RESEARCH ARTICLE

Comparison of thrombomodulin, vWF, and ADAMTS13 levels between preeclampsia and normal pregnancy

Aisha Ahmad, Ghulam Mustafa, Nazish Mazari, Muhammad Asif Naveed

Abstract

Objective: To determine and compare plasma thrombomodulin, von Willebrand factor and von Willebrand factor-cleaving protease levels between pre-eclamptic and healthy pregnant females.

Method: The cross-sectional, comparative study was conducted at the Department of Haematology, University of Health Sciences, Lahore, Pakistan, from November 2019 to December 2020, and comprised pregnant females who were divided into healthy pregnant group A and pre-eclamptic group B. Plasma thrombomodulin and von Willebrand factor-cleaving protease levels were determined by using commercially available enzyme-linked immunosorbent assay kit, and von Willebrand factor level was determined by using immuno-turbidimetric assay kit. Data was analysed using SPSS 25.

Results: Of the 88 participants, there were 44(50%) females with mean age 25.5±6 years in group A and 44(50%) in group B with mean age 26±5 years. Median thrombomodulin level in group B was significantly higher than group A (p=0.003). Median von Willebrand factor-cleaving protease levels were lower in group B compared to group A (p=0.838). A significant difference in von Willebrand factor level was observed between the groups (p=0.038).

Conclusion: Females with pre-eclampsia had significantly higher plasma levels of von Willebrand factor and thrombomodulin than healthy pregnant subjects.

Keywords: Thrombomodulin, vWF, ADAMTS13 protein, Pre-eclampsia. (JPMA 73: 38; 2023)

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Introduction

Pre-eclampsia (PE) is a multi-system gestational disorder that affects 2-8% of all pregnancies across the world.[1] Approximately 12% of mothers die because of pre-eclampsia. Women in developing countries have a 14-fold increased risk of dying from obstetric complications compared to women in the developed world.[2] According to a comprehensive analysis of global mortality, Pakistan is the world's 6th most populous country and has the third-highest maternal, foetal and child mortality burden.[3] Despite significant advancements in the country's healthcare system, Pakistan continues to face several challenges due to its high population growth, newborn and maternal mortality, and a wide range of infectious and non-infectious disorders. In a tertiary care hospital in Sukkur, Pakistan, PE and eclampsia were shown to be among the most important risk factors for hypertensive diseases during pregnancy that may cause maternal and perinatal mortality and morbidity.[4]

The pathogenesis of PE involves inadequate cytotrophoblast invasion, with subsequent abnormal placentation resulting in narrow spiral arterioles leading to placental hypo-perfusion and ischaemia with micro-thrombotic lesions. Hypo-perfused and underdeveloped placentas produce pro-thrombotic and pro-inflammatory substances into maternal circulation during the late maternal stage, producing endothelial dysfunction and thrombosis. Biochemical markers involved in the pathogenesis of PE include the most easily measurable and potentially modifiable risk factors thrombomodulin (TM), von Willebrand factor (vWF) and von Willebrand factor-cleaving protease (vWFCP), which is also known as a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13).[5]

TM is a 557-amino-acid residue long protein encoded by the intron less thrombomodulin (THBD) gene and is uniformly expressed on the vascular endothelium. In healthy people, the plasma levels of TM remain comparatively low. Endothelial injury of any origin causes proteolysis and shedding of endothelial TM, manifesting in a 1.5-2-fold increase in TM plasma levels. This phenomenon is seen in various diseases, such as PE, disseminated intravascular coagulation (DIC), thrombotic thrombo-cytopenia purpura (TTP), scleroderma-associated pulmonary hypertension, and arterial hypertension.[6]

Further, vWF is a massive heterogeneous, sticky glycoprotein which is constitutively produced by the endothelium and stored in Weibel-Palade bodies. The vWF is activated when it binds to sub-endothelial structures

Department of Haematology, University of Health Sciences, Lahore, Pakistan.

