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Evaluation of soluble TNF-like weak inducer of apoptosis (sTWEAK) levels to predict preeclampsia in early weeks of pregnancy

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Title

Evaluation of Soluble TNF-like weak inducer of apoptosis (sTWEAK) Levels to Predict Preeclampsia in Early Weeks of Pregnancy

Short title

sTWEAK in Preeclampsia

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Author Contribution:
SSF conceived the project, analyzed the data and wrote the paper. SS collected and followed study subject, performed the experiments and wrote the paper. EK and GMK wrote the paper. All authors approved the final version before submission and publication.

Abstract

Introduction: Soluble tumor necrosis factor-like weak inducer of apoptosis (sTWEAK) is linked to endothelial dysfunction; a key factor in pre-eclamptic pathogenesis. This study aimed to compare sTWEAK levels during pregnancy to assess for its prognostic ability.

Materials and Methods: Sixty three high risk pregnant women were followed up from 12 weeks of gestation till term. Serum levels of sTWEAK and platelet derived growth factor (PlGF), blood pressure, serum glucose, uric acid, urea/creatinine and liver function tests were measured. Subjects were stratified according to the ACOG criteria as women who developed PE, or PIH or remained normotensive at term. A negative control group of normotensive healthy pregnant women (n=17) was also recruited for comparison.

Results: Baseline sTWEAK levels were lower (4.03±0.37ng/dl) in HR cohort that developed PE and further reduced at term (1.93±0.23ng/dl) as compared to HR subjects who remained normotensive and negative control group (30.53±0.79ng/dl; $p<0.01$). Likewise PlGF levels were significantly lower (74.22±10.11pg/ml) in HR cohort that developed PE ($p=0.013$). At term 39.68% (n=22) HR subjects with low sTWEAK developed PIH and 34.92% (n=24) developed PE. In terms of high risk characteristics observed in the HR group; 73% of the subjects were multiparous, whereas 26.98% reported to have developed PE in previous pregnancies.

Conclusion: sTWEAK levels at early pregnancy weeks were found to be low in high risk females who developed PE at follow up versus normotensive pregnant women. Baseline TWEAK might serve as an independent variable for prediction of pre-eclampsia; however longitudinal studies with larger sample size are required to ascertain the causal relation.

Key words: Hypertension; Pregnancy; Pre-eclampsia; sTWEAK

Introduction

Pre-eclampsia, is characterized by sudden onset of hypertension and proteinuria in women with no preceding hypertensive history. Due to its dormant nature, it possess great threat to maternal as well as fetal wellbeing (1). Globally 5-14% of all pregnancies are complicated by PE and in developing countries it's prevalence is 4-18% (2). PE is a multifactor disorder, starting with placental dysfunction leading to augmented anti-angiogenic response in mother (3). With the loss of balance between pro- and antiangiogenic factors, the maternal endothelial function deteriorates (4). It has also been proposed that normal pregnancy features subservient inflammatory response in itself, however, in PE this response is augmented. The augmented systemic response causes dysfunction in maternal endothelium by involving maternal leukocytes, platelets and also activating pro-inflammatory cytokines (5-7).

For PE, early prediction has always been the primary priority of many clinician and researchers. Previous maternal history and presence of risk factors alone are not reliable for the prediction; therefore more profound monitoring is required for prompt diagnosis and fruitful treatment strategies. Keeping this in view, detection of PE biomarkers in early pregnancy has always been an interesting field of research (8). Placental growth factor (PlGF), pregnancy associated plasma protein A (PAPP-A), Free fetal hemoglobin (HbF), Soluble Endoglin and placental protein 13 (PP-13) are among some of the commonly studied biomarkers for PE pathology (9, 10) (11). Yet the predictive value as a single most important, reliable, and effective marker has not been proven till date (12). Among previously identified predictive serum proangiogenic biomarker related to pre-eclamptic pathogenesis is placental growth factor (PlGF) (13). PlGF is a member of vascular endothelial growth factor (VEGF) family and is highly expressed in placental tissue for healthy placentation (14). The placental angiogenic factors are responsible for proper placental development through neovascularization, cellular remolding and maintaining nitric oxide levels. Lack of these factors seem to play role in defective placentation (15). Serum PlGF levels were found to be reduced in early weeks of pregnancy by multiple studies (16, 17), thus indicating its role in pre-eclamptic pathogenesis.

