Thromboelastography parameters in Italian pregnant women: do antithrombotic drugs change reference values?

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ABSTRACT

This study was carried out to explore hemostasis modifications occurring in pregnant women and thromboelastography profiles in those taking antithrombotic drugs. An exploratory study was carried out in the period from March 2017 to May 2018. Caucasian women from Southern Italy were recruited during a routine obstetric assessment. Participants were divided into four groups: T1 (gestational week <14 weeks), T2 (14–28 weeks), T3 (29–42 weeks) and T4 in the postpartum period. We investigated thromboelastography profile in 19 and 5 women administered with low-molecular-weight heparin or low-dose aspirin, respectively. "MA" value observed in the T1 group was significantly greater than that observed in the T3 and the T4 groups, while "K" in the T1 group was significantly longer than that in the T3 and the T4 groups, indicating a gradual development of a prothrombotic state (in all cases Mann-Whitney U test, $p<0.05$). Significant differences within "R" were observed between the T2 and the T3 and between the T3 and the T4 ("R" parameter) (Mann-Whitney U test, $p<0.05$). "LY30" parameter resulted to be significantly higher in the T1 group (Mann-Whitney U test, $p=0.01$) compared with the T4 one, indicating fibrinolysis decreases throughout pregnancy and until post partum. No significant variations were found in women administered with prophylactic doses of low-molecular-weight heparin. Significantly higher fibrinolysis ($p<0.01$) was observed for "LY30" parameter in women taking low-dose aspirin versus women not taking any treatments. Our data contribute to better interpret thromboelastography profile in the context of peripartum complications, which are often unpredictable and need prompt therapies.

INTRODUCTION

During pregnancy and in post partum, hemostasis, coagulation and fibrinolysis systems show physiological modifications, which result in a hypercoagulable state. Although pregnancy may be a risk factor for thrombosis, nevertheless life-threatening bleeding during delivery represents one of the first causes of maternal mortality in developed countries. In a large WHO systematic analysis, which was carried out to examine maternal death cases, it was estimated that pregnant women mostly die because of major obstetric hemorrhage (MOH).[1] Pre-existing and emerging risk factors, known to predispose to a massive bleeding event, range from abnormal placentation, previous cesarean sections, multiple pregnancies, high body mass index, prolonged labor to congenital or acquired bleeding disorders.[2] Disseminated intravascular coagulation is an important medical problem and is associated with bleeding and maybe a severe complication of MOH.[3] Management of MOH should be based on a prompt decision on treatments. A close monitoring of hemoglobin and clotting factors fluctuations may be an efficacious clinical approach to quickly identify possible bleeding events.[4] Thus, viscoelastic point-of-care testing, such as thromboelastography (TEG), may help to facilitate and optimize clinical decisions. However, there are challenging aspects which should be faced with regards to TEG clinical applications, especially in obstetrics, where reference ranges still need to be definitely determined. We looked at TEG parameters, which separately give information on a global process, as an effort made to define reference ranges values of TEG during pregnancy in a population from Southern Italy.

Furthermore, we show data in a group of pregnant women who were administered with prophylactic doses of low-molecular-weight heparin (LMWH) and/or low-dose aspirin, in order to explore possible modifications during the administration of these drugs.

METHODS

We carried out a time-limited exploratory study in the period from March 2017 to May 2018. We consecutively enrolled 68 pregnant women, recruited during a routine obstetric assessment or in the context of prenatal counseling. A detailed collection of obstetric history was performed to ascertain the presence of any clinically relevant bleeding events that occurred during previous pregnancies. We excluded women with a diagnosis of abnormal platelet functions or congenital bleeding disorders, as

well as twin pregnancies. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were investigated in all women. We recruited four groups of women: T1 included those in the first trimester (gestational week <14 weeks); T2, those in the second trimester (14–28 weeks); T3, those in the third trimester (29–42 weeks); and T4, those who had just delivered (median days after delivery=1 (range 1–3)). Blood samples were collected in 4.5 mL tubes containing 3.8% sodium citrate solution (0.129 M). All TEG assays were started within 30 min, using a TEG 5000 Thrombelastograph Hemostasis Analyzer system (Haemonetics, Braintree, MA, USA). We performed citrate kaolin test and citrate functional fibrinogen test, which require a 340 µL aliquot of whole blood samples pipetted into a warmed cuvette and tested after recalcification with 20 µL of 0.2 M CaCl.[5]

Among enrolled women, 19 and 5 women were being treated by enoxaparin (4000 U/day) or low-dose aspirin (100 mg/day), respectively, and clinical indications were history of gestational vascular complications (GVCs) or venous thromboembolism.

