.5%)	
Education			1.000
High school or lower	6 (15.0%)	6 (15.0%)	
Higher education (higher than high school; including vocational/ high vocational certificate, Bachelor/Master degree, etc.)	34 (85.0%)	34 (85.0%)	
Pre-pregnancy weight (kg)	59.8 ± 13.3	56.8 ± 10.6	0.256
Height (m)	1.57 ± 0.05	1.56 ± 0.06	0.466
Maternal BMI (kg/m^2)	24.1 ± 5.0	23.3 ± 4.4	0.391
Maternal comorbidity	5 (15.0%)	9 (22.5%)	0.390
Preeclampsia screening			1.000
Low risk	35 (87.5%)	35 (87.5%)	
High risk	5 (12.5%)	5 (12.5%)	
Gestational age at diagnosed as GDM (days)	145 ± 53	148 ± 55	0.826
Gestational age at included in trials (days)	202 ± 7	201 ± 7	0.803
CBC at gestational age (day)	205 ± 6	205 ± 7	0.873
Hemoglobin (g/dL)	11.6 ± 0.9	11.8 ± 1.0	0.131
Hematocrit (%)	34.2 ± 2.5	34.7 ± 2.9	0.345
Platelet count (cell/mm^3)	238150 ± 58121	241025 ± 51350	0.817
Mean platelet volume (fL)	10.5 ± 1.1	10.1 ± 0.8	0.087
HbA1C at gestational age (days)	209 ± 13	212 ± 17	0.553
HbA1C level (%)	5.1 ± 0.4	5.1 ± 0.3	0.747
Laboratory tests at gestational age (days)	202 ± 7	202 ± 6	0.803

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Table 3 Primary outcomes by randomized treatment groups.

	Placebo (*n* = 39)	**Metformin** (*n* = 37)	***P*-value**
Maternal blood at 27–31 weeks' gestation			
8-isoprostane levels (pg/mL)	1167 ± 928	1022 ± 458	0.379
Soluble P-selectin (ng/mL)	58.6 ± 11.7	63.1 ± 8.8	0.055
Maternal blood at early labor			
8-isoprostane level (pg/mL)	1232 ± 603	1652 ± 1473	0.105
Soluble P-selectin (ng/mL)	60.6 ± 11.3	68.9 ± 14.4	0.006

metformin group (72.4 ± 12.5 vs 66.3 ± 8.8; *P*-value = 0.017 and 12.1 ± 5.3 vs 9.3 ± 6.0; *P*-value = 0.038, respectively).

Likewise, the birth weight of the newborns in the metformin group was significantly lower (3204 ± 393 vs 3018 ± 364; *P*-value = 0.037).

Discussion

This study provides new insight that metformin does not improve platelet dysfunction in the case of GDM, indicated by no significant difference in MPV, 8IsoP levels, and high P-selectin levels in the metformin group. In contrast to the expected, instead of decreasing P-selectin in the group of metformin, P-selectin was significantly increased, though the explanation of such a finding is unclear. Additionally, our findings consistent with those observed in previous studies were: (i) P-selectin levels are increasing with gestational age, as seen in normal pregnancy (30); (ii) metformin may improve maternal glucose control and result in preventing fetal hyperglycemia and macrosomia. Other parameters including MPVs, also reflective of platelet

