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Research Article

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Significance of Soluble fms-like tyrosine kinase-1 (sFlt-1), Placental growth factor (PlGF) and ratio of sFlt-1:PlGF in Preeclampsia (PE)

Junaid Mahmood Alam, Amna Salman, *Syed Riaz Mahmood

Department of Biochemistry lab services & Chemical Pathology, Liaquat National Hospital, Karachi-74800. Pakistan. Govt Lyari General Hospital, Karachi, Pakistan

Abstract: PE is heterogeneous type of disease, profoundly defined by existence of hypertension and proteinuria, mostly after 20 weeks of gestations. It was reported that placental dysfunction due to PE manifests into alteration of circulating pro-angiogenic or anti-angiogenic mediators, such as placental growth factor (PlGF), and the soluble fms-like tyrosine kinase receptor-1 (sFlt-1). We assessed the importance of sFlt-1/PlGF as a predictive marker for early-onset PE in women at risk of PE with a hypothesis that sFlt-1/PlGF ratio can improve prediction of early-onset PE for women at risk of this condition. Seventy patients were included in the study over period of 10 months with age range 24-42 years. Blood sample were analyzed for serum sFlt-1 and PlGF concentrations and to calculate the sFlt-1/PlGF ratio measured using electro-chemiluminescence (ECL) Elecsys immunoassay technology on a Roche Diagnostics Cobas e411 system (Roche Diagnostics, Basil). sFlt-1 and PlGF manifest different ranges for various gestational week and provided as per manufacturer advise with scientific reference. sFlt-1:PlGF ratio greater than (>) 85.50 is indicator of pre-eclampsia inclusion. Pre-eclampsia was noted in 2 patients with gestational weeks 15-20 whereas in gestational week 21-25, 2 out of 6 in pre-eclampsia group showed sFlt-1:PlGF ratio > 85.40. In gestational weeks 26-31 and 32-37, 3 out of 7 and 4 out 8 in pre-eclampsia group showed sFlt-1:PlGF ratio > 85.50, respectively. Sum of sFlt-1:PlGF ratio in group of pre-eclampsia was calculated as 55.60±10.15 whereas in non-pre-eclampsia it was 20.35±3.4. We determined the diagnostic importance of sFlt-1:PlGF ratio as a predictive marker for early-onset PE in women at risk of PE. Furthermore, it was noted that sFlt-1/PlGF ratio manifestation above > 85.5 can improve prediction of early-onset PE for women at risk of this condition.

Keywords: pre-eclampsia, placental growth factor (PlGF), soluble fms-like tyrosine kinase receptor-1 (sFlt-1)

Introduction : It is well documented that 2%-5% of pregnancies world wide is affected by Pre-eclampsia (PE) [1]. PE is heterogeneous type of disease, profoundly defined by existence of hypertension and proteinuria, mostly after 20 weeks of gestations [2-4]. Clinical outcome of PE ranges from mild to severe complications such as intrauterine growth restrictions (IUGR), HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count), malimplantation of placenta, placental hypoperfusion and systemic endothelial dysfunction, thus making it significant anomaly with consequent fetal-neonatal morbidity and mortality [5-9]. Nonetheless, management of PE patients would be become complicated without knowledge of effective diagnostic entity, or treatment preference [1]. It was reported that placental dysfunction due to PE manifests into alteration of circulating pro-angiogenic or anti-angiogenic mediators, such as placental growth factor (PlGF), and the soluble fms-like tyrosine kinase receptor-1 (sFlt-1) [1,6]. This consequently results in truncation of soluble form of vascular endothelial growth factor (VEGF)

receptor, which neutralizes existing VEGF and PlGF. Therefore it was suggested that PE and its resultant co-morbid occur mostly because of an inadequacy of circulating angiogenic factors [10]. Literature survey showed few recent and past studies exhibiting sFlt-1/PlGF ratio as elevated in patients suffering from preeclampsia with a sensitivity of 89% and a specificity of 97% [1,6,11-13]

In present study we evaluated importance of sFlt-1/PlGF determination as a predictive marker for early-onset PE in women at risk of PE. Furthermore, it was hypothesized that sFlt-1/PlGF ratio can improve prediction of early-onset PE for women at risk of this condition.

