Original Article

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Impact of High Levels of Pregnancy Associated Plasma Protein-A on Pregnancy

Obstetrics and Gynaecology Section

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ABSTRACT

Introduction: Routinely performed aneuploidy screen takes into account free beta human chorionic gonadotrophins (β-hCG), Pregnancy Associated Plasma Protein-A (PAPP-A), nuchal translucency, and various maternal characteristics. Incidental finding of extreme level of PAPP-A can generate anxiety. Association of low PAPP-A has been established with chromosomal anomalies and adverse pregnancy outcome. The effect of high PAPP-A on feto-maternal outcome has not been widely studied.

Aim: The aim of the study was to analyse pregnancy outcome in the group with high maternal PAPP-A, that is >95th centile at the first trimester aneuploidy screen test.

Materials and Methods: The prospective observational study was conducted at Dr. TMA Pai hospital, affiliated to Manipal Academy of Higher Education, Manipal. Each patient visiting antenatal OPD was counselled for first trimester aneuploidy screening during the study period of two years (November 2015 to November 2017). All patients who were registered for delivery at the hospital were taken into the study. Blood samples were taken at 11-13^{+6} weeks of pregnancy. A total of 1500 consecutive pregnancies having first trimester screening were followed up with PAPP-A levels, expressed in Multiple of Medians (MoM). The study group was formed by patients with PAPP-A of more than 95th centile. Only euploid foetuses were considered for the analysis. The pregnancy outcomes were determined in PAPP-A levels of >95th percentiles (3.11 MoM and more) with respect to threatened abortion, preterm delivery, foetal malformations, hypertension in pregnancy, gestational diabetes and growth abnormalities. It was compared with matched control group of PAPP-A between 5th to 95th centile (PAPP-A >0.49 to 3.10). Statistical analysis was performed using Statistical Package for Social Science (SPSS) software version 16 and p-value <0.05 was considered statistically significant.

Results: For our grouped data, the incidence of high PAPP-A was 4.6%, and 95th centile value for PAPP-A was 3.10 MoM. The maximum value of PAPP-A recorded was 8.6 MoM. We found no statistically significant difference in the incidence of threatened abortion, preterm delivery, foetal malformations, large for gestation foetuses, gestational diabetes, and hypertension in pregnancy. The incidence of small for gestation was significantly different across the two groups (p-value<0.05).

Conclusion: High PAPP-A values are seen less frequently. The pregnant woman with structurally normal foetuses should be informed that there is no reason to be anxious for adverse pregnancy outcome due to high PAPP-A.

Keywords: Foetal growth restriction, First trimester aneuploidy screen, Growth abnormality, Macrosomia

INTRODUCTION

Emphasis has been given to the proteins examined during the screening in the first trimester of pregnancy for foetal aneuploidy. These biochemical markers are β-hCG and pregnancy associated plasma protein A (PAPP-A). Variation in the serum levels of such biomarkers can influence the outcome of pregnancy. High levels of free β-hCG and low levels of PAPP-A is found in trisomy 21 [1], while low β-hCG and PAPP-A are observed in trisomy 18 [2]. Apart from association with the chromosomal abnormality, the impact of low PAPP-A on the rates of adverse pregnancy outcome has been well established. The high maternal PAPP-A level is less common, and studied to a lesser extent. The incidental findings of it can even generate anxiety and uncertainty as its association with pregnancy outcome is not well studied. Hence, in the present study, we sought to find the relationship of high PAPP-A with pregnancy outcome.

PAPP-A is a glycoprotein produced by trophoblast syncytium; it appears in maternal blood in early pregnancy (about 32 days), and it increases throughout the gestation until the end of the pregnancy, and the levels reduces rapidly in the postpartum period. PAPP-A regulates Insulin like Growth Factor (IGF) bioavailability, hence, regulates foetal growth and development through its effect on transfer of glucose and aminoacids [3].

In the present study, we aimed to establish the reference range of PAPP-A, i.e., 5th and 95th centile in our population. Secondly, to determine if high PAPP-A levels can predict foetal growth abnormality, preterm labour, diabetes, and to establish its role in risk reduction of pregnancy complications, if exists.

