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Original Research Article

TO STUDY THE ROLE OF SERUM BIOMARKERS COMBINED WITH UTERINE ARTERY DOPPLER IN PREDICTION OF INTRAUTERINE GROWTH RETARDATION

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Abstract

Background: Placentally-mediated fetal growth restriction (FGR) impairs fetal growth, increasing risks of stillbirth, preterm delivery, and lifelong complications. FGR is suspected when fetal measurements fall below the 10th percentile for gestational age. However, some fetuses are constitutionally small but healthy. Identifying placental dysfunction using biomarkers like placental growth factor (PlGF) could improve clinical management. Low PlGF levels may indicate placental FGR. Predictive markers such as PAPP-A and uterine artery Doppler features have shown limited sensitivity, necessitating investigation into combined marker efficacy for predicting intrauterine growth restriction (IUGR). **Materials and Methods:** This prospective observational study was conducted over two years in the Department of Obstetrics and Gynecology at Upper India Sugar Exchange Maternity Hospital, GSVM Medical College, Kanpur. Primigravida women aged over 18 with singleton pregnancies were included. Exclusion criteria were women aged under 18 or over 40, multiparous women, multifetal pregnancies, gross fetal anomalies, and intrauterine death. Data were collected through questionnaires, clinical exams, biochemical marker measurements, ultrasound, and Doppler studies. Statistical analyses included t-tests, Chi-square tests, correlation analysis, and ROC analysis. **Result:** In a study of 100 women, 30 had fetal growth restriction (FGR) and 70 did not. Among FGR cases, 53.3% had late-onset and 46.7% had early-onset FGR. Rural residence and literacy were more common among those with FGR. Significant predictors of FGR included lower PLGF levels and higher uterine artery pulsatility index (UAPI) in the second trimester. Women with FGR had lower birth weights and higher NICU admissions. PLGF was the best marker for predicting FGR. **Conclusion:** All four markers (PAPP-A, PLGF, β-hCG, and UAPI) demonstrated good sensitivity, specificity, PPV, and NPV, making them effective predictors of FGR, and they also have utility for aneuploidy screening. Larger studies are needed to validate these findings and incorporate these biomarkers into routine clinical practice, potentially leading to early prophylactic strategies and improved management of high-risk pregnancies.

INTRODUCTION

Placentally-mediated fetal growth restriction (FGR) is a pathological process that reduces the growth trajectory of a fetus and increases the risk of Stillbirth, preterm delivery, serious neonatal complications, and lifelong sequelae.[1] FGR is clinically suspected when the ultrasound estimated fetal weight or fetal abdominal circumference is below the 10th percentile for gestational age, or serial ultrasounds suggest decreasing growth velocity.[2] However, many fetuses with suspected FGR are small due to constitutional factors and are at low risk for adverse outcomes ("small but healthy" fetuses).[2] Antenatal discrimination of fetuses that are small due to placental dysfunction, rather than constitutionally small, would improve clinical management by focusing care on fetuses that are truly at-risk of the adverse perinatal outcome, reducing surveillance fatigue and unnecessary intervention for pregnancies

with constitutionally-small fetuses.[3] Placental biomarkers such as placental growth factor (PlGF), present in the maternal circulation, may provide an additional clinical tool for identifying placental FGR antenatally. Pilot work by our group suggests that low circulating levels of PlGF may characterize pregnancies complicated by FGR associated with significant placental pathology.[4]

Intrauterine growth restriction (IUGR) remains a major obstetric complication associated with substantial perinatal morbidity and mortality.[5] Furthermore, it has become increasingly clear that IUGR has long-term implications for adult life, as the risk of hypertension, coronary artery disease, and diabetes mellitus in adult life are inversely related to birth weight.[6]

Many researchers have attempted to predict birth weight prenatally using various parameters including ultrasound markers such as uterine artery (UtA) Doppler features and placental volume (PlaV),[7,8] and biochemical markers such as pregnancy-associated plasma protein-A (PAPP-A),[9,10] well as placental protein-13 (PP-13).[11] However, the reported predictive values are not very good, with a sensitivity for detecting IUGR ranging from 16 to 34%. The limitation of these studies is that most of them investigated one marker at a time. It is uncertain whether these markers correlate with each other and which marker or combination of markers gives the best Prediction of birth weight. Another common limitation of these studies is that IUGR or small-for-gestational-age (SGA) was often defined by a population-based growth standard, which has been shown to be inferior to a customized growth standard in representing individual true growth potential as well as reflecting perinatal mortality and morbidity.[12]

Hence the aim of the study was to investigate the relationship between first-trimester and second trimester biochemical (PAPP-A, PLGF) and uterine artery pulsatility index (UAPI) in prediction of intrauterine growth retardation.

