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ORIGINAL ARTICLE

Reference ranges for D-dimer levels in Malaysian women in the three trimesters of pregnancy

Nornattasa Binti MOHMAD SALLIH[1], Indhira SUBBIAH[2], Aishah Binti ALI[3], Nicholas JACKSON[1]

[1]Department of Pathology, University Malaya, Lembah Pantai, 59100, Kuala Lumpur, [2]Department of Pathology, Hospital Sultanah Aminah, 80000, Johor Darul Ta'zim, and[3]Clinical Research Centre, Hospital Sultan Ismail, 81100, Johor Darul Ta'zim.

Abstract

Introduction: Plasma D-dimer levels rise progressively during pregnancy, so one cannot apply normal reference ranges, or the usual cut-off value (500ng/mL), for the exclusion of venous thromboembolism (VTE), in pregnant women. This study was carried out in pregnant Malaysian women in order to build applicable reference ranges for D-dimer. *Materials and Methods:* A cross-sectional study was conducted to measure D-dimer in healthy pregnant women, and a non-pregnant control group, using the quantitative HaemosIL D-dimer HS500 assay. Reference ranges were derived using CLSI 'Robust' methods, and differences between group medians were tested using the Kruskal-Wallis and Mann-Whitney U tests. *Results:* Plasma D-dimer levels were measured in 92 pregnant women (distributed across the three trimesters)and 31 control women. The medians (and reference ranges) in ng/mL were: control 265 (<799); first trimester 481 (<1070); second trimester 1073 (357–1748); 3rd trimester 1533 (771–2410). There were significant differences between the D-dimer levels of each group and each of the other groups (P<0.001). *Conclusions:* Reference ranges for D-dimer in pregnant Malaysian women have been establised by this study. Whether these ranges can be used to determine cut-off levels for the exclusion of VTE at different stages of pregnancy is doubtful, as the levels rise continuously through pregnancy, and some very high outlying values occur in apparently normal near-term pregnancy.

Keywords: Pregnancy, venous thromboembolism, D-dimer, Malaysia, reference range

INTRODUCTION

Many studies have demonstrated that pregnancy is a major risk factor for venous thromboembolism (VTE). It complicates between 1-in-500 to 1-in-2000 pregnancies and is more common post-partum than antepartum.[1] The risk is increased across the whole of pregnancy, but is especially marked in the third trimester. Pulmonary thromboembolism is the third commonest cause of maternal death in Malaysia, being responsible for between 12 and 30% of annual maternal deaths between 2008 and 2015.[2] In the United States of America (2011-2013), United Kingdom (2012-2014) and Japan (2010-2013), pulmonary embolism (PE) as responsible for 9.2%, 8.3% and 7% of maternal deaths respectively.[2]

The signs and symptoms of deep vein thrombosis (DVT) and PE may overlap with the physiological changes of pregnancy (especially dyspnoea and leg swelling) complicating the clinical assessment of possible VTE. Standard investigations for VTE include compression duplex ultrasound (CUS) of the proximal veins for deep vein thrombosis (DVT), and ventilation-perfusion (V/Q) isotope scanning or computed tomographic pulmonary angiography (CTPA) for pulmonary embolism (PE).[3] However, V/Q scanning and CTPA involve radiation to the fetus. Therefore, an objective, rapid and non-invasive test for VTE, which is safe for the fetus, is desirable.

D-dimer is generated by plasmin lysis of fibrin clots, and quantitative D-dimer can be used to exclude DVT and PE.[3] However, plasma D-dimer levels rise progressively during pregnancy, so one cannot apply normal reference ranges, or the usual cut-off value (e.g. 500ng/mL with the HaemosIL assay) for the exclusion of VTE

Address for correspondence: Dr Nornattasa Binti Mohmad Sallih, Department of Pathology, University Malaya, Kuala Lumpur. Tel: 03-79494422. Fax: 03-79492030. Email: mnornattasa1@yahoo.com.my

in pregnant women. Fibrinogen levels also progressively increase through gestation, and are considered part of the hypercoagulable state of pregnancy. [4]However, previous studies showed no correlation between D-dimer and fibrinogen levels in pregnancy, and hence the fibrinogen level cannot assist in the interpretation of the D-dimer level as a predictor of thrombus.[4]

This study was initiated because of the absence of published reference ranges for plasma D-dimer levels in the Malaysian population, especially in pregnant women. This is seen as a first step towards being able to use D-dimer more effectively in pregnancy. However, it should be noted that, because of a lack of standardisation of D-dimer assays, each laboratory should establish their own reference range for their local population, with the reagent and analyser that they are using.

