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Research Article

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Diagnosis of Preeclampsia (PE) on the basis of Soluble fms-like tyrosine kinase-1 (sFlt-1), Placental growth factor (PlGF) and ratio of sFlt-1: PlGF in selected pregnant women

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Abstract *Background*: Two to five percent pregnancies world wide is affected by Pre-eclampsia (PE) due to prolonged hypertension (mostly 20 weeks of gestation) and high urinary excretions. *Aim:* Current study described the determination of sFlt-1/PlGF ratio as a predictive marker for selected group of pregnant women at risk of PE. *Materials and Methods:* Adult female patients with ongoing pregnancy between 20 and 37 gestation weeks, with at least one risk factor and primary diagnosis of PE at the specified gestation period were included in the study. During the study period, 52 patients were included and assessed over period of 10 months with age range 21-38 years. Blood sample was collected in serum clot activator tubes. 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:PlGF ratio were calculated and greater than (>) 85.50 is taken as indicator of confirmed pre-eclampsia. *Results*: In gestational weeks 15-20 of pre-existing PE groups, 5 patients were included in which 1 showed sFlt-1:PlGF ratio > 85.40, whereas in gestational week 21-25 1 out of 3 showed sFlt-1:PlGF ratio > 85.40; in gestational weeks 26-31, 5 out of 6 showed sFlt-1:PlGF ratio > 85.40, where as in 32-37, 6 out of 7 showed sFlt-1:PlGF ratio > 85.50. This depicts the severity of condition and risks in pre-existing PE group. Somewhat similar results were noted in groups with 1st time diagnosis if PE. *Conclusion:* In conclusion, we assessed the diagnostic importance of sFlt-1:PlGF ratio as a predictive biomarker for pregnant women with existing PE and 1st time diagnosed PE. Furthermore, we also noted that some individuals exhibited sFlt-1/PlGF ratio above > 85.5, which can facilitate the improvement in prediction of complications in PE for women at risk

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

1. Introduction

Management of Pre-eclampsia (PE) patients and their co-founded hypertension is complicated matter without familiarity of effective diagnostic entity and treatment preference [1]. Two to five percent pregnancies world wide is affected by Pre-eclampsia (PE) due to prolonged hypertension (mostly 20 weeks of gestation) and high urinary excretions [1-3]. Adverse 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 an anomalous with fetal-neonatal morbidity and mortality [4,5]. Onset of PE at an early stage, which is also referred to as placental PE, arises from a placenta that is under hypoxic conditions with oxidative stress [6], while later stage onset of PE, known as maternal PE, happens due to interaction between a normal placenta and a maternal constitution that is susceptible to metabolic and vascular diseases [7,8].

It is well documented that maternal circulation of biomarkers related to, proangiogenic and antiangiogenic activities is altered in preeclampsia [9,10]. The proangiogenic factor, such as placental growth factor (PlGF) and prohypertensive and antiangiogenic factor such as soluble fms-like tyrosine kinase-1 (sFlt-1) are two biomarkers, that are now considered significant for the diagnosis of PE. Clinical studies evidently showed that there were marked elevation of serum concentration of sFlt-1 and declined serum concentration of PlGF is noted in PE patients and this scale of elevation or decline was correlated with the severity of PE [4]. Particularly, diagnostically, ratio of soluble fms-like tyrosine kinase-1 (sFlt-1) to placental growth factor (PlGF) is actually found to be elevated in preeclampsia and sometimes noted to be raised even before clinical onset of the disease [11-13]. This ratio is now successfully been used in diagnostic set-up to assess prediction of preeclampsia for women at risk [5,12]

Current study described the determination of sFlt-1/PlGF ratio as a predictive marker for selected group of pregnant women at risk of PE.