Correspondence: Muhammad Asif Naveed. e-mail: dr_asifnaveed@hotmail.com

ORCID ID. 0000-0003-2836-4896

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exposed after endothelial injury, or in small arterioles and atherosclerotic arteries with significant shear stresses. After binding of vWF to platelet glycoprotein Ib (GpIb), pro-coagulant platelet-derived micro-particles are generated which further enhance thrombus formation. Of the vWF released into the plasma, the highest molecular weight multimers are the most haemostatically active. The ultra-large multimers, if left unchecked in the circulation, can lead to the formation of vWF-platelet micro-thrombi that can occlude small blood vessels, obstruct blood flow, and eventually lead to end-organ damage.[7]

ADAMTS13 is a circulating metalloprotease which cleaves a single peptide bond (Tyr1605-Met1606) in the core A2 domain of the vWF molecule. This cleavage is required to decrease the size of ultra-large molecular weight multimers of vWF, which are prone to clumping with platelets (PLTs) when subjected to high shear stress in the microcirculation, causing severe thrombotic micro-angiopathies (TMAs). Reduced plasma ADAMTS13 and raised plasma vWF have been reported as risk factors for myocardial infarction (MI), PE and cerebral malaria.[8]

The current study was planned to measure the plasma TM, vWF and ADAMTS13 levels among PE and normotensive pregnant females.

Patients and Methods

The cross-sectional, comparative study was conducted at the Department of Haematology, University of Health Sciences (UHS), Lahore, Pakistan, from November 2019 to December 2020. After approval from the institutional ethics review committee, the sample was raised using convenience non-probability sampling technique from among those in the labour room or the outpatient departments (OPDs) of Lady Willingdon and Jinnah Hospital, Lahore. The sample size was determined using the World Health Organisation (WHO) calculator[9] version 2.0.21 with alpha value 0.05 and power of study 0.9. The formula[10] used was:

$$n = \frac{\left(Z_{1-\beta} + Z_{1-\frac{\alpha}{2}}\right)^2 (\sigma_1^2 + \sigma_2^2)}{(\mu_1 - \mu_2)^2}$$

Z_(1-β)= desired power of study 90%; Z_(1-α/2)= desired level of significance 5%; μ_1= anticipated mean ADAMTS13 in PE females 559ng/mL; μ_2= anticipated mean ADAMTS13 in normotensive pregnant females 680ng/mL; σ_1= standard deviation of mean ADAMTS13 levels in PE females 183; and σ_2= standard deviation of mean ADAMTS13 levels in normotensive pregnant females 172.[11]

Those included were pregnant females who were divided into healthy pregnant group A and age-matched PE group B. Written informed consent was taken from al the subjects. The study excluded females who had chronic hypertension, past history of MI, peripheral vascular disease, cerebrovascular disease, diabetes mellitus (DM), systemic infection, multi-foetal gestation, or hereditary/acquired thrombophilia. Females having the O blood group were also excluded as individuals with type O blood have significantly decreased plasma levels of vWF compared to those with non-O blood types (A, B and AB).[12] PEI was defined as per the American College of Obstetricians and Gynaecologists (ACOG) guidelines[13] as having a systolic blood pressure (SBP) greater than or equal to 140mmHg or a diastolic blood pressure (DBP) greater than or equal to 90mmHg on two occasions at least 4 hours apart in a patient who was previously normotensive and either proteinuria >300mg/24hr or protein/creatinine ratio greater than or equal to 0.3gm in a 24-hour urine sample, the or presence of 1+ urine dipstick protein.

A venous blood sample of 2.7ml was collected from each subject and PLT-poor plasma was extracted by centrifugation for 15 minutes at 3,000g. The extracted plasma sample was transferred into three 0.5ml Eppendorf tubes and stored at -80°C for further laboratory processing. Plasma TM and ADAMTS13 levels were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kits (Genway Biotech Inc. San Diego, US and Bioassay Technology Laboratory (BT LAB), Shanghai, China respectively). Plasma level of vWF was determined by using commercially available immuno-turbidimetric assay vWF kits (STA-LIATEST vWF: Ag, Stago, France).

Data was analysed using SPSS 25. Shapiro Wilk test was used to determine data normality, which revealed that weight, height, body mass index (BMI), haemoglobin (Hb), and PLT count were normally distributed parameters, whereas age, gestational age, SBP and DBP, total leucocyte count (TLC), TM, ADAMTS13 and vWF levels were not normally distributed. The normally distributed parameters were reported as mean and standard deviation, and data not normally distributed was reported as median and interquartile range (IQR). Independent t-test and Mann-Whitney U test were used for the comparison of quantitative parameters, and chi-square and Fisher exact tests were used for comparison of qualitative parameters between the groups. To determine the risk assessment, odds ratio (OR) and regression analysis tests were performed. Pearson's correlation test was applied to check correlation of normally distributed haematological parameters, and Spearman's rho test was applied to determine correlation of not normally distributed haematological parameters. P≤0.05 was considered significant.

