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The use of D-dimer with new cutoff can be useful in diagnosis of venous thromboembolism in pregnancy

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ABSTRACT

Objective: D-dimer testing has an important role in the exclusion of acute venous thromboembolism (VTE) in the nonpregnant population. Establishing D-dimers role in the diagnosis of VTE in pregnancy is hampered because of the substantial increase of D-dimer throughout gestational age.
Study design: In a prospective study we followed 89 healthy pregnant women to establish the reference range of D-dimer for each trimester. D-dimer testing was also performed in 12 women with clinical suspicion of VTE and their results were compared with the established new reference range of D-dimer, and with the recorded ultrasound findings.
Results: In the first trimester, 84% women from reference group had normal D-dimer, in the second 33%, and by the third trimester only 1%, which suggests that D-dimer has no practical diagnostic use in ruling out VTE if the threshold of 230 ng/mL for abnormal is used. All pregnant women with thrombosis who had positive ultrasound findings also had statistically significant elevation of the D-dimer level, considering the established reference range of the corresponding trimester. We found 100% sensitivity of D-dimer test. A women developed thrombosis in the first trimester had 6.7–7.6 time higher level of D-dimer than the mean value in the reference group, and in the third trimester thrombotic women had 2.0–3.8 time higher level of D-dimer, $p < 0.0001$.
Conclusion: D-dimer test with the new threshold for: the first of 286, the second of 457 and the third trimester of 644 ng/mL can be useful in diagnosis of pregnancy related VTE.

1. Introduction

Pregnancy is a major risk of thrombosis, and thromboembolic complications are the leading causes of maternal as well as fetal morbidity and mortality in developed countries [1–4]. The overall incidence of VTE is 1 per 1000 pregnancies and risk of thrombosis is increased throughout pregnancy and is particularly high after the delivery [5]. The clinical diagnosis of VTE in pregnancy is unreliable, because of the fact that since problems such as leg swelling and discomfort are common features of normal pregnancy.

The primary diagnostic tool for DVT is compression ultrasonography [6]. This test does not reliably detect isolated iliac vein thrombosis that occurs relatively frequently during pregnancy, or calf vein thrombosis [7]. Failure to identify VTE places the mothers life at risk, while unnecessary treatment will expose her not only to anticoagulants but also will label her as having had a VTE and this can have an impact on her future healthcare [8].

Therefore, objective, quick, non-invasive and safe test with respect to the fetus are required. Although D-dimer testing has an important role in the exclusion of VTE out of pregnancy, inclusion of D-dimer in algorithms of VTE diagnosis in pregnancy has not been adequately studied. Establishing D-dimers role in the diagnosis of VTE in pregnancy is hampered because of substantial increase of D-dimer throughout gestational age [9].

The objective of our study was to investigate level of D-dimer during each trimester of pregnancy and to establish the reference range for each trimester. We also performed D-dimer tests in 12 women with clinical suspicion of VTE and compared their results with the reference range of the reference group, and with the recorded ultrasonography findings to define sensitivity and correlation with the ultrasonography findings.

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2. Patients and methods

In a prospective study, from January 2007 to October 2008, 107 pregnant women in early pregnancy, age 18–40 years (median 31 years), were initially referred to our Center of Hemostasis Research to be investigated using hemostasis tests, with purpose to establish the reference range for D-dimer during pregnancy.

Including criteria: Healthy pregnant women without personal or family data about thrombosis were included in the study. With the purpose to minimize the possible influence of some diseases on D-dimer level, pregnant women with: diabetes, SLE, chronic hypertension, hepatic or renal diseases were excluded from the study. Before inclusion in the follow up study thrombophilia testing was performed in all 107 pregnant women. After thrombophilia testing was performed, 18 pregnant women with thrombophilia presence were excluded from the study, and 89 pregnant women were followed up during their pregnancy as the reference group.

Laboratory testing: At the first visit in their first trimester (8–12 weeks of gestation) history data were recorded and informed consent was obtained from all participants. After recording their history during the first visit, blood was drawn for hemostasis tests: prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, factor VIII, thrombophilia and D-dimer testing. The laboratory analyses for thrombophilia testing included the assessment of antithrombin, protein C, protein S, presence of lupus anticoagulans (LA), activated protein C resistance (APC-R). For the detection of thrombophilia and other hemostasis tests, IL tests (Instrumentation Laboratory, Milan, Italy) were used. For D-dimer testing HemosIl D-dimer HS (IL), rapid automated quantitative latex-based immuno-agglutination assay was used, with reference value up to 230 ng/mL [10]. Analyses were performed on IL Coagulometars ACL 6000 and Elite Pro (Instrumentation Laboratory, Milan, Italy). DNA analyses for FV Leiden, PT-G20210A and MTHFR C677T mutations were detected by polymerase chain reaction as previously described [11].