MATERIALS AND METHODS

This was a cross-sectional study of healthy pregnant Malaysian women and a control group of non-pregnant Malaysian women. Although the number of subjects needed to derive the reference ranges was calculated as 23 per group by power sampling calculation,[5] 30 subjects from each trimester were studied.This was to allow for a 30% dropout (which might occur, for example, if the patient was later found to have gestational toxaemia, diabetes, or multiple pregnancy).

We defined 'healthy pregnant women' as those who were non-obese (BMI <30kg/m^2) at booking, and had singleton pregnancies, diastolic blood pressure<85mmHg and no proteinuria.[6] We only included pregnant women who were para 5 or less, with no more than one spontaneous abortion in their life time. Our exclusion criteria included: multiple pregnancy; history of VTE; current/past smoking; recent trauma, burns, immobility or surgery; known Systemic Lupus Erythematosus (SLE); impaired glucose tolerance; hypertension; liver/renal disease; or active malignancy. We could not obtain pre-conception or late post-partum samples from our patients, and due to time constraint and the short half-life of the reagent used,[7] we could not follow a cohort of patients through their pregnancies. Some samples were taken from a cross-section of women at different stages of gestation. The control women were of the same age (18-48 years old), para 5 or below, and with no more than one abortion in their lifetime. The controls were selected, with informed consent, from amongst the female staff of the Department of Pathology at Hospital Sultanah Aminah, Johor Bahru: they had to be non-obese, and not on hormone therapy (with the same exclusion criteria).The study was approved by the Medical Research Ethics committees of both University Malaya and the Malaysian Ministry of Health, and was carried out in accordance with the principles of the Declaration of Helsinki 1975 (as revised 2008).

Convenient random sampling[8] was carried out within an antenatal clinic held in an urban health clinic in Johor state, southern peninsular Malaysia, during June and July 2016. Patients gave informed consent to participate in this study. Demographic details were obtained together with information on risk factors for VTE. Blood was collected with their routine clinic venepuncture, and anticoagulated with a 1:9 citrate: blood ratio. Samples were then transported at room temperature to the haematology laboratory in Hospital Sultanah Aminah, Johor Bahru. The D-dimer assay was performed within 4 hours of collection using the HaemosIL HS 500 assay on an ACL Top machine.[9] Although the gold standard quantitative D-dimerassay is the ELISA, latex microparticle turbidometric immunoassays (such as the HS500) are more rapid and convenient.[10] The HaemosIL HS500 has been clinically validated, and is licensed by the Food and Drugs administration (FDA), for use in VTE exclusion and correlates well with other D-dimer assays.[11,12]

The 'Robust method' was used for the derivation of reference intervals as outlined by the Clinical and Laboratory Standards Institute.[13, 14] Comparisons between the median D-dimer levels of the different trimesters were performed using the Kruskal-Wallis analysis of variance and the Mann-Whitney U test.[15]

RESULTS

Pregnant patients were divided into three approximately equal groups between the first, second and third trimesters. Samples were analysed from 101 pregnant ladies, but only 92 results were included in the derivation of the reference ranges (one was rejected due to twin pregnancy from subsequent ultrasound, four because of subsequent impaired maternal glucose tolerance test, and four because they were categorised as extreme outliers in the third trimester, see below). Demographic data of the subjects is shown in Table 1.

First, normality tests and Grubb's outlier screening test were carried out on each of the

TABLE 1: Demographic details of study subjects

		Pregnant subjects (n=101)		Control subjects (n=31)	
		n	(%)	n	(%)
Age (years)	< 26	49	49	15	47
	27 – 48	52	51	16	53
BMI (kg/m²) (at booking if pregnant)	<18.5	5	5	3	7
	18.5– 25	69	69	19	63
	25– 30	27	27	9	30
Parity	Primigravida*	41	40	3	10
	Para 2 – 5	60	60	6	17
	Nulliparous			22	73
Gestational age	<13w +6d	31	31	-	-
	14w – 27w+6d	34	34	-	-
	28w – 40w	36	36	-	-