Blood samples for TEG assays were collected 12 hours after the heparin injection or ASA administration.

STATISTICAL ANALYSIS

Data obtained in the four groups were compared to verify the existence of possible differences across trimesters and between trimesters and post partum.

Mann-Whitney U non-parametric test was used to analyze statistical differences between TEG parameters; a non-parametric Spearman coefficient was calculated to investigate the presence of any correlation between TEG parameters and PT and aPTT. Mann-Whitney U non-parametric test was used to examine statistical differences in prophylaxis groups. Data on TEG performed in Italian pregnant women, especially in those who undergo pharmacologic antithrombotic prophylaxis, are scarce. Thus, we did not perform a power analysis because we had no data on which we would have based calculations. Furthermore, we carried out a time-limited exploratory study in the period from March 2017 to May 2018 that allowed us to consecutively enroll 68 women.

Statistical analysis was performed employing the SPSS statistical software, whereas graphs were made using GraphPad Prism V.8.0 for Windows (GraphPad Software, San Diego, CA, USA; www.graphpad.com).

RESULTS

Ten women were enrolled during their first trimester (T1 group), 16 within second trimester (T2 group), 18 in their third trimester (T3 group) and 24 in post partum (T4 group). All were Caucasian and came from Southern Italy. Clinical and laboratory features are illustrated in table 1. All women showed clotting tests within normal ranges. Most of them experienced previous pregnancies (n=45) and gave birth by vaginal delivery in the index pregnancy (table 1).

Among those experiencing previous pregnancies, 12 (26.6%) had early or late pregnancy losses, 3 (6.6%) pregnancy-related vein thrombosis, 1 (2.2%) intrauterine fetal growth restriction (IUGR) and 1 (2.2%) pregnancy-related hypertensive disorder; no relevant bleeding episodes during pregnancy/peripartum were reported. Only two GVCs were observed in the index pregnancies: one IUGR and one premature rupture of membranes (table 1). In both cases, TEG traces were not significantly different from those observed in uncomplicated pregnancies (data not shown). Values of TEG parameters measured in all the trimesters and post partum are summarized (online supplementary table 1). We observed significant differences between groups ($p<0.05$, Mann-Whitney U test). More in detail, we found the value of "angle" measured in T1 significantly smaller than that in T2 and "K" time in T1 was significantly longer than that observed in T2 ($p<0.01$, figure 1A,B), indicating a different kinetic of clot formation between these trimesters. Moreover, we found that clot strength in the first trimester was significantly weaker than that observed in the third one, as demonstrated by a significantly shorter "MA" found in T1 compared with T3 ($p<0.05$, figure 1C).

We also observed a statistically significant difference between TEG parameters measured in the first trimester and post partum. The "angle" measured in T1 was smaller than that observed in the T4 group, and "K" time measured in the T1 group resulted significantly longer than in the T4 ($p<0.01$, figure 1D,E); furthermore, the "MA" observed in T1 group was significantly shorter than that found in T4 ($p<0.05$, figure 1F).

Lysis rate observed at 30 min in T1 group was significantly different from that observed in T4 ($p=0.01$, Mann-Whitney U test) ($p<0.05$, figure 1G), and this rate progressively decreased throughout pregnancy and post partum (online supplementary graph 1). These data provide support to the concept that pregnancy is characterized by hypercoagulability and decreased fibrinolysis.

