Original Article

Maternal Pregnancy Associated Plasma Protein-A Levels in Late First Trimester as a Predictor of Miscarriage- A Cross-sectional Study

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ABSTRACT

Indroduction: Miscarriage is the most common complication of pregnancy. Defective implantation is one of the common causes of miscarriage. Pregnancy Associated Plasma Protein-A (PAPP-A) is secreted from syncytiotrophoblast and it enables trophoblast invasion. Few studies have shown association of PAPP-A with miscarriage. However, there is limited data available to establish the role of PAPP-A as a predictive marker of miscarriage, especially in Indian population.

Aim: To determine the potential of maternal PAPP-A level estimation in asymptomatic women in late first trimester (10-13 weeks) with viable foetus in predicting subsequent miscarriage.

Materials and Methods: This was an observational, cross-sectional study conducted from November 2016 to April 2018 at University College of Medical Science and Guru Teg Bahadur Hospital, Delhi, India. Asymptomatic pregnant women (N=500) at 10-13 weeks of gestation were recruited from an antenatal clinic after confirmation of foetal viability. A 2 mL of blood sample was collected and serum PAPP-A level was measured. Independent t-test and Chi-square test was used to compare continuous data and Mann-Whitney U test was used to compare PAPP-A Multiple of Median (MOM). Logistic regression was used to estimate risk of miscarriage.

Results: Out of 500 participants, 9 were lost to follow-up. From remaining N=491, 32 (6.5%) women had a miscarriage. PAPP-A levels were significantly decreased in miscarriage group compared to ongoing pregnancy group with median MOM 0.116 (0.080-0.17) and 1.25 (0.665-3.249) respectively (p-value <0.001). PAPP-A MOM value of ≤10th percentile sensitivity and specificity of detection of miscarriage was 81.25% and 94.98% and at ≤5th percentile sensitivity and specificity was 40.62% and 97.82%, respectively. Lower the percentile cut-off of serum PAPP-A value, higher was the specificity and positive predictive value for prediction of miscarriage. By applying logistic regression we found that if PAPP-A MOM decreases by 1 unit the chances of miscarriage increased by 1.2 times. By this model 63.2% of cases could be explained (Nagelkerke R Square=0.632). For prediction of pregnancies likely to miscarry, the area under Receiver Operator Characteristic (ROC) curve (95% CI) was 0.969 (0.955-0.983).

Conclusion: Low serum PAPP-A levels from asymptomatic women in late 1st trimester is a good predictive marker of miscarriage.

INTRODUCTION

Miscarriage is the most common complication of pregnancy and causes anxiety, sadness, guilt and mental stress to women who have miscarriage. Miscarriage is defined as clinically recognised pregnancy loss before 20 weeks of gestation [1]. It constitutes about 15% of clinically recognised pregnancy while it's occurence among all fertilisation are around 30-50%. Of all the miscarriages, approximately 80% occur before 12 weeks of gestation. Approximately, 50% of abortions occur due to chromosomal abnormality and rest occur due to suboptimal implantation [2]. During early pregnancy, vaginal bleeding most commonly originates from placenta and is the outcome of defective placentation. Studies suggest that improper implantation is an important cause of spontaneous abortion [3]. Interaction of various proangiogenic and antiangiogenic factors, matrix metalloproteinase like Vascular Endothelial Growth Factor (VEGF), Placental Growth Factor (PlGF), Macrophage Inhibitory Cytokine-1 (MIC-1), Flt-1, Alpha Fetoprotein (AFP), PAPP-A are involved in regulation of normal placentation [4,5].

The PAPP-A is produced by syncytiotrophoblast and decidual cells and detected in maternal serum, placental tissue amniotic fluid and coelomic [6]. It is a Zinc (Zn) metalloproteinase with an elongated Zn-binding motif which has been identified as insulin- like growth factor binding protein-4 proteinase [7]. It starts appearing in maternal serum after 5th week of pregnancy and it's levels continue to increase with time. PAPP-A level increases exponentially with doubling time of 3-4 days during 1st trimester of pregnancy. Peak levels of PAPP-A are observed at the end of pregnancy and levels are down regulated subsequently at the time of delivery [7].

Maternal serum PAPP-A level is affected by gestational age and maternal characteristics including weight, racial origin, cigarette smoking, diabetes mellitus and method of contraception. PAPP-A level is more in twin pregnancy [8]. It is used in screening of Down's syndrome. PAPP-A is known to be insulin-like growth factor binding protein-4 protease. Therefore, low levels of PAPP-A are associated with higher levels of insulin-like growth binding protein leading to lower levels of free insulin like growth factor 1 and 2. At maternal foetal interface, Insulin-like Growth Factor-2 (IGF-2) bioavailability mediated by PAPP-A enables trophoblast invasion into maternal decidua, steroidogenesis, and glucose and amino acid transport into chorionic villous cytotrophoblast [7].

