Research Article

The role of maternal serum placental growth factor level in predicting delivery within two weeks in pre eclamptic women

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Abstract

Background: Placental growth factor (PlGF) is an angiogenic factor, a secondary marker of placental dysfunction in preeclampsia, with known low plasma levels in the disease.
Objectives: to evaluate the diagnostic accuracy of plasma PlGF concentrations in women presenting with suspected preeclampsia before 35 weeks' gestation in determining the need for delivery.
Patients & Methods: A prospective study was done at Al Yarmouk Teaching hospital at the Department of Obstetrics and Gynecology, Between the 15th of March 2014 to 14th of January 2015 all pregnant women with gestational age of (20 weeks-37 weeks), who were admitted because of suspected preeclampsia were included in the study. Detailed history and examination was done. Two blood samples were obtained, first sample was for biochemical markers The other for Placental Growth Factor assay.
Results: The outcome was delivery of confirmed preeclampsia within 14 days. Of 100women, 47(47%) developed confirmed preeclampsia. PlGF <5th centile had high sensitivity (0.95; 95% confidence interval, 0.87–0.99) and negative predictive value (0.88; 0.83–0.97) for preeclampsia within 14 days; specificity was lower (0.59; 0.41–0.74). Area under the receiver operating characteristic curve for low PlGF (0.87, standard error 0.03) for predicting preeclampsia within 14 days was greater than all other commonly used tests in women presenting with suspected preeclampsia ($P<0.001$ for all comparisons).
Conclusion: Women presenting before 37 weeks' gestation with suspected preeclampsia, low PlGF has high sensitivity and negative predictive value for preeclampsia within 14 days.

Key words: Placental growth factor, Hypertension, Pregnancy

INTRODUCTION

Preeclampsia is a multisystem disorder affecting 8% of pregnant women [1, 2] .The pathophysiology of preeclampsia still unclear, despite intensive research. One of the favored hypotheses is that preeclampsia is generated by shallow invasion of the extra villous trophoblast followed by an incomplete remodeling of the maternal vascular structures which leads to uteroplacental insufficiency and fetal growth retardation [3–5] Insufficient invasion seems to lead to an altered placental angiogenesis, thus implicating causal importance in the origin of preeclampsia. An imbalance of angiogenic and growth factors at the maternal-fetal interface and a consecutive imbalance of these factors in maternal blood might lead to the clinical symptoms of hypertension and proteinuria [6–9]. An imbalance between the factors promoting angiogenesis such as vascular endothelial growth factor or placental growth factor (PlGF) and the factors antagonizing angiogenesis such as soluble fms-like tyrosine kinase 1 (sFLT1) plays a fundamental role in the pathogenesis of preeclampsia [10 , 11]. PlGF serum levels of patients with preeclampsia are significantly lower than the levels in non-preeclampsia pregnancies [11]. At present, the women at risk of preeclampsia are identified on the basis of epidemiological, clinical and anamnestic risk factors. Until now, there has been no clinically useful screening test to predict the development of preeclampsia in the early phases of pregnancy [12]. The purpose of all the screening tests for preeclampsia must be the detection of a high-risk group as early as possible in pregnancy and to offer a prophylactic treatment to the women at high risk. . Although the pathologic origins of preeclampsia likely occur during placentation, the clinical signs and symptoms typically do not emerge until after 20 weeks

of gestation and only 38% of women had both hypertension and proteinuria before the development of eclampsia [13, 14] A diagnosis of PE based on blood pressure and proteinuria has a positive predictive value of approximately 30% for predicting PE-related adverse outcomes[12]

Since women with suspected hypertensive disease are routinely monitored every 2 weeks, the test must be applicable for a subsequent 14-day window to impact management strategies. The primary aim of this study was to evaluate the diagnostic accuracy of plasma PlGF concentrations in women presenting with suspected preeclampsia before 35 weeks' gestation in determining the need for delivery.