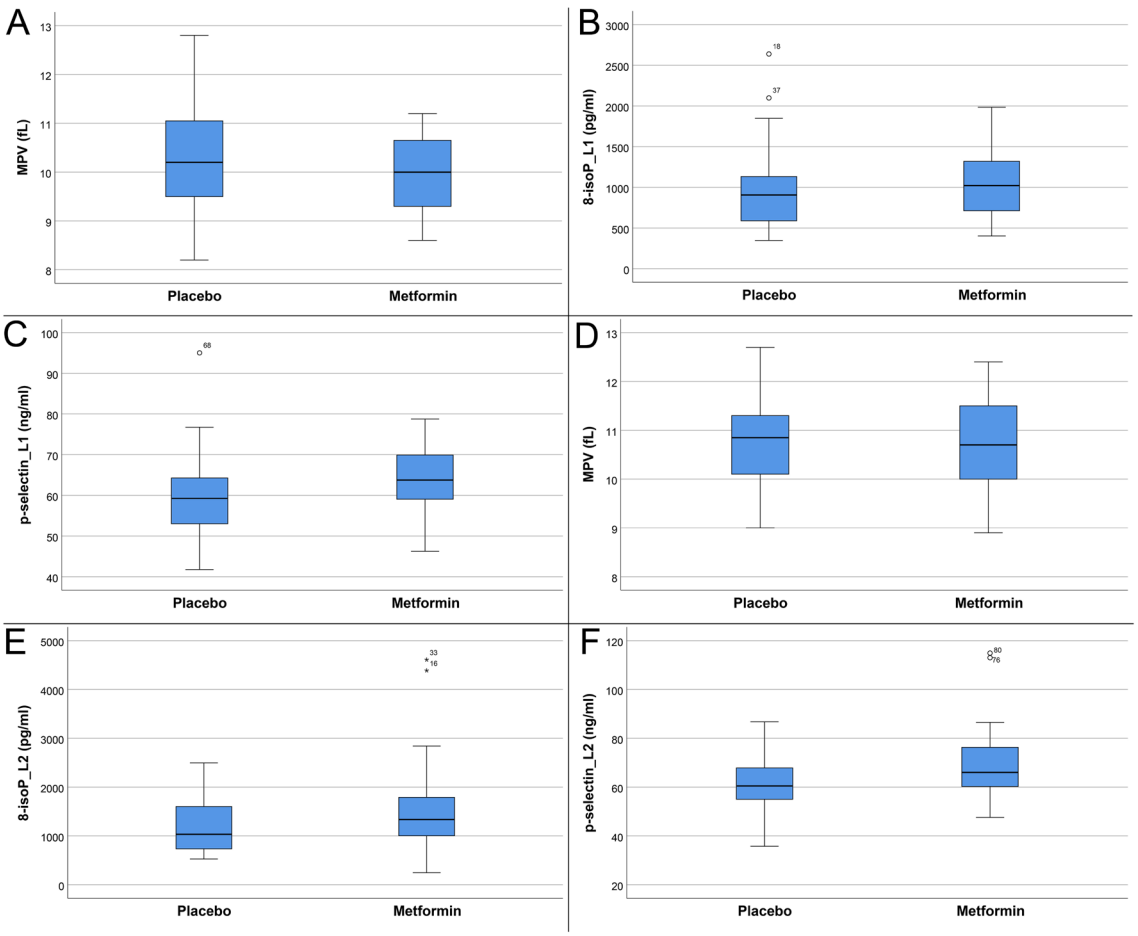

Figure 2
Comparisons of MPV, 8-isoprotane, and P-selectin levels between the two groups at baseline (A, B, and C) and after treatment (D, E, and F).

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Table 4 Secondary outcomes by randomized treatment group.