Results

Of the 88 participants, there were 44(50%) females with mean age 25.5±6 years in group A and 44(50%) in group B with mean age 26±5 years. Demographic and haematological parameters of the overall sample were noted, and inter-group comparison Showed that SBP, DBP, BMI, TM and vWF values differed significantly (p<0.05), while ADAMTS13 levels were not significantly different (p=0.838) (Table 1).

Parity, miscarriage history, family history of PE, past history of foetal growth restriction, and past history of foetal death after 22 gestational weeks were significantly different between the groups (p<0.05), while clinical parameters, such as, Rhesus (Rh) blood group, and past history of pre-term delivery showed no significant difference (Table 2).

Within group B, a significant positive correlation was found between TM and vWF levels (p=0.005). Non-significant correlation of ADAMTS13 levels was observed with vWF levels in group B (p=0.418) (Table 3).

Furthr, BMI (OR: 1.39, 95% CI: 1.18-1.67, p<0.001) and vWF (OR: 1.00, 95% CI: 1.00-1.01, p=0.049) significantly increased the risk of developing PE in univariate regression analysis. Multivariate logistic regression model showed BMI and vWF as significant PE risk factors (Table 4).

Table-1: Demographic and haematological parameters in pre-eclamptic (PE) and healthy pregnant female subjects.

Study Variables	Total (n=88)	Healthy Pregnant (n=44) Mean±SD/ Median (IQR	PE Pregnant (n =44) Mean±SD/ Median (IQR)	*p*-value
Age (years)	26 (5)	25.5 (6)	26.0 (5)	0.814[b]
Gestational Age (weeks)	34 (18)	34.0 (6)	34.0 (5)	0.990[b]
Systolic Blood Pressure (mmHg)	130 (50)	110.0 (10)	160.0 (14)	<0.001[b]
Diastolic Blood Pressure (mmHg)	85 (40)	70.0 (0)	110.0 (10)	<0.001[b]
Body Mass Index (BMI) kg/m^2	26.2±3.7	24.5±2.7	27.9±3.7	<0.001a
Haemoglobin (g/dl)	10.7±1.4	10.9±1.1	10.5±1.6	0.193[a]
Total Leucocyte count (x10^3/µl)	9.5±2.2	9.2±1.3	9.7 (3)	0.341[b]
Platelet count (x10^3/µl)	255.8±70.3	248.7±48.2	262.9±87.0	0.347[a]
Thrombomodulin levels (pg/ml)	975.5 (802)	836.5 (635)	1115 (949)	0.003[b]
ADAMTS13 levels (ng/ml)	256 (186)	260 (159)	240 (256)	0.838[b]
Von Willebrand Factor %	230.1±99.2	209.0±103.2	227.5 (166)	0.038[b]

[a]=Independent student t-test, [b]=Mann-Whitney U-test and p≤0.05 was considered statistically significant; SD: Standard deviation, IQR: Interquartile range, ADAMTS13: a disinterring and metalloproteinase with a thrombospondin type 1 motif, member 13.

Table-2: Clinical and family history of pre-eclamptic (PE) and healthy pregnant female subjects.

Clinical and Family History	Categories	Healthy Pregnant (n=44)	PE Pregnant (n=44)	Odd Ratio	95%CI	Chi square *p-value*
Parity Difference	Nulliparous	8 (18.2%)	21 (47.7%)	4.109	1.56-10.82	0.003
	Primiparous + Multiparous	36 (81.8%)	23 (52.3%)			
Miscarriages history	Yes	Nil (0.0%)	6 (13.6%)	0.463	0.37-0.58	0.026*
	No	44 (100%)	38 (86.4%)			
Rh Blood Group	Positive	41 (93.2%)	39 (88.6%)	0.571	1.28-2.55	0.713*
	Negative	03 (6.8%)	05 (11.4%)			
H/O PE in previous pregnancies	Yes	Nil (0.0%)	19 (43.2%)	0.362	0.26-0.49	<0.001*
	No	44 (100%)	25 (56.8%)			
H/O PE in mother/sister	Yes	01 (2.3%)	09 (20.5%)	0.090	0.01-0.75	0.015*
	No	43 (97.7%)	35 (79.5%)			
Preterm history	Yes	Nil (0.0%)	02 (4.5%)	0.488	0.39-0.61	0.494*
	No	44 (100%)	42 (95.5%)			
Foetal growth restriction history	Yes	Nil (0.0%)	6 (13.6%)	0.463	0.37-0.58	0.026*
	No	44 (100%)	38 (86.4%)			
Previous foetal death after 22 gestational weeks	Yes	Nil (0.0%)	05 (11.4%)	0.470	0.37-0.59	0.055*
	No	44 (100%)	39 (88.6%)			