Materials and Methods

Patients and setting: This multi-centric, prospective, non-interventional study was conducted from Feb 2017 to Nov 2017 at Department of Clinical Biochemistry and Chemical Pathology, Liaquat National Hospital, Karachi-Pakistan. Informed consent was obtained from each patient before start of their inclusion. Samples collected from Lyari general hospital, local maternity clinics and known subjects attending our lab collection center.

Inclusion and exclusion criteria: Established protocols described earlier were used for inclusion and exclusion of patients [1,6]. Adult patients with ongoing pregnancy between 20 and 37 gestation weeks and with at least one risk factor were included in the study. Women with a confirmed diagnosis of PE at the specified gestation period were excluded from the study. During the study period, 82 patients were included, out of which 12 were unable to follow-up and thus were excluded. Thus final count of patients was 70 that were included in the study over period of 10 months with age range 24-42 years.

Analysis of Preeclampsia markers PlGF & sFlt-1: Blood sample was collected in serum clot activator tubes during routine check-up to determine serum sFlt-1 and PlGF concentrations and calculate the sFlt-1/PlGF ratio. Samples were centrifuged, serum obtained and stored at –20°C until analysis. sFlt-1 and PlGF concentrations were measured using electro-chemiluminescence (ECL) Elecsys immunoassay technology on a Roche Diagnostics Cobas e411 system (Roche Diagnostics, Basil). sFlt-1 and PlGF manifest different ranges for various gestational week and provided as per manufacturer advise with scientific reference. sFlt-1:PlGF ratio greater than (>) 85.50 is indicator of pre-eclampsia inclusion.

Statistical analysis: Data from patients' clinical histories and sFlt-1 and PlGF for PE were collected and analyzed. All the data in this study are presented as mean ± SD. Two way ANOVA and student's t test was conducted to analyze continuous variables. P value below 0.05 was considered statistically significant.

Results

Results are summarized in tables 1 and 2. Total number of patients included in the study was 70, 23 with pre-eclampsia and 47 without pre-eclampsia. Average age of patients was 33.10 yrs, with 34.50 ± 10.15 yrs in pre-eclampsia group and 28.65 ±12.20 yrs in without pre-eclampsia group. Clinical characteristics of all patients were reviewed and logged (Table 1). Majority patients in pre-eclampsia group and some in without pre-eclampsia groups manifested abnormal uterine artery Doppler = 21 (30.00%), had multiple pregnancy = 28 (40.00%), BMI was >30 kg/m² = 32 (45.71%), with previous pre-eclampsia = 14 (20.00%), showed vascular intra-uterine growth restriction = 12 (17.14%), suffering from thrombophilia = 10 (14.28%) and presented with pre-existing proteinuria =16 (22.85%). Patients were grouped according to gestational weeks (Table 2) and assessed for PlGF, sFlt-1and ratio. Pre-eclampsia was noted in 2 patients with gestational weeks 15-20 whereas in gestational week 21-25, 2 out of 6 in pre-eclampsia group showed showed sFlt-1:PlGF ratio > 85.40). In gestational weeks 26-31 and 32-37, 3 out of 7 and 4 out 8 in pre-eclampsia group showed sFlt-1:PlGF ratio > 85.50, respectively. Sum of sFlt-1:PlGF ratio in group of pre-eclampsia was calculated as 55.60±10.15 whereas in non-pre-eclampsia. It was 20.35±3.45 with significant of P <0.01, manifesting diagnostic efficacy of sFlt-1:PlGF ratio.

Discussion

Present study described the diagnostic importance of PlGF and sFlt-1 in patients suffering from preeclampsia. Sub-grouping was done according to gestational weeks in our study that made easier for us to correlate alteration in

PlGF, sFlt-1 or their ratio to progression of pre-eclampsia. As gestational week progressed so does positive outcome of PlGF:sFlt-1 ratio predicting pre-eclampsia in 2 patients during gestational weeks 15-20, 3 out of 7 in weeks 26-31 and 4 out of 8 in weeks 32-37, with sFlt-1:PlGF ratio > 85.50. Sum of sFlt-1:PlGF ratio in group of pre-eclampsia was noted to be as 55.60±10.15 whereas in non-pre-eclampsia it was 20.35±3.45 with significant of P <0.01, thus displaying diagnostic efficacy of sFlt-1:PlGF ratio. Recent and past studies are in agreement with the outcome presented in current cohort [1,6 11-14]. Furthermore, reported studies advocated that automated estimation of sFlt-1:PlGF ratio is a dependable tool for the diagnosis of pre-eclampsia [6, 12-16].

