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Plasma D-dimer reference ranges in pregnant Nigerians

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Abstract

Background: The use of D-dimer as a screening test for thromboembolic disorders has not been validated in pregnancy thus necessitating further studies. This cross sectional study was carried out among Pregnant Nigerians at the Nigerian Air Force Hospital Ante-natal Clinic, Ikeja, Lagos.

Objective: This study was to determine local reference ranges for plasma D-dimer in both pregnant and non-pregnant females and compare differences in both populations.

Methods: Structured questionnaires were administered on a total of 365 participants which included 71 apparently healthy non-pregnant females, 64 women in the first trimester of pregnancy (≤13 weeks gestation); 65 women at the second trimester of pregnancy (14-26 weeks), and 82 pregnant females at the third trimester of pregnancy (>=27 weeks). Citrated blood was collected for estimation of prothrombin time (PT), activated partial thromboplastin time (aPTT), and D-dimer estimation by ELISA method. Aspartate aminotransferase (AST) and alanine aminotransferase enzymes (ALT) were estimated using the Hitachi chemistry analyzer. Reference ranges were estimated non-parametrically using the Reference Value Advisor V 2.1.

Results: Median D-dimer level for non-pregnant females was 190ng/mL, while the median D-dimer levels for pregnant female in 1st, 2nd, and 3rd trimester were 485ng/ml; 620ng/mL; and 1185ng/mL respectively. Reference ranges were calculated to be 86-494ng/mL; 338-624ng/mL; 451-799ng/mL and 665-1262ng/mL for non-pregnant females, 1st, 2nd, and 3rd trimester of pregnancy respectively.

Conclusion: A diagnostic algorithm for venous thromboembolism (VTE) in pregnant women which combines clinical suspicion with elevated plasma D-dimers levels above estimated reference range for each trimester should precede definitive formal imaging.

Keywords: *D-dimer, Venous thromboembolism, Pregnancy.*

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Résumé

Introduction : L'emploi du D-dimer comme un crible réactif pour les désordres thromboemboliques n'a pas été validé dans la grossesse ainsi nécessitant des études ultérieures. Cette étude à cross-section était menée parmi les Nigérianes Enceintes à la Clinique Anténatale de l'Hôpital des Forces de l'Armée de l'Air Nigériane, Ikeja, Lagos.

Objective : Cette étude était pour déterminer les ranges de référence locale pour le plasma D-dimer dans les deux groupes de femmes enceintes et non-enceintes et compare les différences dans les deux populations.

Méthode : Questionnaires structurés étaient administrés sur un total de 365 participants qui comprenait apparemment 71 femmes non-enceintes saines, 64 femmes dans leurs premier trimestre de grossesse (≤13 semaines de gestation) ; 65 femmes a leurs deuxième trimestre de grossesse (14-26 semaines), et 82 femmes enceintes a leurs troisième trimestre de grossesse (≥27 semaines). Sang citrate était recueilli pour estimation de temps prothrombine (TP), temps thromboplastine partiel activé (TTPa), et estimation D-dimer par la méthode ELISA. Amino-transférase aspartique (ATS) et enzymes Amino-transférase alanine (ALT) étaient estimés en utilisant l'analyseur chimie Hitachi. Les ranges de référence étaient estimées non-paramétriques en utilisant le Conseiller de Valeur Référence V 2.1.

Résultats : Le niveau médian D-dimer pour les femmes non-enceintes était 190ng/mL, tandis que les niveaux médian D-dimer pour les femmes enceintes dans le 1er, 2eme, 3eme trimestre étaient respectivement 485ng/mL, 620ng/mL, et 1185ng/mL. Les ranges de référence étaient calculées a être 86-494ng/mL, 338-624ng/mL, 451-799ng/mL et 665-1262ng/mL pour les femmes non-enceintes, 1er, 2eme, 3eme trimestre de grossesse respectivement.

