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Research Article

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Risk factors in pre-existing Preeclampsia (PE) in patients with 1st time and multiple pregnancies: Independent diagnostic efficiency of fms-like tyrosine kinase-1 (sFLt-1), placental growth factor (PlGF) and as a profile with ratio of sFlt-1:PlGF

Junaid Mahmood Alam, Sarah Sughra Asghar, *Syed Riaz Mahmood, Shazia Noureen, Ishrat Sultana

Department of Biochemistry lab services & Chemical Pathology, Liaquat National Hospital, Karachi-74800. Department of Anatomy, Sir Syed Institute of Medical Sciences, Karachi. Pakistan. Govt Lyari General Hospital, Karachi, Pakistan (correspondence author: dr_jmalam@hotmail.com)

Abstract Incidence of Pre-Eclampsia (PE) in mothers with multiple pregnancies as compared to those with singleton pregnancy is a known co-morbid for adverse outcome. Therefore our study presented here provides information regarding determination of sFlt-1 and PlGF individually and sFlt-1:PlGF ratio as a predictive marker of PE for selected group of pregnant women with multiple and singleton pregnancies. Adult patients with ongoing pregnancy between 20 and 37 gestation weeks, with at least one pregnancy (N = 24) and multiple (N = 14; atleast two) pregnancies with manifestation of PE risk factor were included in the study. During the study period, 38 patients were included and assessed over period of 7 months with age range 28-38 years. sFlt-1 and PlGF concentrations (pg/ml) were measured in serum using electro-chemiluminescence (ECL) Elecsys immunoassay technology on a Roche Diagnostics Cobas e411 system (Roche Diagnostics, Basil). sFlt-1 and PlGF ranges (pg/ml) for various gestational week were followed as per provided by manufacturer with scientific reference. sFlt-1:PlGF ratio were calculated and greater than (>) 85.50 is taken as indicator of pre-eclampsia inclusion. For patients with Pre-eclampsia and multiple pregnancies in gestational weeks 20-31, 7 out of 9 showed sFlt-1:PlGF ratio > 85.40 whereas in gestational week 32-37, 9 out of 11 showed sFlt-1:PlGF ratio > 85.50. For patients with Pre-eclampsia and singleton pregnancy in gestational weeks 20-31 and 32-37, 5 out of 9 patients showed sFlt-1:PlGF ratio > 85.40. Occurrence of number of patients exhibiting higher sFlt-1:PlGF ratio was more in patients with multiple pregnancies than the singleton ones, thus exhibiting occurrence of incidence as significant ($P < 0.01$, $P < 0.001$) with moderate to high levels of alteration in concentrations of sFlt-1 and PlGF in both groups of multiple and singleton pregnancies. Thus, after considering all resultant data from selected cohorts, we strongly recommend that sFlt-1:PlGF ratio and/or individual sFlt-1 and PlGF should be used as predictive biomarker, most importantly in nulliparous women with 1st time pregnancy and women with multiple pregnancies

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

1. Introduction

It is well documented in earlier studies that problematic placenta, in-vitro fertilization (IVF) or artificial fertilization or reproductive technologies (AFT/ART), in addition to donations of ova, eggs, advanced maternal age, unhealthy life style leading to obesity are some of the co-morbid or reasons for having higher incidence of Pre-Eclampsia (PE) in mothers with multiple pregnancies as compared to those with singleton pregnancy [1-5]. Furthermore, managing PE patients and their co-founded hypertension is a known complicated matter, and becomes more troublesome if effective diagnostic entity and treatment preference are not familiarized [6]. Thus 2 to 5 percent pregnancies are reported to be with PE, emerging from prolonged hypertension (mostly 20 weeks of gestation) and high urinary excretions [7-9]. But still, studies done so far are not enough to evaluate the performance of biomarkers for properly diagnosing PE in single or multiple pregnancies [10-12].

