Original Research Article

Role of dual biomarkers and uterine artery doppler study in predicting PIH and IUGR in antenatal patients registered in a tertiary care centre

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ABSTRACT

Background: The incidence of IUGR is between 3 to 7%, whereas that of Hypertensive disorders of pregnancy is about 10% of pregnant women around the world. These conditions are associated with a high rate of perinatal morbidity and mortality, posing a need for the detection of the potential causes of maternal and foetal morbidity and mortality and for the prediction of these conditions early during pregnancy.
Methods: Patients with first antenatal visit before 10 weeks gestation underwent routine NT scan between 11–13 weeks with bilateral UAD-RI of the maternal uterine arteries. The placental volume was assessed. Serum dual biomarker test (β hCG and PAPP-A) was performed after this scan and analysed as multiples of median (MoM). Blood pressure was recorded at every ANC visit till 2 weeks after delivery. Neonatal head and chest circumference, birth length and weight were recorded.
Results: The mean values of PAPP-A levels of Non-PIH and PIH groups were 1.32±0.91 MoM and 0.68±0.39 MoM respectively, showing statistically significant difference. The serum PAPP-A levels showed statistically significant difference between Non-IUGR and IUGR groups (1.24±0.87 MoM and 0.46±0.20 MoM respectively).
Conclusions: In our study, PAPP-A level is observed as a good indicator for possible prediction of PIH and IUGR whereas levels of β hCG and UAD-RI were not good predictors. The role of Placental volume in prediction of IUGR needs to be explored further with larger sample size. Future studies are needed with a larger group with inclusion of measurement of PI values.

Keywords: Dual biomarkers, IUGR, PIH, Uterine artery doppler - resistivity index (UAD-RI)

INTRODUCTION

Hypertension is the one of the common medical problem complicating nearly 10% of pregnancies.[1] The national incidence of Pregnancy Induced Hypertension (PIH) is 15.2% in Indian population. PIH is said to occur when there is a rise of blood pressure to ≥ 140/90 mm Hg during gestation, as per the ISSHP.[2]

In the hypertensive disorders' spectrum in pregnancy, preeclampsia and eclampsia are responsible for about 10.0% of maternal deaths, annually, with an additional 20-30 women suffering significant morbidity for every

maternal death.[3] Foetuses of these mothers are at greater risk of IUGR, prematurity and intrauterine demise.[4]

IUGR – intra-uterine growth restriction, also known as foetal growth restriction (FGR) is defined as the pathologic inhibition of intrauterine foetal growth and failure of the foetus to achieve its normal growth potential.[5]

IUGR, with an incidence between 3 to 7%, is diagnosed in antenatal period if, sonographically estimated foetal weight <10th percentile for gestational age or with a documentation of foeto-placental insufficiency.[6,7]

Increased foetal and neonatal mortality and morbidity as well as adult-onset pathological conditions are also often attributed to IUGR due to associated epigenetic changes.

Currently no treatment is available that can reverse the pathophysiologic events associated with preeclampsia and so also for IUGR.[8] Amongst the several screening methods Uterine artery Doppler (UAD) ultrasound is used for the indirect assessment of uteroplacental circulation from early gestation.[9] UAD is considered as a potential screening tool for detection of preeclampsia and IUGR.[10,11]

β human chorionic gonadotropin (hCG) hormone secreted by the placenta has been linked to foetal growth whereas Pregnancy-associated plasma protein-A (PAPP-A) is a known promotor of angiogenesis.[12,13] According to Poon *et al.* (2009, 2010), when the pregnant women first time visit the hospital, i.e., at 11-13 weeks gestational age, sonographic and biochemical testing may be carried out for gestational hypertensive disorders.[14-16] Patil et al (2014) observed low levels of PAPP-A in first trimester with infant death, IUGR, preterm birth and pre-eclampsia in chromosomally normal foetuses.[13] They also observed raised PAPP-A levels and nuchal translucency to be associated with specific structural abnormalities and genetic syndromes.

