Comparative Study Between soluble FMS like-Tyrosine kinase1 and Neurokinin in prediction of preeclampsia

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Abstract: Pre-eclampsia (PE) clinically known to be presented in 7–10% of gestations and a well-known leading factor in maternal mortality and morbidity Preeclampsia is accompanied with a changed maternal pattern of circulating placentally originating proteins controlling angiogenesis. **Methodology:**100 cases were recruited in this study 50 normotensive (control group) and 50 hypertensive (study group) gestations the biomarkers of interest were FMS Like Tyrosine Kinase1 and Neurokinin B investigated at 28 weeks and at 32 weeks from patients at El sayed galal and Elhussein h ospitalsduring the p eriodfrom April2017 t oOctober 2017. Blood sample was collected from the patients to investigate for the FMS tyrosine kinase 1 and Neurokinin B using enzyme immune assay. **Results**: Comparison between normal women and those who developed PET in all women using students (t) test, showing only one variable having statistical difference which is NK level at 32 weeks p value =0.0001compares between hypertensive and control group as regards development of maternal and fetal complications using Chi square test showing no statistical significant difference as regard fetal complications p value =0.5, and maternal complications p value= 0.7. compares laboratory investigations between Patients who developed PET and normal cases in all study participants using students (t) test showing statistical significant difference in 2 variables only which are serum creatinine and BUN with p values =0.03 and 0.02 consequently. **Conclusion**: Our conducted study reveal possible optimistic approaches for managing pre-eclampsia as regards screening, diagnoses and treatment opening new advanced pathways for future development of more efficient management and resulting in improved clinical practice guidelines.

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1. Introduction:

Preeclampsia (PE) clinically known to be presented in 7–10% of gestations and a well-known leading factor in maternal mortality and morbidity**.** The core disorder that underlies preeclampsia is vasospasm. Vasoconstriction of the blood vessels increases resistance to blood flow and consequently hypertension. Vasospasm and injury to the vascular endothelium in association with local hypoxia results in hemorrhage, necrosis, and other end-organ damage of severe preeclampsia. **(1-5)**

Normally vascular response to angiotensin II infusion and other vasopressors is reduced in normotensive pregnancy**.** Reduced response to angiotensin II may be caused by vascular endothelium secretion of vasodilatory prostaglandins such as prostacyclin. Findings and data obtained from previous extensive research from variable studies implemented imply that preeclampsia may be linked powerfully and significantly at molecular and cellular levels with improperly increased synthesis of prostaglandins with vasoconstrictor action e.g thromboxane. **(6-10)**

Previous authors demonstrated the raised vascular response to pressor hormones in patients with near the beginning of preeclampsia development. Pathophysiologic explanation to the mode of raised response to vasopressors may be due to distorted ratios of thromboxane and prostacyclin. **(11-15)**

Neurokinin B (NKB) has been categorised as neurotransmitters being found in discrete neurons and immune cells. **(16-20)**

This class of neuropeptides have been involved in various pathways of biological functions, e.g smooth muscle contraction, vascular reactivity, pain transmission, neurogenic inflammation and the provocation of the immune system. **(21-25)**

That hypothesis was tested and demonstrated when the placenta, a tissue devoid of nerves, was found to be a source of NKB gene expression. (**26-30)**

The NK3 receptor is primarily activated by the tachykinin peptide hormone Neurokinin B (NKB)

which is the most dominant natural agonist for the NK3 receptor. NKB has been suggested to influence as a cornerstone mechanism in the naturally normal physiologic human reproductive path and in potentially life threatening clinical scenarios e.g preeclampsia. **(31-35)**

Preeclampsia is acompanied with a changed maternal pattern of circulating placentally originating proteins controlling angiogenesis**,** e.g sFlt-1 (soluble fms-like tyrosine kinase 1) and PlGF (placental growth factor).. Placental soluble fms-like tyrosine kinase 1 (sFlt1), an antagonist of VEGF and placental growth factor (PlGF), is upregulated in preeclampsia, raising systemic levels of sFlt1 that decrease after birth. Increased circulating sFlt1 in patients with preeclampsia is linked in studies with reduced circulating levels of free VEGF and PlGF, causing endothelial dysfunction in vitro that can be rescued by exogenous VEGF and PlGF. Interpretations from various research studies imply that increased circulating sFlt1 contributes to the pathogenesis of preeclampsia. (**36-40)**

In pregnancies predestined to develop PET, cytotrophoblastic endovascular invasion is trivial, leading to a imperfect uteroplacental circulation and consequent placental ischemic changes. (**41)**