2. Materials and Methods

2.1 Patients and study setting: A prospective, non-interventional study was conducted from Feb 2018 to Nov 2018 at Department of Clinical Biochemistry and Chemical Pathology, Liaquat National Hospital, Karachi-Pakistan and Govt Lyari General Hospital, Karachi. 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.

2.2 Inclusion and exclusion criteria: Established protocols described earlier were used for inclusion and exclusion of patients [13-16]. Adult patients with ongoing pregnancy between 20 and 37 gestation weeks, with at least one risk factor were included in the study. Women with a confirmed diagnosis of PE at the specified gestation period were preferred. During the study period, 52 patients were included and assessed over period of 10 months with age range 21-38 years.

2.3 Immunoassay Analysis of Preeclampsia markers PlGF & sFlt-1: Blood sample was collected in serum clot activator tubes. 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 were calculated and greater than (>) 85.50 is taken as indicator of pre-eclampsia inclusion.

2.4 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.

3. Results

Results are summarized in Tables 1 and 2. Total number of patients included in the study was 52, 21 with existing pre-eclampsia and 31 with 1st time diagnosis of pre-eclampsia. Average age of patients was 34.50 ± 10.15 yrs in existing pre-eclampsia group and 29.65 ±10.25 yrs in 1st time pre-eclampsia group. Clinical characteristics of all patients were reviewed and presented in Table 1. Abnormal uterine artery Doppler was noted in 28.84% patients; Multiple pregnancy, 46.15%; BMI >30 kg/m² 36.53%; Previous pre-eclampsia, 40.38%;Vascular intra-uterine growth restriction, 28.84%; Thrombophilia, 19.23%; Pre-existing proteinuria, 42.30%; Hepatic anomalies, 23.07%; Age >35 years,30.76%; Nephropathy, 21.15% and diabetic or abnormal glycemic state in 26.92% patients. Patients were grouped according to gestational weeks (Table 2) and assessed for PlGF and sFlt-1 concentration and PlGF: sFLt-1 ratio. In gestational weeks 15-20 of pre-existing PE groups, 5 patients were included in which 1 showed sFlt-

1:PlGF ratio > 85.40, whereas in gestational week 21-25 1 out of 3 showed sFlt-1:PlGF ratio > 85.40; in gestational weeks 26-31, 5 out of 6 showed sFlt-1:PlGF ratio > 85.40, where as in 32-37, 6 out of 7 showed sFlt-1:PlGF ratio > 85.50. This depicts the severity of condition and risks in pre-existing PE group. Similarly in group of patients with 1st time suspected PE diagnosis, 1 out of 5 showed sFlt-1:PlGF ratio > 85.40, 1 out of 4 showed sFlt-1:PlGF ratio > 85.40; 5 out of 9 and 6 out of 13 showed sFlt-1:PlGF ratio > 85.40, in gestational weeks 15-20, 21-25, 26-31 and 32-37, respectively. In this groups also, with progressing gestational weeks, percentage of patients with higher PlGF:sFlt-1 ratio increased, suggesting not only diagnostic importance of PlGF;sFlt-1 ratio but also the onset of pre-PE.

Table 1. Distribution of patients (n = 52 with age range 21-38 yrs) included according to the inclusion criteria [14]

Clinical Characteristics	Distribution = n	Distribution = %
Abnormal uterine artery Doppler	15	28.84
Multiple pregnancy	24	46.15
BMI >30 kg/m²	19	36.53
Previous pre-eclampsia	21	40.38
Vascular intra-uterine growth restriction	15	28.84
Thrombophilia	10	19.23
Pre-existing proteinuria	22	42.30
Hepatic anomalies	12	23.07
Age >35 years	16	30.76
Nephropathy	11	21.15
Diabetic or abnormal glycemic state	14	26.92