Thus, it is possible from a biological standpoint to speculate that the obstetric conditions that are associated with inadequate trophoblastic invasion in first trimester such as spontaneous abortion, low birth weight, Foetal Growth Restriction (FGR), and preeclampsia would be associated with low PAPP-A [5,9]. International studies have investigated the serum levels of PAPP-A in patients who have miscarriage but in literature [5-7,9], limited data is available regarding serum PAPP-A as a predictor of miscarriage in our population. Thus, current study was planned to

assess maternal serum levels of PAPP-A at early gestation to predict miscarriage in Indian population.

MATERIALS AND METHODS

This was an observational, cross-sectional study conducted in the Department of Obstetrics and Gynaecology in collaboration with the Department of Biochemistry from November 2016 to April 2018 at University College of Medical Science and Guru Teg Bahadur Hospital, Delhi, India. Institutional Ethical Committee approval was obtained and written informed consent was obtained. Pregnant women presenting to Outpatient Department (OPD) of Obstetrics and Gynaecology were recruited.

Sample size calculation: Considering median MOM PAPP-A level in miscarriage group as 0.23 and interquartile range was (0.12-0.48) and median MOM of 1.0 and interquartile range was (0.46-1.72) in normal group in the study of Tong S et al., to estimate this difference that was 0.77 in median MOM levels at type 1 error alpha=5% and power (P)=80% sample of 18 subjects in each group were required [5]. In our setup, overall incidence of miscarriage in clinically recognised pregnancy is around 15%. Approximately, 80% of miscarriage occurs before 12 weeks of gestation. In this study, authors enrolled pregnant women between 10-13 weeks of gestation. Thus, considering 5% of miscarriage rate at this gestation and 5% of loss of follow-up, so authors screened a sample of 500 asymptomatic subjects at 10-13 week period of gestation.

Inclusion criteria:

- Maternal age between 20 to 34 years.
- Normal Body Mass Index (BMI) of 18.5-24.9.
- Singleton pregnancy at 10-13 weeks period of gestation.
- Spontaneous conception.
- Ultrasound confirmed intrauterine pregnancy, internal os closed and normal cervical length.

Exclusion criteria:

- Pregnancy with anomalous foetus detected in first trimester or any time during follow-up.
- History of any medical disorders such as diabetes mellitus, hypertension, asthma, antiphospholipid antibody syndrome, thyroid dysfunction, liver and renal disease and any collagen vascular disease.
- Diagnosed uterine anomaly and cervical insufficiency or any uterine surgeries like myomectomy, septoplasty, adhesiolysis for Asherman's syndrome except previous caesarean section.
- Personal history of smoking or any other substance abuse.
- Women unwilling to participate.

Selection of group 1: Women who had spontaneous abortion (miscarriage) before 20 weeks of pregnancy and they satisfied the inclusion and exclusion criteria.

Selection of group 2: Normal healthy subjects who carried their pregnancy beyond 20 weeks period of gestation and satisfied the inclusion and exclusion criteria.

A total of 500 pregnant women were enrolled from antenatal OPD on the basis of inclusion criteria at 10-13 week of gestation. An informed written consent was taken from all the subjects after enrollment. Confirmation of gestational age was done on the basis of definite last menstrual period (with 3 previous cycles regular) and first trimester ultrasonography using Crown Rump Length (CRL).

All subjects enrolled were interviewed to collect information on age, address, occupation, religion, education, socio-economic status (Kuppuswamy socio-economic classification 2017) [ANNEXURE-1], substance abuse (excluded from study) in a well designed questionnaire.

A 2 mL of peripheral blood sample in plain vial was collected at the time of enrollment along with routine antenatal investigation, clotted sample was centrifuged at 3000 rpm for 15 minutes and separated serum was stored at -20°C in Department of Biochemistry for analysis of PAPP-A level in all subjects. Serum samples of all subjects was evaluated for PAPP-A levels by PAPP-A Enzyme Linked Immunosorbent Assay (ELISA) Kit (DRG International, Inc., USA) which was based on solid phase ELISA (sandwich ELISA).

STATISTICAL ANALYSIS

Microsoft Excel (version 2013) and statistical software Statistical Package for the Social Science (SPSS) for windows (version 20.0) was used for data presentation and statistical analysis. PAPP-A levels were calculated in MOM. Independent t-test and Chi-square test was applied to compare continuous data that is normally distributed like age, BMI and socio-demographic profile. Mann-Whitney U test was applied to compare data that was not normally distributed like PAPP-A MOM. Logistic regression was used to estimate risk of miscarriage.