MATERIALS AND METHODS

Study Design and Setting: The present study was conducted in the Department of Obstetrics and Gynecology at Upper India Sugar Exchange Maternity Hospital, GSVM Medical College, Kanpur. This prospective observational study spanned over a period of two years.

Study Population: The study included primigravida women who met the following criteria: Aged over 18 years, Presenting with a singleton pregnancy, Attending the outpatient department (OPD) in their first trimester, Available for the entire study duration, Provided written informed consent to adhere to the study protocol.

Exclusion criteria included: Women aged under 18 or over 40 years, Multiparous women, Those with multifetal pregnancies, Pregnancies with gross fetal chromosomal or structural anomalies, and Pregnancies complicated by intrauterine fetal death.

Initial Assessment: A comprehensive questionnaire was administered to collect demographic data, obstetric history, past medical history, family history, blood group, and body mass index (BMI). Clinical examination including height, weight, and blood pressure measurements.

Biochemical Marker Measurement: Blood samples were collected to measure maternal serum free β-hCG and PAPP-A levels using the Elecsys analyzer. The measured values were converted to Multiples of the Median (MoM) specific for gestational age by comparing them to median values for the gestational age at the time of sampling. Maternal serum PLGF was measured using the Quidel Triage PLGF Test, a fluorescence immunoassay. The test was performed with the Quidel Triage Meter, which quantitatively determines PLGF levels in EDTA-anticoagulated plasma specimens.

Ultrasound Examination: An ultrasound examination was performed using the Voluson Pro V ultrasound machine. Fetal nasal bone (NB) and nuchal translucency (NT) were measured. NT measurements were obtained using FMF (Fetal Medicine Foundation) software, ensuring standardization and accuracy.

Uterine artery Doppler ultrasonography was performed according to the ISUOG Practice Guidelines (2013). The patient was asked to empty her bladder and then positioned supine. A trans-abdominal ultrasound probe was used to measure crown-rump length or biparietal diameter and to localize the placenta. A midsagittal section of the uterus was obtained, identifying the cervical canal. The probe was moved laterally to visualize the paracervical vascular plexus, and color Doppler was used to identify the uterine artery as it ascended to the uterine body. Measurements were taken at the point before the uterine artery branched into the arcuate arteries on both sides. The pulsatility index (PI) was recorded bilaterally, and the mean PI was calculated. The presence of an early diastolic notch was also noted.

Participants were followed up in the third trimester for the following assessments: Growth Scan: Measurement of abdominal circumference (AC) and estimated fetal weight (EFW), Color Doppler Ultrasonography: Assessment of umbilical artery PI, Measurement of cerebroplacental ratio (CPR) <5th percentile, Detection of abnormal flow patterns, including the presence of absent end-diastolic flow (AEDF) or reversed end-diastolic flow (REDF). Data on the mode of delivery, neonatal outcomes, and APGAR scores at 1 and 5 minutes were collected.

Statistical Analysis: Data were analyzed using SPSS version 20.0. Continuous variables were expressed as mean ± standard deviation (SD) and compared using the Student's t-test. Categorical variables were expressed as frequencies and percentages and analyzed using the Chi-square test or Fisher's exact

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test. Correlation analysis was performed to determine the relationships between biochemical markers, ultrasound markers, Doppler indices, and pregnancy outcomes using Pearson correlation coefficients. Receiver Operating Characteristic (ROC) curve analysis was conducted to evaluate the predictive accuracy of the biochemical and ultrasound markers for adverse pregnancy outcomes. The area under the curve (AUC) was calculated to determine the diagnostic performance. A p-value of <0.05 was considered statistically significant.