Recently, a new serum marker, soluble tumor necrosis factor like weak inducer of apoptosis (sTWEAK), has been proposed to be altered in the maternal blood. sTWEAK is a multipurpose cytokine which is involved in conducting diverse biological events, like cellular multiplication, growth, migration, angiogenesis, cell differentiation, apoptosis and inflammation by instigating expression of multiple pro-inflammatory cytokines. Furthermore, in peculiar it appears to perform critical role in tissue repair and wound healing (3). TWEAK is universally expressed as Type II trans-membrane protein of 35-kDA which after cleaving, shed functionally active soluble 18-kDA processed factor called as sTWEAK (18). sTWEAK is widely expressed in many different tissues (19). Overall data suggests that sTWEAK may have physiological as well as pathological responses in tissues. On one hand it is known to induce proliferation of endothelial cells in vitro and angiogenesis in vivo and on other hand triggers the production of pro-inflammatory cytokines (20). Binding of sTWEAK to its receptor results in activation of any one of the three effector pathways; a) proliferative, b) inflammatory or c) apoptotic, in which the inflammatory pathway being the most dominant one. The other 2 pathway activation depends on the cellular integrity. With intact cellular physiological mechanisms along with absence of inflammatory process, the effector pathway will be the proliferative pathway, however in the presence of pro-inflammatory cytokines (TNF-α, IFN γ), the predominant pathway is the apoptotic one (21). In this context, Donohue and group have demonstrated the in vivo stimulatory effect of sTWEAK on angiogenesis in human endothelial cells (3, 22).

Since pregnancy is the condition comprising of both angiogenesis and mild inflammation; there might be possibility of sTWEAK's involvement in these processes at the time of placentation. Further any alteration in the levels of sTWEAK and PlGF in the beginning of pregnancy may lead to development of PE. Keeping the literature in view, we proposed that there might be close relation of pre-eclamptic pathogenesis to disrupted angiogenic system and endothelial dysfunction caused by low sTWEAK. Hence, this study aimed to compare sTWEAK levels during pregnancy to assess for its prognostic ability.

Methods:

This prospective study was conducted from January 2017 to March 2018. The study was approved by the Intuitional Review Board (IRB) of Jinnah Post Graduate Medical Centre (JPMC) (Ref: NO.F.2-81/GENL-2017-IRB/15107/JPMC) and Aga Khan University (4523-BBS-ERC-16) in collaboration with Taj Medical Complex, Karachi. The minimum sample required for this study was 60 subjects with a 95% confidence interval and a 4% frequency of outcome factor in the population (23). A total of n=137 pregnant women were initially enrolled from the obstetrical clinics of these hospitals; out of these n=80 subjects were successfully followed till the end of study and were included in this manuscript.

Out of the study cohort; 63 subjects were classified as high risk pregnant women (HR group). The HR status was based on the presence of any of the criteria: first pregnancy/ family history of PE/ multiple gestation/ previous history of PE/ maternal age up to 35 years / BMI of >35 kg/m^2 and or presence of chronic hypertension. These subjects were followed up from 12 weeks of gestation till term (observed for development of PE/HTN). Pre-eclampsia diagnoses was based on the American College of Obstetricians and Gynecologists (ACOG) criteria as follows:- values of systolic blood pressure 140mm/Hg or higher and diastolic blood pressure 90mm/Hg or higher after 20 weeks of gestation with or without dipstick proteinuria (0.3gm/l or $>1^+$) were taken as a reference (24). The remaining (n=17) females who did not show any proteinuria or blood pressure derangements were labeled as pregnant negative controls. For all study subjects females with chronic systemic disease (cardiovascular, urogenital, immunological, endocrinological), renal disease, previous history of complication of pregnancy such as abortion, intra-uterine fetal demise, antenatal bleeding were excluded from the study.