Table 1 Clinical and laboratory data of the whole sample (n=68)

Patients' features	Values
Age, years*	34 (22–42)
BMI*	22.9 (16.4–31.2)
Prothrombin time, INR*	0.95 (0.9–1.1)
Activated partial thromboplastin time, ratio*	0.94 (0.8–1.2)
Blood group, O vs non-O	29/39
Thrombophilia yes, n (%)	7 (10.3)†
Gravidity*	2 (1–7)
Parity*	0 (0–6)
Previous GVCs/vein thrombosis	14/3
Previous CS, n	15
Index pregnancy	
LMWH/aspirin, n	19/5
GVCs	2
Cesarean section, n (%)	27 (39.7)
Vaginal delivery, n (%)	41 (60.3)
Gestational weeks at delivery*	38 (33–41)
Birth weight*, g	3295 (1600–4100)
Newborn sex, male/female	26/42

*Values are provided as median and (range).
†Five women were factor V Leiden and 2 prothrombin mutation heterozygous carriers.
BMI, body mass index; CS, cesarean section; GVC, gestational vascular complication; INR, international normalized ratio; LMWH, low-molecular-weight heparin.

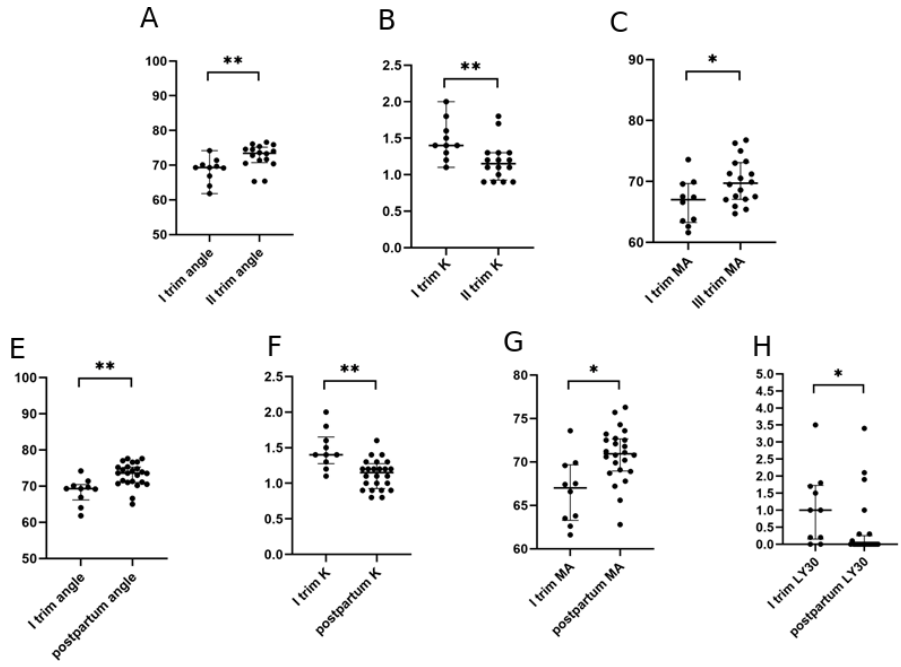

Figure 1 (A) Dot plot displaying differences within "angle" parameter between first and second trimesters. (B) Dot plot displaying differences within "K" parameter between first and second trimesters. (C) Dot plot displaying differences within "MA" parameter between first and third trimesters. (D) Dot plot displaying differences within "angle" parameter between first trimester and post partum. (E) Dot plot displaying differences within "K" parameter between first trimester and post partum. (F) Dot plot displaying differences within "MA" parameter between first trimester and post partum. (G) Displaying differences within "LY30" parameter between first trimester and post partum. *p<0.05, **p<0.01.

Statistically significant differences were also observed with regard to the "R" value. Indeed, women in T2 showed a significantly shorter "R" than those in T3 (p<0.05, Mann-Whitney U test; online supplementary graph 2a). A significantly longer "R" was observed in T3 in comparison with T4 (p<0.05, Mann-Whitney U test; online supplementary graph 2b), suggesting that in the third trimester, rate of fibrin formation may be lower in comparison with post partum.