* Primigravida if pregnant; Para 1 if a control subject. w - weeks, d - days.

three study groups and the control group.[16] No outliers were detected in the first and second trimester groups, or in the control group. However, outliers were detected in the third trimester, and further analysis, using Rosner's Extreme Studentized Deviate Test for multiple outliers,[17] identified four high outliers (Fig. 1a), which were excluded from the derivation of the reference ranges. By excluding these extreme outliers the D-dimer values in the third trimester also followed a normal distribution (Fig. 1b).The four outlying patients had very high D-dimer levels (3331-6354ng/mL), but were clinically unremarkable (normal BMI, aged 20-28 years, and an uncomplicated antenatal/puerperal course without VTE). However, they were all at the end of the third trimester (37-40 weeks of gestation).

The main results, our proposed 95% reference ranges for quantitative D-dimer in healthy pregnant and non-pregnant Malaysian women, are shown in Table 2 and Figure 2. An increasing proportion of subjects had D-dimer values > 500ng/mL in successive stages of pregnancy, from 38% in the first trimester to 100% of the subjects in the third trimester. This study also found significant differences between the median D-dimer levels of each group and the medians of all the other groups (P<0.001).

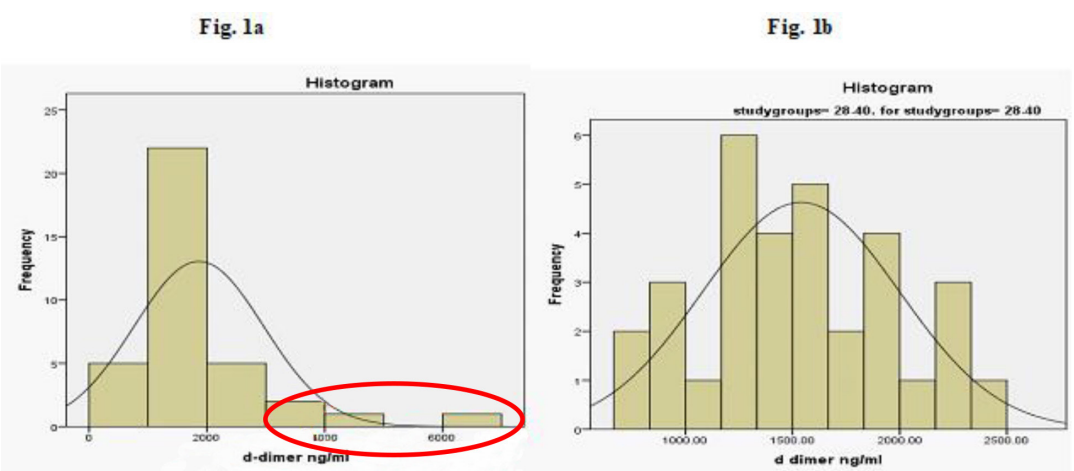

FIG. 1: D-dimer levels in 3rd trimester – removing outliers. Bar chart histogram and distribution curves of D-dimer levels in the third trimester group, before (1a) and after (1b), excluding the high outliers in the third trimester (circled in Fig. 1a).

TABLE 2: D-dimer levels in 3 trimesters of pregnancy and control

Group	N	D-dimer (ng/mL) (Median)	D-dimer (ng/mL) (Reference range)	D-dimer>500 ng/mL N (%)
Non-pregnant Control	31	265	<799	5 (16%)
First trimester (<13weeks+6 days)	29	481	<1070	11 (38%)
Second trimester (14 weeks to27 weeks +6 days)	31	1073	357-1748	30 (97%)
Third trimester (28 weeks to 40 weeks +6 days)	32	1533	771-2410	32 (100%)

Median D-dimer levels and reference ranges (central 95% of values) for the control group and the three trimesters of pregnancy. The final column showed the number (and %) of those in each group whose D-dimer level was above the manufacturer's cut off for exclusion of VTE in non-pregnant patients (500 ng/mL).

