Research Article

Profile of D-dimer in Uncomplicated Pregnancy

Profil D-dimer Kehamilan tanpa Komplikasi

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Abstract

Objective : To obtain the profile of D-dimer in uncomplicated pregnancy.

Methods : A cross sectional study was done on 90 uncomplicated pregnant women consisted of 30 women in each trimester and 30 healthy, nonpregnant women as control group from July to August 2012. D-dimer level was measured by particle enhanced immunoturbidimetry method using Innovance D-dimer and Sysmex CA 1500 in the Department of Clinical Pathology, Dr. Cipto Mangunkusumo Hospital, Jakarta.

Results : All women in the control group showed normal D-dimer level (<0.,5 mg/L FEU). The median and range of D-dimer level in the 1st trimester, 2nd trimester, and 3rd trimester were 0.42 mg/L FEU and 0.1-1.07 mg/L FEU, 0.97 mg/L FEU and 0.6-3.34 mg/L FEU, and 1.56 mg/L FEU and 0.69-3.75 mg/L FEU, respectively. Increased D-dimer level was found in 27% of pregnant women in 1st trimester, 87% in 2nd trimester, and 100% in 3rd trimester.

Conclusions : Increased D-dimer level was found in 27% of pregnant women in 1st trimester, 87% in 2nd trimester, and 100% in 3rd trimester. The range of D-dimer level in the 1st trimester was 0.1-1.07 mg/L FEU, in the 2nd trimester was 0.6-3.34 mg/L FEU, and in the 3rd trimester was 0.69-3.75 mg/L FEU.

Keywords : D-dimer, trimester, uncomplicated pregnancy.

Abstrak

Tujuan : *Untuk mendapatkan profil D-dimer pada kehamilan tanpa komplikasi.*

Metode : *Penelitian potong lintang dilakukan pada 90 perempuan hamil tanpa komplikasi yang terdiri atas 30 perempuan pada tiap trimester dan 30 perempuan sehat yang tidak hamil, sebagai kelompok kontrol dari bulan Juli sampai Agustus 2012. Kadar D-dimer diukur dengan cara particle enhanced immunoturbidimetry menggunakan reagen Innovance® D-dimer dan koagulometer Sysmex® CA 1500 di Deparemen Patologi Klinik, Rumah Sakit Umum Pusat Nasional Dr. Cipto Mangunkusumo, Jakarta.*

Hasil : *Seluruh perempuan dalam kelompok kontrol mempunyai kadar D-dimer dalam batas normal (<0.,5 mg/L FEU). Median (rentang) kadar D-dimer pada trimester pertama, kedua, dan ketiga berturut-turut 0.42 mg/L FEU (0.1-1.07 mg/L FEU), 0.97 mg/L FEU (0.6-3.34 mg/L FEU), dan 1.56 mg/L FEU (0.69-3.75 mg/L FEU). Peningkatan kadar D-dimer ditemukan pada 27% perempuan hamil trimester pertama, 87% trimester kedua, dan pada 100% trimester ketiga.*

Kesimpulan : *Peningkatan kadar D-dimer ditemukan pada 27% perempuan hamil trimester pertama, 87% trimester kedua dan 100% pada trimester ketiga. Rentang kadar D-dimer level pada trimester pertama adalah 0.1-1.07 mg/L FEU, pada trimester kedua 0.6-3.34 mg/L FEU, dan pada trimester ketiga 0.69-3.75 mg/L FEU.*

Kata kunci : *D-dimer, kehamilan tanpa komplikasi, trimester*

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INTRODUCTION

The incidence of Venous Thromboembolism (VTE) in pregnant women is 4-5 folds higher than nonpregnant women.[1] Obstetric complications that associated with thromboembolisms are recurrent abortus, preeclampsia, and fetal growth retardation.[2] Increased risk of VTE in pregnancy is associated with venous stasis due to uterus enlargement and hypercoagulable state.[1,3] Many experts think that hypercoagulable state in pregnancy is anaturally occurring mechanism to anticipate bleeding during labour.[3]