Variable	**Placebo** (*n* = 39)	**Metformin** (*n* = 37)	***P*-value**
Maternal outcomes			
Antepartum complications			
Preterm labor	0 (0.0%)	3 (7.5%)	0.077
Maternal anemia	3 (7.5%)	3 (7.5%)	1.000
Pregnancy-induced hypertension	2 (5.0%)	1 (2.5%)	0.556
Other conditions	3 (5.0%)	3 (5.0%)	1.000
Monitoring blood sugar throughout antenatal care			
Fasting blood sugar (mg%)	86 ± 6	87 ± 5	0.965
2-hr postprandial sugar (mg%)	102 ± 7	101 ± 8	0.531
Maternal weight at giving birth (kg)	72.4 ± 12.5	66.3 ± 8.8	0.017
Total maternal weight gain (kg)	12.1 ± 5.3	9.3 ± 6.0	0.038
CBC at gestational age that giving birth (weeks)			
Hemoglobin (g/dL)	12.5 ± 1.3	12.7 ± 1.0	0.578
Hematocrit (%)	36.7 ± 3.6	36.6 ± 2.3	0.901
Platelet count (cell/mm^3)	223428 ± 45084	225066 ± 53006	0.893
Mean platelet volume (fL)	10.8 ± 0.9	10.7 ± 0.9	0.564
Mean platelet volume change (fL)	0.51 ± 0.48	0.70 ± 0.49	0.137
Maternal clotting time			0.435
Grade 0 (clot)	34 (85.0%)	28 (70.0%)	
Grade 1 (partially clot)	1 (2.5%)	2 (5.0%)	
Grade 2 (not clot)	–	–	
Route of delivery			0.419
Normal delivery	24 (60.0%)	28 (70.0%)	
Vacuum or Forceps extraction	5 (12.5%)	2 (5.0%)	
Cesarean delivery	10 (25.0%)	6 (15.0%)	
Estimate blood loss (ml)	338 ± 272	245 ± 233	0.154
Perineal third degree tear	2 (5.0%)	2 (5.0%)	1.000
Postpartum 75-gm OGTT			0.642
No DM	14 (35.0%)	16 (40.0%)	
Impaired fasting glucose	15 (37.5%)	12 (30.0%)	
DM	1 (2.5%)	1 (2.5%)	
Neonatal outcomes			
Gestational age at birth (days)	274 ± 8	270 ± 8	0.059
Fetal growth restriction	1 (2.5%)	2 (5.0%)	0.556
Macrosomia (>4000 g)	2 (5.0%)	0 (0.0%)	0.152
Cord blood clotting time			0.612
Grade 0 (clot)	32 (80.0%)	29 (72.5%)	
Grade 1 (partially clot)	2 (5.0%)	1 (2.5%)	
Grade 2 (not clot)	–	–	
Gender of newborn			0.546
Male	21 (52.5%)	19 (47.5%)	
Female	18 (45.0%)	17 (42.5%)	
Birth weight (gm)	3204 ± 393	3018 ± 364	0.037
Low APGAR score (<7)			
At 1 minute-of-life	2 (5.0%)	0 (0.0%)	0.152
At 5 minute-of-life	–	–	
At 10 minute-of-life	–	–	
Neonatal blood glucose (mg%)			0.359
Hypoglycemia	0 (0.0%)	1 (2.5%)	
Normal blood glucose	39 (97.5%)	35 (87.5%)	
Hyperglycemia	–	–	
Neonatal hematocrit (%)	56.1 ± 6.3	56.7 ± 4.8	0.672
Anemic status			0.414
Anemia	1 (2.5%)	0 (0.0%)	
Normal	37 (92.5%)	35 (87.5%)	
Polycythemia	1 (2.5%)	1 (2.5%)	
Needs for neonatal blood transfusion	1 (2.5%)	0 (0.0%)	0.240
Hyperbilirubinemia			0.243

(*Continued*)

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Table 4 (Continued).

Variable	Placebo (n = 39)	Metformin (n = 37)	*P*-value
No	37 (92.5%)	32 (80.0%)	
Yes, needs for phototherapy	2 (5.0%)	4 (10.0%)	
Yes, needs for total exchange transfusion	1 (2.5%)	0 (0.0%)	
Respiratory distress syndrome	3 (7.7%)	4 (11.1%)	0.611
NICU admission	3 (7.5%)	6 (15.0%)	0.288
Total length of stay (day)	3.4 ± 2.1	3.3 ± 1.4	0.755

dysfunction, clotting time, and 8IsoP as an oxidative stress marker were also not significantly different between the two groups.

Note that both groups were under good glycemic control with diet and lifestyle modifications, as indicated by fasting blood and 2-h postprandial glucose levels throughout pregnancy, even in the placebo group. The findings signify that baseline of the metabolic status of both groups was relatively good. Thus, the effect of further treatment with metformin may be less obvious because of other intervention effects (diet and lifestyle modifications). Our findings could not be applied to pregnant women with overt DM who have had already vascular impairment before pregnancy. Moreover, the dosage of metformin used in our study was relatively low for pregnant women (500 mg/day), different from other studies which usually use 1500–2000 mg/day. This may also decrease the effect of metformin on platelet dysfunction if it exists.

Our finding of higher P-selectin levels in both groups was in agreement with that observed in normal pregnancy with advancing gestational age. In normal pregnancy, P-selectin concentrations were significantly higher in the second and third trimesters of pregnancy when compared with that of non-pregnant women sampled in parallel (30).

