Original Article

Maternal–Placental Growth Factor and the Identification of Fetuses with Placental Intrauterine Growth Restriction

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Abstract

Objective: This study was designed to discriminate between fetal growth restriction that is placentally mediated and constitutionally small fetuses depending on the measurement of placental growth factor (PlGF) in the maternal circulation. **Study Design:** This was a prospective case–control study. **Settings:** This study was conducted at the Department of Gynecology and Obstetrics at Al-Yarmouk Teaching Hospital. **Patients and Methods:** The study included 100 cases (11 placental intrauterine growth restriction [IUGR] and 89 constitutionally small) with singleton pregnancies. Serum PlGF was measured by ELISA technique. Concentration less than the 5th percentile for normal pregnancy was considered a positive PlGF test. **Results:** A positive PlGF test was found in 10 out of the 11 placental growth restriction cases and in 4 out of the 89 constitutionally small fetuses. PlGF can differentiate between IUGR due to placental dysfunction from constitutionally small fetuses with 90.1% sensitivity and 95.5% specificity. **Conclusion:** PlGF may serve as a promising tool to identify placental IUGR antenatally.

Keywords: Fetus, growth, placenta

Introduction

Intrauterine growth restriction (IUGR) represents a complex obstetric problem, affects approximately 10%–15% of pregnant women, and refers to a condition in which a fetus is unable to achieve its genetically determined potential size.[1] The diagnosis is usually done antenatally, but some of these fetuses, especially unscreened, may be detected only in the neonatal period. It is important to recognize growth-restricted fetuses because this condition is associated with significant perinatal morbidity and mortality. Both Royal College of Obstetricians and Gynaecologists and American Congress of Obstetricians and Gynecologists have adopted the definition of IUGR as fetal weight <10th percentile.[1,2] Small-for-gestational age (SGA) complicates 25% of pregnancies in developing countries and 4%–8% of pregnancies in developed countries.[3,4] One of the main functions of the placenta is to deliver oxygen and nutrients to the fetus. Failure to deliver an adequate supply of nutrients by the placenta to the fetus is called placental insufficiency which results in IUGR.[5] Placental insufficiency affects up to 3% or more of all pregnancies and accounts for many cases of IUGR. The pathophysiology of IUGR is not well defined. The relative decrease in placental mass and function can result in the development of IUGR.[6] Placental growth factor (P1GF) is one of the vascular endothelial growth factor (VEGF) groups close to VEGF-A.[7] It has potent pro-angiogenic effects and is produced mainly by the placenta.[8] In an uncomplicated pregnancy, PlGF levels rise until the 32nd week of pregnancy and then fall till delivery.[9] Levels are significantly lower in pregnancies where preeclampsia develops before the 37th week with or without IUGR.[10] In recent years, extensive studies were done looking for a role of PlGF in the development of preeclampsia.[11,12]

Recent information about the levels of these factors in other types of hypertension in pregnancy found that free PlGF measured before 34 weeks may predict preterm delivery, but there is little information to date on their capability to detect fetal outcomes such as IUGR through measurement of placental function.[13,14]

Patients and Methods

After the approval of the project by the Committee of Obstetrics and Gynecology, the Arab Board of Medical Specialization, we

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proceed to collect serum samples from patients admitted to the Obstetrics and Gynecology unit at Al-Yarmouk teaching hospital.

All patients admitted with suspected IUGR or SGA were enrolled in this study and, according to the prospective case–control study design applied, patients were enrolled between January 11, 2015, and January 15, 2016. The study included patients with singleton pregnancy at 26 weeks and more with IUGR suspected by Doppler ultrasound, and we excluded patients with uncertain date, those with no early pregnancy booking, patients with active labor or received steroid within the last 48 h, babies with structural or chromosomal abnormalities, preterm spontaneous rupture of membranes, and maternal medical comorbidities such as diabetes, hypertension, and sickle cell anemia.

The antenatal definition of IUGR adopted in this study was fetal abdominal circumference (AC) less than the 10th percentile for gestational age identified by antenatal abdominal sonography or birth weight less than the 10th percentile with either absent/reversed umbilical artery end-diastolic flow.