An informed written consent form was signed by each subject. Their demographic data, medical and obstetrical history and examination were recorded at the time of enrolment on a predesigned form. Serum samples were obtained and analyzed for complete blood count, random blood glucose, uric acid, urea/creatinine and liver function test. Freshly voided early morning mid-stream urine sample was obtained for estimation of proteinuria. The serum sTWEAK concentration was determined by using Human sTWEAK ELISA Kit (Cat. No. H1911 by Glory Science Co, Ltd Belgium) and PlGF (kit cat #DPG00 by R&D systems USA) according to the provided protocol. In

high risk and control group, blood samples were collected a) at baseline (12-16weeks) and b) follow-up (28-36 weeks gestation).

Statistical analysis was conducted by SPSS version 23.0. A descriptive statistical analysis of continuous variables was performed. Data on continuous variables i.e. biophysical (age, height, weight, blood pressure etc.) and biochemical (Serum sTWEAK, PlGF, blood glucose, serum uric acid, serum creatinine, etc.) parameters were expressed as Mean ± standard deviation (SD) or standard error of mean (SEM) whereas data on categorical variables were presented as absolute number and percentages. Statistical comparisons were performed by using student t-test, paired sample t-test and Mann Whitney-U-test for continuous/quantitative variables, chi-square or Fisher exact test for categorical variables. In all statistical analysis only p-value < 0.05 was taken as significant.

Results

The detailed results of this study are shown in Tables 1-3. Table 1 shows the demographic distribution of study cohort. Mean age, BMI, weight, hemoglobin levels and blood glucose parameters were matched for each group therefore no difference was seen ($p>0.05$). Alkaline phosphatase levels were slightly raised in HR (243.38±78.36 mg/dl) as compared to control group (167.0±120.22 mg/dl; $p=0.039$). Serum uric acid and urea showed no difference among the groups ($p>0.05$). In terms of high risk characteristics observed in the HR group; 73% study subjects were multiparous, whereas 26.98% reported to have developed PE in previous pregnancies.

Table 2 shows the systolic and diastolic blood pressure of study subjects stratified according to the HR outcome. Baseline blood pressure reading of each subject was within normal range; whereas subjects who developed PIH (n=24) or PE (n=22) had significantly higher blood pressure readings than normotensive HR subjects (n=17) and negative control group (n=17) ($p<0.01$). The urine dipstick assay for urine protein and glucose showed 22% of HR subjects with positive proteinuria while 3.2% were positive for glycosuria varying degrees.

The assessment of baseline sTWEAK levels revealed a lower value (4.03±0.37ng/dl) in subjects that developed PE and were further reduced at term (1.93±0.23ng/dl) in comparison to normotensive HR subjects and negative control group ($p<0.001$). Similar trend was observed for baseline and follow up sTWEAK levels of HR subjects who developed PIH ($p<0.001$). Likewise, PlGF levels were significantly low in HR cohort that developed PE (74.22±10.11ng/dl) or PIH (89.38±8.38ng/dl) as compared to normotensive HR (101.0±12.13ng/dl) and negative controls (109.82±7.83ng/dl) ($p=0.013$) (Table 3). The pregnancy outcomes of these HR subjects were as follows: 2 IUGR; 3 IUD's; 19 LSCS; where the remaining 24 were delivered via simple vaginal deliveries.

Discussion:

Recent advancements in pre-eclamptic management has upgraded the level of safe pregnancy and to some extent reduced the mortality and morbidity in developed countries, however in developing countries there is still a need to improve the pregnancy outcome by early detection of pregnancy complications. In this study, sTWEAK has emerged as a promising contemporary biomarker for early pre-eclamptic prediction in women having risk factors such as multiple pregnancies, previous history of PE and age of up to 35 year. The study reports that a considerable number of pregnant subjects with remarkably lower sTWEAK levels at baseline and follow up developed either pre-eclampsia or pregnancy induced hypertension near term. This finding reinforces the assumption that low sTWEAK is related with the progression or disease severity (21). Therefore, it is plausible that this finding may give a novel insight into the ability of sTWEAK to identify high risk subjects during early pregnancy weeks for the first time that may develop PE later. This finding is in consensus with the only available published study which found decreased serum sTWEAK concentration in pre-eclamptic women as compared to controls (p-value 0.04) (3). However, there is a slight difference in reported concentrations that may be attributed to a different population, different time of sample collection and difference assay protocol. Moreover, in this study sTWEAK levels were slightly lower (but not below 9ng/dl) in normotensive HR subjects in comparison to pregnant controls, which could be due to the presence of risk factors influencing the general condition of the patients. In addition, these subjects had a normal PlGF level that might have compensated for the changes in pregnancy.