Previous studies showed that metformin protects diabetic patients from oxidative stress, leading to improving platelet dysfunction and a decrease in P-selectin (31). Nevertheless, women with overt DM and GDM have different vascular health. Patients with overt DM are usually associated with some degree of endothelial cell damage and stress oxidation whereas GDM usually develops in pregnant women with healthy vascular endothelial cells. It develops in response to human placental lactogen (HPL), the placental hormone of powerful insulin resistance. Theoretically, GDM is a temporary metabolic derangement of insulin resistance, not involving endothelial cell damages and platelet dysfunction. It usually completely disappears after delivery, as supported by a 75-g glucose tolerance test at 6 weeks after birth in this study. Therefore, we hypothesize that metformin does not improve vascular oxidative stress and platelet dysfunction in cases of healthy vessels. However, metformin may help prevent maternal and fetal hyperglycemia, improving glucose utilization leading to avoiding fetal macrosomia, as reflected by a significant reduction in fetal weight and maternal weight in late pregnancy as presented in Table 3. Convincingly, probably different mechanisms in improving fetal hyperglycemia result in significantly lower fetal size, theoretically preventing fetal macrosomia.

However, GDM may be different from DM type 2 (pre-gestational DM) which is usually associated with endothelial damage, healthy vascular endothelium, or less oxidative stress and inflammation. Therefore, oxidative stress is not as severe as seen in overt DM, and the effect of metformin in improving oxidative stress is not so obvious. Additionally, the levels of oxidative stress markers in both groups were not different, indicated by no significant difference in the 8IsoP levels in the two groups.

Random error might have occurred. The baseline vascular health might be different. The metformin group had a relatively higher level of P-selectin before treatment, though not significant (P-value = 0.062). With advancing gestational age, both groups had an increase in P-selectin levels. Higher levels in late gestation may be easier to express differences. Possibly the significance was untrue because of the baseline difference in P-selectin levels. However our results including high P-selectin, and no difference in 8IsoP levels and MPV, strongly suggests that metformin does not improve platelet dysfunction in cases of GDM.

We hypothesize that, with a larger sample size, P-selection and oxidative stress agents may be correlated with subtle endothelial cell damage in some cases and maybe a predictor of future development of overt DM among women with GDM.

The limitations of this study included (i) no long-term follow-up; (ii) glycemic control was also interfered from diet and lifestyle modification, not only metformin; Though diet and lifestyle are always difficult for perfect comparison, we believe that the advantage of a randomized control trial in terms of allocating the known and unknown confounders

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to the two groups equally was unlikely that diet and lifestyle could affect the interpretation of the comparison; (iii) Metformin dosage was relatively low for pregnancy, relatively short period of high insulin resistance secondary to placental HPL, possibly inadequate to show a small metabolic effect; (iv) possible random error, resulting in higher, though not significant, baseline levels of P-selectin in the metformin group; (v) Sample size was relatively small for secondary objectives. Strengths of this study were: (i) nature of its parallel-group double-blind randomized control trials; (ii) the comparisons were performed at two timeline periods; (iii) obstetric outcomes, neonatal outcomes, and postpartum follow-up to evaluate glucose status were also assessed to compare secondary outcomes.

In conclusion, metformin, in addition to diet and lifestyle modifications, does not seem to improve oxidative stress and platelet dysfunction. Nevertheless, metformin significantly reduces fetal weight in women with GDM, theoretically preventing macrosomia. To the best of our knowledge, this is the first trial to study the effect of metformin on platelet function in pregnant women with GDM.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Institutional review board statement
The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by Research Ethics Committee, Faculty of Medicine, Chiang Mai University (Study Code: OBG-2562-06696, Approval date: 8 August 2019).

Informed consent statement
Informed consent was obtained from all subjects involved in the study.

Data availability statement
The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Author contribution statement
P H: conceptualization, proposal development, acquisition of data, data validation, manuscript writing; R S: investigation, acquisition of data, data validation, manuscript final approval; T J: laboratory analysis, investigation, final approval; S K: laboratory analysis, investigation, final approval; C C: laboratory analysis, investigation, final approval; N C: laboratory analysis, investigation, final approval; T T: data validation, formal analysis, and manuscript editing, final approval; P J: conceptualization, data validation, acquisition of data, manuscript writing, final approval. All authors have read and agreed to the published version of the manuscript.

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