Conclusion : Un algorithme diagnostique pour VTE dans les femmes enceintes qui combine suspicion clinique avec niveaux de plasma D-dimer élevées au dessus des ranges de référence estimées pour chaque trimestre devrait précéder l'image formelle définitive.

Mots Clé : *D-dimer, Thrombose-embolisme veineuse, Grossesse.*

Introduction

D-dimer antigen is a unique marker of fibrin degradation that is formed by the sequential action

of three enzymes; thrombin, factor XIIIa and plasmin. While other assay systems detect structures related to acute coagulation activation, D-dimer assays detect proteolytic fragments of thrombi released after the thrombotic event has ended [1]. D-dimer testing has been used prominently in the exclusion of acute deep vein thrombosis (DVT) in the non-pregnant population [1,2,3] Furthermore, the clinical prediction rule (CPR) for the assessment of clinical pre-test probability for venous thromboembolism (VTE) currently in use in some centres, has not been validated in pregnancy [2]. In a recent study, incidence of venous thromboembolism (VTE) in pregnancy was reported to be highest in pregnant women above the age of 35years, in the third trimester of pregnancy, and relatively high among hospitalized pregnant women [3].Pulmonary embolism was reported to be the 4th leading cause of maternal mortality in a review by Ngwan and Swede (2011) in Jos Nigeria [4]. The need for simple diagnostic method for evaluation of VTE risk then becomes imperative [3]. But the use of Plasma D-dimer in the diagnosis of VTE in pregnancy has been limited by marked variations in plasma levels and dearth of local data on reference ranges in normal pregnancy. Some of the pivotal diagnostic tests for DVT and pulmonary embolism (PE) recommend that pregnant women with D-dimer > 500ng/mL (by the ELISA method), should undergo formal imaging. However, this reference value is without consideration for the stage of pregnancy, and more so are largely Caucasian values [5-7]. Most of the data on the effectiveness and safety of various other diagnostic tests for VTE such as compression (Doppler) ultrasonography, and spiral computed tomography are not fully documented in pregnancy [8]. Also, contrast Venography, though the gold standard for diagnosis of DVT, is time-consuming, expensive, and risky in pregnancy [2,9]. Therefore in view of these challenges, it is imperative to carefully screen and select pregnant patients with the highest indices for VTE in our environment before undergoing these definitive investigations, and to subsequently follow up after an initial treatment response.

D-dimer assay still remains a first line and safer screening method in the algorithm for the diagnosis of VTE in pregnancy when combined with detailed clinical evaluation [8,17].However, there is need for standardization of results because of the variability in D-dimer assays from various test kits [9]. Stein *et al.* (2004), demonstrated that a negative D-dimer test by the ELISA method is as diagnostically useful as a negative computed tomography (CT) or a negative compression ultrasonography study (CUS) in excluding PE and DVT, respectively [10]. In another study by Wells PS *et al.* (2003), patients with a Wells clinical prediction rule score of less than 2 points and a negative D-dimer test, were less likely to have venous thrombo-embolism during follow-up than were patients with a negative ultrasound examination [9]. Testing for plasma D-dimer has emerged as an excellent non-invasive triage test for patient with suspected DVT [2]. The test has a high negative-predictive value (NPV) for DVT up to 99.5% when properly used [2,8]. Expert opinion and systematic review of D-dimer as a diagnostic tool for VTE, indicated that all non-pregnant patients with D-dimer concentration > 500ng/mL (by ELISA method), may be diagnostic for DVT [10]. In pregnancy, diagnosis of DVT and pulmonary embolism (PE), pose considerable diagnostic, safety, and therapeutic challenge. Pregnancy itself is an independent risk factor for thrombo-embolism, being a state of hypercoagulability caused by the increased levels of natural anticoagulant protein-C[12]. The incidence of VTE probably increases 2 to 4 fold when a woman becomes pregnant and is higher after a caesarean delivery than a vaginal delivery [12]. Deep vein thrombo-embolism of the lower extremities during pregnancy occurs at a rate of 0.5 to 3.0 per thousand pregnancies [8]. Venous stasis resulting from pressure of the gravid uterus on the inferior vena-cava and decreased venous flow velocity and increased diameter of the deep leg veins may further predispose a pregnant woman to develop DVT [7]. Despite its relatively low incidence, DVT may lead to pulmonary embolism. However, in PE as with DVT, D-dimer testing when used as part of a diagnostic algorithm that incorporates the determination of greatest probability may result in earlier detection of cases.