The clinical manifestations of VTE are Deep Vein Thrombosis (DVT) and Pulmonary Embolism (PE). The accuracy of VTE diagnosis solely based on clinical manifestations is low. Venography is the gold standard in the diagnosis of DVT, but this procedure is invasive, and now compression ultrasonography and D-dimer are used as a modality in the diagnosis of DVT after clinical probability has been determined.[4]

D-dimer is a degradation product of cross-linked fibrin by the action of plasmin during fibrinolysis process. Since cross-linked fibrin is formed as the end product of coagulation process, D-dimer can be used as a marker of coagulation activation as well as fibrinolysis.[5] Elevated D-dimer level is found in many conditions where activation of coagulation occurs such as VTE, disseminated intravascular coagulation (DIC), malignancy, sepsis, postoperative condition, post-traumatic injury, myocardial infarction, heart failure, liver diseases, pregnancy, and in elderly. Since increased D-dimer is very sensitive but not specific for VTE, normal level of D-dimer isused to exclude the diagnosis of VTE.[5]

Measurement of D-dimer level in pregnancy is vital to predict VTE and associated obstetric complications. However, this approach is hampered due to increased D dimer in normal pregnancy.[3] D-dimer level in Caucasian women using MDA® Immunoturbidimetric, Organon Teknika reported that D-dimer level increased above normal value starting from first trimester in pregnancy.[6] At second trimester normal D-dimer level only found in 22% of pregnant women, and in the third trimester all pregnant women showed increased D-dimer level.[6]On the other hand who measured D-dimer in Nigerian women using ELISA technique with Technozym®, Technoclone GmDH reported that 63.3% of pregnant women had normal D-dimer values (0-200 ng/mL),[7] 26.7% of the pregnant women had elevated D-dimer values (201-499 ng/mL), while 10.0% were found to be at risk of thrombosis (D-dimer >500 ng/mL). This discrepancy may be caused by different population race and different method in measuring D dimer. To date, there is no data regarding the D-dimer level in Indonesian pregnant women. This situation prompts us to determine D-dimer level in Indonesian uncomplicated pregnant women in each trimester. The objective to study is to obtain the profile of the D-dimer level of uncomplicated pregnancy.

METHODS

This study had been approved by the Ethics Committee, Faculty of Medicine, Universitas Indonesia, Dr. Cipto Mangunkusumo Hospital (No.396). Written informed consents were obtained from all subjects. This study was designed as a cross-sectional study and was performed from July to August 2012.

Subject and Control

Based on results, the minimum number of subjects in each trimester was 29 [6]. In this study 30 subjects in each trimester wererecruited from the Obstetric clinic in Dr. Cipto Mangunkusumo Hospital, and from several Community Health Centres in Matraman, Jatinegara, Cakung, and Pulogadung. Inclusion criteria were pregnant women on the first, second, and third trimester. Control group were 30 healthy, nonpregnant women of the same reproductive age that were recruited from students and laboratory technician. Exclusion criteria were hypertension, leg oedema, seizures, leukocyte count more than 12 000/mL, proteinuria, and diabetes mellitus. Additional exclusion criteria for the control group were positive in pregnancy test, inflammation, and oral contraceptive users. Samples collection were done consecutively.

Samples and Measurement

Samples for this study comprised blood and urine. Blood was collected by veni puncture at fossa cubiti, 3 mL of blood was mixed with

trisodium citrate 0.109 M as an anticoagulant for D-dimer determination and 3 mL was mixed with K_3EDTA for hematology examination. Citrate bloodwas centrifuged at 1500 x g for 15 minutes to obtain platelet-poor plasma for D-dimer measurement. Hematology examination was done using hematology analyzer Sysmex XE-2100. Proteinuria and glucosuria were examined by semi quantitative method using AIM URI-TEST®according to manufacturer instruction.