Earlier and recent studies showed that circulation of biomarkers in pregnant individuals is related to proangiogenic and antiangiogenic activities and thus gets distorted in preeclampsia [13,14]. One of the proangiogenic factor known as placental growth factor (PlGF) and an important antiangiogenic factor which is soluble fms-like tyrosine kinase-1 (sFlt-1) are now considered significant for the diagnosis of PE [15-19]. Clinical evaluation investigations in maternal PE notified that there were marked alteration in both sFlt-1 and PlGF levels with specific elevation in sFlt-1 and declined concentration of PlGF [17-19]. Moreover, this scale of elevation or decline was observed to be correlated with the starkness of PE [9,17] and ratio of both biomarkers sFlt-1:PlGF can be successfully used to diagnose and assess prediction of preeclampsia for women with multiple pregnancies at risk [1,10,18-20].

PE is a known clinical syndrome with set of co-morbid ranging from mild to severe intrauterine growth restrictions, HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count), mal-implantation of placenta, placental hypoperfusion and systemic endothelial dysfunction [17,18]. Therefore our study presented here provides information regarding determination of sFlt-1 and PlGF individually and sFlt-1:PlGF ratio as a predictive marker of PE for selected group of pregnant women with multiple and singleton pregnancies.

2. Materials and Methods

Study setting and period: A prospective, non-interventional study was conducted from Dec 2018 to June 2019 at Department of Clinical Biochemistry and Chemical Pathology, Liaquat National Hospital, Karachi-Pakistan and Govt Lyari General Hospital, Karachi. Samples collected from Lyari general hospital, local maternity clinics and known subjects attending our lab collection center.

Criteria for Inclusion and exclusion of patients: Established protocols described earlier were used for inclusion and exclusion of patients [1,21-24]. Adult patients with ongoing pregnancy between 20 and 37 gestation weeks, with at least one pregnancy (N = 24) and multiple (N = 14; atleast two) pregnancies with manifestation of PE risk factor were included in the study. During the study period, 38 patients were included and assessed over period of 7 months with age range 28-38 years.

Immunoassay determination of placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 biomarkers (s-Flt-1): Blood samples were collected in serum clot activator tubes. Samples were centrifuged, serum obtained and stored at –20°C until analysis. sFlt-1 and PlGF concentrations (pg/ml) were measured using electro-chemiluminescence (ECL) Elecsys immunoassay technology on a Roche Diagnostics Cobas e411 system (Roche Diagnostics, Basil). sFlt-1 and PlGF ranges for various gestational week were followed as per provided manufacturer with scientific reference. sFlt-1:PlGF ratio were calculated and greater than (>) 85.50 is taken as 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.

3 Results

Results are summarized in Tables 1. Average age of total number of 38 patients was 38.45 yrs whereas BMI (kg/m^2) was 28.15 kg/m^2. Patients in gestational 20-31 weeks were n = 18, whereas in 32-37 weeks n = 20. Determination

of Systolic (mmHg) 128.20±13.20 and Diastolic (mmHg) 87.20±12.05 blood pressures showed higher levels, suggesting abnormal homeostasis. Mean sFlt-1 level of all 38 patients was 5456.10±254.10, PlGF = 364.65±90.15 and sFlt-1:PlGF ratio was 14.98±9.10. Patients with Pre-eclampsia and multiple pregnancies were 14, with mean **age** 37.65 ± 12.25 yrs and BMI 29.10 ± 9.35 (kg/m^2). In gestational weeks 20-31, 7 out of 9 showed sFlt-1:PlGF ratio > 85.40 whereas in gestational week 32-37, 9 out of 11 showed sFlt-1:PlGF ratio > 85.50. BP was on higher side 138.70±13.10 (mmHg) and 88.15±13.70 (mmHg), respectively for Systolic and Diastolic. Mean sFlt-1 level was 8756.45±206.10 pg/ml and that of PlGF 135.35±95.30 pg/ml whereas ratio was 64.85±11.25. Patients with Pre-eclampsia and singleton pregnancy were 24, with mean age 30.15 ±11.45 yrs and 28.75 ± 11.15 BMI kg/m^2. In gestational weeks 20-31 and 32-37, 5 out of 9 patients showed sFlt-1:PlGF ratio > 85.40. BP was on higher normal 129.35±16.10 and 84.65±11.90 (mmHg) respectively for Systolic and Diastolic. Mean sFlt-1 level was 5998.10±398.30 pg/ml and PlGF was 231.25±100.10 pg/ml with ratio of 25.96±14.05. Comparison of number of patients exhibiting higher sFlt-1:PlGF ratio showed that difference of occurrence of incidence was significant (P< 0.01) at moderate to high levels (< 0.001) of alteration on concentrations of sFlt-1 and PlGF in both groups of multiple and singleton pregnancies (Table 1). Notably, percentage of patients with higher PlGF:sFlt-1 ratio in both gestational groups seems to be increased, more in patients with multiple pregnancies than in groups with singleton pregnancy, suggesting not only diagnostic importance of PlGF;sFlt-1 ratio but also the onset of PE.