Pregnancy is a physiological condition with up regulation of many plasma proteins including D-dimer; this makes it necessary to re-evaluate the use of Plasma D-dimer as asimple screening method for exclusion of DVT by determining appropriate reference ranges for plasma D-dimer parameter in each of the trimesters of pregnancy. The objective of this study is to determine reference ranges for plasma D-dimer among apparently healthy pregnant and non-pregnant black females in Nigeria.

Materials and methods

This cross sectional study was carried out at the Nigerian Air Force Hospital, Ikeja, Lagos, Nigeria, among women attending routine ante natal care, after

obtaining ethical approval from the Nigerian Air force Medical Services. Structured questionnaires were administered on a total of 365 age matched participants of which 282 (77.2%) successfully completed the study. These included 71 apparently healthy non-pregnant females, who were female naval officers recruited from the barrack; 64 women in the first trimester of pregnancy (≤13 weeks gestation); 65 women at the second trimester of pregnancy (14-26 weeks) and 82 pregnant female at the third trimester (>=27 weeks). Those with positive history and clinical evidence of past or present thrombo-embolic disorders, cardio-respiratory disorders, metabolic and chronic inflammatory conditions, history of bleeding disorders,use of contraceptives and non-steroidal anti-inflammatory drugs, smokers, and alcoholics were excluded from the study and also Injury requiring hospitalization or an emergency room management or special non-orthodox treatment within 4 weeks; surgery within the previous 4 weeks ; current infection with fever >38 °C; active menstruation; strenuous exercise within 12 hours were also excluded. All participants were those screened negative for Human immune deficiency virus (HIV), Hepatitis B (HBsAg) and Hepatitis C from hospital records. A total of 4.5ml citrated blood (0.105M) was collected and plasma stored at -40°C after centrifugation(1500g for 15 minutes),for estimation of prothrombin time (PT), activated partial thromboplastin time (aPTT) using TS 4000 coagulation analyzer (Tianjin MD pacific, China), and D-dimer ELISA test using Cusabio D-Biotech Human D-dimer ELISA kit(**CUSABIO biotech** Co., Ltd, Wuhan, China). Blood collected in a 1ml EDTA tube was used to estimate platelet count using the Sysmex KX-21 haematology analyser (Sysmex Incorporation, Japan) within 1 to 2hours of sample collection. Aspartate aminotransferase (AST) and Alanine aminotransferase enzymes were estimated using the Hitachi chemistry analyzer (Roche diagnostics, Germany) within 1 to 2 hours of sample collection. All participants with abnormal PT, aPTT, Platelet count, AST and ALT were excluded from the calculation of reference ranges for plasma D-dimers.

Statistical analysis

Data entry and analyses were performed using the IBM SPSS version 20 (SPSS Inc. Chicago). Kolmogrov-Smirnov test was used to determine data normality at $p>0.05$. Level of association was estimated by Chi-square statistics with significant association set at $p<0.05$. Reference intervals were estimated using the Reference Value Advisor V 2.1 [13] which showed the distribution pattern of values (dot-plot, histograms and QQ plots) for visual inspection , tested normality of distributions according to Anderson-Darling, and estimates reference ranges for non-parametric distribution within 2.5th and 97.5th percentile as recommended by the International Federation of Clinical Chemistry (IFCC) [14]. PT, aPTT, INR, AST and ALT values were normally distributed while plasma D-dimer levels and platelet counts were skewed and subsequently expressed as median values and ranges. Differences in median values of D-dimers in the study groups were compared using Kruskal-Wallis analysis of variance with significant level set at $p<0.05$.