Maternal venous blood was collected antenatally in 10 cc tubes with EDTA, and the samples were obtained through centrifugation at 2500 rounds/min for 20 min at 5°C and kept at –80°C until the time of examination. Laboratory staff were blind to the clinical diagnosis of all patients. The kit used was the Cusabio®(Cusabio Technology LLC -USA) human PlGF enzyme-linked immunoassay kit which is based on biotin double antibody sandwich technology to assay human PlGF. Staff of the laboratory were blind to clinical and pathology data and clinicians were blind to PlGF results. We followed patients after delivery, and all babies were examined by a neonatologist and were conformed either IUGR or SGA according to the definition and the diagnosis correlated with the result of PlGF.

Placentas were collected at delivery. Placental weight was measured and biopsies of parenchymal villi (1 cm^3 each) were taken in random from the margin and central part of the placenta and fixed in 10% formalin. An experienced pathologist, blind to clinical outcomes and PlGF levels, examined the placental tissue recording for lesions such as malperfusion, fetal stromal and villous maldevelopment, fetal thrombotic vasculopathy, intraplacental hematoma chorioamnionitis, and abruption.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 23 (Armonk, NY: IBM Corp).

- Continuous data were presented as mean and standard deviation
- Independent *t*-test (two tailed) or Mann–Whitney U-test was used, according to the normality of continuous data, comparing them between the study groups
- The categorical data were represented by frequency and percentage tables
- Pearson's Chi-square test was used to assess statistical association between the categorical data and the study groups
- Receiver operator characteristic (ROC) curves were used to assess the sensitivity and specificity of PlGF
- A level of $P < 0.05$ was considered statistically significant.

Results

Results showed that mean age, gravidity, parity, and abortion did not vary significantly with the study groups.

Participants' systolic and diastolic blood pressure readings also did not express a significant association with the study groups ($P = 0.276$ and $P = 0.217$, respectively) [Table 1]. $P < 0.05$ was considered statistically significant.

It is clearly shown that a median of 29 weeks and interquartile range of 26–31 weeks for patients who delivered a newborn with placental intrauterine growth retardation (PIGR) are low compared to a median of 37 weeks and interquartile range of 35–38 weeks for patients who delivered a constitutionally small fetus, this difference was statistically significant ($P < 0.001$) [Table 2 and Figure 1].

It was also apparent that the median for gestational age at delivery is significantly changing between the study groups ($P < 0.001$).

The median for birth weight also expresses a significant change among the aforementioned study groups ($P < 0.001$).

PlGF serum levels obtained from mothers in this study also show a significant difference between both study groups with notable increment in the readings of the constitutionally small fetuses' study group ($P < 0.001$).

Both fetal AC and umbilical artery resistive index reveal a considerable difference between the two study groups, with notable higher measurements of ACs in constitutionally small fetuses and also higher readings in umbilical artery resistive

Table 1: Maternal characteristics of the study groups ($n=100$)

Variables	Placental intrauterine growth restriction ($n=11$)	Constitutionally small fetuses ($n=89$)	P
Age (years), mean±SD	28.1±6.2	29.1±5.6	0.401
Gravidity, mean±SD	2.6±2	2.4±1.7	0.664
Parity, mean±SD	1.4±1.8	1.3±1.6	0.905
Abortion, mean±SD	0.2±0.6	0.1±0.3	0.206
Systolic BP (mmHg), mean±SD	120.1±4.9	118.6±4.1	0.276
Diastolic BP (mmHg), mean±SD	80.1±4.6	77.8±4.2	0.217

Mann-Whitney U-test. BP: Blood pressure, SD: Standard deviation

Table 2: Perinatal outcome characteristics of the study groups

Variables	Placental intrauterine growth restriction ($n=11$)	Constitutionally small fetuses ($n=89$)	*P*
Gestational age at sampling (weeks), median (IQR)	29 (26-31)	37 (35-38)	<0.001*
	28.2±4.7	35.8±3.8	
Gestational age at delivery (weeks), median (IQR)	31 (29-33)	38 (37-39)	<0.001*
	35.8±3.6	38±1.6	
Birth weight (g), median (IQR)	2000 (1600-2450)	2548 (2021-2910)	<0.001*
	2442.8±884.3		
Placental growth factor (ng/L) median (IQR)	31.5 (11.7-40)	142.5 (97.5-195.5)	<0.001*
	29.1±17.5	171.5±119.3	
Abnormal placental pathology (%)	11 (100)	0 (0)	<0.001*
Abdominal circumference (percentile for gestational age)	2.5 (1-5)	6 (3-10)	<0.001*
Umbilical artery RI (percentile for gestational age)	81.2 (56.7-97.2)	73.6 (42.2-88.9)	<0.001*