Currently, no research data is available regarding the role of sTWEAK in pre-eclamptic pathogenesis. However, several previous studies have identified sTWEAK as a potent inducer of angiogenesis, acting as a mitogenic factor for human endothelial cells (25). It is also found to be linked with endothelial dysfunction in non-dialysis chronic kidney disease, diabetic and renal transplant patients (26). Decreased sTWEAK concentrations were also detected in conditions like atherosclerosis, coronary artery disease and peripheral arterial disease (27). Another group linked lower sTWEAK to inflammatory

changes in gestational diabetes (GDM) and insulin resistance (28); however in this study only 2 cases of GDM were observed.

The current study also reports a reduced maternal serum PlGF level along with reduced sTWEAK levels in early pregnancy indicating its link to pre-eclamptic pathogenesis. Maternal PlGF involvement in proper placentation through effective angiogenesis has been proved previously (29). PlGF works as a mitogenic factor for endothelial cells and its levels are high throughout the pregnancy (14), therefore, the reduced levels of PlGF may predict pre-eclampsia (30). However, studies have proposed that PlGF alone has a limited predictive capacity (31). The predictive value of most of the biomarkers working as a single entity is not satisfactory unless a combination of markers are used (9). Since this study suggests sTWEAK ability to predict PE as an independent marker, introducing the new combination of sTWEAK and PlGF may enhance the screening capabilities for the disease in early weeks of pregnancy.

The connection of sTWEAK with the pre-elcamptic pathogenesis can be explained by its behavior as an inflammatory cytokine. Since pregnancy is considered as a low grade inflammatory condition (32); sTWEAK physiological or pathological nature, may have some contribution in pre-eclamptic pathogenesis, owing to the fact of exaggerated maternal inflammatory response in pre-eclamptic pregnancy (33). Published data also suggest the role of sTWEAK in neovascularization (25), there might be a possibility that deficiency of sTWEAK in the blood is responsible for defective angiogenesis in placenta leading to PE as seen in this study.

In Pakistan, the overall estimated incidence of intra uterine deaths is around 5.22% (34) which is linked to cases of antepartum hemorrhage, hypertensive disorders of pregnancy (preeclampsia and eclampsia), mismanagement of labor and diabetes etc. In this study, the number of IUD's reported was 3 (4.76%) and IUGR was 2 (3.17%) in the PE/PIH group, which is comparable to the available data (35, 36).

This study was however limited on commenting on the prediction of early onset and late onset PE as the number of patients in this study design was limited. Additionally, it is uncertain whether sTWEAK is superior in predicting PE alone or in coalition with other angiogenic factors such as PlGF. This ensues for the requirement of more

prospective studies in assessing the role of sTWEAK alone and comparing its prognostic performance when combined with other biomarkers. Despite the limitations, this is perhaps the first prospective study on the role of sTWEAK in diagnosing PE and these findings may open ways for future researchers in assessing the molecular events that lead to the disease pathogenesis.

Conclusion:

sTWEAK levels at early pregnancy weeks were found to be low in high risk females who developed pre-eclampsia at follow up versus normotensive pregnant women. Baseline TWEAK might serve as an independent variable for prediction of pre-eclampsia; however longitudinal studies with larger sample size are required to ascertain the causal relation.

Conflicts of interest: none declared.