Ethical Considerations: The study was approved by the Institutional Ethics Committee of GSVM Medical College, Kanpur. Written informed consent was obtained from all participants. Confidentiality of participants' data was maintained throughout the study.

RESULTS

In our study out of total 100 women 30 women were found having FGR while 70 women were without FGR. Among 30 women with FGR, majority 53.3% (N=16) of the women had Late Onset FGR while 46.7% (N=14) of the women had Early Onset FGR. The mean age of the both the group was almost similar that is 27.41 ± 4.70 years with FGR and 27.63±4.84 years. Majority of women belong to literate class who developed FGR. Majority of women belong to Rural area (66.7%) who were found having FGR [Table 1].

The study found no significant differences in baseline characteristics such as mean BMI, systolic and diastolic blood pressure, hemoglobin, blood sugar, serum bilirubin, SGPT, and SGOT levels between women with and without Fetal Growth Restriction (FGR) (p>0.05). In the first trimester, mean levels of PAPP-A, β-hCG, NT, and NB were similar between both groups, although a significant relationship was found between PAPP-A levels and FGR (p<0.05). In the second trimester, women with FGR had significantly lower PLGF levels and higher UAPI compared to those without FGR (p<0.05). Third trimester assessments showed significant relationships between abdominal circumference, estimated fetal weight, and FGR (p<0.05). Additionally, 13.3% of women with FGR had CPR <5th percentile, and 20% had AEDF/REDF, indicating significant correlations between color Doppler findings and FGR. Labor induction was more common in women with FGR (73.3%) compared to those without FGR (18.6%). The mode of delivery varied, with 12% of women with FGR having vaginal deliveries and 18% having LSCS, compared to 40% vaginal and 30% LSCS in women without FGR. Preterm delivery occurred more frequently in women with FGR (66.7%) than in those without FGR (50%). Women with FGR had significantly lower birth weights (1855±721 g) compared to those without FGR (2529±402 g) (p=0.001). APGAR scores <7 at 5 minutes were seen in 26.7% of women with FGR and 32.8% of those without FGR, while NICU admissions >48 hours were higher in the FGR group (73.3%) compared to the non-FGR group (32.8%) [Table 2].

The cut-off values of UtA PI, maternal serum PlGF, and PAPPA in the first-trimester of 100 low risk pregnant women for prediction FGR were 2 , 51 pg/ml and 2.3mIU/ml respectively. UtA-PI was truly positive for FGR in 14 cases and falsely negative in 16 cases. Consequently the sensitivity of UtA PI for prediction FGR was 46.67%, specificity 88.57%, positive predictive value (PPV) 63.6%, and negative predictive value (NPV) 79.48%. Maternal serum PlGF was truly positive in 16 cases of FGR and falsely negative in 14 cases. Consequently its sensitivity for prediction of FGR was 53.3%, specificity 91.42%, PPV 76% and NPV 85.33%. Similarly sensitivity for PAPP-A prediction of FGR was 43.3, specificity 92.85, PPV 72.2% and NPV 79.26% [Figure 1].

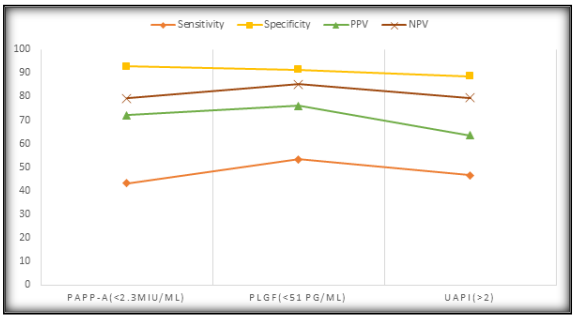

Figure 1: Sensitivity, specificity, PPV AND NPV of PAPP-A, PLGF (PG/ML) and UAPI in the prediction of FGR fetuses.

The Area Under the Curve (AUC) is the measure of the ability of a classifier to distinguish between classes and is used as a summary of the ROC curve. For PAPP-A, the area under the ROC curve (AUC): 0.709 (95% CI: 0.609 to 0.795), p=0.0005. For PLGF, the area under the ROC curve (AUC): 0.717 (95% CI: 0.616 to 0.804), p=0.0005. For UAPI, the area under the ROC curve (AUC): 0.690 (95% CI: 587 to 0.781), p=0.002. The higher the AUC, the better the performance of the model at distinguishing between the positive and negative classes. According to the study carried out, the highest AUC is for PLGF. Therefore, we can say that PLGF is a better marker for predicting the FGR. Better amongst other four markers belonging to 1st trimester and 2nd trimesters [Figure 2].