MATERIALS AND METHODS

A prospective observational study was conducted for two years i.e., from November 2015 to November 2017 at Dr. TMA Pai hospital, Udupi, affiliated to Manipal Academy of Higher Education and approved by ethical committee of Manipal University, Manipal. The screening population consisted of 1500 women with singleton pregnancies who consented for first trimester aneuploidy screening at 11-13^{+6} weeks of gestation. Informed consent was taken from patients in Kannada and English languages about the study enrolment. Inclusion criteria were singleton pregnancies booked in the first trimester and consented for first trimester aneuploidy screening and willing to participate. Exclusion criteria were multiple pregnancies, chromosomal anomaly, lethal congenital anomaly, pre-gestational diabetes, chronic hypertension, chronic renal disorders, cardiac disorders and congenital viral infections.

Gestational age was based on last menstrual period with previous regular cycles and corresponding scan findings in first trimester

ultrasound {Crown-Rump Length (CRL)} between 7-10 weeks of gestation. The disparity in CRL up to four days was considered acceptable. Patient's age, height, weight, the presence of above mentioned medical illness, and other demographic characteristics were noted.

Three to four millilitres of venous blood sample was collected. The serum was separated and analysed at biochemistry laboratory of Kasturba Hospital, Manipal. The test was carried out by automatic analyser machine by Electrochemiluminiscence immunoassay (ECLIA) with Cobas e601 analyser. The results were calculated by Cobas e software and corrections were made according to weight, smoking and diabetes status. Measuring range for PAPP-A was 4-10,000 mIU/L. Appropriate dilution was carried out for PAPP-A of more than measuring range. The risk calculation was performed using software Sswd Lab version 5.0, and was given as MoM.

From the available cohort, 5th and 95th centile for PAPP-A values were obtained. All the women with PAPP-A of more than 95th centiles were selected as cases and women with 5th- 95th centile serum PAPP-A were taken as controls. The foetal aneuploidy risk was calculated and risk of 1:250 or higher was taken as high risk for chromosomal abnormality for Down syndrome and risk of 1:100 was reported as screen positive for trisomy 18 and 16. Definitive testing was suggested for high risk of aneuploidy. The results suggestive of euploid were continued with respective groups. Women detected with major anomalies at second trimester scan were given option for termination of pregnancy. Pregnancy outcome information was obtained for Spontaneous Abortion (SA), Preterm Delivery (PTD), Large for Gestational Age (LGA), hypertension in pregnancy, gestational diabetes, oligohydramnios, Small for Gestational Age (SGA), mode of delivery and low birth weight baby.

LGA was defined as birth weight above the 90th centile for the gestational age [4]. SA was determined as a loss of a pregnancy before 20 weeks of gestation [5]. PTD was defined as delivery before 37 completed weeks of gestation. Hypertension in pregnancy was determined as a blood pressure of more than 140/90 mm of Hg at least twice, after six hours of interval after 20 weeks of pregnancy with or without proteinuria. Isolated low liquor was defined as amniotic fluid of less than 7 cm after 28 weeks gestation in absence of any other foetal or maternal pathology for our study. Premature Rupture of Membrane (PROM) was defined as rupture of membranes after 37 weeks of gestation but before labour starts. SGA was defined as birth weight below the 10th centile for the gestational age [6]. We used centile charts from Callen's book of Ultrasound [7].

STATISTICAL ANALYSIS

Statistical analysis was performed using Statistical Package for Social Science (SPSS) software version 16 and p-value <0.05 was considered statistically significant. Chi-square test was used to show differences between the study groups. The Student's t-test was used for the comparison of means. Fisher's-exact test was used when appropriate. Odds Ratio (OR) with 95% CI was calculated for certain outcomes.

RESULTS

Our cohort consisted of PAPP-A values between 0.50 and 8.6 MoM; range between 1079–113333 mIU/ml. Seventy patients (4.6%) with high PAPP A were found amongst screened 1500 pregnant women in the first trimester. The PAPP-A values of 0.49 and 3.10 Multiples of Median (MoM) were respective values for 5th and 95th centiles in our populations. The 1st, 3rd, 90th and 99th centile PAPP-A values were 0.324, 0.45, 2.4, 4.8 MoM respectively. PAPP-A value of >3.10 MoM were taken as cases and 200 women with normal PAPP-A (0.50 – 3.09 MoM) were considered as controls. We got 45 cases (64%) with values between 3.10-3.99 MoM, 17(24%) had 4-4.99 MoM and 8 (12%) had more than 5MoM. Our cohort had an age range of 18-38 years.