These features are apparent in the gross and microscopic features of placentas obtained from preeclamptic patients. **(42, 43)**

Biopsies studied at microscopic levels obtained from preeclamptic placentas reveal narrow and constricted vessels as a consequence of scarce trophoblastic cellular invasion of maternal decidual arterioles. **(44-47)**

Rational to expect that, patients with predisposition to vascular dysfuntion, e.g diabetes mellitus, thrombophilias, systemic lupus erythematosus, and chronic hypertension, show greater liability and risk to develop preeclampsia. **(48)**

Pregnancies with an enlarged placental mass and relatively less placental blood flow are also at increased liability and show greater risk. (**49)**

Furthermore, disturbance of uterine blood flow causing placental insufficiency and preeclamptic changes has beenpresented in animal models. **(50)**

Widespread research studies performed in the laboratories imply and demonstrate possibiliy that variations in oxygen tension may control cytotrophoblastic cellular invasion. **(29, 30)**

Recent research performed show, hypoxia-inducible transcription factors have been appearing to be particulary elevated in preeclamptic placentas. **(31, 32)**

In addition, the gene expression profiles of preeclamptic placentas appear to siulate those from villous explants exposed to hypoxic effects and placentas obtained from women who gave birth at high altitudes. **(33)**

Hypoxia even though could be present in preeclamptic placentas, it is still a matter of debate whether this is a primary or secondary mechanism in the phenomenon of disease developmental process. **(34).** Abnormalities in placentation and associated hypoxia are beleived to cause the emergence of soluble factors that have influence and manipulative fuctional effect on the maternal vasculature to provoke endothelial dysfunction and the clinical symptoms of preeclampsia. **(35)**

Table (1) Comparison of Patient's characteristics of both groups

Varaible	Hypertension Group	Normal (Control) Group	P value*
Age	30.3±4.4	30.7±4.8	0.66 (NS)
20-35	42	41	
> 35	8	9	
Parity	Mode 1	Mode 1	0.49 (NS)
Primigravida	9	14	
Para1	17	15	
Multiparity	24	21	
Lab. Tests			
Hb	10.2±1.2	10.3±0.8	0.79 (NS)
Hct	41.2±4.8	39.1±6.5	0.08 (NS)

*Student's t-test

The above table compares age ,parity hb ,hct between both hypertensive and normal groups showing no statistical significant difference.

*Student's t-test

2. Methodology:

100 cases were recruited in this study 50 normotensive (control group) and 50 hypertensive (study group) gestations the biomarkers of interest FMF Like Tyrosine Kinase 1 and Neurokinin B were investigated at 28 weeks and at 32 weeks from patients at El sayedgalal and Elhussein hospitals duringt h e perio dfrom Apri l201 7t oOctober 2017. Blood sample was collected from the patients to investigate for the FMS tyrosine kinase 1 and Neurokinin B using

enzyme immune assay, in addition to full history taking and clinical assessmentthe following subjects were excluded.

The data obtained have been edited coded and then entered into the computer. Kolmogorov_Smirnov test was applied for normality prior analysing the numerical data followed by univariate analysis and transformation and comparison between levels of sflt1 and Neurokinin in both groups was performed statistically by using Mann Whitney test.

3. Results:

Table (2) Comparison of Laboratory investigations in women who developed PET in all study participants

Variable	Developed PET	Normal	P value*
Hb	10.3±1.1	10.2±1	0.9 (NS)
Hct	40.2±5.1	40.1±6.3	0.9 (NS)
Platelets	144±42.5	159.8±77.8	0.2 (NS)
ALT	26.4±12.2	20.3±8.9	0.008
AST	28.9±11.7	27.4±10.4	0.3 (NS)
Creatinine	1.1±0.3	0.9±0.3	0.03
BUN	13.3±3.7	15.2±4.5	0.02

*Student's t-test

The above table displays and compares laboratory investigations between Patients who developed PET and normal cases in all study participants using students t test showing statistical significant difference in 2 variables only which are serum creatinine and BUN with p values =0.03 and 0.02 consequently.

Table (3)Comparison of Patients who developed fetal and maternal complications in cases and control groups

Variable	Hypertension group	Control group	P value*
Fetal	20/50	17/50	0.5 (NS)**
FGR	20/50	17/50	
NICU admission of FGR	15/20	7/17	
CTG abnormalities	15/20	9/17	
FW Doppler	20/20	17/17	
NICU admission	22/50	15/50	
Maternal	23/50	21/50	0.7 (NS)***
ICU Admission	13/23	14/21	
PPH	10/23	7/21	

*Chi square test

** Chi test 0.39

***Chi test 0.16

The above table compares between hypertensive and control group as regards development of maternal and fetal complications using Chi square test showing no statistical significant difference as regard fetal complications p value =0.5, and maternal complications p value= 0.7.