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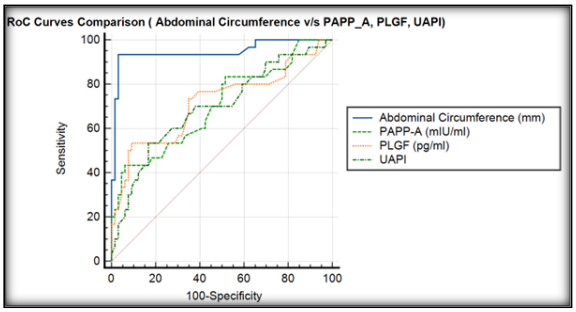

Figure 2: ROC analysis for the AUC for AC, PAPP-A, PLGF, and UAPI.

Univariate analysis showed that Fetal Growth Restriction significantly correlated with the first-trimester PAPP-A. Fetal Growth Restriction also significantly correlated with second-trimester PLGF and UtA-PIscore, but not free β-hCG of fetal biometric parameters [Table 3].

Table 1: Comparison of Socio-demographic characteristics of the women with or without FGR.

Characteristics	With FGR (N=30) Mean ± SD, or n (%)	Without FGR (N=70) Mean ± SD, or n (%)	P-value
Maternal age (years)	27.41 ± 4.70	27.63±4.84	0.531
Residential Area			
Urban	10 (33.3%)	35 (50%)	0.045
Rural	20 (66.7%)	35 (50%)	
Education status			
literate	21 (70%)	42 (60%)	0.985
Non-literate	9 (30%)	28 (40%)	

Table 2: Comparison OF Feto-maternal characteristics of the women with or without presence of FGR.

Characteristics	With FGR (N=30) Mean ± SD, or n (%)	Without FGR (N=70) Mean ± SD, or n (%)	P-value
Physical			
BMI (Kg/m2)	26.37±5.38	26.38±5.35	0.202
Maternal BP (mmHg)			
Systolic	114.82±7.97	114.82±7.97	0.149
Diastolic	71.85±5.49	71.78±5.46	0.241
Lab Investigations			
HB (g/dl)	12.43±9.51	12.40±9.42	0.231
blood sugar (mg/dl)	120.57±6.5	120.79±6.5	0.127
serm - il(mg/dl)	0.82±0.20	0.83±0.21	0.321
SGPT (U/litres)	32.26±9.5	32.16±9.5	0.105
SGOT (U/litres)	28.7±7.8	28.8±7.74	0.224
1ST TRIMESTER			
PAPP-A (mIU/ml)	33.81±17.43	33.65±17.66	0.000
NT SCAN (mm)	2.56±0.26	2.55±0.26	0.650
NB SCAN (mm)	1.54±0.22	1.53±0.22	0.424
β-hCG (ng/ml)	40.22±6.35	40.13±6.32	0.224
2nd Trimester			
PLGF (pg/ml)	47.46±19.49	48.96±18.27	0.000
UAPI	2.16±1.72	2.09±1.72	0.001
3rd trimester			
Growth scan			
Abdominal Circumference (mm)	225.95 ± 12.79	225.28 ± 8.36	0.012
Estimated fetal weight	1.65±0.24	1.66±0.24	0.001
colour doppler			
UMBLICAL ARTERY PI	1.09±0.28	1.09±0.28	0.000
CPR>5th percentile	4 (13.3%)	0 (0%)	0.002
AEDF/REDF	6 (20%)	0 (0%)	0.000
Labour			
SPONTANEOUS	8 (26.7%)	34 (48.6%)	0.021
INDUCED	22 (73.3%)	13 (18.6%)	0.001
Mode of delivery			
VAGINAL	12 (40.0%)	40 (57.1%)	0.214
LSCS	18 (60.0%)	30 (42.9%)	0.032
Pre-term delivery <37 weeks	20 (66.7%)	35 (50%)	0.024
Birthweight (g)	1855 ± 721	2529 ± 402	0.001
Stillbirth	5 (16.7%)	0 (0%)	0.000
Neonatal outcome			
APGAR <7 at 5 min	8 (26.7%)	3 (4.3%)	0.001
NICU admission >48 h	22 (73.3%)	23 (32.8%)	0.001

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Table 3: correlation between markers with FGR.