Linking UAD study to the booking tests (Nuchal scan and Dual biochemical markers) could probably be able to identify a high-risk group, which in turn would help in initiating prophylactic therapies as early as 12–14 weeks into the pregnancy.

METHODS

This was a prospective, observational study carried out in the department of 'Obstetrics and Gynaecology' of a private tertiary care centre. Approval for the study was obtained from the Institutional Ethics Committee Review Board in 2017. Antenatal women visiting OPD, with first visit before 10 weeks of gestation and the same falling within 1st November 2017 and 30th April 2019 were enrolled. Those patients with pregnancy between 11-13 weeks, confirmed by LMP and early scan, with a single live intrauterine pregnancy after having given written informed valid consent were included. Patients with age ≥40 years, multi-foetal pregnancy, pre pregnancy BMI ≥30.0, with pre-existing hypertension or infections, smoking, drinking habits or other addictions were excluded.[17] Patients developing hypertension before 20 weeks and those lost to follow up or with diagnosis of IUFD were excluded from analysis. The studies undertaken by Narang S. et al and Spencer K. et al were used as reference for calculating the sample size.[18,19] The target sample size was not less than 30 patients. Of the 35 patients enrolled in the research study, 3 were lost to follow up and 1 fell in the exclusion criteria, thus 4 were excluded. Data analysis of the remaining 31 patients was carried out.

The enrolled patients underwent additional UAD study (RI values of the right and left maternal uterine arteries) and placental volume assessment (calculated by measurements of length, width, and thickness) with routine NT scan and Dual biomarker test between 11.0-13.0 weeks.[20-22] The data of dual biomarkers (β hCG and PAPP-A) was converted in multiples of median (MoM) for analysis.[23]

Blood pressure was recorded at every ANC visit. Required ANC care was provided and follow up was advised with ultrasound scans at 20, 28 and 36 weeks. Maternal BP before and up to 2 weeks after delivery was recorded. Date and mode of delivery, the head and chest circumference along with birth length in centimetres and weight of the neonate in grams were recorded. APGAR score of the new-born at the end of 1, 5 and 10 mins was noted. Any need for admission of the baby to NICU was documented.

Relevant data was analysed by statistician using Microsoft Excel's in-built functions and two sample t-test was performed using Analysis Tool-pack Add-In, provided in Microsoft Excel.

RESULTS

The results were analysed in terms of demographic data and outcomes along with the UAD-RI screen, Dual Biomarkers and Placental volume.

The outcomes of interest in the study were the development of PIH and IUGR.

Table 1: Frequency of outcomes in terms of PIH and IUGR.

Outcome	Frequency	%
PIH	6	19.35
IUGR	2	6.45

The outcomes evaluated were found to occur in 22.58 % of patients. One patient (3.2% of total number of patients studied) had both PIH and IUGR adverse effects.

The RI was recorded separately for the right and left uterine arteries and mean RI calculated. The values recorded are depicted in tabular form.

Table 2: Average UAD RI in both Ut arteries.

UAD (Doppler values) RI	Mean ± SD
Right	0.66±0.15
Left	0.66±0.14

One patient had notching in unilateral uterine artery- in this case, the right, while 1 had bilateral notching of Ut arteries. However, in the study none of these 2 patients developed any complications of PIH or IUGR.

The mean value of β hCG in terms of MoM was 1.16±0.89 whereas that for PAPP-A was 1.18±0.86 for the entire patient group.

The average placental volume recorded in the study was 46.62±16.11 cc.

The mean values of the Dual biomarkers in MoM, UAD-RI, Placental volume in cc. in PIH, Non PIH and IUGR and Non IUGR groups are given in the Table below.

Table 3: Comparison of mean values of various parameters and their outcomes.

Parameters	PIH	Non PIH	IUGR	Non IUGR
PAPP-A	0.68±0.38	1.32±0.91	0.46±0.2	1.24±0.87
β hCG	0.95±0.65	1.22±0.95	1.0±0.64	1.18±0.92
UAD- RI avg.	0.60±0.17	0.68±0.1	0.57±0.095	0.67±0.12
Placental volume	43.2±16.16	47.44±16.32	24.35±8.13	48.15±0

The details of the master-chart record are available in the dissertation submitted by the author to the Maharashtra University of Health Sciences.[24]

There was a statistically significant correlation between low PAPP-A levels and development of PIH and IUGR.