Determination of D-dimer Level

Determination of D-dimer level was performed using Innovance® D dimer Kit, Sysmex consisted of Innovance D-dimer Reagent which contains polystyrene particle coated with monoclonal mouse antibodies against D-dimer, Innovance D-dimer Buffer is buffer saline solution which contains detergent and carbohydrate polymer, Innovance D-dimer Supplement is buffer saline solution which contains blocking reagent to inhibit nonspecific reaction against heterophilic antibodies such as rheumatoid factor and anti-mouse antibody, Innovance D-dimer Diluent is buffer saline solution which contains detergent to dilute sample, control, and calibrator. Principle of Innovance® D-dimer assay is particle-enhanced immunoturbidimetric assay. D-dimer in the plasma reacted with specific monoclonal antibody-coated bead and resulted in agglutination and increased turbidity. Increased turbidity was measured by Sysmex CA1500.[8,9] Before measuring D-dimer in plasma samples, precision and accuracy tests were done by measuring Innovance® D-dimer control 1 (normal control) with assign value 0.26 – 0.38mg/L FEU and Innovance® D dimer control 2 (abnormal control) with assign value 2.22 – 3.32 mg/L FEU.

Statistical Analysis

The result of precision test was analyzed by calculating the mean, standard deviation and Coefficient of Variation (CV). Accuracy was analyzed by calculating the difference between the smallest and largest value obtainedfrom the target value.

Data analyses of D-dimer of pregnant and control groups were done based on data distribution. Distribution of continuous data was analyzed.[10]Data with normal distribution would be presented as mean and standard deviation, while data with abnormal distribution would be presented as median and range. The difference between groupswith normal distribution was analyzed by one way ANOVA. If there was a significant difference, ANOVA was followed by Post-Hoc analysis. If abnormal distribution was obtained, logarithmic transformation should be done and the distribution oftransformed data would be checked. If data distribution was still abnormal, then analysis was used to analyze the difference between groups.[11] Statistical analyses were done using SPSS version 12.

RESULTS

The results of within run precision test using Innovance® D-dimer normal control and abnormal control revealed CV 2.5% and 1.36%, respectively. While the results of accuracy test revealed d=(-12,5%) – (-6.2%) for normal control and d= (-0.9%) – (5.0%) for abnormal control. Data distribution of D-dimer in the pregnant group as well as in control group were abnormal; thus, data were presented as median and range from minimum to maximum (Table 1).

Table 1. The Level of D-dimer in Pregnant Women and Control Group

Group	n	Median (mg/L FEU)	Range (min-max) (mg/L FEU)
Control	30	0.21	0.11 – 0.50
1st trimester	30	0.42	0.17 – 1.07
2nd trimester	30	0.97	0.31 – 3.34
3rd trimester	30	1.56	0.69 – 3.75

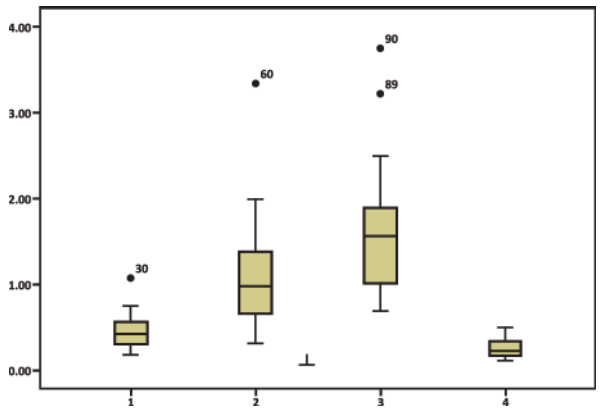

Figure 1. Boxplot of D-dimer Level in Pregnant Women and Control Group

Based on 0.5 mg/L Fibrinogen Equivalent Unit (FEU)as the upper limit of normal range, increased D-dimer level was found in 8 out of 30 (27%) pregnant women in first trimester, 26 out of 30 (87%) pregnant women in second trimester, and all (100%) pregnant women in third trimester (Table 2). There was nosubject who showed increased D-dimer level in the control group.