Pregnant women were seen in the second trimester (22–24 weeks of gestation), the third trimester (32–34 weeks of gestation), and for the last time 6–8 weeks after delivery. At this visit, blood was drawn for D-dimer testing. According to the prior recommendation of Morse [12], who established the reference range for their local population of the pregnant women, we calculated the new cutoff for each trimester, as a mean value of D-dimer for all group plus one standard deviation.

Twelve pregnant women who had clinical suspicion of VTE were included in the study as thrombosis group. All women included in thrombosis group were admitted to Gynaecology and Obstetrics Clinic, where was the therapy included (low molecular weight heparin in doses of 200 IU/kg). For all women blood samples were taken for D-dimer testing during the first, second and the seventh day of admission. In women with confirmed DVT, D-dimer testing was done once weekly during anticoagulant therapy treatment. Women with unconfirmed VTE were discharged from hospital and they were referred to regular control of D-dimer in 2 weeks until the termination of the pregnancy. To point the presence of thrombosis process the Colour Doppler sonography test was used in all of them. For the detection of deep venous thrombosis of legs, the linear transducer of 6–8 MHz was used and for pelvic veins transducer of 3–5 MHz was used. Doppler sonography was repeated after 7 days, and in women with established deep venous thrombosis after 1, 3 and 6 months. The following clinical history data were used: age, gestational week of thrombosis, data about personal or family presence of thrombosis, hormonal stimulation before pregnancy and records of Doppler sonography that confirmed or rejected presence of acute deep vein thrombosis. Reference range of D-dimer for each trimester, obtained from healthy pregnant women, was used in comparison with the results obtained from women with suspicion of VTE. After delivery (3 months) all women from thrombosis group were tested on thrombophilia presence. Institutional approval for the study was granted by The Clinics Ethics Committee in accordance with internationally accepted ethical standards (The Helsinki Declaration of 1964, as revised 1975,1983 and 1989).

3. Statistical analysis

Descriptive statistics such as the mean, standard deviation (SD) and range were calculated for each variable. To test statistical difference in D-dimer concentration between each trimester in reference group, and difference between D-dimer concentration between reference group and thrombosis group, Student's *t*-test was used. p values less than 0.05 were considered statistically significant.

4. Results

Prospective study included 89 healthy pregnant women in their early pregnancy, and 12 pregnant women with clinical suspicion of acute VTE. The baseline characteristics of the study population are shown in Table 1. Two women had miscarriages in the 17th and the 18th gestation week, and were excluded from the study during the second trimester. During the third trimester (the 32nd gestation week), the D-dimer level rise of 1078 ng/mL was recorded, during preeclampsia as pregnancy complication, in one pregnant woman and she was also excluded from the study.

Hemostasis test results performed in the reference group showed significant increases of D-dimer concentration during pregnancy and no abnormal coagulopathies. The mean D-dimer concentration in the first trimester in the amount of 222 ng/mL, ranging 121–474, in the second trimester of 326, ranging 171–733, and in the third of 475 ng/mL, ranging 206–890, indicate a 46% increase of D-dimer concentration, from 12 to 24, and from 24 to 34 weeks of gestation. The difference of D-dimer level between the first and the second and between the second and the third trimester is statistically significant $p < 0.0001$ (Student's *t*-test). In the first trimester, 75 of 89 (84%) women had a normal D-dimer. The number of women with normal D-dimer decreased to 29 of 87 (33%) in the second trimester, and by the third trimester 1 of 86 (1%) women had normal D-dimer. The mean D-dimer concentration after delivery in the amount of 223 ng/mL (ranging 110–390) showed no statistically significant difference between the first trimester and after the delivery, $p = 0.933$ (Table 2).

In Table 3 the concentrations of D-dimer in healthy pregnant women and in 10 pregnant women who developed DVT are shown. Two pregnant women developed thrombosis in the 9th and in the 12th week of gestation, and D-dimer of 1500 ng/mL and 1691 were

Table 1
Characteristics of the study population.