RESULTS

Out of 500 participants, nine were lost to follow-up till 20 weeks. Out of 491 participant, 32 (6.5%) women had miscarriage forming group 1 of study population and rest 459 women continued their pregnancy beyond 20 weeks taken as group 2. Study population largely comprised of females between 20-24 years of age (n=300, 61.1%) followed by between 25-29 years of age (n=162, 32.99%) and rest between 30-34 years (n=29, 5.91%). Mean age of study population was 24.08±2.92 years.

The mean maternal age among participants of group 1 was (23.25±1.54 years), which was no different from those in group 2 who had ongoing pregnancies 24.14±2.98 years (p-value=0.092). Most of women in both groups were primigravida. A total of 46.9% cases in group 1 and 45.5% cases in group 2 were primigravida. Women in both groups were well matched with reference to religion, education, occupation and socio-economic status [Table/Fig-1].

Characteristic	Group 1 n=32 n (%)	Group 2 n=459 n (%)	p-value
Age (Mean)	23.25±1.54	24.14±2.98	0.092
BMI (kg/m²) (Mean)	21.40±1.39	21.59±1.71	0.546
Religion			
Hindu	25 (78.1)	355 (77.3)	0.918
Muslim	7 (21.9)	104 (22.7)	
Socio-economic status			
Upper	0	3 (0.7)	
Upper middle	8 (25)	72 (15.7)	
Lower middle	11 (34.4)	198 (43.1)	0.648
Upper lower	13 (40.6)	184 (40.1)	
Lower	0	2 (0.4)	
Education			
Illiterate	0	17 (3.7)	
Primary/Middle	5 (15.6)	151 (33)	
High	19 (59.4)	192 (41.8)	0.321
Intermediate	5 (15.6)	52 (11.3)	
Graduate/Profession	3 (9.4)	47 (10.2)	
Occupation			
Housewife	32 (100)	457 (99.6)	0.932
Working	0	2 (0.4)	
Parity			
Primigravida	15 (46.9)	209 (45.5)	
2nd gravida	11 (34.4)	174 (38)	0.746
3rd or more	6 (18.7)	76 (16.5)	

[Table/Fig-1]: Comparison of socio-demographic characteristics between group 1 and group 2.
*p-value <0.05 was considered significant, Chi-square test (95% CI) was used for religion, education, socio-economic status, occupation and parity. Independent t-test was used for mean of age and BMI

The median MOM of PAPP-A for abortion group was 0.116 (0.080-0.170) and for viable pregnancy beyond 20 weeks was 1.25 (0.665-3.249) with p-value <0.001 which was highly significant [Table/Fig-2].

Outcome group	Median (MOM)	p-value
Group 1 (n=32): Spontanoeus abortion	0.116 (0.080-0.170)	<0.001
Group 2 (n=459): Pregnancy beyond 20 week	1.25 (0.665-3.249)	

[Table/Fig-2]: Comparison of serum PAPP-A value between group 1 and group 2. *p-value <0.05 was considered significant, Mann-whitney test

The 10th percentile and 5th percentile of PAPP-A MOM value was 0.184 and 0.099, respectively. At PAPP-A MOM value of ≤10th percentile sensitivity and specificity of detection of miscarriage was 81.25% and 94.98%, respectively. Sensitivity and specificity for miscarriage at PAPP-A MOM value of ≤5th percentile was 40.62% and 97.82%, respectively. Lower the percentile cut-off of serum PAPP-A value higher was the specificity and positive predictive value for prediction of miscarriage [Table/Fig-3].

PAPP-AMOM in centiles	Sensitivity	Specificity	Positive Predictive Value (PPV)	Negative Predictive Value (NPV)
≤10th Percentile (0.184 MOM)	81.25%	94.98%	53.06%	98.64%
≤5th Percentile (0.099 MOM)	40.62%	97.82%	56.52%	95.9%

[Table/Fig-3]: Performance characteristic of PAPP-A for prediction of miscarriage.

By applying logistic regression authors found that if PAPP-A MOM decreases by 1 unit there was 1.2 times higher chances of spontaneous abortion. Total 63.2% of cases could be explained by this model (Nagelkerke R Square=0.632) [Table/Fig-4].

