D-dimer reference range in each trimester of Pregnancy-Need to detect Venous Thromboembolism of Pregnancy

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Abstract

This study carries significance as the medical literature has paucity of published reference ranges of maternal plasma D-dimer during all the trimesters of a normal pregnancy including the postpartum period in Southeast Asia in pregnant females.

INTRODUCTION:

Normal pregnancy is often referred to as a physiological hypercoagulability state.The changes include increased thrombotic activity, which is due to increase in the plasma coagulation factor activities. These mainly include Factor I,VII, VIII, IX, X and XII along with decrease in the concentration of the natural clotting inhibitor protein S and by intensified process of platelet adhesion and platelet aggregation. [(1,2)]

The high procoagulation activity during normal pregnancy (from conception until delivery), results in increased fibrin turnover (increased concentrations of D-dimer, a recognized marker of activation of fibrinolysis), [(3)] and thus increased D-dimer does not necessarily mean any existence of hyper fibrinolysis (as in non-pregnant state). [(4,5,6)]

Pregnancy puts women in a high-risk group for developing VTE especially in the puerperium with an estimated 20 times increase in relative risk. Approximately 80% of venous

thromboembolic events during pregnancy are deep venous thrombosis (most commonly in the left leg) and 20% are pulmonary emboli. [7-9]

The Wells Pre-test probability criteria for DVT and PE do not include pregnancy as a risk factor.Thus, VTE diagnosis in pregnancy/ puerperium is a great challenge for clinicians. [6]

None of the present diagnostic algorithms has been validated on pregnant women.Radiation imaging modalities like Computed tomography pulmonary angiogram (CTPA) and lung ventilation/perfusion scans (V/Q)along with Pre Test Probability and D-dimer values, which can be well used in non-pregnant statefor diagnosis of VTE, cannot be used in pregnancy due to increased risk of developmental damage to the fetus.[10]

Therefore, can D-dimer values in each trimester and post-partum in normal pregnancy which are usually high above the usual threshold can be used to rule out presence of VTE?.Can D-dimer along with pre-test probability can be used as quick, non-invasive and a safe test for VTE in pregnancy with respect to the foetus.

This study carries significance as the medical literature has paucity of published reference ranges of maternal plasma D-dimer during all the trimesters of a normal pregnancy including the postpartum period in Southeast Asia in pregnant females.

Material & Method:

1. SUBJECT SELECTION:

   Pregnant females aged between 20-35 years from 11 weeks- 13 weeks period of gestation were included in the study at the start after strictly implementing the exclusion criteria (Table 1)

   Out of total 100 pregnant females, initially screened, 39 were selected,among them, 14 were lost to follow up (Due to COVID). Samples were collected from 25 booked pregnant who did not develop any complications during pregnancy or postpartum period.

2. SAMPLE DRAWN:

5 ml of whole blood was collected in 3.2% sodium citrate.

A. First sample- At the time of booking (first trimester) 11-13 weeks.

B. Second sample- Second trimester- 24-26 weeks.

C. Third sample- Third trimester – 34-36 weeks.

D. Fourth sample- Four weeks postpartum.

The plasma stored at -70 ºC was thawed at 37 ºC in water bath. D dimer was then assayed on ACL Elite pro Automated analyser which works on the principle of Latex enhanced turbimetric immunoassay. The test was carried out as per the operating protocol by the manufacturer. The statistical analysis was done on SPSS version 21 software.

RESULTS:

A total of 39 subject's blood samples were collected in 3.2 % sodium citrate vial for D- dimer in this study. 14 patients were lost to follow up and hence were excluded from the study. Samples from 25 patients (4 samples each) were tested for D dimer. Of these, samples from 8 pregnant women could not be included due to error message shown by machine. Thus 4 samples obtained at appropriate times collected from 17 pregnant women were finally available for the study. The following observations were noted.