PATIENTS AND METHODS

A prospective study was done at Al Yarmouk Teaching hospital at the Department of Obstetrics and Gynecology, the study was approved by the Iraqi Council of Medical specialization (Iraqi scientific committee of Obstetrics and Gynecology). Between the 15th of March 2014 to 14th of January 2015 all pregnant women with gestational age of (20 weeks-37 weeks), who were admitted because of suspected preeclampsia were included in the study. Criteria contributing to suspicion of clinical diagnosis of pre-eclampsia (PE) includes, *de-novo* elevated blood pressure, aggravation of pre-existing hypertension *de-novo* protein in urine

, aggravation of pre-existing proteinuria, epigastric pain, excessive edema /severe swelling (face, hands, feet) headache, Visual disturbances, Sudden weight gain (>1 kg/week in third trimester) PE-related findings, elevated liver enzymes, low platelets, (Suspected) intrauterine growth restriction, abnormal uterine artery Doppler (mean PI>95th centile in second trimester and/or bilateral notching). Those with confirmed preeclampsia were excluded.

Included patients were classified as preeclampsia, gestational hypertension, and chronic hypertension. Gestational hypertension is defined as blood pressure >= 140/90 mmHg on two occasions according to the classification of the International Society for the Study of Hypertension in Pregnancy at least 4 hours apart after 20 weeks of gestations without significant proteinuria at the time of evaluation.

Chronic hypertension is defined as the presence of hypertension prior to 20 weeks of gestation or known before pregnancy. Isolated proteinuria is defined as > = 300 mg/24h without hypertension at time of evolution. Low platelets are defined as < 150×10^5 /L and elevated liver enzymes as aspartate aminotransferase (AST) or alanine aminotransferase (ALT) > 2normal, eg, > 80 IU/mL.

Generalized edema is defined as de novo edema of the face, upper and lower limbs.

Preeclampsia defined as the association of hypertension (more than 140/90 mm Hg in seated position with cuff of sphygmomanometer at the level of the heart) and significant proteinuria (300 mg or more 24 hr urine sample). PlGF is classified as:

- Very low (<12 pg/ml)
- Low (< 5th centile) or<100pg/ml
- Normal (>=5th centile) ≥100pg/ml [15]

After taking verbal consent from the patient, explanation of the nature of the study was done. Detailed history and examination was done. Two blood samples were obtained, first sample was for biochemical markers (Blood urea, serum creatinine, liver enzymes, platelet count, PCV and urine for proteinuria. The other for Placental Growth Factor assay. Samples were obtained, centrifuging of them was done and they were stored at (-2C) till time of examination.

We followed the patient who later developed confirmed PE and those who remained without complete picture of PE. Collected data about time of delivery since admission and the outcome of babies were analyzed.

Placental growth factor assay was done by ELISA kit Cusabio. China CSB-EO470

Primary analysis was the diagnostic accuracy of low plasma PlGF (<5th centile for gestational age) to predict need to delivery for preeclampsia within 14 days of testing in women with suspected preeclampsia before 35 weeks' gestation. The secondary analyses included women presenting later (35–36+6; ≥37 weeks), or by using a lower threshold (<12 pg/mL)

Statistical analysis

Each questionnaire assigned a serial identification number. The data were reviewed, cleaned with double check entry into the computer using Statistical Package for Social Sciences (SPSS) version 20.

The Shapiro–Wilk test was used to test continues variables for their normality of distribution, the test revealed that plasma placental growth factor level and fetal birth-weights in the present study were not normally distributed as the test p-value for all variables was analyzed

The data presented as mean, median, standard deviation, inter quartile range, frequency and percentages tables and box-plot and clustered bar charts.

Receiver Operator (ROC) curve was used to assess the sensitivity and specificity of placental growth factor test in prediction of closeness of the birth after first inclusion in the resent study. Level of p – value less than 0.05 was significant.