We then performed comparison of TEG parameters according to the antithrombotic treatment. We compared women treated by LMWH (n=19) or those taking low-dose aspirin (n=5) with women not taking any antithrombotic treatments (n=44). No statistically significant differences were found between women in prophylaxis with LMWH and women not taking any antithrombotic drugs. On the other hand, we found statistically significant differences for "LY30" parameter between women taking low-dose aspirin and those not taking any antithrombotic drugs (p<0.01, Mann-Whitney U test; online supplementary graph 3).

DISCUSSION

The present study focused on physiological hemostatic changes occurring during pregnancy and aimed at contributing to set reference ranges of TEG-derived parameters measured in Italian pregnant women. Our data confirm and extend those previously reported[6] and contribute to better define reference ranges in pregnant women.[7]

Furthermore, the study explores possible changes in those taking antithrombotic drugs.

Most of the statistically significant differences were observed between the first trimester and the postpartum period. "K", "angle" and "MA" provide information on clot kinetic and strength. Specifically, lower and higher values of the "K" and "angle" parameters are mainly associated with a hypercoagulable state, as well as higher values of the "MA" parameter. We observed that women in the first trimester have a reduced clot kinetic and strength, respectively, compared with those observed post partum. These differences were also observed when the first trimester was compared with the second and the third ones, respectively. We confirm previous data showing that in the second half of pregnancy and post partum,[8] the trend of prothrombotic state increases, as demonstrated by the "R" values. Clinical data document that incidence rate ratio of venous thromboembolism in pregnant and puerperal women is higher in the last weeks of pregnancy and in the first week after delivery.[9] Furthermore, our findings substantiate those obtained by other authors,[8 10] showing that the hypercoagulable state in pregnancy gradually develops, based on the "K", "angle" and "MA" values throughout pregnancy and post partum. In agreement with previous studies, lysis rate measured at 30 min (LY30) showed that fibrinolytic activity decreases throughout pregnancy.[8 10] This reflects the increased expression—mainly by placenta—of PAI-2 levels.[11]

As previously reported, no correlation between TEG and plasma-based clotting tests was observed. This can be due to a substantial difference in the methodological approach to the haemostatic process.

We then examined the effect of LMWH and low-dose aspirin on TEG-derived parameters. We were not able to identify any significant differences between women administered with LMWH and those who did not take any antithrombotic drugs. The present data are consistent with those previously reported in a study carried out—using ROTEM technology—in 82 women taking LMWH. Indeed, in that study, a maximal clot firmness was shown without any modification of clot formation time.[12] On the other hand, we confirm previous *in vitro* findings showing that only supratherapeutic anti-factor Xa levels are detected by viscoelastic tests.[13] Actually, we enrolled only women taking prophylactic doses of LMWH, thus based on the aforementioned findings, we did not expect changes in TEG profile.

With regards to aspirin, a significantly higher lysis rate was detected in pregnant women taking low-dose aspirin compared with those not taking any drugs.

As previously reported, we find that low-dose aspirin may induce a higher lysis rate in pregnant women, without exhibiting any influences on other TEG parameters.[14] In 1992, a study was published reporting comparison of TEG parameters measured in pregnant and non-pregnant women before and after the aspirin administration.[14] In that study, fibrinolysis rate was not measured and all the remaining TEG parameters did not significantly change after aspirin administration in pregnant and non-pregnant women. Similarly, we did not find influence of aspirin on TEG parameters. When we analyzed the fibrinolysis rate, we found it significantly higher in those women taking low-dose aspirin. We speculate that aspirin may have an effect on thrombus stability and impact on fibrinolysis, inducing biochemical modifications of fibrinogen. Actually, it has been reported that aspirin may intensify fibrinolysis, increasing acetylation of fibrinogen and, in turn, fibrin clot permeability.[15]

Our data contribute to add knowledge on viscoelastic modifications in pregnant women who are frequently prescribed with antithrombotic drugs because of GVCs. They could help in the interpretation of TEG profile in the context of peripartum thrombotic and bleeding complications, which are often unpredictable and need a prompt and efficacious approach.

Contributors GLT drafted and designed the work. ADL, FC and PV performed TEG assays. FC, GF and EC analyzed and interpreted data. PV and EG selected patients. AO, MM and EG conceived the work and revised it critically for important intellectual content. All authors approved the final version of the work submitted.

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