The Chi square test was applied and p≤0.05 was considered significant; *p-value generated from Fisher exact test; CI: Confidence interval, Rh: Rhesus, H/O: History of.

Discussion

Over the last several years, many studies have been done on biochemical markers involved in PE pathogenesis.[14] Among those biochemical markers, TM, vWF and ADAMTS13 have been shown to be the most easily measurable and potentially modifiable risk factors.[15,16] Hence, the current study focussed on these three markers.

The median gestational age and the median plasma levels of TM in the current study were in line with published results.[17] The possible cause of higher serum levels of soluble TM is widespread endothelium activation and damage in PE.[18] Boffa et al. also reported similar findings.[19] Hence, TM has the potential to be used as an early predictor of PE in high-risk women.[20]

In the present study, there was a significant difference in plasma vWF antigen levels between PE pregnant (227.5%) and healthy pregnant (209%) subjects. Univariate and multivariate analyses also identified vWF as a potential PE risk factor (OR: 1.00, 95% CI: 1.00-1.01, p=0.048). Dandan Zhang et al. observed that vWF levels increased during pregnancy, and the current study results were consistent with such findings.[21]

The current study also matched the findings of Aref and Goda et al., who revealed that PE pregnant females had significantly higher vWF levels than healthy pregnant females. The increased vWF levels in PE might be due to the

Table-3: Correlation of demographic and haematological parameters of pre-eclamptic (PE) females with one another (n =44).

Parameters		**Hb (g/dl)**	**TLC** (x10^3/ µL)	**PLT** (x10^3 µL)	**TM** (pg/ml)	**ADAMTS13** (ng/ml)	**vWF (%)**
Age (years)	*r*/rho	**0.305**[b]	-0.119[b]	**-0.370**[b]	0.055[b]	-0.011[b]	0.141b
	p-value	**0.044**	0.441	**0.013**	0.723	0.944	0.360
Hb (g/dl)	*r*/rho	1	0.184[b]	-0.063[a]	-0.333[b]	0.055[b]	0.090[b]
	p-value		0.232	0.683	0.834	0.724	0.563
TLC (x10^3/µL)	*r*/rho	0.184[b]	1	-0.058[b]	-0.001[b]	0.153[b]	0.070[b]
	p-value	0.232		0.710	0.996	0.321	0.652
PLT (x10^3 µL)	*r*/rho	-0.063[a]	-0.058[b]	1	0.069[b]	-0.027[b]	**-0.383**[b]
	p-value	0.683	0.710		0.656	0.862	**0.010**
TM (pg/ml)	*r*/rho	-0.333[b]	-0.001[b]	0.069[b]	1	0.069[b]	**0.416**[b]
	p-value	0.834	0.996	0.656		0.655	**0.005**
ADAMTS13 (ng/ml)	*r*/rho	0.055[b]	0.153[b]	-0.027[b]	0.069[b]	1	-0.125[b]
	p-value	0.724	0.321	0.862	0.655		0.418
vWF (%)	*r*/rho	0.090[b]	0.070[b]	**-0.383**[b]	**0.416**[b]	-0.125[b]	1
	p-value	0.563	0.652	**0.010**	**0.005**	0.418	
Gestational Age (weeks)	*r*/rho	0.000[b]	-0.027[b]	0.083[b]	-0.061[b]	0.191[b]	-0.011[b]
	p-value	0.999	0.864	0.592	0.695	0.214	0.942
SBP (mmHg)	*r*/ rho	0.154[b]	**0.545**[b]	-0.078[b]	-0.002[b]	0.023[b]	0.116[b]
	p-value	0.319	**<0.001**	0.615	0.992	0.882	0.454
DBP(mmHg)	*r*/rho	0.190[b]	**0.478**[b]	-0.139[b]	-0.012[b]	0.070[b]	0.187[b]
	p-value	0.216	**0.001**	0.367	0.936	0.652	0.224
BMI	*r*/rho	0.066[a]	-0.066[b]	**0.416**[a]	0.089[b]	-0.058[b]	-0.261[b]
	p-value	0.670	0.671	**0.005**	0.565	0.708	0.087