RESULT

The present study revealed the following results; of the 100 participated patients 47 (47%) of them were diagnosed to have preeclampsia, while 29 (29%) of them had gestational hypertension, 16 (16%) had chronic hypertension and 8 (8%) had isolated proteinuria. As shown in figure 1 below.

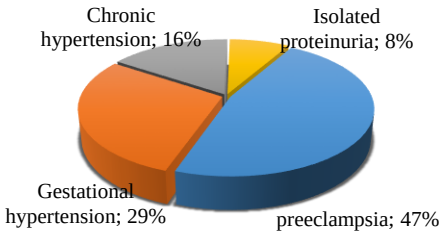

Figure 1. Categories of the participant patient according to their diagnosis, n=100.

The study also revealed that, 64 (64%) of them gave birth within two weeks and 36(36%) delivered after two weeks. As shown in figure 2 below.

Figure 2. Patient categories according to their time of delivery after inclusion in the study, (n=100).

Table 1 below illustrates the characteristics of the patients according to the time of their delivery.

The results revealed that neither ages of the participant nor their gestational ages were showing statistically significant differences (p=0.455, p=0.193) respectively.

Participants' systolic blood pressure at the time of their inclusion in the study also showed no significant differences between the two groups (p=0.087).

In the other hand; diastolic blood pressure of those delivered within two weeks of the inclusion was significantly higher than those delivered later (more than two weeks), (p=0.001).

As well; more than one half (57.8%) of the patients' group who delivered earlier were nulliparous, compared to about on thirds of those delivered after two weeks of the inclusion, this difference showed to be statistically significant (p=0.009).

Table 1. The patients with suspected NOHL classified according to the PTA in the speech frequencies 500-2000 Hz.

Characteristics of patients	Within 2 weeks (n=64) Mean±(SD)	>2 weeks (n=36) Mean±(SD)	p-value [a]
Maternal age (years)	29.9±(5.5)	29.0±(5.8)	0.455
Systolic bl. pressure mmHg	157.2±(16.0)	151.4±(16.1)	0.087
Diastolic bl. pressure mmHg	103.1±(10)	93.5±(11.1)	0.001*
Gestational age at inclusion (Weeks)	31.0±(4.9)	32.6±(6.3)	0.193
	No. (%)	No. (%)	p-value [b]
Nulliparous	37 (57.8)	11 (30.6)	0.009*
Nulliparous Singleton	34 (91.9)	10 (90.9)	0.918
Nulliparous Twins	3 (8.9)	1 (9.1)	

a=independent t-test (two tailed), b=Pearson's chi-square, SD=standard deviation, * significant at α<0.05.

Table 2 below illuminates the comparison of pregnancy's outcomes of the patients between those delivered within two weeks of the inclusion and those who delivered later on. The present study stated that; there was no significant difference neither in fetal numbers nor in modes of delivery between those delivered earlier and those who delivered later than two weeks of the inclusion, (p=0.781, p=0.31) respectively.

Regarding proteinuria; about two thirds 42 (65.6%) of the females delivered earlier than two weeks had positive proteinuria, in comparison to about one third 13 (36.1%) of those delivered beyond two weeks after their inclusion, this difference was shown to be statistically significant (p=0.004).

Significantly higher percentages of stillbirth had been appeared among those with early delivery as compared to those delivered after two weeks of inclusion in the present study, (p=0.022). Preeclampsia is prevalent in more than one half 38 (59.4%) of the patients who delivered prematurely (within 2 weeks), compared to only one fourths of those delivered after two weeks, this difference had shown to be statistically significant (p=0.001).

As for the gestational age at the delivery it had been revealed that; the majority of those delivered after two weeks of inclusion 32 (88.9%) their gestational age was 34 weeks or older, compared to 38 (59.4%) of those delivered within two weeks of inclusion in the present study, this difference was statistically significant (p=0.002).