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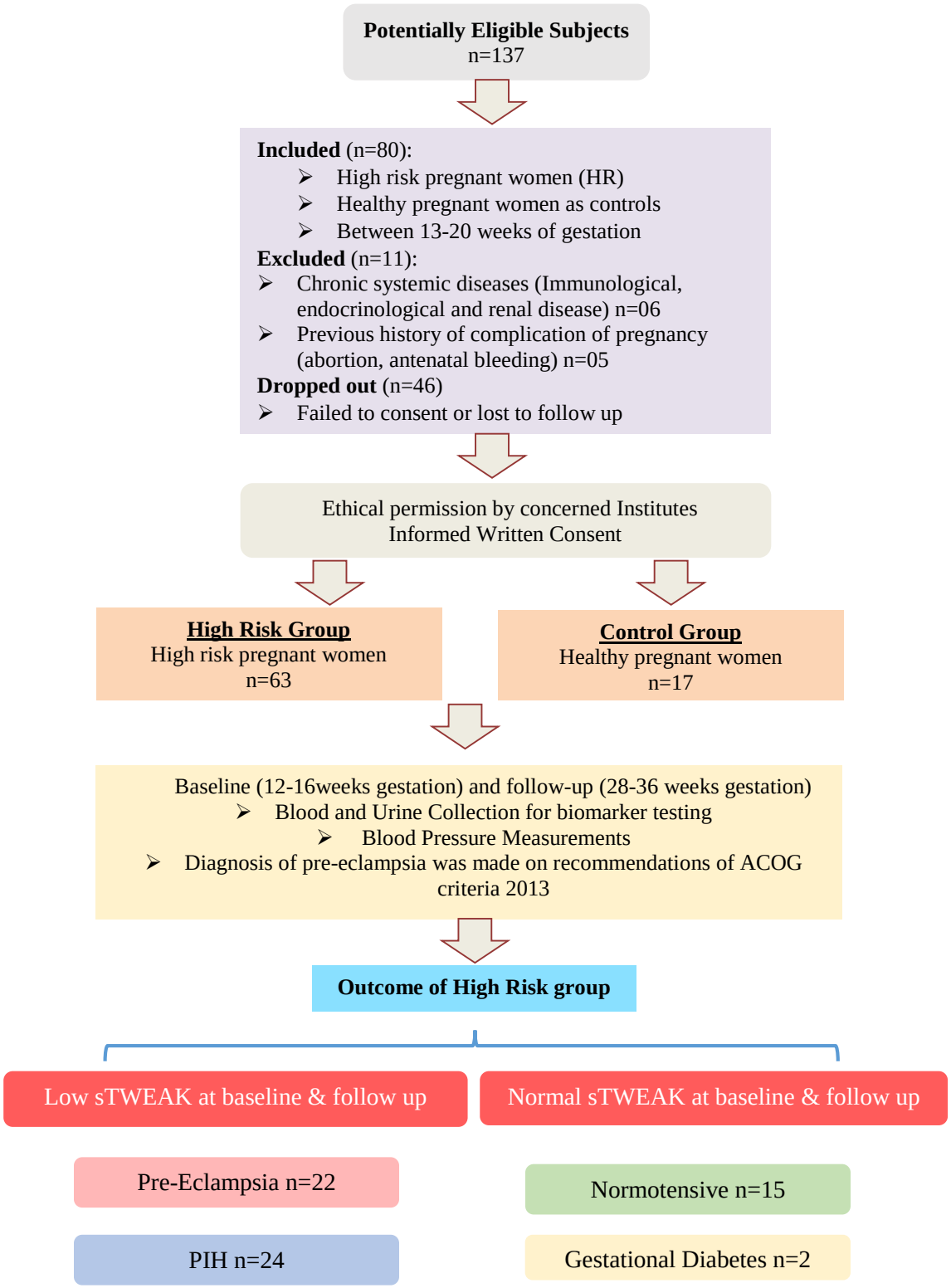