Results

Of the 365 study participants, 83 subjects were excluded from the data analysis due to incomplete data and abnormal clotting profiles and liver function test results. Of the 282 study participants analyzed, 211 (74.8%) were pregnant and 71 (25.2%) were not pregnant. Of those pregnant, 30.3% (n=64), 30.8% (n=65), and 38.9% (n=82) were in the first, second and third trimester of pregnancy respectively. Mean gestational age for women ±SD in 1st, 2nd and 3rd

Table 1: Mean age, parity and mean gestational age of participants

Parameters	Non-pregnant control	Trimester of pregnancy		
	-	1st trimester	2nd trimester	3rd trimester
Number of subjects: n (%)	71(25.2)	64(30.3)	65(30.8)	82(38.9)
Mean Age± SD(years)	30±5.2	27.8±5.0	30.2±4.8	29.7±3.9
Mean Gestational Age (weeks)	-	8.0±2.4	19.6±3.6	32.1±3.7
Parity: n (%)				
a. Primigravida	-	31(48.4)	34(52.3)	39(47.6)
b. Multigravida	-	33(51.6)	31(47.7)	43(52.4)

trimester were 8.0±2.4 weeks, 19.6±3.6 weeks, and 32.1±3.7 weeks respectively. Distribution of Prim gravida and multigravida were similar across the groups.

The mean D-dimer level for normal healthy non-pregnant female of child bearing age was 217±108.7ng/mL, while the mean values for pregnant female in 1st, 2nd, and 3rd trimester were 448±63.2ng/mL; 561±100ng/mL; and 924±238.2ng/mL respectively. The median D-dimer level for non-pregnant female of child bearing age was 190ng/mL, while the median values for pregnant female in 1st, 2nd, and 3rd trimester were 485ng/mL; 620ng/mL; and 1185ng/mL respectively. Reference range for each group was calculated to be 86-494ng/mL; 338-624ng/mL; 451-799ng/mL and 665-1262ng/mL for non-pregnant females, 1st, 2nd, and 3rd trimester of pregnancy respectively. Using reported cut-off values for healthy subjects (500ng/mL), median D-dimer level differ significantly between trimesters of pregnancy (p=0.00) with statistically significant association between D-dimer levels and duration of pregnancy (χ^2=244; p=0.000). The analyzed study participants had platelet count, PT, aPTT, AST and ALT that were within normal physiological range and showed no significant variations throughout pregnancy (p>0.05)

Discussion

Pregnancy has long been identified as a thrombogenic state [8, 12]. Clinicians are daily confronted with the problem of early identification and diagnosis of thrombo-embolic phenomenon which has accounted for increased morbidity and mortality especially in developing countries where advanced facilities for early diagnosis and management pose serious challenge. In non-pregnant individuals, use of D-dimer estimation remains the gold standard for excluding thromboembolism [10]. In a comparative study of 241 selected subjects by Galipenzo *et al.* [11], the prevalence of pulmonary embolism in the entire population was 23.6% using computed tomography. The combination of unlikely probability using the dichotomized Wells clinical decision rule and D-dimer levels, also occurred in 23.6% thus implying a 100% concordance [11]. Reports have indicated relatively higher values for D-dimer among blacks and African-Americans compared to the Caucasian population [15]; these reports make it imperative to establish local reference range for D-dimer in order to enhance its screening potential in our environment, a fact which was further highlighted in this study, where mean plasma D-dimer in both control and study groups were observed to be significantly higher than mean values obtained by Morse *et al.* among Caucasian population studied.