Table 1: Clinical characteristics, demography, serum sFlt and PlGF concentrations (pg/ml), and sFlt-1/PlGF ratios of PE patient groups with multiple and 1st time pregnancies

Parameters	**Total number of patients**	**Patients with Pre-eclampsia and multiple pregnancies**	**Patients with Pre-eclampsia and1st time pregnancies**	**P < 0.05**
Numbers of patients	38	14	24	< 0.01
Age (years)	Average 38.45	37.65 ± 12.25	30.15 ±11.45	NS
BMI (kg/m^2)	Average 28.15	29.10 ± 9.35	28.75 ± 11.15	NS
		Gestational week (Pregnancy term)		
20-31 weeks	18	9 (7 showed sFlt-1:PlGF ratio > 85.40)	9 (5 showed sFlt-1:PlGF ratio > 85.40)	< 0.01*
32-37 weeks	20	11 (9 showed sFlt-1:PlGF ratio > 85.50)	9 (5 showed sFlt-1:PlGF ratio > 85.40)	< 0.01*
		Blood Pressure		
Systolic (mmHg)	128.20±13.20	138.70±13.10	129.35±16.10	NS
Diastolic (mmHg)	87.20±12.05	88.15±13.70	84.65±11.90	NS
		sFlt-1 and PlGF results and its Ratio		
sFlt-1 pg/ml	5456.10±254.10	8756.45±206.10	5998.10±398.30	< 0.001
PlGF pg/ml	364.65±90.15	135.35±95.30	231.25±100.10	<0.01
sFlt-1:PlGF ratio	14.98±9.10	64.85±11.25	25.96±14.05	<0.001

Data is significant when P< 0.05. Results are expressed as Mean ± SD. *comparison as per number of patients exhibiting sFlt-1:PlGF ratio > 85.40.

4. Discussion

PE in multiple pregnancies has been seen as a threat to both maternal and fetal health. Reasons for PE in multiple pregnancies might be higher placental mass, mature age, altered homeostasis, assisted versus natural conception etc [1,11]. Advanced gestational age with mature age of the mother 35 years plus, in addition to twin or multiple pregnancies would also be a co-morbid for onset of PE and higher levels of sFlt-1: PlGF ratio. Nulliparous pregnancies were also noted to be an influential factor in increasing sFlt-1 levels as compared to those with consecutive normal pregnancies [20]. Moreover, such pregnancies, even its 1st time, were characterized by significantly increased sFlt-1 levels compared with second pregnancies [1,20]. Hydatidiform moles, pre-existing PE is another pathological reason documented by researchers which might cause raised sFlt-1 and sFlt-1:PlGF ratio

[25,26]. It was pointed out that chief basis of sFlt-1 placental overproduction is considered hypoxia associated with placentation failure [24,25]. Nonetheless several previous studies have reported that not only due to hypoxia, trophoblast cells also produces sFlt-1 under various stress conditions independently, including due to influence of inflammatory cytokines [25-27].