DISCUSSION

We have studied D-dimer levels in pregnant Malaysian women with uncomplicated singleton pregnancies. We derived reference ranges for non-pregnant women (control group), and for women in the three trimesters of pregnancy, after strict inclusion/exclusion criteria, using standard methods, including statistically valid methods

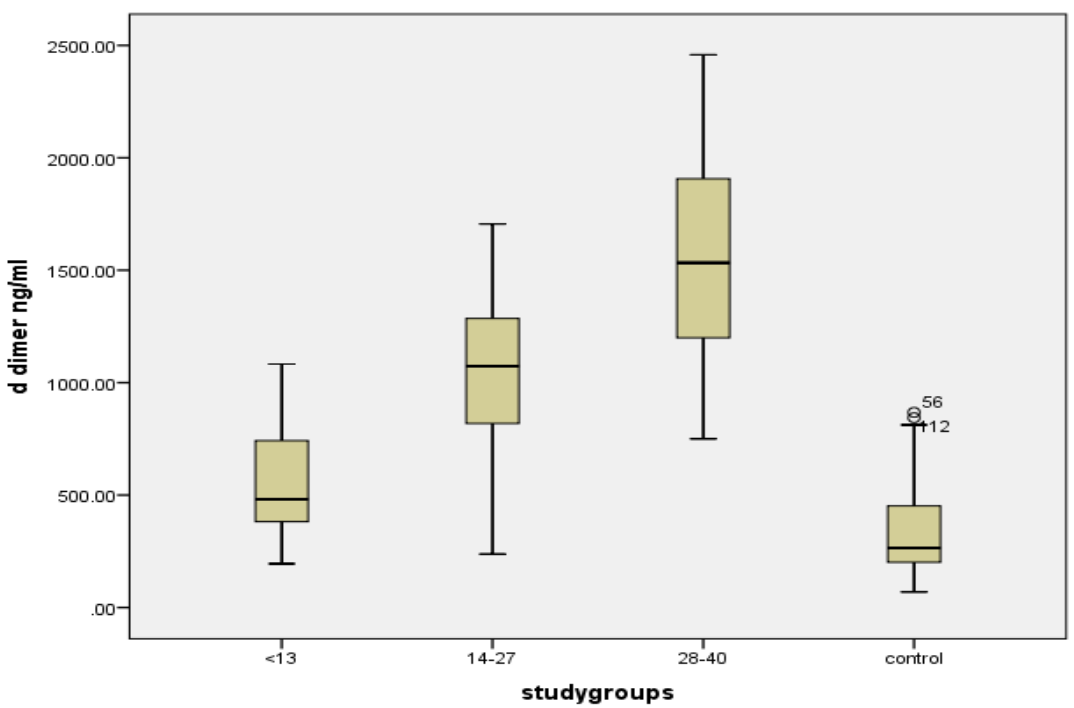

FIG. 2: D-dimer levels in the three trimesters of pregnancy. Whisker plot of D-dimer levels (median, with shaded boxes showed25thand 75th centiles, and 95% confidence intervals) for the first, second and third trimester patients and the control group. The median value of each group was significantly different from all the other groups by the *Kruskal-Wallis* test and pairwise *Mann Whitney U test* (p <0.001).

for the elimination of the few extreme outlying values in the third trimester which might have unduly skewed the reference ranges. Our study showed the presence of statistically significant rises in D-dimer levels from the non-pregnant state to the first trimester, and then as the patient passed into the second and again into the third trimester. There have been several studies of D-dimer levels in normal healthy pregnancies, and our results are compared with theirs in Table 3. All the studies showed D-dimer levels rising progressively, two-to-six fold, through pregnancy, although not all have shown a statistically significant difference between the non-pregnant state and the first trimester.[18,19,20] The wide discrepancy between D-dimer values in different studies is likely to be due to the different assays and analysers used, rather than geographical or ethnic differences, although these have not been studied systematically. Our results will be useful for the interpretation of D-dimer results in pregnant Malaysian women, especially in laboratories using the HaemosIL HS 500 assay.

Of note there were four patients, all beyond 37 weeks of gestation, with very high D-dimer levels ranging from 3331 to 6354 ng/mL. They were completely well during that clinic visit, remained asymptomatic of VTE, completed their pregnancies uneventfully, and gave birth by spontaneous vaginal deliveries without complications. This suggests that there is a continuum of rising D-dimer levels throughout a healthy pregnancy, and especially into the immediate pre-partum period, as has been shown by two other studies.[21,22] Hence, very high D-dimer levels, in clinically healthy women near term, should not be a cause for alarm.