	Healthy pregnant women (reference group) ($N = 89$)	Women with suspicion of VTE during pregnancy ($N = 12$)	p
Age, median (range)	31 (20–40)	29 (19–39)	0.4789
Number of previous pregnancy	138	6	
One	37	5	
Two	25	1	
More than two	17		
Previous DVT, *n*	0	1	

DVT (deep venous thrombosis).

Table 2
D-dimer concentration and number of pregnant women with D-dimer up to 230 ng/mL (cutoff out of pregnancy) in reference group.

Week of gestation	8–12	22–24	32–34	6–8 w. postpart.
D-dimer (ng/mL)				
Mean ± SD	222 ± 64	326 ± 131	475 ± 169	223 ± 59
Range	121–474	171–733	206–890	110–390
Women with D-dimer up to 230 ng/mL[a], *n* (%)	75 (84)	29 (33)	1 (1)	76 (88)
N	89	87	86	86

$p < 0.0001$ difference between first and second, second and third trimester. $p = 0.933$ difference between the first trimester and after delivery.
[a] Cutoff for exclusion of DVT out of pregnancy.

Table 3
D-dimer (ng/mL) in reference group and in women who developed DVT during pregnancy.

Week of gestation	D-dimer concentration	Healthy pregnant women	Women with confirmed DVT	*p*
8–12	Mean ± SD	222 ± 64	1596 ± 95	<0.0001
	Range	121–474	1500–1691	
22–24	Mean ± SD	326 ± 131	1330 ± 700	<0.0001
	Range	171–733	524–1784	
32–34	Mean ± SD	475 ± 169	1157 ± 374	<0.0001
	Range	206–890	922–1818	
N		89	10	

DVT (deep venous thrombosis).

recorded. In four women who developed thrombosis during the second trimester, the mean D-dimer concentration of 1330 ng/mL, ranging 524–1784 was recorded. In the group of pregnant women who developed thrombosis during the third trimester, the mean D-dimer concentration of 1157 ng/mL, ranging 922–1818, was recorded. The difference between the reference group and the women with DVT was statistically significant, $p < 0.0001$. Women with the first trimester thrombosis had 6.7–7.6 time higher level of D-dimer than the mean value in the reference group. In women with the second trimester thrombosis, 1.6–5.4 time higher level of D-dimer was recorded compared with the mean value in the reference group, and the third trimester thrombotic women had from 2.0 to 3.8 time higher level of D-dimer than the mean value in the reference group, $p < 0.0001$ (Student's *t*-test). In two pregnant women due to suspicion of VTE in the 26th and in the 32th week of gestation, D-dimer concentration of 414 and 472 ng/mL, respectively, were recorded. The obtained results were within reference range considering D-dimer concentration in the reference group, and no statistically significant difference was observed between this women and the reference group (Fig. 1). The presence of VTE was excluded based on ultrasonography and scintigraphy findings in both cases.

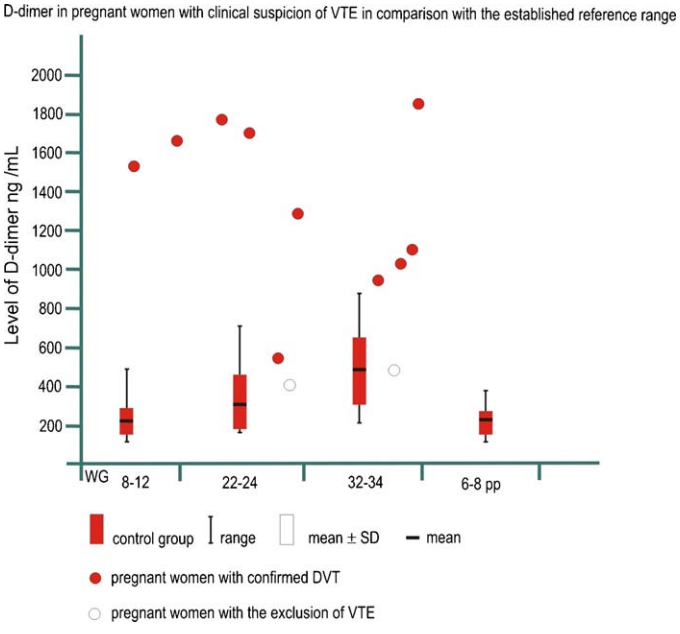

Fig. 1. D-dimer in pregnant women with clinical suspicion of VTE in comparison with established reference range.