Figure 1: Schematic Research Methodology and Outcome of High risk Subjects

291 **Table 1: Baseline Data of the study Cohort**

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		High risk group n=63	Negative Control n=17	P value
Age (year)		27.05± 5.89	30.65 ± 9.40	0.474
Weight (kg)		68.65±19.34	62.71±14.22	0.488
Body mass index (kg/m^2)		27.01±7.02	24.14±5.30	0.248
Hemoglobin (g %)		11.2±1.20	11.35±1.01	0.334
Random Blood Glucose (mg/dl)		96.24±23.66	98.45±10.27	0.840
SGPT (U/L)		21.73±10.7	10.00±1.22	0.040
Alkaline Phosphatase (U/L)		243.3±78.36	167.0±120.22	0.039
Total Bilirubin (mg/dl)		0.571±0.21	0.30±0.11	0.145
Direct Bilirubin (mg/dl)		0.13±0.03	0.10±0.02	0.601
Serum Uric Acid (mg/dl)		3.91±1.09	3.50±1.00	0.540
Serum Urea (mg/dl)		16.45±5.76	12.00±2.45	0.260
Serum Creatinine (mg/dl)		0.60±0.12	0.80±0.11	0.250
Parity	Primi	17 (26.98)	14 (82.23)	<0.01
	Multi	46 (73.01)	2 (11.76)	
Previous History of PE		17 (26.98)	--	<0.001
No of fetus	Singleton	60 (95.23)	17 (100)	0.621
	Multiple	3 (4.76)	--	

Values expressed as Mean ± SEM and absolute values and percentages in parenthesis. Comparison between groups was made by Man Whitney U test, Chi square statistics or Fischer exact test. Statistically significant as compared to normotensives $p<0.05$

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295 **Table 2: Serial Blood Pressure Measurements, Proteinuria and Glycosuria in HR &**
296 **Control Groups**

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Blood Pressure In Different Gestational weeks	High Risk Group (n=63)			Negative Control (n=17)	p value
	Developed PE (n=22)	Developed PIH (n=24)	Normotensive (n=17)		
Systolic Baseline (mmHg) at 12 weeks	120.00 ±17.45	118.10±15.84	114.70 ± 7.98	110.59±13.44	0.124
Diastolic Baseline (mmHg) at 12 weeks	78.64 ± 14.57	75.48±13.98	74.38 ± 8.92	72.35±9.701	0.059
Systolic Follow up at (mmHg) 28 weeks	159.50 ± 17.00	121.00±4.55	112.35 ± 9.70	109.09±10.41	0.007
Diastolic Follow up at (mmHg) 28 weeks	93.81 ± 16.57	89.25 ± 11.20	75.29 ± 7.99	82.05±8.80	0.412
Systolic Follow up at (mmHg) 32 weeks	133.64 ± 16.84	127.62 ± 13.43	111.76 ± 9.51	108.22±5.89	0.009
Diastolic Follow up at (mmHg) 32 weeks	85.50 ± 6.048	85.59 ± 9.735	79.62 ± 3.54	79.55±8.45	0.052
Urine Dipstick Analysis for Protein and Glucose					
	High Risk Group (n=63)		**Negative Control (n=17)**		
Urine Protein					
0	49 (77.9)		15 (88.23)		
1+	5 (7.9)		2 (11.76)		
2+	2 (3.2)		--		
3+	7 (11.1)		--		
Urine Glucose					
0	61 (96.8)		17 (100)		
1	2 (3.2)		--		

Values expressed as Mean ± SEM or as absolute values and percentages in parenthesis. Comparison between groups was made by Man Whitney U test. Statistically significant as compared to normotensive and control $p<0.05$.

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Table 3: sTWEAK and PlGF Levels in subjects stratified based on HR group outcomes

Biomarkers	High Risk Group (n=63)			Negative Control (n=17)	p-value
	Developed PE (n=22)	Developed PIH (n=24)	Normotensive (n=17)		
sTWEAK Baseline (ng/dl)	4.03 ± 0.37	5.80 ± 0.56	13.29 ± 0.70	15.10± 0.64	<0.001
sTWEAK Follow up (ng/dl)	1.93 ± 0.23	8.35 ± 0.78	10.13 ± 1.10	30.53± 0.79	<0.001
PlGF (pg/ml)	74.22± 10.11	89.38 ± 8.38	101.0 ± 12.13	109.82 ± 7.83	0.013
Values expressed as Mean ± SEM. Comparison between groups was made by T Test, Man Whitney U test. Statistically significant as compared to normotensives p<0.05					

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