In non-pregnant groups, exclusion of VTE is safe with adherence to validated algorithms, e.g. the Wells pre-test clinical probability score and the D-dimer value.[23] However, a validated clinical pre-test probability tool for use in pregnant women does not yet exist.[24] It can be seen that the rising D-dimer concentration through pregnancy complicates the diagnosis of VTE. Higher D-dimer mean levels in women with confirmed VTE during pregnancy compared to control women in the same trimester were found in one study. The VTE patients had mean values of 1596ng/mL (vs 222ng/mL in normal women) in the first trimester, 1330 ng/mL vs 326 ng/mL in the second trimester, and 1157 ng/mL vs 475 ng/mL in the third trimester.[19] In all three trimesters, there was very little overlap in the range of D-dimer levels between those with VTE and those without. However, the prospective clinical utility (positive and negative predictive value) of the pregnancy-related reference ranges was not studied.[15] While a threshold of more than 230 ng/mL, suggested by Kovac*et al*.[19] was labelled as abnormal, a 'normal' level was observed in only 84% of women in their first trimester, 33% in the second trimester, and1% in the third trimester. Min Wang in China, using >500 ng/mL as abnormal, found only 85% normal in the first trimester, 29% in the second trimester and 4% by the third trimester (compared to 62%, 3% and nil, respectively, in our study).[20] These results undoubtedly explain why the manufacturers of D-dimer assays¬do not recommend using the non-pregnant cut-off value in pregnancy. It may also be the reason why most professional bodies do not recommend the use of D-dimer for the exclusion of VTE in pregnancy.[25]

It seems unlikely that D-dimer will be any more usable as a positive predictive test for VTE in pregnancy than it is in the non-pregnant state, especially in the late third trimester when the reference range is high, and very high outlying values can occur even in apparently VTE-free women.[26] Whether a useful negative cut-off value can be derived must also be open to doubt, as the rise in D-dimer values appears to be a continuous process throughout pregnancy, and is not divided into neat ranges for the three trimesters. It seems likely that there would have to be more than three divisions in the pregnancy which would then make for difficulties if the gestational age is less than certain. In addition, before a negative predictive test can be recommended for use in pregnancy, a prospective study of women with suspected VTE in pregnancy would need to be carried out. If this trial required women to be divided into groups with gestational ranges of only a few weeks, then such trials would become impossibly large to carry out.

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TABLE 3: Comparison of current study with previous publications on D-dimer levels in pregnancy

Study	Assay	Mean or median D-dimer level (range) (ng/mL)					Comment
		Control	1st trimester	2nd trimester	3rd trimester	Peri-partum / Postpartum	
Chabloz *et al*., 2001[21] (Switzerland)	Vidas	-	NS (139-602)	NS (291-1231)	NS (489-2217)	NS(678-5123)	Median (central 80%)
Morse, 2004[18] (United Kingdom)	IL Test TM	140 (82-198)	191 (166-216)	393 (321-465)	544 (448-640)	-	Mean (± 2SD)
Kovac *et al*., 2010[19] (Serbia)	HaemosIL HS	223 (110-390) (postpartum)	222 (121-474)	326 (171-733)	475 (206-890)	-	Mean (± 2SD)
Kawaguchi *et al*., 2013[27] (Japan)	Own latex assay	-	54 (<430) 82 (8-79)	122 (<926) 178 (11-345)	181 (<1095) 248 (12-484)	213 (<1032) 265 (71-459)	Median (central 99%)
Wang *et al*., 2013[20] (China)	STAGO-R	17 (<56)	20 (<66)	68(<229)	133 (<312)	-	Median (central 90%)
Murphy *et al*., 2015[28] (Republic of Ireland)	Biopool auto	-	149 (60-470)	376 (184-801)	599 (299-1305)	668 (329-1538)	Median (central 90%)
Current study, 2017 (Malaysia)	HaemosIL HS 500	265 (<799)	481 (<1070)	1073 (357-1748)	1533 (771-2410)	-	Median (central 95%)

NS = Not stated.

Author Contributions

Nornattasa Binti Mohmad Sallih - conceived and carried out the study, analysed the data, and wrote the paper.
Indhira Subbiah - co-supervised the project, including the acquistion and analysis of patient samples in Johor, and helped to edit the paper.
Aishah Binti Ali - provided statistical advice for the analysis of the data.
Nicholas Jackson - supervised the project, helped analysed the data, and co-wrote the paper.

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