[Table/Fig-1] shows the group characteristics, where age, first trimester weight, BMI, CRL and β-hCG value of study and control group was comparable. The mean sampling period was 12.0 weeks in study group and 12.3 weeks in control group (p-value 0.001). The study group had 47(57%) nulliparous patient and control group had 129 (64.5%) nulliparous patients (p-value 0.86).

Characteristics observed	Study group (PAPP-A >3.11 MoM) (n=70) Mean±SD	Control group (PAPP-A >0.491-3.10 MoM) (n=200) Mean±SD	p-value
Maternal age (years)	27.71±3.34	27.48±3.80	0.41
First trimester weight (kg)	53.37±11.2	52.64±10.69	0.64
Weight gain in pregnancy (kg)	10.5±4.15	11.05±4.16	0.34
BMI (kg/m^2)	22.24±3.96	21.77±4.16	0.41
Gestational weeks at FTS	12.06±0.55	12.31±0.47	0.00
PAPP-A value (MoM)	3.95±0.93	1.45±0.60	0.00
Crown rump length (mm)	58.53±10.44	60.06±6.31	0.15
β hCG (MoM)	1.31±1.03	1.06±0.92	0.69

[Table/Fig-1]: Characteristics of study population in both groups.
PAPP-A: Pregnancy associated plasma protein-A; BMI: Body mass index; FTS: First trimester combined screening; Mom: Multiple of median

[Table/Fig-2] shows the incidence of pregnancy complications. There was no statistically significant difference in the incidence of threatened abortion and preterm delivery in both the groups. We found one major and minor malformations in the study group and three major and seven minor foetal anomalies in control group, here p-value-0.81, was insignificant. The minor soft markers were choroid plexus cyst [2], small atrial septal defect [2], perimembranous ventricular septal defect, polydactyly, single umbilical artery [2], hypospadias, renal pelvicalyceal dilatation etc. The incidence of hypertension in pregnancy and intrauterine growth restriction was lower in the study group, which was statistically insignificant. The incidence of diabetes and oligoamnios was almost same in both the groups. We did not find any case of late 1st or 2nd trimester abortion in any group. The incidence of PROM was lower in the study group than in the control group, which was statistically insignificant.

Complications/ Events	Study group (n=70) (%)	Control group n =200 (%)	Chi-square value	p-value
Threatened abortion	8 (11%)	18 (9%)	0.35	0.55
Preterm delivery	8 (11%)	24 (12%)	0.01	0.89
Pregnancy associated hypertension	3 (4.2%)	21 (10.5%)	2.4	0.11
IUGR	3 (4.2%)	21 (10.5%)	2.4	0.11
Diabetes	6 (8.5%)	15 (7.5%)	0.083	0.77
Isolated low liquor	7 (10%)	19 (9.5%)	0.149	0.90
PROM	2 (2.8%)	15 (7.5%)	1.89	0.16

[Table/Fig-2]: Complications during pregnancy in both groups.
IUGR: Intrauterine growth restriction; PROM: Premature rupture of membrane

[Table/Fig-3] mentions the outcome of pregnancy in both the groups. Here we found mean gestational age at delivery was same. The mean birth weight was marginally different i.e., 3 kg and 2.9 kg in study group and control group. We found a low incidence of SGA and high incidence of LGA in the study group. However, statistically significant difference was found for SGA (birth weight <10%) only, OR-0.31 (95% CI – 0.11-0.85), suggested risk reduction of SGA with high PAPP-A. The difference

in the incidence of LSCS and neonatal complications were not statistically significant between both groups. We had five cases of prolonged neonatal stay for respiratory distress syndrome [2], and hyperbilirubinemia in neonate [3].