*Significant at 0.05 level by Mann-Whitney U-test, RI: Resistive index. IQR: Interquartile range

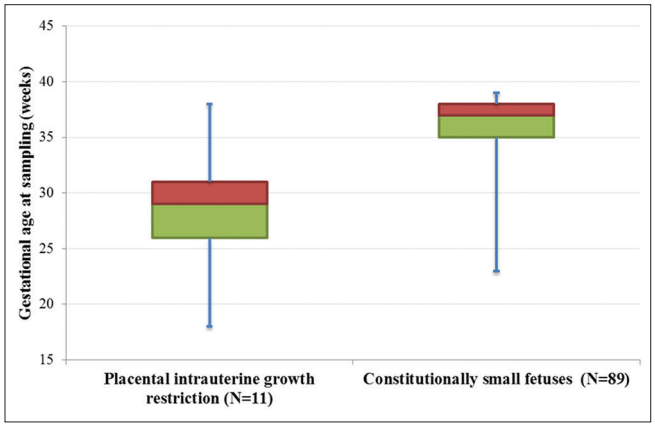

Figure 1: Box plot graph comparing gestational age at sampling between the study groups

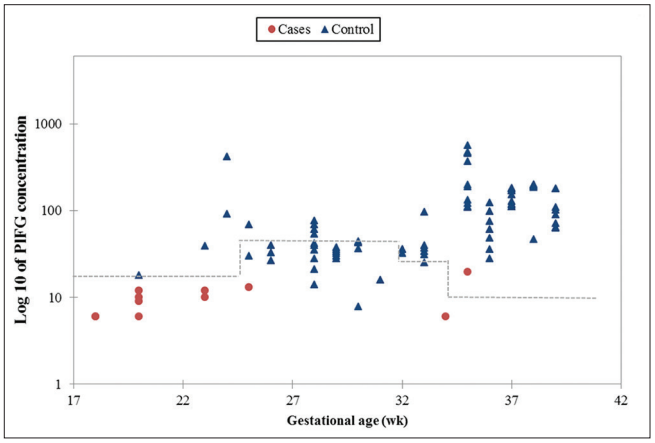

Figure 2: Placental growth factor concentrations in the circulation of women with placental intrauterine growth restriction fetuses and normal pregnancies at the time of sampling (gray dotted line represents the 5th centile according to gestational age)

index were conveyed by ultrasonography for mothers who delivered an infant with PIGR.

It is clearly evident that PlGF (median = 31.5 ng/ml and interquartile range [11.7–40 ng/ml]) for those patients who delivered a newborn with placental IUGR is low compared to PlGF (median = 142.5 ng/ml and interquartile range [97.5–195.5 ng/ml]) for patients who delivered a constitutionally small fetus which was statistically significant (Mann–Whitney nonparametric test, $P < 0.001$) [Table 2 and Figure 2].

As shown in Table 3, 10 out of the 11 patients who delivered a newborn with PIGR and 4 out of the 89 patients who delivered a constitutionally small fetus had a positive serum level of PlGF. On the other hand, 1 out of the 11 patients who delivered a newborn with PIGR and 85 out of the 89 patients who delivered a constitutionally small fetus had a negative plasma level, and this difference was statistically significant ($P < 0.001$).

Table 4 shows the comparison of PlGF test and its sensitivity and specificity in the prediction of delivery with intrauterine growth retardation.

The calculated cutoff point between the two groups was 61.2 ng/ml, those of lower PlGF level was considered positive for predicting IUGR delivery with sensitivity of 90.1 (85.6–97.6) and positive predictive value of 71.4 (67.2–77.5).