Table 2. The Proportion of Women with Increased D-dimer Level

Group	Proportion of subjects with increased D-dimer level (%)
Control group	0
1st trimester	27
2nd trimester	87
3rd trimester	100

After logarithmic transformation, it was found that datadistribution of D-dimer in all trimester was normal. The result of one way ANOVA revealed that p = 0.00, so Post-Hoc analysis was done. Post-Hoc analysis between the control group, first trimester, second trimester, and third trimester revealed p=0.00.

DISCUSSION

The results of within run precision using normal and abnormal control revealed CV=2.5% and 1.36%, respectively. These values were still lower than allowable CV mentioned by the manufacturer, i.e.: 4.1% for normal control and 1.4% for abnormal control.

The results of accuracy test indicated d=(-12.5%) - (-6.2%) using normal control and d=(-0.9%) - (5.0%) using abnormal control. Both values were still lower than allowable value mentioned by manufacturer 18.75% for normal control and 19.85% for abnormal control. Based on precision and accuracy, Ddimer measurement in this study is valid.

The level of D dimer in the control group showed that median was 0.21 mg/L FEU with range 0.11 – 0.50 mg/LFEU. Increased D dimer level was not found in the control group. In pregnant women median and range of D dimer level in the first, second, and third trimester were 0.42(0.17– 1.07)mg/L FEU, 0.97(0.31 – 3.34) mg/L FEU, and 1.56(0.69 -3.75)mg/L FEU,respectively. Comparisonof the results of this study with data from the literature presented in table 3.

It was shown that the increased D dimer level during pregnancy was obtained by other studies. The difference of D dimer level in pregnancy, as shown in table 3 might be caused bydifferent method, reagent, cutoff value, and population. The study who measured D- dimer level in the same samples of pregnant women using 5 different reagents confirmed that different reagents and methods caused variability of D-dimer level (Table 4).[15]

Table 3. Comparison of the Results of This Study with Data from the References

Author Country	Method	Reagent	Cut off	1st trimester mg/L or µg/mL	2nd trimester mg/L or µg/mL	3rd trimester mg/L or µg/mL
Kline[6] et al US	Immunoturbidimetric	MDA	0.5	0.58±0.36*	0.83±0.46*	1.16±0.57*
Kawaguchi[11] et al Japan	Latex agglutination	Mitsubishi Kagaku Iatron Inc, Tokyo	1.0	0.82±0.79*	1.78±1.67*	2.48±2.36*
Reger[12] et al Hungary	Not mentioned	IL, Milano	0.250	0.250 (0.152-0.607)**	0.309(0.191-0.874)**	0.541(0.260-1.036)**
Hedengran[13] et al Denmark	Latex agglutination	STA Liatest D-DI	0.5	0.2–0.9	0.2–1.5	0.4--2.8
Elst[5] et al Belgium	ELFA	Vidas, Biomerieux	0.5	0.18–0.30	0.18-1.29	0.28-2.08
Morse[14] et al UK	Not mentioned	IL- Test™ D dimer	0.280	0.191±0.025*	0.393±0.072*	0.544±0.096*
This Study Indonesia	Immunoturbidimetric	Innovance, Sysmex	0.5	0.42(0.17 – 1.07)**	0.97(0.31 – 3.34)**	1.56(0.69 – 3.75)**

*mean±SD, ** median (range)

Table 4. Median and Range of D-dimer Level at Pregnancy Determined by Various Methods[15]