Table 2: Plasma D-dimer, clotting profile and liver enzymes among participants

Parameters	Control n=71	1st trimester n=64	2nd trimester n=65	3rd trimester n=82	*P – value*
Mean D-dimer (ng/mL)±SD	217±108.7	448±63.2	561±100	924±238.2	
Median(range)	190(0-512)	485(234-580)	620(350-677)	1185(557-1355)	**0.00
Reference range(ng/mL)	86-494	338-624	451-799	665-1262	
% subject with D-dimer value <500ng/mL	97.2	84.4	33.8	0	
% subject with D-dimer value >500ng/mL	2.8	15.6	66.2	100	χ^2=244; P=0.00
MedianPlatelet count x10⁹/L (range)	248(150-330)	274(102-295)	236(90-315)	195(140-318)	**0.65
Mean PT±SD(seconds)	13.51±1.43	12.04±0.22	12.18±0.15	12.17±0.16	*0.38
Mean INR±SD	1.05±0.01	1.05±0.01	1.05±0.01	1.05±0.01	*0.99
Mean aPTT±SD(seconds)	35.01±0.75	36.85±0.68	36.56±0.72	35.31±0.86	*0.87
AST±S.D(IU/L)	21.5±0.99	21.5±1.33	21.6±2.2	21.5±1.6	*0.89
ALT±S.D(IU/L)	16.8±0.93	15.6±0.94	18.7±2.7	15.5±1.3	*0.15

***Key**: *p-value for ANOVA; χ^2 (Chi- square); ** p-value for Kruskal-Wallis analysis of variance; PT (prothrombin time); aPTT (activated partial prothrombin time) AST (aspartate transaminase); ALT (Alanine transaminase); SD (standards deviation).*

Median plasma D-dimer level among control subjects was 190ng/mL.This agrees with work done by Jeremiah *et al* [16] among 60 non-pregnant and 120 pregnant Nigerian females in port Harcourt using the ELISA method where median plasma D-dimer was estimated to be 118.5ng/mL among non-pregnant controls[16]. Suspicion of thromboembolism becomes real in normal individuals when plasma D-dimer level begins to rise above 500 ng/mL using ELISA assay method [9].Though age has been closely associated with varying levels of D-dimer, the control subjects and pregnant female subjects were well matched for age. In a study by Christopher *et al.* [15], the mean plasma D-dimer did not vary significantly between the 3rd and 4th decade of life [15]. In our study, most of the pregnant females were in their late twenties and early thirties. Therefore, the reference ranges for D-dimers in our study will be applicable to a wider range of pregnant females in our environment for exclusion of possible VTE.

Progressively elevated D-dimers were observed throughout pregnancy in our study population which may be attributed to increase procoagulant activity characterized by elevated coagulation factors, due to increased synthesis or increased activation by thrombin which has been documented to be maximal around term and thus pose serious difficulty in diagnosing venous thromboembolism in pregnancy [17]. This study tends to suggest that using the conventional cut off level of 500ng/mL as proposed by earlier researchers [9, 17], 97% of non-pregnant females in this study would have D-dimer concentration within the safe limit, whereas the pregnant females would progressively fall outside this range from 84.8% in first trimester to 33.8% and 0% in the 2nd and 3rd trimester respectively. In this study, the estimated reference range using values corresponding to 2.5th and 97.5th percentile for apparently healthy non pregnant women, and pregnant women in the 1st, 2nd and 3rd trimester of pregnancy will be: 86-494ng/mL; 338-624ng/mL; 451-799ng/mL and 665-1262ng/mL respectively. Morse *et al* [18] attempted to establish normal range for D-dimers across the different trimesters of pregnancy among 48 pregnant Caucasians and 34 non-pregnant females [18]; the study supports our submission on the progressive increase in plasma D-dimer throughout pregnancy. However, there is a dearth of local information on reference ranges for plasma D-dimers in pregnancy. As reported by Jeremiah *et al* [16] and variously cited in literature, no significant variations were reported in platelet count, clotting profiles and liver enzymes of pregnant women in this study which further reduces the chance for false positive D-dimer results in this study [15,19-21].

Conclusion

A diagnostic algorithm based on empirical evidence and plasma D-dimer levels above the reference rangefor a given trimester of pregnancy, should serve as basis for selectively referring pregnant women for further definitive investigations.

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