Table(4) Comparison of Patients who developed pre-eclampsia in all women

Variable	Developed PET	Normal	P value*
Age	31.6±4.9	29.7±4.2	0.05 (NS)
Parity	1.7±1.2	1.3±1.1	0.09 (NS)
FMS level at 28 wks	11.2±9.5	9.9±8.2	0.5 (NS)
FMS level at 32 wks	18.8±9.3	17.4±9.9	0.5 (NS)
NK level at 28 wks	24.2±7.9	26.7±9.3	0.2 (NS)
NK level at 32 wks	58±16	45.1±14.9	0.0001

*Student's t-test

The above table displays and compares between normal women and those who developed PET in all women using students t test , showing only one variable having statistical difference which is NK level at 32 weeks p value =0.0001

4. Discussion:

This study reveals and displays the following findings as regards the role of FMS and NK levels in detecting and predicting the onset preeclampsia in both normal and hypertensive gestations which is considered essential to reduce maternal and fetal mortality and morbidity in the future management of PET.

As regards comparison between study categories for age, parity, Hemoglobin, Hematocrit between both hypertensive and normal groups showed no statistical significant difference. This is in harmony and coherence with other research studies such as that performed by YANYAN LIU in which a total of 60 women in the third trimester of gestation were subjected for the study while in our study we recruited 100 patients dividing them into 2 groups hypertensive group and normal group and following them to observe whom developed PET while YANYAN LIU categorized his study subjects as follows, 40 gestations with PE (study cases) and 20 normotensive gestations (healthy controls). They were further categorised into three sets: the 20 normotensive gestations (Group 1); 20 gestations with mild PE (Group 2); 20 gestations with severe PE (Group 3). **(50, 51)**

In our research we measured NK, FMS levels at 28, 32 weeks in both study categories and observed those for development of PET. While YANYAN LIU research group measured the plasma levels of NKB and Urotensin 2 simultaneously by enzyme-linked immunosorbent assay.

Their study group concluded the following that in comparison with controls, serum levels of NKB were significantly higher in women with mild or severe PE (p50.01 for both groups), levels of Urotensin 2 were significantly higher in women with mild or severe PE (p50.01 for both groups). Moreover, there was a positive correlation between plasma levels of NKB and Urotensin 2 in preeclamptic women (r¼0.783, p50.01).

Similarly in our study we displayed that comparing patients who developed PET and normal patients in cases group FMS and NK levels at 28 and 32 weeks using Students t- test showed statistical significant difference in, NK level at 32 weeks, with p value =0.001. Additionally in our study patients who developed PET and normal patients in control group, were compared as regards age, parity, FMS and NK levels at 28 and 32 weeks using Students (t-) test showing statistical significant difference only in 3 variables FMS Level at 28 weeks p value=0.02, FMS level at 32 weeks p value =0.01, NK Levels at 32 weeks p value =0.0008. The difference between bothresearch groups methodologies exist in number of cases recruited and serum markers differs in which our study group used FMS levels while (**50-53).**

YANYAN LIU used urotensin 2 however in addition he categorized the patients differently by classifying them into mild and severe preeclampsia. Both studies shows prominent significance of possible future role of serum markers for PET prediction. Similarly Stefan Verlohren et al approached the following research in which he goaled to establish gestational phase tailored cutoff values for the implementation of the soluble fms-like tyrosine kinase-1 (sFlt-1)/placental growth factor (PlGF) ratio as a diagnostic tool for preeclampsia in the clinical management, he conducted a multicenter case–control research recruiting a total of 1149 patients. He reported normal serum values of sFlt-1, PlGF, and the sFlt-1/PlGF ratio relying on the analysis of a total sum of 877 cases with uneventful gestational outcome.

A total number of 234 patients with preeclampsia and a matched cohort composed of 468 patients with normal gestational outcome were put in comparison, and sFlt-1 and PlGF were anlysed and calculated using an automated platform. Separate cutoff values for the sFlt-1/PlGF ratio were determined for the early (20weeks+33+6 weeks) and the late gestational phase (34 weeks–delivery). For each of the 2 gestational phases, 2 independent cutoffs framing an equivocal zone were costumed: the first cutoff value with aiming for high sensitivity, and the second aiming for high specificity.