Outcome	Study group n=70	Control group n=200	Chi-square value	p-value
Mean gestational age at delivery (Weeks±SD)	38.3±1.27	38.3±1.28		0.975
Mean weight at birth (Kg±SD)	3.0±0.42	2.9±0.47	--	0.12
SGA<10thcentile	4 (6.5%)	36 (18%)	6.20	0.012
SGA <5th centiles	2 (2.8%)	20 (10%)	3.5	0.063
LGA	5 (7.9%)	6 (3%)	2.3	0.125
Neonatal prolonged stay	1 (1.4%)	4 (2%)	0.16	0.68

[Table/Fig-3]: Outcome of pregnancy in both groups.
SGA: Small for gestational age; LGA: Large for gestational age

DISCUSSION

The incidence of high PAPP-A in our study was 4.6%. it was noted high (7.9%) in the study by Timmerman E et al., [8]. For our study, 95th mean centile value (SD) was 3.95±0.93 MoM, the same is mentioned as 3.9 MoM at 11 weeks of gestation [9]. In the FASTER trial, median 95% value achieved was 2.51 MoM [6].

The incidence of threatened abortion was almost same (11% vs 9%) in study and control group respectively, which is in agreement to worldwide incidence [10]. We did not have late 1st trimester and early second trimester abortion. We had four major foetal anomalies, which were severe ventriculomegaly (hydrocephalus) with PAPP-A levels of 3.4 MoM in the study group and control group, three anomalies noted were (Double outlet right ventricle, vermian agenesis, and severe bladder outlet obstruction with hydronephrosis). All the four couple with major anomaly opted for termination of pregnancy, hence were not included in the study. Foetal severe ventriculomegaly has not been reported with high PAPP-A levels. Hence, it is incidental or associated with high PAPP-A could not be confirmed.

The incidence of hypertension in pregnancy and Intra-uterine growth restriction was less in the study group (4.2% vs. 10.5% in control group), which suggests the beneficial effect of high PAPP-A on effective placentation. The incidence of Diabetes in pregnancy was almost same in both the groups (8.5% and 7.5%), which was in agreement to the population incidence mentioned in various studies from South India (6.5 to 17%) [11]. The incidence of isolated low liquor is almost similar in both the groups (10% and 9.5%), shows higher PAPP-A doesn't have influence on amniotic fluid levels. PROM was seen less in the study group (2.8%), while it was much higher (7.5%) in control group, which was statistically insignificant. This can be due to improved strength of membranes with higher PAPP-A. However, further studies are required to support the same. The incidence of PROM has been mentioned up to 10% [12], and our study has similar incidence in control group.

The mean gestational age was almost similar in both the groups, suggesting higher PAPP-A levels doesn't affect the duration of pregnancy. The mean birth weight was higher in the study group. However, the difference was not statistically significant. We observed a linear correlation of birth weight with increasing PAPP-A levels, which was in agreement with the study by Habayeb O et al., [13]. Even SGA was seen in high PAPP-A group, but the incidence was low in the study group, and the difference was statistically significant (p-0.012). The trend of Large For Gestation (LGA) was observed with increasing PAPP-A levels [14].

The analytical method used was ECLIA, which is precise in measurement, recent and reliable method. Other modalities are time-resolved fluorescent assay using a Delfia analyser, a Kryptor analyser, and enzyme-linked immunosorbent assay on blood spot [15]. We believe that high PAPP-A means more cleavage of Insulin like Growth Factor Binding Protein (IGFBP), leading to high bio available IFG 1 and 2 levels, improving growth and metabolism, and better invasion of trophoblast to spiral arterioles. Hence, the possibility of macrosomia and less risk of hypertension in pregnancy and SGA.

LIMITATION AND STRENGTH

The small sample size and non-randomized study pattern are limitations of the study. It represents south west coastal Karnataka population; the results cannot be implied to other parts of India. This is the first ever study from one of the highly populated country of the world, i.e. India and it confirms the findings of previous study. We recommend to look at PAPP-A MoM done at FTS for aneuploidy test as it may provide insight on recurrence of the complication in risk population and risk of certain events in low risk group.

CONCLUSION

PAPP-A assessment is a routine component of first trimester screening and as it provides information at no extra cost, it can be used to assess foetal well-being and pregnancy complications. Higher PAPP-A levels suggest the benign course of pregnancy. It can be an early manifestation of the healthy foetus in the absence of genetic abnormality. However, further studies are needed to prove its utility in hypertension in pregnancy.

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