Also it had been revealed that the median of the birth-weight of the newborn fetuses was higher for patients delivered beyond the two weeks (2.9 kilograms), compared to (2.45 kilograms) for patients delivered within two weeks since the inclusion in the recent study, this difference was statistically significant (p=0.006).

The levels of placental growth factor in the plasma [Median=31 pg/ml and Inter-quartile range (16-49 pg/ml)] for patients delivered within two weeks, which is low as compared to [Median=57 pg/ml and Inter-quartile range (21.6-180 pg/ml)] for patients delivered beyond the two weeks since the inclusion in the recent study, this difference was statistically significant (Mann-Whitney non-parametric test, p<0.001).

More than one thirds 22 (34.4%) of patients delivered within two weeks had very low level of placental growth factor, compared to only 3 (8.3%) of the patients delivered after two weeks since inclusion in the recent study. While, 28 (77.8%) of the patients delivered after two weeks had normal level of placental growth factor, compared to 29 (45.3%) of patients delivered within two weeks since inclusion in the recent study, this differences showed to be statistically significant (p=0.004).Table 2

It is also illustrated in figure 3 below as the area under the curve for placental growth factor was higher and more predictive with higher sensitivity than other parameters like uric acid, platelet count, systolic and diastolic blood pressure.

Table 2. Pregnancy outcomes of the participated patients according to their time of delivery after inclusion in the study, (n=100).

Pregnancy outcomes	Within 2 weeks (n=64) No. (%)	>2 weeks (n=36) No. (%)	p-value [a]
Pregnancy			
Singleton	**58 (90.6)**	**32 (88.9)**	**0.781**
Twins	**6 (9.4)**	**4 (11.1)**	
Positive Albumin in urine	**42 (65.6)**	**13 (36.1)**	**0.004***
Stillbirth	**15 (22.3)**	**2 (5.6)**	**0.022***
Mode of delivery			
Vaginal delivery	**27 (42.8)**	**19 (52.8)**	**0.31**
Caesarean section	**37 (57.2)**	**17 (47.2)**	
Intrauterine growth restriction (IUGR)	**32 (50.0)**	**10 (27.8)**	**0.031***
Preeclampsia	**38 (59.4)**	**9 (25.0)**	**0.001***
Gestational age at delivery			
≥ 34 weeks	**38 (59.4)**	**32 (88.9)**	**0.002***
<34 weeks	**26 (40.1)**	**4 (11.1)**	
Parameters	**Median (IQR)**	**Median (IQR)**	**p-value [b]**
Birth weight of the fetus (kg)	**2.45 (0.95-2.98)**	**2.90 (2.6-3.0)**	**0.006***
Placental growth factor in the plasma (pg/ml)	**31 (16-49)**	**57 (21.6-180)**	**<0.001***
Placental growth factor levels	**No. (%)**	**No. (%)**	**p-value [a]**
Very low PlGF <12 pg/ml	**22 (34.4)**	**3 (8.3)**	**0.004***
Low PlGF <5th centile	**13 (20.3)**	**5 (13.9)**	
Normal ≥ 5th centile	**29 (45.3)**	**28 (77.8)**	