Parameter	Pearson correlation coefficient (r)	P
1ST TRIMESTER		
PAPP-A (mIU/ml)	-0.384**	0.000
NT SCAN (mm)	0.047	0.650
NB SCAN (mm)	-0.083	0.424
2nd trimester		
β-hCG (ng/ml)	0.125	0.224
PLGF (pg/ml)	-0.503**	0.000
UAPI	0.341**	0.001

DISCUSSION

In the present study, the mean age of both group was almost similar that is 27.41 ± 4.70 years in women with FGR and 27.63±4.84 in women without FGR. Likewise, the findings of our study a reference study by Li et al., (2016) has reported maternal age of 27.12±3.12 years in cases while it was 28.67±3.51 years for controls.[13]

Similar to our results FGR was more prevalent in women belonging to rural areas in the study conducted by Leite et al., (2019).[14]

The findings were found to be consistent with a reference study by Li et al., (2016) reported that at the time of blood sampling there was no statistically significant difference in maternal age, body mass index and gestational age, between the women that developed pre-eclampsia and the normal controls.[13]

The 1st trimester findings in both the group were almost similar and difference in their relations were found insignificant. There was significant difference found between both the group for PAPPA-A as $p<0.05$. Screening for pre-eclampsia and fetal growth restriction by uterine artery Doppler and PAPP-A at 11–14 weeks' gestation in study by Pilalis et al., (2007).[15] Prediction of birth weight by fetal crown–rump length and maternal serum levels of pregnancy associated plasma protein A in the first trimester as proved by Leung et al., (2008).[16]

UAPI was significantly higher in women with FGR compared to women without FGR. These studies were consistent with studies done by Martin et al., (2001) and Papageorghiou et al., (2001).[17,18]

Maternal serum PlGF was significantly lower in women with FGR as compared to women without FGR. Regarding distribution of 3rd trimester findings. Placental growth factor as a marker of fetal growth restriction caused by placental dysfunction as proved by Benton et al., (2016).[19]

Also, the importance of the cerebroplacental ratio in the evaluation of fetal well-being in SGA and AGA fetuses proved by DeVore et al., (2015).[20]

As women with FGR were induced due to colour doppler changes and after induction some of them were taken for LSCS for indication of fetal distress. These results were also proved by a study conducted by Nardozza et al., (2017).[21]

Likewise, the findings of our study the birthweight was significantly lower in cases as compared to controls ($p<0.001$). There was significant relation found between APGAR <7 at 5 min and NICU admission >48 h with FGR as $P<0.05$. These results were also proved by a study conducted by Nardozza et al., (2017) which showed a higher rate of perinatal morbidity in fetus with FGR.[21] Baschat et al., (2011) studied Neurodevelopment following fetal growth restriction.[12]

CONCLUSION

This study highlights the enhanced predictive value of combining serum biomarkers and Doppler studies for Fetal Growth Restriction (FGR). PAPP-A emerged as the most significant first trimester predictor, while β-hCG was not significant. In the second trimester, both PLGF and UAPI were significant predictors, with higher UAPI and PAPP-A levels in early-onset FGR and PLGF having the highest ROC area. FGR was more common among rural and illiterate women, with many showing umbilical artery Doppler changes in the third trimester, often requiring induction before 37 weeks and sometimes cesarean sections due to fetal distress. FGR was associated with increased perinatal morbidity, including low APGAR scores and prolonged NICU admissions. Severe anemia was the most prevalent medical cause of FGR. All four markers (PAPP-A, PLGF, β-hCG, and UAPI) demonstrated good sensitivity, specificity, PPV, and NPV, making them effective predictors of FGR, and they also have utility for aneuploidy screening. Larger studies are needed to validate these findings and incorporate these biomarkers into routine clinical practice, potentially leading to early prophylactic strategies and improved management of high-risk pregnancies.

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