5. Discussion

Although D-dimer testing has important role in the exclusion of VTE out of pregnancy, inclusion of D-dimer in algorithms for diagnosis VTE in pregnancy has not been adequately studied [9]. Establishing D-dimers role in the diagnosis of VTE in pregnancy is hampered because of substantial increase of D-dimer throughout gestational age [13,14]. However, the interpretation of the D-dimer level depends on the test used to perform the assay and the used cutoff values [15]. In our study the quantitative latex immunoagglutination D-dimer assay was used with reference value up to 230 ng/mL (out of pregnancy) [10].

The aim of our study was to establish the reference range of D-dimer per each trimester during pregnancy and the following step was comparison with the results obtained from pregnant women with clinical suspicion of VTE. It is most probably the first time that D-dimer testing was used to aid in the diagnosis of VTE in pregnancy with the new threshold, using comparison with the previously established reference range, obtained from healthy pregnant women in their pregnancy.

Our study shows a change towards a hypercoagulable state with the increased concentration of blood coagulation factors and D-dimer concentration which was confirmed in previous studies [16,17]. As expected we found the increase of the D-dimer concentration during pregnancy, with the highest level during the third trimester. The mean D-dimer concentration in the first trimester of 222 ng/mL, in the second of 326 and in the third of 475 ng/mL, indicates a 46% increase of D-dimer concentration, from 12 to 24, and from 24 to 34 weeks of gestation. Our results showed that in the first trimester, 84% women had normal D-dimer, in the second 33%, and by the third trimester 1% of women

had normal D-dimer, which suggests that D-dimer has no practical diagnostic use in ruling out VTE if the threshold for abnormal 230 ng/mL is used.

In the study of Kline et al. 50 pregnant women were included and tested during each trimester of pregnancy using D-dimer assay (MDA immunoturbodimetric Assay, Organon Teknika) with reference range of 0.5 mg/L (out of pregnancy). They showed that in the first trimester 50% of women had D-dimer level within the reference range (out of pregnancy), in the second only 22%, and in the third trimester 0%, which is similar to our results [18].

In Morse's study, there were 48 pregnant healthy women and 34 women out of pregnancy as a control group to establish a normal range of D-dimer levels in pregnancy. He also used IL D-dimer assay for testing all participants and his new reference range for the second trimester <465 ng/mL and for the third <640 ng/mL are similar with our results, for the first trimester <286 ng/mL, for the second <457 ng/mL and for the third <644 ng/mL [12].

In pregnant women who developed deep venous thrombosis during pregnancy, D-dimer testing was performed during the period of clinical suspicion of DVT. Comparison of the results obtained from women with suspicion to DVT with previously established reference range showed statistically significant differences. When we compared D-dimer values obtained from thrombotic women with a new cutoff, ratio in the first trimester was 5.1–5.9, 1.2–3.4 in the second and in the third trimester it was 1.5–2.8, which points to the lower specificity of D-dimer test decreasing as pregnancy progresses.

Chan et al. also pointed to the lower specificity of D-dimer during the second and especially the third trimester. In their study the qualitative Red Blood Cell Agglutination assay (Simply RED assay) was used during the investigation of a study group of 145 pregnant women [19].

In 10/12 cases, positive results of D-dimer correlated with ultrasonography findings. Even though in one case we had positive D-dimer test and negative ultrasound, clinical findings required the introduction of the low molecular weight heparin (LMWH). Repeated D-dimer test showed significantly increased level of D-dimer and findings of ultrasound performed after 7 days, showed acute venous thrombosis of the left femoropoplitealis vein. In this case positive D-dimer test points to the development of DVT before the visualization of thrombotic process using ultrasound. This case points to the fact that clinical utility of D-dimer measurement in individual patients should be interpreted in conjunction with the clinical risk factor and clinical condition.

In the remaining two pregnant women with clinical suspicion of VTE D-dimer concentrations were in reference range compared with the reference group. In both cases presence of VTE was excluded using radiological procedures (scintigraphy and ultrasonography). There was a good correlation between the results of D-dimer testing and radiological procedures. In all women who developed thrombosis, confirmed with ultrasonography findings, D-dimer test was positive, sensitivity of D-dimer was 100% and this assay has demonstrated a negative predictive value (NPV) of 100%.

The concentration of D-dimer in women with confirmed DVT showed the tendency to decrease during the anticoagulant therapy use. The initial D-dimer concentration, which was significantly elevated in comparison with the new reference range dropped after 4 weeks to the level which is expected for corresponding trimester. In two pregnant women with unconfirmed thrombosis process, the level of D-dimer showed expected gradual elevation throughout the pregnancy, which was within the new reference range.