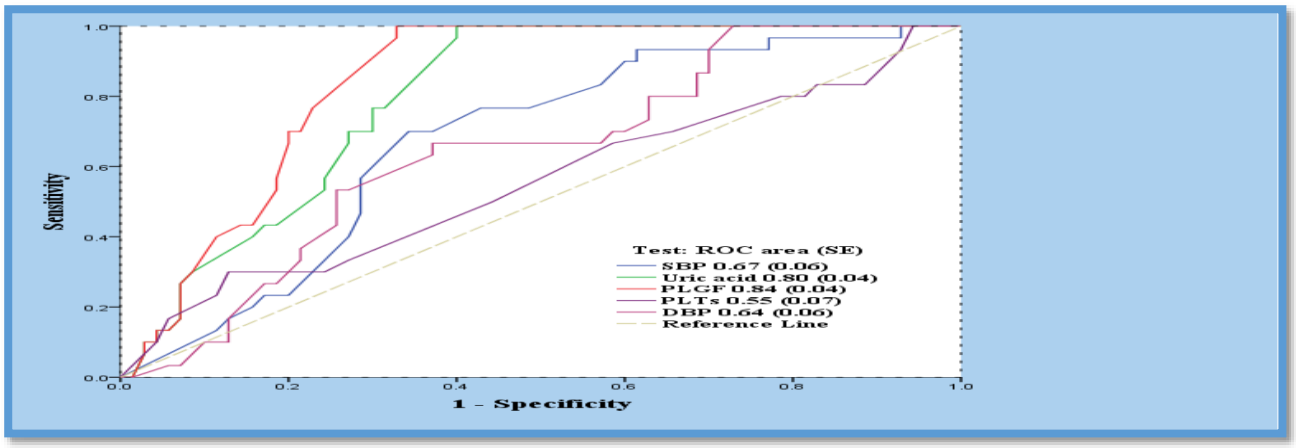

Figure 3. Receiver-operator curve to assess sensitivity of PlGF, SBP,DBP, platelets and uric acid in predicting patients with preeclampsia needed to deliver within 2 weeks after inclusion in the study, (n=100).

Table 3. Statistics test performance for Low PlGF in Predicting Adverse Outcomes, n=100.

Level of placental growth factor	Gestation at enrollment (weeks, days)		
	$<35^{+0}$ (n=55)	35^{+0} - 36^{+6} (n=17)	$\geq 37^{+0}$ (n=28)
$<5^{th}$ centile for	Preeclampsia requiring delivery within 14 days		
Sensitivity	0.95 (0.87–0.98)	0.76 (0.59–0.80)	0.61 (0.49–0.68)
Specificity	0.59 (0.41-0.74)	0.65 (0.54–0.77)	0.73 (0.65–0.81)
Positive predictive value	0.45 (0.36–0.55)	0.68 (0.55–0.79)	0.72 (0.63–0.83)
Negative predictive value	0.88 (0.83–0.97)	0.69 (0.57–0.84)	0.70 (0.66–0.78)
Positive likelihood ratio	2.0 (1.5–2.5)	2.0 (1.5–2.8)	2.3 (1.7–3.2)
Negative likelihood ratio	0.05 (0.02–0.18)	0.40 (0.31–0.70)	0.52 (0.41–0.73)
PlGF <12 pg/ml	Preeclampsia requiring delivery within 14 days		
Sensitivity	0.65 (0.55–0.78)	0.47 (0.38–0.59)	0.42 (0.36–0.58)
Specificity	0.94 (0.84-0.98)	0.85 (0.74–0.97)	0.83 (0.75–0.93)
Positive predictive value	0.72 (0.66–0.85)	0.75 (0.67–0.84)	0.72 (0.63–0.85)
Negative predictive value	0.85 (0.80–0.96)	0.59 (0.47–0.74)	0.60 (0.54–0.72)
Positive likelihood ratio	4.1 (3.2–7.5)	2.3 (1.6–3.1)	2.1 (1.5–2.9)
Negative likelihood ratio	0.4 (0. 2–0.6)	0.67 (0.47–0.87)	0.65 (0.51–0.78)

Table 4. Time for delivery (days) from day of inclusion according to the level of PlGF for patients with preeclampsia, n=47.