1. Therewasnosignificantcorrelationbetweenage of pregnant femalesandDdimerlevels. (Table 2)

2. Therewasno significantcorrelation betweenparity&Ddimerlevels.(Table3)

3. Mean value of d-dimer in each trimester ofpregnancy and4 weekspost-partum(n=17) were 314.76 ng m/ml,370.29 ng m/ml,418.59 ngm/ml and 272.18 ng m/ml in 1^{st} ,2^{nd},3^{rd} trimester and 4 weeks post-partum respectively. (Table 4) The difference of D -

dimer values were statistically different between the three trimesters and post-delivery 4 weeks.

4. Comparison of Mean and Reference ranges in current study and previous publications on D-dimer levels in Pregnancy. (Table 5)
5. Pattern of D-dimer values when cut off is kept at >500 ng/ml instead of 255 ng/ml as given in kit insert of reagent. (Table 6)
6. Comparison between D-dimer values in 17 subjects with normal pregnancy with pregnant cases with DVT.

Discussion: A normal pregnancy is characterized by changes in hemostasis towards hypercoagulationduetoalteredlevelsof coagulation factors, venous stasis and some vascular damage. Abnormalhemostasisleadstomorevenousthromboembolisminpregnantfemalesas compared tononpregnant women.

VTEdiagnosis in apregnant womenneeds following points to be considered.

1. Pregnant cannotbeinvestigatedwithimagingmodalitiesduetoriskofexposureofthefetustoradiations,leadingtoincreasedriskofteratogenesisandcarcinogenesis.
2. ThesignandsymptomsofDVTandPEoverlapwith physiologicalchanges of pregnancy (especially dyspnea and leg swelling) complicatingthe early clinical assessment.

3. Since D dimer levels increase with gestational age, its conventionalcutoffof500ng/ml(FEU)todiagnose VTEisoflimitedvalueinpregnantwomen.

Variousstudiesdone in normal pregnant propose a highercut offs of D-dimer, while others advocate use of gestational agespecificvalues.

For gestational age specific D-dimer, weregistered39pregnantwomenatthebeginningofthestudy., finally 4sampleseachfrom17womenwereevaluated for Ddimer levels.

The sample size was small in our study similar to two of the studies where subjects were 18 and 20 respectively,(11,12)

A study done on 24 pregnant women with expected normal pregnancy,compared the D-dimer levels with 10 non pregnantwomen and 33women with complicatedpregnancy .(13)

However many studies with larger sample size were also conducted(Choi et al, Katerine et al, Mirjana et al, Yuji et al and WS Chan et al)(14,15,16,17) .

In order to derive reference ranges for the pregnant population, we selected pregnant females in the age group of 20-35 years. There were two reasons for it, one this is the most common age of pregnancy in south east Asia .(18) and secondly the increaseofD-dimervalues with age 100% of the women >40 yrs had higher D dimer levels ascomparedto44%and43%womenaged20yearsand30years respectively.(12)

Few of studies are done for D-dimer levels in healthy pregnancies and our results as compared with theirsin table 5, All the studies,showed the D-dimer levels rising progressively in pregnancy, and a downfall later in post-partum period.The wide discrepancy between D-dimer values in different studies may be likely due to different assaysand analyzers used, rather than geographic or ethnic differences.

According to

BritishCommitteeofStandardsinHematology guidelines (19,20) thecut-offvaluetoexcludeVTEneedstobeconfirmedlocallyinminimumof

200subjects in laboratory. However, this approach is not possible in alllaboratoriesandthusthemanufacturercutoffmaybeused.

In the present study, the manufacturer cut off value for VTE at 255ng/ml was exceeded in 76.5% of the patients in 1st trimester, 88.2%of the patients in 2nd trimester and 76.5 % of the patients in 3rd trimester and 53% (6-8 weeks post partum).If, the cut off is raised to >500ng/ml (as in non pregnant),it shows that 12% in first trimester,18 % in second trimester,35% in third trimester and 5% in post-partum have values >500ng/mlas in Table 6. These results showthat why manufacturers do not recommend to us the non-pregnant cut-off value of D-dimer in pregnant.

Two recent prospective studies (21,22) showed that when the cut off values of D-dimer were taken as < 500ng/ml, along with WellsPre test Probability (23) of low,

intermediate or unlikely in pregnant, the safety of D-dimer use to exclude VTE in pregnant patients holds great promises.