Table 1: Distribution of patients (n = 70-age range 24-42 yrs) included according to the inclusion criteria [1]

Clinical Characteristics	Distribution = n	Distribution = %
Abnormal uterine artery Doppler	21	30.00%
Multiple pregnancy	28	40.00%
BMI >30 kg/m²	32	45.71%
Previous pre-eclampsia	14	20.00%
Vascular intra-uterine growth restriction	12	17.14%
Thrombophilia	10	14.28%
Pre-existing proteinuria	16	22.85%
Hepatic anomalies	10	14.28%
Age >40 years	13	18.57%
Nephropathy	10	14.28%
Diabetic or abnormal glycemic state	15	21.42%

Table 2: Demographical data, clinical characteristics, serum sFlt PlGF concentrations, and sFlt-1/PlGF ratios of patients

Parameters	Total number of patients	Patients with Pre-eclampsia	Patients without Pre-eclampsia	P < 0.05
Numbers of patients	70	23	47	< 0.01
Age (years)	Average 33.10	34.50 ± 10.15	28.65 ±12.20	<0.01
BMI (kg/m^2)	Average 23.85	25.45 ± 8.60	21.40 ± 10.15	NS
Gestational week (Pregnancy term)				
15-20 weeks	15	2	13	
21-25 weeks	23	6 (2 showed sFlt-1:PlGF ratio > 85.40)	17	
26-31 weeks	20	7 (3 showed sFlt-1:PlGF ratio > 85.40)	13	
32-37 weeks	12	8 (4 showed sFlt-1:PlGF ratio > 85.50)	4	
Blood Pressure				
Systolic (mmHg)	113.15±13.10	135.25±10.35	111.30±12.20	NS
Diastolic (mmHg)	78.20±15.15	85.40±12.65	73.45±11.55	NS
sFlt-1	5025.45±350.10	9102.30±380.40	3118.25±400.10	< 0.01
PlGF	325.10±98.40	201.45±85.40	450.75±100.45	<0.01
sFlt-1:PlGF ratio	22.25±8.10	55.60±10.15	20.35±3.45	<0.01

In a recent study carried out in a peri-natal center, the researchers reported that the ratio of sFLt-1 and PlGF is a useful marker to either rule in or rule out pre-eclampsia in specific high risk patients with correlated negative predictive values [1]. An earlier prognosis study demonstrated that negative predictive value of sFlt-1:PlGF was 100% for ruling out PE within one week [1, 12]. The case to use ratio of sFlt-1:PlGF as a predictive biomarker to

rule-in or rule-out PE onset was not only to avoid unnecessary hospital stay but also to indentify pregnant women at high risk of developing PE and requiring monitoring.

Anomalies, such as hypertensive disorders or gestational hypertension, observed in our study as well, is a risk that can occurs occasionally during pregnancy, especially in overweight populous. Such anomalies, inclusive of pre-existing hypertension with or without proteinuria always represents a complex clinical scenario, where ruling out PE becomes essential [1,6,11-14,17]. Moreover, significantly higher ratio of sFLt-1:PlGF was reported in patients with PE as compared with patients with chronic and gestational hypertension [14]. Thus this makes sFlt-1:PlGF ratio a significant marker in ruling out pregnancy-related hypertensive disorders and onset of PE [1,12,17].

As stated earlier, clinical presented associated with pre-eclampsia is multi-dimensional and thus sometimes make it difficult for the obstetricians to properly diagnose and manage [18]. Existing guidelines to diagnose PE is solely dependent on blood pressure and proteinuria assessments that has always been of low accuracy due to dependence on variable co-morbid [6]. Nonetheless, significant studies by several scientists through various cohorts, including our study, to assess sFlt-1:PlGF ratio as a positive predictor for onset of PE, resulted in success [1,12,13,17]. Therefore establishment of sFlt-1:PlGF as a diagnostic biomarker is feasible, clinical dependable, helpful in improving prediction reliability of adverse PE-related co-morbid and easy to interpret.

Conclusion

In conclusion, we determined the diagnostic importance of sFlt-1:PlGF ratio as a predictive marker for early-onset PE in women at risk of PE. Furthermore, it was noted that sFlt-1/PlGF ratio manifestation above > 85.5 can improve prediction of early-onset PE for women at risk of this condition.

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