Level of placenta l growth factor	Gestation at enrollment (weeks, days)		
	$<35^{+0}$ (n=26)	35^{+0} - 36^{+6} (n=8)	$\geq 37^{+0}$ (n=13)
Very low PlGF <12 pg/ml	7 (9-19)	2 (5-8)	2 (7-9)
Low PlGF <5th centile	10 (19-27)	4 (7-13)	1 (3-5)
Normal ≥ 5th centile	33 (45-81)	5 (9-18)	1 (5-7)

DISCUSSION

In this prospective study of 100 pregnant women presenting with suspected preeclampsia, low plasma PlGF (lower than the fifth centile for gestation) or less than 100pg/ml had very high sensitivity and very low negative predictive value for pinpointing those women who actually had the disorder and would need delivery within 14 days. We found that the test was most accurate

in the earlier stages of pregnancy. Less than 35 weeks, the sensitivity of the assay in predicting the need for delivery within 14 days was 0.95 (95% CI 0.87–0.98) and its negative predictive value was 0.88 (95% CI 0.83–0.97),between 35 and 36 weeks' gestation, the sensitivity of low PlGF in predicting the need for delivery within 14 days was 0.76 (95% CI 0.59–0.80) and its negative predictive value was 0.69 (95% CI 0.57–0.84)and at 37 weeks or more, the test's sensitivity was lower at 0.61 (95% CI 0.49–0.68) and its negative predictive value was 0.70 (95% CI 0.66–0.78).And that's important for the woman, it's important for the doctor, and for the health service The real importance of the test is to flag the women who need greater surveillance, and on the other hand avoid unnecessary hospital admission for low risk. . Maternal plasma PlGF declines in the latter half of the third trimester, reducing test performance at >35 weeks' gestation; an ideal test would maintain separation between preeclampsia cases and other women, which is probably cannot be achieved by using a single biomarker at all gestations. More accurate determination of very low PlGF values (less than the current limit of detection of 12 pg./mL) could be useful; however, the high clinical sensitivity reported in this study relates to the prspecified threshold of <5th centile(low PlGF, or PlGF <100pg/m:) rather than very low PlGF .The PlGF test was significantly better than all other commonly used tests, such as diastolic and systolic blood pressure, alanine transaminase, uric acid and proteinuria, in determining preeclampsia requiring delivery within 14 days, when used alone or in combination (P<0.001 for all comparisons). Lucy C. *et al* in a prospective study of pregnant women between (20-35) weeks gestation who were suspected to have PE, they found that low PlGF (< 5th centile for gestation) had high sensitivity and negative predictive value for patients with PE who should be delivered within 14 days. PlGF test was better than other currently used tests and presented a great help in the management of such women [(15)]. Jeanne S. *et al* in a prospective study for 96 pregnant women who were suspected PE or IUGR, PlGF was measured, adverse outcome were identified (sever PE, SGA < 10th centile, elective delivery for maternal or fetal complication < 34 weeks). They observed that PIGF was lower for patients with PE and was markedly lower for patients with adverse outcome, so PlGF helps to identify women who will experience an adverse outcome and those who will not within time period of 15 days [(16)].

Attila M. *et al* observational study for women less than 35 weeks gestation, single maternal blood sample was taken and PlGF level was measured. Results were compared with last Doppler ultrasound measurement of fetal flow prior to delivery. Positive PlGF test was found with abnormal fetal flow which required delivery. In conclusion PIGF test provided useful information before 35 weeks gestation to identify fetuses requiring urgent delivery and those at risk of later adverse outcome not identified by fetal flow Doppler ultrasound [(17)].

In our study women with suspected (PE)and high diastolic blood pressure were significantly having lower level of PlGF .Patrcia G. *et al* studied the levels of angiogenic factors and their relation with PE in regard to increased diastolic blood pressure. Samples of pregnant women (33-35) weeks gestation were collected, blood pressure was measured and preeclampsia was define according to the National High Blood Pressure Education Program Working Group (NHB-PEPWG), that to say (systolic blood pressure > 140 mm Hg, diastolic blood pressure > 90 mm Hg). PlGF was measured by ELISA, they observed that PlGF was reduced significantly in high diastolic blood pressure in PE patients compared to normotensive patients [(18)]