According to these studies at 3 months follow up for thromboembolic risk in low and intermediate risk cases (wells Pre Test Probability criteria) when D-dimer values were < 500 ng/ml, was just 2/981 and 1/312.

These observations were perfectly in line with the recent recommendations from the International Society of Thrombosis &Hemostasis, suggesting that the upper bound of the 3-month VTE risk should be below 2% in diagnostic strategies for VTE.(24) In our study, although we did notclinically categorize (wells criteria) still the exclusion criteria and follow up at 4 weeks and 12 weeks follow up showed that,all the 17 patients did not develop VTE.

As can be observed in table 5, there is no a consensus about the D-dimer values in different trimesters of pregnancy. On the other hand, a general trend of increasing D-dimer values with each trimester and fall post-partum is seen. When the D-dimer values

fromnormal pregnant females was compared with that of pregnant females with DVT (X).In this study, the pregnant females with DVT in first trimester had 7-7.6 times higher values of D-dimer than the mean D-dimer value in the first trimester normal pregnant females. When compared with the D-dimer values in second trimester in pregnant with DVT,it was 1.6-5.4 times higher than the values in second trimester normal pregnant females. Lastly, in third trimester the D-dimer values in the pregnant with DVT was 2-3.8 times higher than the normal pregnant females.

When we compared the D-dimer values obtained in our study with the values from pregnant females with DVT in different trimesters from the study (X), and statistically analyzed (unpaired t test)the values were statistically significant. Table 6.

Thus although we had a small sample size, still when compared with known cases the values are statistically significant.

Conclusion: Thus, a reference range of D-dimer for normal pregnant women is required before utilizing D-dimer test along with Pre Test Probability to diagnose and detect high risk events of VTE in pregnancy.

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Table 1: List of exclusion criteria for pregnant females to be included in final study group.

Sno	**EXCLUSION CRITERIA AT THE TIME OF REGISTRATION**	**EXCLUSION CRITERIA DURING PRESENT PREGNANCY**
1	Age < 20 years> 35 years	Gestational diabetes
2	Family or personal history of thromboembolic disorder	Preeclampsia/ Eclampsia
3	Morbid obesity (BMI >40 Kg/m2)	Abruption placenta
4	Family or personal history of bleeding disorder	. Cholestasis of pregnancy
5	Infection with fever (>38 C)	Acute fatty liver of pregnancy
6	History of Autoimmune disorders	Intrauterine growth restriction
7	History of liver or kidney disease	Still birth
8	If taking any anticoagulant (oral or parenteral)	Inability to return to the hospital due to geographical inaccessibility
9	History of any recent surgery	
10	History of diabetes mellitus/ hypertension	
11	Previous obstetric complications (still birth/ Intrauterine growth restriction/ spontaneous abortion/ abruption placenta/ gestational diabetes/ preeclampsia/ eclampsia).	

TABLE 2: DISTRIBUTION OF D DIMER ACCORDING TO AGEOFTHEPATIENTS (N=17)

	Agecategory	N	Mean	Std. Deviation	Minimum	Maximum	P value
1sttrimester	20-25	5	257.60	74.718	135	319	0.093
	26-30	7	293.43	61.161	188	368	
	31-35	5	401.80	155.938	255	577	
	Total	17	314.76	111.648	135	577	
2ndtrimester	20-25	5	393.80	136.959	272	625	0.284
	26-30	7	303.29	59.601	238	402	
	30-35	5	440.60	224.850	227	757	
	Total	17	370.29	149.474	227	757	
3rdtrimester	20-25	5	524.80	204.390	194	733	0.295
	26-30	7	332.57	96.005	205	520	
	30-35	5	432.80	295.913	204	929	
	Total	17	418.59	206.438	194	929	
4week	20-25	5	301.40	128.436	187	520	

postpar tum	26-30	7	231.00	43.882	183	293	0.323
	30-35	5	300.60	96.996	231	470	
	Total	17	272.18	91.974	183	520	