Table 4: PAPP-A v/s PIH.

PAPP-A		
t-Test: two-sample assuming unequal variances		
	PIH	
	No	Yes
Mean	1.32	0.68
Variance	0.82896	0.1516
Observations	22	6
Hypothesized mean difference	0	
Df	20	
t Stat	2.5509	
P(T<=t) one-tail	0.00952	
t Critical one-tail	1.72472	
P(T<=t) two-tail	0.01904	
t Critical two-tail	2.08596	

The difference in two group values is statistically significant at confidence level α=0.05. Thus, PAPP-A is a potential indicator of PIH.

However, since the variability in the Non-PIH group is high, it is difficult to propose a flag-post PAPP-A level for predicting PIH. Value of less than 1.0 MoM would be an indication of likelihood of impending PIH.

Table 5: PAPP-A v/s IUGR.

PAPP-A		
t-Test: two-sample assuming unequal variances		
IUGR		
	No	Yes
Mean	1.238462	0.46
Variance	0.757302	0.0392
Observations	26	2
Hypothesized mean difference	0	
Df	6	
t Stat	3.526569	
P(T<=t) one-tail	0.00621	
t Critical one-tail	1.94318	
P(T<=t) two-tail	0.012419	
t Critical two-tail	2.446912	

The difference in two group values is statistically significant at confidence level α = 0.05. Thus, PAPP-A is potential indicator of IUGR. PAPP-A levels of less than 0.6 MoM would be an indication of impending IUGR outcome. However, since the variability in the Non-IUGR group is high, it is difficult to propose a flag-post PAPP-A level for predicting IUGR.

Table 6: Placental volume v/s IUGR.

Placental volume, cc		
t-Test: two-sample assuming unequal variances		
IUGR		
	No	Yes
Mean	48.15862	24.35
Variance	237.9118	66.125
Observations	29	2
Hypothesized mean difference	0	
Df	2	
t Stat	3.706263	
P(T<=t) one-tail	0.032853	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.065706	
t Critical two-tail	4.302653	

Though the difference in two group values is not statistically significant at α = 0.05, the low p-value is indicative of weak evidence towards rejection of Null hypothesis. The role of low placental volume conjointly with low PAPP-A MoM values could be valuable indicator of IUGR outcome.

Mean UAD-RI, serum β hCG-mean MoM value and placental volume showed no statistically significant correlation between the PIH and non PIH groups.

Serum PAPP-A levels were on the lower side in PIH and for IUGR group than the normal population in the study with statistically significant difference in the marker level.

Mean UAD-RI, serum β hCG-mean MoM value and Placental volume values did not exhibit statistically significant correlation in patients who developed IUGR and for the patients without IUGR.

DISCUSSION

Our study recorded the median age of women who developed PIH to be 27.5 yrs and those who did not develop PIH to be 30 yrs, same as that of all participants. Study published by Muti et al. (2015) showed median age for occurrence of PIH to be 29.0 yrs in their sample. According to this study, affected women were older than the unaffected ones.[25]

In our study, the mean RI values for the sample were 0.66±0.15 of the right uterine artery and 0.661±0.14 for the left. The mean RI value of right and left arteries was 0.66±0.12. A prospective cohort study comprising singleton pregnancies published by Scandiuzzi et al. in 2016 reported Right Ut A RI value as 0.69±0.13, and 0.69±0.12 was reported for the left. The mean RI for both arteries was 0.69 ± 0.1. [26] These values are similar to our findings.

Based on observations in the present study we observed a statistically significant difference in PAPP-A levels of Non-PIH and PIH patient groups. The observed mean value of PAPP-A levels in Non-PIH group was 1.32±0.91 MoM, whereas in PIH group was 0.68±0.39 MoM. The results are in agreement with a prospective cohort study published by Moety et al. (2016), to evaluate the first trimester placental function in predicting PE and IUGR which revealed that PAPP-A levels were significantly reduced in patients who developed these complications.[27] Since data dispersion in the non-affected sample is high, as indicated by relatively large Standard Deviation values observed in our study, a clear flag-post value of PAPP-A level to indicate forthcoming PIH complication is difficult to establish.