Among the limitations of this study, that should be considered is a small number of patients (only 12) with suspicion of pregnancy related VTE. In this respect, it should be considered as a pilot study.

In conclusion, our results showed that D-dimer test with the new threshold for: the first of 286, the second of 457 and the third trimester of 644 ng/mL, can be useful in diagnosis of pregnancy related venous thromboembolism. A negative D-dimer test may be helpful if ultrasonography findings are normal, whereas a positive D-dimer test requires additional diagnostic testing. Because of several advantages such as the fact that D-dimer testing is quick, relatively non-invasive, does not harm the fetus, its role in pregnant women should be furthermore investigated in a larger prospective study.

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References

[1] Greer I. Diagnosis prevention and treatment of gestational venous thromboembolism. In: Brenner B, Marder V, Conard J, editors. Womens issues in thrombosis and haemostasis. Martin Dunitz Ltd., a member of the Taylor & Francis Group; 2002. p. 182–97.
[2] Brenner B. Haemostatic changes in pregnancy. Thromb Res 2004;114:409–14.
[3] Bremme K. Haemostasis in normal pregnancy. In: Brenner B, Marder V, Conard J, editors. Womens issues in thrombosis and haemostasis. Martin Dunitz Ltd., a member of the Taylor & Francis Group; 2002. p. 151–65.
[4] Bremme K, Ostlund E, Almqvist I, et al. Enhanced thrombin generation and fibrinolytic activity in the normal pregnancy and the puerperium. Obstet Gynecol 1992;80:132–7.
[5] Ginsberg JS, Brill-Edwards P, Burows RF, et al. Venous thrombosis during pregnancy: leg and trimester of presentation. Thromb Haemost 1992;67:519–20.
[6] Ginsberg JS, Hirsh J, Rainbow AJ, Coates G. Risk to the fetus of radiologic procedures used in the diagnosis of maternal venous thromboembolic disease. Thromb Haemost 1989;61:189–96.
[7] Kaeron C, Julian JA, Newman TE, Ginsberg JS. Noninvasive diagnosis of deep venous thrombosis. McMaster Diagnostic Imaging Practice Guidelines Initiative. Ann Intern Med 1998;128:663–77.
[8] Greer I. Inherited thrombophilia and venous theomboembolism. Best Pract Res Clin Obstet Gynaecol 2003;17(3):413–25.
[9] Eichinger S. D-dimer testing in pregnancy. Semin Vasc Med 2005;5(4):375–8.
[10] Scarvelis D, Palareti G, Toulon P, et al. HemosIL D-dimer HS assay in diagnosis of deep vein thrombosis and pulmonary embolism. Results of a multicenter management study. J Thromb Haemost 2008;6:1973–5.
[11] Djordjevic V, Rakicevic LJ, Spasic M, et al. FV Leiden, F II G20210A MTHFR [677C] mutations and first venous thromboembolism during pregnancy and puerperium. Vojnosanit Pregl 2004;62:201.
[12] Morse M. Establishing a normal range for D-dimer levels through pregnancy to aid in the diagnosis of pulmonary embolism and deep vein thrombosis. J Thromb Haemost 2004;1202–4.
[13] Clarc P, Brennand J, Conkie JA, et al. Activated protein C sensitivity. Protein C, protein S and coagulation in normal pregnancy. Thromb Haemost 1998;79:1166–70.
[14] Kjellberg U, Anderson NE, Rosen S, et al. APC resistance and other haemostatic variables during pregnancy and puerperium. Thromb Haemost 1999;81:527–31.
[15] Marik PE, Plante LA. Venous thromboembolic disease and pregnancy. N Engl J Med 2008;359:2025–33.
[16] Chabloz P, Reber G, Boehlen F, et al. TAFI antigen and D-dimer levels during normal pregnancy and at delivery. Br J Haematol 2001;115:150–2.
[17] Bombeli T, Mueller PR, Fher J. Coagulation activation markers do not correlate with the clinical risk of thrombosis in pregnant women. Am J Obstet Gynecol 2001;184:382–9.
[18] Kline JA, Williams GW, Hernandes-Nino J. D-dimer concentrations in normal pregnancy: new diagnostic thresholds are needed. Clin Chem 2005;51:825–9.
[19] Chan WS, Sanjeev C, Lee A, et al. A red blood cell agglutination D-dimer test to exclude deep venous thrombosis in pregnancy. Ann Intern Med 2007;147:165–70.