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

Parameters	Total number of patients	Patients with existing Pre-eclampsia	Patients with 1st time Pre-eclampsia	P < 0.05
Numbers of patients	52	21	31	< 0.01
Age (years)	Average 32.20	34.50 ± 11.30	29.65 ±10.25	<0.01
BMI (kg/m^2)	Average 27.85	28.55 ± 8.60	27.50 ± 12.35	NS
Gestational week (Pregnancy term)				
15-20 weeks	10	5 (1 with sFlt-1:PlGF ratio > 85.40)	5 (1 with sFlt-1:PlGF ratio > 85.40)	
21-25 weeks	14	3 (1 showed sFlt-1:PlGF ratio > 85.40)	4 (1 showed sFlt-1:PlGF ratio > 85.40)	NS
26-31 weeks	16	6 (5 showed sFlt-1:PlGF ratio > 85.40)	9 (5 showed sFlt-1:PlGF ratio > 85.40)	< 0.01
32-37 weeks	12	7 (6 showed sFlt-1:PlGF ratio > 85.50)	13 (6 showed sFlt-1:PlGF ratio > 85.40)	< 0.01
Blood Pressure				
Systolic (mmHg)	121.18±15.10	136.55±11.20	113.20±15.45	NS
Diastolic (mmHg)	86.10±13.75	87.45±14.80	85.75±12.40	NS
sFlt-1	6563.25±341.20	9995.50±375.55	7998.55±401.30	< 0.01
PlGF	355.20±88.55	135.35±89.40	138.15±101.75	<0.01
sFlt-1:PlGF ratio	18.48±7.10	74.05±10.15	57.95±13.45	<0.001

Data is significant when P< 0.05. Results are expressed as Mean ± SD

4. Discussion

In our current study presented here, we described the diagnostic significance of individual assessment of PlGF and sFlt-1 and their ratio in female pregnant patients suffering from 1st time and pre-existing preeclampsia. Women were sub-grouped according to gestational weeks for proper correlation of elevation or any decline in PlGF, sFlt-1 or its ratio to evaluate the progression or existence of pre-eclampsia. We noted that as gestational weeks progressed, positivity of PlGF:sFlt-1 ratio increased with increase in its predicting capacity for pre-eclampsia. In patients with pre-existing PE, in gestational weeks 15-20 of 5 patients were included in which 1 showed sFlt-1:PlGF ratio > 85.40, whereas in gestational week 21-25 1 out of 3 showed sFlt-1:PlGF ratio > 85.40; in gestational weeks 26-31, 5 out of 6 showed sFlt-1:PlGF ratio > 85.40, where as in gestational weeks 32-37, 6 out of 7 showed sFlt-1:PlGF ratio > 85.50. This depicts the severity of condition, diagnostic efficacy of PlGF:sFlt-1 ratio and risks in pre-existing PE group. Furthermore, in group of patients with 1st time suspected PE diagnosis, 1 out of 5 showed sFlt-1:PlGF ratio > 85.40, 1 out of 4 showed sFlt-1:PlGF ratio > 85.40; 5 out of 9 and 6 out of 13 showed sFlt-1:PlGF ratio > 85.40, in gestational weeks 15-20, 21-25, 26-31 and 32-37, respectively. Here also, with progressing gestational weeks, percentage of patients with higher PlGF:sFlt-1 ratio increased, suggesting not only diagnostic importance of PlGF;sFlt-1 ratio but also the onset of pre-PE. Many recent and past studies are in agreement with the outcome presented in our presented cohort [1-5, 15].

In a recent study carried out in a large multicenter cohort with suspected cases PE, the researchers noted that performing PlGF test and availability of it induced reduction in the time to clinical confirmation of pre-eclampsia, which of great necessity [1]. Wherever this PlGF test was implemented as a mandatory during 15-30 gestational weeks, the researchers have noted a lower incidence of adverse outcomes of pregnancies even in the presence of PE, consistent with adoption of well-targeted, improved supervision, as recommended in the clinical management algorithm for clinicians [1]. Adoption of PlGF testing or PlGf:sFlt-1 ratio in women with suspected pre-eclampsia is thus advocated strongly by the results of this study. The researchers and clinicians of this study successfully demonstrated the accuracy of PlGF tests but also managed to correlate co-morbid and management regiments regarding pregnant women with PE.