TABLE 3: DISTRIBUTION OF D DIMER ACCORDING TOPARITY

Parity			1st trimester	2nd trimester	3rd trimester	4 weeks postpartum	P value
Nulliparous	N		11	11	11	11	0.016
	Mean		315.0000	401.9091	451.2727	273.2727	
	Std.Deviation		95.05262	176.15246	236.02843	90.65329	
	Minimum		188.00	227.00	194.00	183.00	
	Maximum		565.00	757.00	929.00	520.00	
	Percentiles	25th	267.0000	272.0000	283.0000	219.0000	
		50th (Median)	310.0000	324.0000	349.0000	262.0000	
		75th	319.0000	594.0000	637.0000	293.0000	
2nd pregnancy	N		6	6	6	6	
	Mean		314.3333	312.3333	358.6667	270.1667	
	Std.Deviation		147.71008	56.62744	135.26221	103.08330	

	Minimum		135.00	238.00	205.00	183.00	0.281
	Maximum		577.00	390.00	559.00	470.00	
	Percentiles	25th	225.0000	257.5000	234.2500	206.2500	
		50th (Median)	280.5000	312.5000	335.0000	236.5000	
		75th	412.7500	365.2500	495.2500	328.2500	
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Table 4: MEAN VALUE OF D-DIMER IN EACH TRIMESTER OFPREGNANCY AND4 WEEKSPOSTPARTUM(n=17)

		1sttrimester	2ndtrimester	3rdtrimester	4weekpostpartum	P value
						0.005
Mean(ng/ml)		314.76	370.29	418.59	272.18	
Std.Deviation (ng/ml)		111.648	149.474	206.438	91.974	
Minimum (ng/ml)		135	227	194	183	
Maximum(ng/ml)		577	757	929	520	
Percentiles	25th	261.00	268.00	263.50	216.50	
	50th (Median)	302.00	321.00	344.00	245.00	
		-3836			3832	

Table 5: Comparison of Mean and Reference ranges in current study and previous publications on D-dimer levels in Pregnancy. D-Dimer (ng/ml)

Sno	Author	Study population	instrument	Journal /year	Age group	1st trimester/ Range	2st trimester/ Range	3st trimester / Range	6-8 weeks post partum
1	Mirjana et al	89	Instrumentation laboratory (IL)	2009	18-40	222 (121-474)	326 (171-733)	475(206-890)	223(110-390)
2	Aldona et al (64)	37	Enzyme linked fluorescence assay	2020	25-44	376 (247-505)	688 (252-1124)	1082 (646-1168)	Not done
3	Nornattasa (69)	101	ACL top machine	2019	18-48	481 (<1070)	1073 (357-1748)	1533 (771-2410)	Not included
4	Tang et al	Metanalysis (30 Studies,15514)	variable	2018	18-44	570ng/ml (430-710)	980ng/ml (750-1210)	1480ng/ml (1810-1770)	790ng/ml (430 1160)
5	Our study	18	ACL Elite pro	2022	20-35	314 ng/ml (261-338)	370 ng/ml (268-396)	418 ng/ml (263-539)	223 ng/ml(216 287)

Table 6: Pattern of D-dimer values when cut off is kept at >500 ng/ml instead of 255 ng/mlas given in kit insert of reagent.

Cut off value of D-dimer	1ST Trimester	1ST Trimester	1ST Trimester	Post partum
>255ng m/ml	76%	88.2 %	76%	53 %
>500 ng/ml	12%	18%	35%	5%

Table 7: Comparison between D-dimer values in 17 subjects with normal pregnancy with pregnant cases with DVT.

1st trimester							
group	Group I (OUR VALUES) (1st trimester)	Group II (Confirmed cases of DVT in preg ±) Marjana et al(1st trimester	t	df	95% Confidence Interval	P value	
N	17	10	30.36	25	-1369 to -	<.0001	

Mean± SD	313.76 ± 111.64	1596 ± 95			1195.28		
SEM	27.07	30					
2nd trimester							
N	17	10	5.52	25	-1318.42 to -601.57	<.0001	
Mean± SD	370 ± 149.47	1330±700					
SEM	36.25	221.36					
3RD Trimester							
N	17	10	6.64	25	-966.09 to -508.72	<.0001	
Mean± SD	418.6±206.43	1156 ±374					
SEM	50	118.27					