He displayed the following parameters between 20 week and 33+6 weeks, the cutoff values at ≤33 and ≥85 resulted in a sensitivity/specificity of 95%/94% and 88%/99.5%, respectively. A sFlt-1/PlGF ratio of ≤33 had the lowest likelihood of a negative test (0.05; 95% confidence interval, 0.02–0.13), while values ≥85 had the highest likelihood of a positive test (176; 95% confidence interval, 24.88–1245). Following 34 weeks, the cutoffs at ≤33 and ≥110 yielded a sensitivity/specificity of 89.6%/73.1% and 58.2%/95.5%, respectively.

The usage to use multiple cutoff values was implied by their research group for the early and late gestational phase augmented the diagnostic precision of the sFlt-1/PlGF ratio as a diagnostic tool for preeclampsia. The difference between our research conducted and **Stefan Verlohren et al** is that the number of cases was larger in Stefan study group which increases study power of obtained results however Stefan used sFlt-1/Pl GF ratio while our group compared and contrasted two serum markers separately FMS, NK at two specific gestational age points of time which are 28 weeks and 32 weeks.

A prominent author pointed and displayed a growing interest in serum PET markers **Chun Lam,** research studies imply that PET and its major phenotypes, high blood pressure and proteinuria, are due to excessive circulating soluble fms-like tyrosine

kinase-1 serum level concentrations. Soluble fms-like tyrosine kinase-1 is an endogenous antiangiogenic protein that is synthesized by the placenta and functions by neutralizing the proangiogenic proteins vascular endothelial growth factor and placental growth factor.

High serum soluble fms-like tyrosine kinase-1 and low serum free placental growth factor and free vascular endothelial growth factor have been observed in preeclampsia. Abnormalities in serum levels of these circulating angiogenic proteins are not only existing during clinical preeclampsia but also preceed clinical symptoms by several weeks. For that reason, this raises the option of measuring circulating angiogenic proteins in the blood and the urine as a diagnostic and screening tool for preeclampsia. **(50-54)**

An interesting additional finding by **Sharon E. Maynard et al** study group using research study on experimental animals they observed the following that raised circulating levels sFlt1 in cases with preeclampsia is linked with reduced circulating levels of free VEGF and PlGF, resulting in endothelial dysfunction in vitro that could be rescued by exogenous VEGF and PlGF. Additionally, VEGF and PlGF basis microvascular relaxation effect of rat renal arterioles in vitro that is blocked by sFlt1.

Lastly, administration of sFlt1 to pregnant rats triggers hypertension, proteinuria, and glomerular endotheliosis, the common pathological events of PET. These findings propose that excessive circulating levels of sFlt1 contribute to the pathological development of PET.

Similarly on experimental animals **Suzanne D. Burke et al** study group showed and presented the following augmented vasoconstrictor sensitivity and serum elevations in soluble fms-like tyrosine kinase 1 (sFLT1), a circulating antiangiogenic protein, go before clinical presentations, signs and symptoms of PET.

They reported that increased expression of *sFlt1* in pregnant mice triggered angiotensin II sensitivity and hypertension by disrupting and impairing endothelial nitric oxide synthase (eNOS) phosphorylation function and augmenting oxidative stress in the vascular system. Administration of the NOS inhibitor l-NAME to pregnant mice recapitulated the angiotensin sensitivity and oxidative stress observed with *sFlt1* over expression.

Sildenafil, an FDA-approved phosphodiesterase 5 inhibitor that augments NO signaling, reversing *sFlt1*-induced hypertension and angiotensin II sensitivity in the preeclampsia mouse model. Sildenafil treatment also improved uterine blood flow, reduced uterine vascular resistance, and improving fetal weights in contrast and comparison with untreated *sFlt1*-expressing mice. (**53,54)**

Finally, sFLT1 protein expression inversely associated with decreasing in eNOS phosphorylation in placental tissue of human preeclampsia cases. These findings sustain the fact that endothelial dysfunction due to raised circulating sFLT1 may be the primary triger causing augmented vasoconstrictor sensitivity that characteristically features preeclampsia and imply that aiming and targeting sFLT1-induced pathways may be an opportunity for managing PET and improving fetal outcomes. **(54**)

5. Conclusion:

All previously mentioned studies in addition to our conducted study reveal possible optimistic approaches for managing preeclampsia as regards screening, diagnoses and treatment opening new advanced pathways for future development of more efficient management and resulting in improved clinical practice guidelines.

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