On the other hand, specificity was 95.5 (83.5–97.8) and negative predictive value was 98.8 (93.1–99.7).

These values are considered conclusive for this test which appeared highly sensitive and highly specific for prediction of fetuses with placental IUGR.

Figure 3 shows the area under the curve of 0.981, which is highly suggestive of test sensitivity and high positive likelihood ratio as mentioned above.

"Area under the ROC curve represents the probability that a randomly chosen diseased subject is (correctly) rated or ranked with greater suspicion than a randomly chosen nondiseased subject."

Discussion

This study involved the selection of 100 pregnant women with uncomplicated singleton pregnancies who delivered small

Table 3: Results of placental growth factor for the study and control groups

Test results	Placental intrauterine growth retardation	Constitutionally small fetuses	*P*
Positive	10	4	<0.001*
Negative	1	85	
Total	11	89	

*Significant at 0.05 level by Chi-square test

Table 4: Sensitivity and specificity of placental growth factor test in intrauterine growth restriction

	Values
Cutoff value	61.2
Sensitivity (95% CI)	90.1 (85.6-97.6)
Specificity (95% CI)	95.5 (83.5-97.8)
PPV (95% CI)	71.4 (67.2-77.5)
NPV (95% CI)	98.8 (93.1-99.7)
Accuracy (95% CI)	95.0 (84.6-97.5)

CI: Confidence interval, PPV: Positive predictive value, NPV: Negative predictive value

newborns. It has been shown that PlGF levels in maternal serum may have the potential to identify placental IUGR antenatally.

The recognition of fetuses with placental-fetal growth retardation (FGR) was done using Cusabio® human PlGF enzyme-linked immunoassay kit technique to detect low maternal serum PlGF (concentration below the 5th percentile for gestational age for a normal pregnancy) and this method had shown a specificity of 95.5% and also high sensitivity of 90.1% with negative predictive value of 98.8% and positive predictive value of 71.4%.

High sensitivity, high negative predictive value, and low negative ratio (0.02) all mean that a normal PlGF concentration can be a useful test for identification and exclusion of IUGR.

The area under curve of 0.981 showed that low maternal serum PlGF level is more useful than other parameters such as AC or umbilical artery resistance index and gestational age for detection of placental FGR antenatally. And that, normal placental PlGF is more reassuring than umbilical artery resistance index (RI) and AC to follow expected management with better neonatal outcome and these findings have been supported by good evidence from other studies.[15]

In our study, we excluded women with hypertension in order to avoid cases of abnormal PlGF due to hypertensive disorders as it has been found that PlGF decreases in circulation of women with hypertension, especially before 34 weeks of gestation (early-onset preeclampsia), and these required delivery within 2 weeks of their clinical diagnosis.[16]

It is evident upon searching for a similar comparative study in the published medical literature that studies evaluating PlGF in normotensive pregnancies with placental FGR are limited

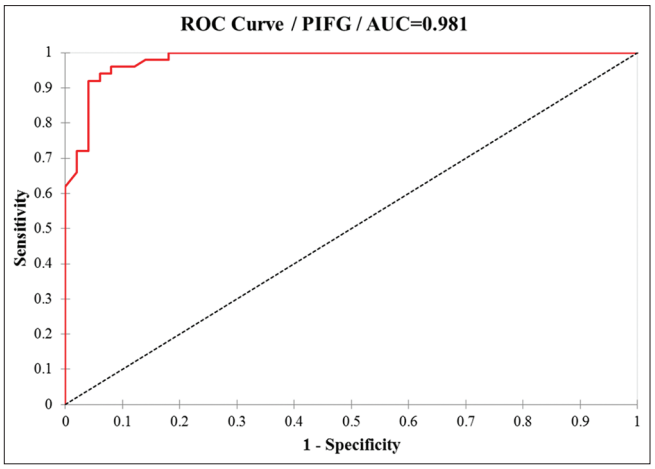

Figure 3: Receiver operator curve for in the detection of placental intrauterine growth restriction cases

and this may be due to the fact that these studies depended more on birth weight to define FGR.[17]

Furthermore, majority of those studies have neither investigated PlGF levels and placental IUGR nor looked at it in isolation from preeclampsia, another obstetric complication with placental implications.