Reagents (unit)	Method	1st trimester	2nd trimester	3rd trimester
Vidas® (µg/mL FEU)	ELFA	0.6 (0.15 – 1.35)	0.71 (0.55 – 0.95)	1.48 (0.97 – 2.05)
Asserachrome® (µg/mL FEU)	MicroELISA	0.65 (0.19 – 1.17)	0.66 (0.51 – 0.89)	1.25 (0.84 - 2.19)
Dimertest® (µg/mL FEU)	Latex agglutination	0.27 (0.21 – 0.34)	0.42 (0.29 – 0.70)	0.82 (0.47 – 1.04)
Liatest® (µg/mL FEU)	Latex agglutination	0.48 (0.22 – 1.06)	0.52 (0.33 – 0.82)	0.99 (0.67 – 1.77)
Innovance D-dimer (µg/mL FEU)	Immunoturbidimetry	0.80 (0.21- 1.51)	0.93 (0.74 – 1.17)	1.56 (1.10 – 2.67)

The results of our study in the second and third trimester as shown in table 3 were similar with the result when using Innovance D-dimer (the same reagents that were used in this study).This indicated that D dimer level was not affected by population race.[15]

Many studies reported that D-dimer level increased during pregnancy, some studies revealed that increased D-dimer was started at the first trimester, but the others started at the second trimester. In our study increased D-dimer level was found in 27% of pregnant women in the first trimester, 87% in the second trimester and 100% in the 3rd trimester. It means that activation of coagulation started from the first trimester of pregnancy and was progressively increased. This result that increased D-dimer was started in the first trimester, found in 78% in the second trimester and all pregnant women in the third trimester.[6] Increased D-dimer was found in 21.2% in gestational week 4-13, 59.4% in gestational week 14-27, 85.2% in gestational week 28-35, and 92.3% in gestational week 36-42.[11] On the other hand, reported that increased D dimer was found in more than 25% of pregnant women in gestational week 13-20, and by weeks 36-42, all of the pregnant women had D-dimer level above threshold.[13]

This discrepancy may be caused by different specificity of D-dimer reagent and different unit in expressing the result. The performance of each D-dimer reagent differs, depending on the specificity of the monoclonal antibodies used in the kit, variability in the cutoff value to identify positive results, and heterogeneity of the patient population[16]. Monoclonal antibodies used in the kit recognize neoepitopes on D-dimer that are not expressed on D domains of non-cross-linked fibrin. In addition, up to now, there is no international standard as a reference in measuring D-dimer level.

The result of our study indicated that coagulation cascade had been activated since the first trimester. This condition might be caused by thromboplastin substances released by placenta, which trigger coagulation cascade by activating factor VII.[17]As reported by many authors, changes in the balance of coagulation and fibrinolysis occur in pregnancy.[1,17,18] Increased level of some coagulation factors such as fibrinogen, factor VII, VIII, X, and von Willebrand factor, and reduced protein S, a natural anticoagulant, result in hypercoagulability in pregnancy.[1,17] In addition, fibrinolytic activity is depressed in pregnancy due to increased plasminogen activator inhibitor 1 and plasminogen activator inhibitor 2.[1,17,18]All of these changes cause an imbalance of hemostasis, which favours to thrombotic tendency.

In many studies, all pregnant women in the third trimester showed increased D-dimer level and the highest D-dimer level was found in the third trimester. It means that activation of coagulation reached the peak at the third trimester.

The impact of this study is the upper limit of the normal range of 0.5 mg/L FEU cannot be used as a cutoff value to rule out thrombosis in pregnancy. It is recommended to determinea new cutoff value of D dimer in pregnancy that can be used to exclude VTE.

The strength of this study is the first study in Indonesia which determine D dimer level in pregnancy while the weakness of the study measures D dimer only performed using 1 reagent while there are many reagents available in the market in Indonesia.

CONCLUSION

Increased D-dimer level in pregnant women was started in the first trimester, and it was found in 27% of pregnant women in the first trimester, 87%in the second trimester, and in 100% in the third trimester. To exclude VTE in pregnancy, determination of a new cutoff value of D-dimer is recommended.

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