[a]Correlation coefficient (*r*) and *p*-values are generated by Pearson Correlation co-efficient; [b]Correlation coefficient (rho) and p-values are generated by Spearman's Rho Correlation coefficient; *p*-value≤0.05 was considered statistically significant; Hb: Haemoglobin, TLC: Total leukocyte count, PLT: Platelet, TM: Thrombomodulin, ADAMTS13: A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, BMI: Body mass index.

Table-4: Univariate and multivariate regression analysis for risk factors involved in pre-eclampsia (PE).

Variable	**OR**	**95%CI**	***p*-value**
Univariate Regression Analysis			
Age (Years)	0.987	0.90-1.08	0.777
Hb (g/dl)	0.813	0.60-1.11	0.192
TLC (x103/µL)	1.177	0.95-1.46	0.144
PLT (x103 µL)	1.003	0.10-1.00	0.343
BMI	1.394	1.18-1.65	**<0.001**
TM (pg/ml)	1.000	1.00-1.00	0.258
ADAMTS13 (ng/ml)	1.000	1.00-1.00	0.987
vWF (%)	1.005	1.00-1.01	**0.049**
Multivariate Regression Analysis			
BMI	1.405	1.18-1.67	**<0.001**
vWF (%)	1.005	1.00-1.01	**0.048**

OR: Odds ratio, CI: Confidence interval, Hb: Haemoglobin, TLC: Total leukocyte count, PLT: Platelet, TM: Thrombomodulin, ADAMTS13: A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13, BMI: Body mass index, vWF: von Willebrand factor.

widespread occlusion of placental and renal arterioles. The vWF aids PLT adhesion and aggregation under high shear stress conditions and it could be used as a marker to assess endothelial dysfunction because it is released from the vascular basement membrane.[22]

In the current study, median ADAMTS13 levels in the PE group were lower (240ng/ml) than in healthy ones (260ng/ml), but the difference was not significant (*p*=0.838). The findings were in line with earlier findings.[11] One study showed that the possible reason for the reduction in plasma levels of ADAMTS13 in PE pregnant women could be that it is required for the cleavage and clearance of ultra-large vWF from circulation.[23]

The correlation of vWF with TM was noted in the PE group, which matched earlier results, which in addition suggested that the possible reason for the correlation of these markers is the vascular endothelial disturbance and dysfunction which is recognised to be a central process in PE.[24] The current study observed an inverse and weak correlation of vWF with ADAMTS13 in PE and healthy groups. Similar findings were reported by Vicktoria Bitsadze et al. who also proposed that the decreased levels of ADAMTS13 could be due to its increased consumption in the cleavage of vWF in PE.[25]

In the current study, a number of PE risk factors were identified, including BMI, nulliparity and personal or family history of PE. These risk factors were also reported earlier.[26]

Although PE aetiology is not well understood, there has been a lot of work done to identify potential risk factors that could lead to PE. Some earlier studies had also found an association between vitamin B12 levels and PE in pregnant women.[27] Further research is required in this area.

The current study has limitations. Due to limited funds and laboratory resources, only TM, vWF and ADAMTS13 biomarkers could be investigated. Other endothelial dysfunction markers, like plasminogen activator inhibitor-1, plasma angiogenic markers, such as placental growth factor (PlGF), and other inflammatory cytokines, including tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) or IL-8, have also been cited in literature[28] which the current study did not explore.

Conclusion

Plasma vWF and TM levels were significantly raised in PE pregnant females, but ADAMTS13 levels were not significantly different between the groups.

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Author Contribution:

MAN, GM, NM: Study Design, drafted the work, supervised, final approval of research.
AA, GM: Data collection, literature search, experimental work, analysis, critical revision.
AA, GM, NM: Manuscript writing, literature search, critical revision, accuracy and integrity of the work, proofreading.
AA, GM, MAN: Study Design, drafting the work, data analysis, interpretations, accuracy and integrity of the work, proofreading, final approval.