This study arranges to demonstrate the true usefulness of the maternal serum level of PlGF and its ability to identify growth restriction because of a pathological placenta. We try to achieve that by grouping women with fetuses with placental IUGR and comparing them with women with constitutionally small fetuses.

The majority of previous studies showed low levels of PlGF in SGA neonates, but the low predictive or diagnostic performance may be related to populations studied that involved both true cases of growth-restricted and constitutionally small fetuses.[18]

By surfing many related publications we could establish many comparative results, so in that scope Samantha J. *et al*[19] who did case control study of 16 cases (9 placental IUGR, 7 constitutionally small) PIGF positive when concentration was (< 5th centile for gestational age for normal pregnancy), so PIGF identified placental IUGR from constitutionally small fetuses and this finding come in similar agreement with the results reported in our study.

In another preliminary study, also published by Benton *et al*. which evaluated placental morphology quantitatively in a group with suspected IUGR to determine if low PlGF has a relation with abnormal placental morphology, it was concluded that low PlGF was associated with a greater incidence of abnormal morphology in pregnancies with suspected IUGR, so it could serve as a test to detect abnormal placental function antenatally in these pregnancies, besides that the study clearly remarks that future work is ongoing.[20] The findings stated by Benton *et al*. in their preliminary study also agreed with our final findings that demonstrate the significant association

between the maternal serum level of PlGF and abnormal placental pathology.

In our study, we used placental pathological examination (the pathologist was blind about the results about PIGR) and the findings demonstrate the presence of placental lesions in association with placental dysfunction which ultimately revealed the correlation between histological lesion of placenta and placenta under perfusion, which has further strengthened our study. This has been supported by other studies that show similar correlation.[21]

In a double-blind prospective study presented by Chaiworapongsa *et al*. who enrolled 96 women for suspected preeclampsia or IUGR, and measured serum levels of PlGF at enrollment, there was a significant low level of PlGF among women with suspected preeclampsia or IUGR.[22] And by concluding this statement also, our study comes in agreement with the observations of Chaiworapongsa *et al*., since our data analysis emphasizes the high specificity, with reasonable sensitivity of the maternal serum level of PlGF in anticipating the placental variant of IUGR.

Ghosh *et al*. published a cohort study on 722 women with singleton pregnancies, from an antenatal clinic, with serum PlGF levels estimated at 20–22 weeks of gestation, the study finally revealed that maternal serum PlGF level estimation in early second trimester could be useful in predicting the development of early-onset IUGR and early-onset preeclampsia.[23]

Results outlined by Ghosh *et al*. properly agreed with our study feedback in the prediction of placental IUGR by using the antenatal serum level of PlGF as a detective tool.

Gomez-Roig *et al*. in a longitudinal, prospective, and case-controlled study that was conducted over a period of 24 months, within this design the At-risk pregnancies involving small-for-gestational-age (SGA) fetuses, IUGR, gestational hypertension (GH), or PE were investigated, analyzing umbilical artery doppler placental findings and maternal PlGF levels determined at the time of diagnosis. The results elucidate the association between the low plasma level of placental growth factor in the pregnancies with an outcome of placental fetal growth retardation, also the low PlGF may indicate the severity of fetal compromise in placental disease. In comparison to the result conducted by Gomez-Roig *et al.* in this study our study as well had pointed to the helpfulness of PIGF not only in the diagnosis of placenta FGR but also in the prediction of the severity of the condition and this has been supported by other studies.[24] Moreover, our study also agreed that the low PlGF identified women destined to deliver within a shorter period of time, so the reassurance of a normal PlGF may support expectant management to improve neonatal outcomes.

Conclusion

Our data suggest that maternal serum PlGF is useful to differentiate between fetuses with placental abnormalities from small healthy fetuses and this ability to discriminate can further improve the handling of fetus which is deemed to be risky and avoid interventions for women with pregnancies with constitutionally small, healthy fetuses, and hence, maternal serum PlGF is a promising test for delineation of growth-restricted fetuses due to placental disease from normal small neonates.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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