In our study β hCG levels did not show any significant statistical correlation with PIH. The volume of placenta in PIH patients was marginally lower than the normal population mean. The difference in placental volume of PIH and Non-PIH patients was not statistically significant.

In present study, the serum PAPP-A levels showed statistically significant difference between Non-IUGR and IUGR groups. The observed PAPP-A levels of Non-IUGR group were 1.24±0.87 MoM, whereas that of IUGR group were 0.46±0.20 MoM. Since data dispersion in non-IUGR i.e., normal patients' sample is high, as indicated by relatively large 'Standard Deviation' value, a clear flag-post value to predict imminent case of IUGR is difficult to establish.

There was no statistically significant difference in mean levels of the biomarker β-hCG between IUGR and Non-IUGR groups. The mean level of β-hCG in IUGR patients (1.0±0.64 MoM) was marginally lower in comparison to Non-IUGR patients (1.18±0.92 MoM). This is in line with study published by Dugoff et al. who evaluated pregnancies at 11-14 weeks of gestation and proved that maternal serum free β-hCG was mildly reduced in pregnancies that subsequently developed IUGR.[28] Overall, the predictiveness of free β-hCG is unsatisfactory.[29-31]

Placental volume in the present study showed a p=0.07, with respect to development of IUGR. Placental volume of the Non-IUGR and IUGR outcome group were well separated - 48.16 and 24.35 cc respectively. Though the two-sample t-test did not show statistically significant difference at α=0.05 level of significance (p=0.07), evidence for failing to reject Null hypothesis is weak.

The limitations of our study were the small sample size, cost constraints and non-availability of advanced ultrasonography techniques. A larger sample size with a possibility of more patients with adverse pregnancy outcomes would be helpful in determining the statistical corelation between UAD indices and serum biomarkers and the development of PIH and IUGR. Low placental volume observed in cases of IUGR, though not statistically significant, could be further studied with 3D colour doppler for accurate measurement using larger sample size.

Recent advances in biochemical markers includes study of angiogenic and antiangiogenic factor levels that play profound role in pathophysiology of PIH. Angiogenic factors include VEGF, PlGF, whereas the antiangiogenic factors playing major role are sFlt-1 and soluble Endoglin. However, there is a severe cost constraint in utilization of these factors in low resource settings. Future studies are needed with inclusion of these newer biomarkers like sFlt -1, PlGF levels and their individual as well as combined correlation in prediction of PIH in a cost-effective manner.

CONCLUSION

The prospective observational study was conducted in our hospital to study predictive ability of Uterine Artery Doppler RI values, placental volume and levels of bio marker β-hcg and PAPP-A during 11th to 13th week of pregnancy to identify increased risk of PIH and IUGR.

The adverse pregnancy outcomes in our study, evaluated in terms of PIH and IUGR, occurred in 22.58% of patients.

In the IUGR patients, levels of PAPP-A showed statistically significant difference compared to non-IUGR. Serum PAPP-A levels were on the lower side (0.68±0.38) in PIH than the normal population (1.32±0.91) in the study and were statistically significant.

The results of our study thus indicate, that biomarker PAPP-A level is a good indicator for possible prediction of PIH and IUGR. Levels of β hCG and UAD-RI were not found to be good predictors of the condition. Placental volume can be further explored in view of observed p value being close to level of statistical significance (p=0.07, α=0.05).

The study highlights the use of combined approach using clinical data and routine tests like serum dual markers and ultrasound parameters in early prediction of PIH and IUGR. This in turn will be useful in initiation of prophylactic management early in the course of pregnancy to prevent these complications. The combined approach using clinical data, serum biochemical markers and biophysical parameters (ultrasound parameters, arterial blood pressure) promises increased predictive relevance.

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