In another recent study carried out in China with pregnant women and confirmed PE, ratio of PlGF: sFlt-1 and its diagnostic and prognostic accuracy were assessed [4]. The study noted very interesting and promising results. The study observed ratio of sFlt-1/PlGF in early onset PE subgroup as significantly higher than other clinical groups and control subjects, in addition to their observation of noticing sFlt-1/PlGF ratio as higher in late onset PE subgroup. The sFlt-1/PlGF ratio was also noted to be significantly increased in the PE group compared with autoimmune disease and uncomplicated proteinuria pregnancies. They concluded that maternal sFlt-1/PlGF ratio is an efficient biomarker in the diagnosis and differential diagnosis of PE. This ratio can be used to predict the timing of delivery for PE pregnancies as well. Some recent studies, other than presented here and in agreement with our findings also reported the use of the sFlt-1/PlGF ratio in the prediction and diagnosis of PE [12,18,19].

Yet another recent study in Switzerland was reported regarding good economic impact of assessing PlGF: s-Flt-1 ratio in pregnant women with PE and is in agreement with our results and diagnostic efficacy of this ratio [9]. Previously, similar type of studies was earlier reported from UK, Germany and Italy [20-22] respectively. The clinicians concluded that by introducing the sFlt-1/PlGF ratio as mandatory diagnostic test into clinical practice for women with suspected preeclampsia would provide cost effective hospital management, largely by refining the capability to rule out preeclampsia and thereby reducing long or unnecessary hospitalization [9].

It is known act worldwide that key allocation of maternal and perinatal morbidity and mortality (e.g. Nepal) is attributed to presence of preeclampsia/Eclampsia in pregnant women [17,23]. In routine clinical performance, mostly in under developed or impoverished countries, screening for preeclampsia is made by measuring blood pressure and quantification of protein in the urine and no other specialized test is used. Interestingly, at present no standard practice is followed in the world for laboratory testing in early pregnancy that can predict the occurrence of preeclampsia however PlGF and sFlt-1 tests are available since many years [9,17]. Therefore, recently a study was carried out in Nepal to compare the serum sFlt-1:PlGF ratio in pregnant Nepalese women suspected of preeclampsia and those without it. The outcome of their study showed sFlt-1 and PlGF ratio as significantly higher in women with

preeclampsia (31.6 ± 9.6) than in the controls (3.2 ± 1.3). Similarly, diastolic blood pressure was noted to be significantly linked (p-value 0.000) with the sFlt-1:PlGF ratio in women with preeclampsia [17]. Interestingly, the significantly higher ratio (35.51 ± 8.1 versus 25.4 ± 8.7) was noted in women with preeclampsia who developed complications than the group of women with preeclampsia who remained without complication during their entire pregnancy, thus advocating not only the diagnostic and but prognostic importance of PlGF:sFlt-1 ratio.

5. Conclusion

In conclusion, we assessed the diagnostic importance of sFlt-1:PlGF ratio as a predictive biomarker for pregnant women with existing PE and 1st time diagnosed PE. Furthermore, we also noted that some individuals exhibited sFlt-1/PlGF ratio above > 85.5, which can facilitate the improvement in prediction of complications in PE for women at risk. Noteworthy studies by several scientists in recent years through various cohorts, including our studies (previous and current one), to assess sFlt-1:PlGF ratio as a positive predictor not only for the onset of PE, but also to forecast development of complication of PE resulted in better outcome and promising conclusion. Therefore it is strongly suggested that sFlt-1:PlGF ratio should be considered as diagnostic biomarker as it is feasible, clinical reliable, supportive of prediction reliability of adverse PE-related co-morbid and simple to interpret.

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