Our presented study described determination of sFlt-1 and PlGF individually and sFlt-1:PlGF ratio as a predictive marker of PE for selected group of pregnant women having multiple and singleton pregnancies with related co-morbid and information. Patients with Pre-eclampsia and multiple pregnancies were 14 and in gestational weeks 20-31, 7 out of 9 showed sFlt-1:PlGF ratio > 85.40 whereas in gestational week 32-37, 9 out of 11 showed sFlt-1:PlGF ratio > 85.50. BP in gestational week was on higher side for Systolic and Diastolic. However, patients with Pre-eclampsia and singleton pregnancy were 24, and in gestational weeks 20-31 and 32-37, 5 out of 9 patients showed sFlt-1:PlGF ratio > 85.40. BP was on higher normal 129.35±16.10 and 84.65±11.90 (mmHg) respectively for Systolic and Diastolic. Comparative analysis of number of patients exhibiting higher sFlt-1:PlGF ratio showed that difference of occurrence of incidence as within a group was significant at moderate ($P< 0.01$) to high levels (< 0.001), means higher incidence level in patients with multiple pregnancies as compared to singleton. Alteration in concentrations of sFlt-1 and PlGF in both groups of multiple and singleton pregnancies (Table 1) was also significantly different. As suspected, percentage onset in patients with higher PlGF:sFlt-1 ratio in both gestational groups seems to be more, as higher numbers in patients with multiple pregnancies than in groups with singleton pregnancy. This strongly suggests not only diagnostic importance of PlGF;sFlt-1 ratio but also for the onset of PE and its prognosis.

Many recent and past studies are in agreement with the outcome presented in our presented cohort [1,10-12,20,25-27]. A pervious study hypothesized that hypothesis that nulliparous women might have increased sFlt-1 levels as compared to multiparous women, suggesting an overall increase in relative antiangiogenesis during first pregnancies thus raising sFlt-1 and PlGF levels [20]. Analyses of blood through multiple methods showed their sFlt-1 at significantly higher level in first as compared to their second pregnancies. Furthermore, interestingly, considerable interaction between ethnicity and pregnancy order was noted viz Hispanic women manifested higher sFlt-1 levels than white women during their first pregnancy [20,28]. It was argued that nulliparous women might have increased risk of PE and thus the subsequent elevated sFlt-1 secretion in first versus second pregnancies.

In a study completed in recent past determined sFlt-1 and ratio of sFlt-1:PlGF in twin pregnancies exhibiting onset of PE, with sFlt-1 higher levels and elevated sFlt-1/PlGF ratio [1]. However PlGF levels were noted to be declined as compared to patients with twin gestations and normal pregnancy outcome. Interestingly, in this cohort, sFlt-1/PlGF ratio did not differ between twin and singleton pregnancies with PE. The study confirmed that with an uneventful outcome, sFlt-1 levels and sFlt-1/PlGF ratio were increased, but no differences were noted in PlGF concentration were found when compared with that of singleton controls. Thus they advocated that there were significant differences in the serum marker levels in singleton vs twin pregnancies, suggesting probably of onset of PE and eventful or adverse outcome of pregnancy in later case.

Another recent study explored the frequency of pre-eclampsia which was noted to be sum of 3.7% such that 3.4% in singleton pregnancies and 11.8% in twin pregnancies ($P<0.0010$) which is highly significant. Pre-eclampsia in twin pregnancies was found out to be three to fourfold more than within the singleton pregnancies [29]. Therefore, authors concluded that with many other known risk factors that can affect twin pregnancy, twin pregnancy itself lingered on to be an independent risk factor for pre-onset of PE. Gestational hypertension another risk factor and inception was 1.7% in singleton pregnancies and 2.2% in twin pregnancies [29,30]

5. Conclusion

We determined the diagnostic importance of sFlt-1:PlGF ratio, independent efficacy of sFlt-1 and PlGF assessment as a predictive biomarker for onset of PE in singleton and multiple pregnancies. It was also observed that certain individuals exhibited sFlt-1/PlGF ratio above > 85.5, more in patients with multiple pregnancies than singleton one ($P<0.001$), thus suggesting better prediction capability of onset or occurrence PE for women at risk. Thus, after

considering all resultant data from selected cohorts, we strongly recommend that sFlt-1:PlGF ratio and/or individual sFlt-1 and PlGF should be used as predictive biomarker, most importantly in nulliparous women with 1st time pregnancy and women with multiple pregnancies.

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