Nulliparous women with suspected preeclampsia were more significantly prone to have low level of PlGF than multiparous this is probably because preeclampsia is more common in nulliparous. Robert N. *et al in a* cross sectional study on nulliparous women, they observed that serum placental growth factor was reduced in abnormal pregnancy relative to control subjects as early as 15 – 19 weeks of gestation in preeclampsia with small for gestational age (SGA) neonates [(19)], also Francois A. *et al* who did prospective study on nulliparous women from 11 – 13 weeks gestation, combined the relation between uterine artery Doppler and serum placental biomarkers (PlGF). They observed that there was relation of clinical characteristics and first trimester maternal serum PlGF provided an accurate screening for early onset PE in nulliparous women [(20)]although these studies done at earlier gestational age than our study.

Regarding our result in delivery of small gestational age neonate with low PlGF (P value 0.006) which is agreed by Roberto Romero *et al* in a case control study included pregnant women grouped as Patient with uncomplicated pregnancy who delivered appropriate for gestational age (AGA) neonates, patient who delivered SGA but did not developed preeclampsia and Patient who developed preeclampsia. They observed that patients who destined to develop PE (term or preterm) and those who delivered small for gestational age (SGA) had lower plasma concentration of PIGF than those with normal pregnancy throughout gestation, also there were no significant differences in the plasma concentration of sVEGFR-1 between patients destined to deliver SGA and those with normal pregnancy.[(21)] Tjoa M. *et al* in a

prospective study of 72 pregnant patients they observed that between (17 – 21) weeks of pregnancy significant low level of PIGF was found in plasma of women with IUGR [22]. Samantha J. *et al* incase control study of 16 cases (9 placental IUGR, 7 constitutionally small).The PlGF positive when concentration was (< 5th centile for gestational age for normal pregnancy), was found in IUGR fetuses so PlGF identified placental IUGR from constitutionally small fetuses [23].

Some studies have demonstrated that PlGF concentrations begin to decrease from 9-11weeks before the onset of preeclampsia, with greatest reductions during the 5 weeks before the onset of hypertension or proteinuria ([24-26] Sohrabi N. *et al* collected samples from pregnant mothers (8-12) week gestation. PlGF was measured, the result were compared between the patients who later developed PE and those with normal pregnancy. They observed that there was significant difference in PlGF between those who would develop PE from those who would not. So serum level of PlGF in 1st trimester of pregnancy can be used to predict the occurrence of PE [27].

These alterations in the PlGF level are more pronounced in women with early-onset preeclampsia, especially before the 26th week of pregnancy [28, 29]. The main problem of all these screening tests is that the strategies to develop prophylaxis are limited. Beyond 20 weeks of pregnancy, the only option is to improve pregnancy care by allowing closer and carful prenatal monitoring, recognition of preeclampsia earlier in the disease course and administration of steroids for fetal lung maturation [30]. The diagnostic and predictive value of the soluble fms-like tyrosine kinase-1), a trophoblast derived antiangiogenic factor sFlt1/PlGF ratio in patients at risk of placenta-related disorders, i.e. preeclampsia (PE), HELLP syndrome, IUGR and stillbirth, has been show in the recent literature and estimation of the sFlt-1/PlGF ratio has become an additional tool in the management of these disorders, primarily PE. [15]

In this study we measured PlGF once at time of admission and we observe the correlation with the clinical features, repeat measurements of the PlGF or sFlt-1/PlGF ratio are suggested by some studies to improve individual risk assessment in these patients, but this has to be proven by further studies. To date, the use of sFlt-1, PlGF or the sFlt-1/PlGF ratio has not been incorporated into official guidelines. In this study, we have aimed to give good clinical practice guidance for implementation of this method into the management of pregnant women. Use of the sFlt-1/PlGF ratio may help to optimize care by improving management of women with suspected preeclampsia.

Conclusion

Evidence is building in support of the utility of PlGF as an accurate and specific marker identifying the underlying cause of disease, placental dysfunction. A proposal has been made for the definition of pre-eclampsia to include PlGF as a marker of placental dysfunction.

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