	Coefficient	p-value	R^2
MOM	-0.125	<0.001	0.632

[Table/Fig-4]: Risk estimation for miscarriage.
*p-value <0.05 was considered significant, logistic regression

For prediction of miscarriage, the area under ROC curve (95% CI) was 0.969 (0.955-0.983). By using ROC optimal PAPP-A MOM, cut-off for detection of miscarriage was 0.251 by using Youden's index. At this cut-off sensitivity 92.6% and specificity 96.9%. Positive predictive value 47.6% and negative predictive value 99.7% [Table/Fig-5].
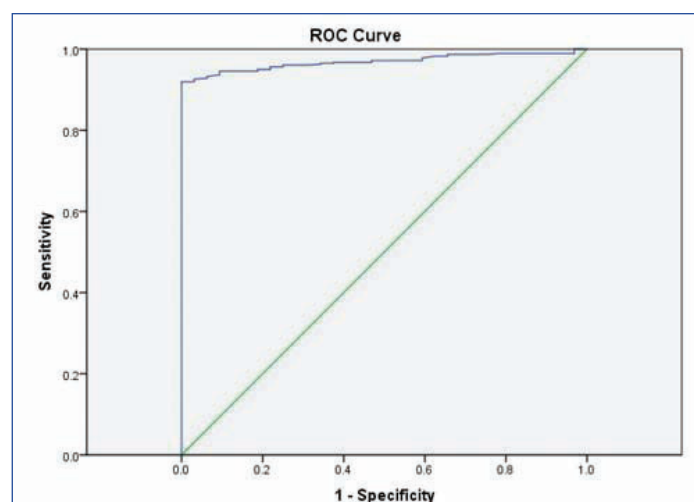
[Table/Fig-5]: ROC of PAPP-AMOM for prediction of miscarriage.
Area under curve 0.969 (0.955-0.983)(significant)

DISCUSSION

In present study, both groups of miscarriage and viable pregnancy beyond 20 weeks were comparable in terms of age, socio-demographic profile, BMI. The MOM of PAPP-A in abortion group was 0.116 (0.080-0.170) compared to viable pregnancy beyond 20 weeks was 1.25 (0.665-3.249) with p-value <0.001 which was significantly lower. Similar finding was observed by Tong S et al., Santolaya-Forgas J et al.,

Ong CY et al., and Spencer K et al., [Table/Fig-6] [5,6,10,11]. In current study, the performance of PAPP-A for the prediction of miscarriage at different percentile of PAPP-A MOM of study population was observed and it was observed that specificity of test for prediction of miscarriage increases with fall of PAPP-A value. Similar result observed by Dugoff L et al., in his FASTER trial [Table/Fig-6] [12].

Studies	Total subjects (n)	Abortion (n)	PAPP-A MOM	p-value	ROC curve	Sensitivity at 5th percentile	Specificity at 5th percentile
Ong CY et al., [10]	5584	54	0.755	0.033	NA	NA	NA
Spencer K et al., [11]	54722	531	0.893	<0.001	NA	14.8%	NA
Dugoff L et al., [12]	34217	294	NA	<0.001	NA	12.9%	94.9%
Tong S et al., [5]	782	21	0.23	<0.001	0.63 (0.50-0.77)	NA	NA
Present Study	491	32	0.116	<0.001	0.969 (0.955-0.983)	40.6%	97.8%

[Table/Fig-6]: Comparison of PAPP-AMOM value in miscarriage group with different study [5,10-12].

In present study, authors found that with decreasing PAPP-A MOM value, risk of abortion increases. By decreasing one unit of PAPP-A MOM risk of miscarriage increased by 1.2 times. This finding was supported by studies in literature by Barrett SL et al., Ong CY et al., Spencer K et al., Dugoff L et al., [9-12]. Barrett SL et al., found relative risk of miscarriage 4.7 at PAPP-A value <0.3 compared to >0.3. Ong CY et al., found 1% miscarriage rate, and 20.4% of miscarriages were associated with PAPP-A levels <10th centile. Spencer J et al., found that 5th centile odds ratio was increased by three fold in those with fetal loss before 24 weeks. Dugoff L et al., found odds ratio for miscarriage at <10th, <5th, <1st percentile were 1.95, 2.5 and 5.22, respectively. For prediction of pregnancies likely to miscarry, the area under ROC curve [Table/Fig-5] (95% CI) for PAPP-A was found to be 0.969 (0.955-0.983) in present study compared to study by Tong S et al., they found 0.63 (0.50-0.77) for PAPP-A [5]. We found specificity and sensitivity of PAPP-A in predicting miscarriages was 96.9% and 92.6%, respectively whereas negative predictive value of 99.7% as compared to study conducted by Tong S et al., wherein specificity was 90% and sensitivity was 44% [5].

As PAPP-A tests are routinely performed for screening of Down's syndrome in the first trimester. The same test done on a single visit, at the time of dating by sonography and screening for Down's syndrome can be of value to predict miscarriage. Determination of role of PAPP-A as first trimester marker for miscarriage will help in counselling and prognostication of patient.

Limitation(s)

The current study was limited by relatively small number of study population (n=491) limited to one hospital. Analysis of PAPP-A level in large population was not possible in current settings because of time and financial constraints. Despite this significantly low serum PAPP-A levels were found in miscarriage group compared to viable pregnancy.

CONCLUSION(S)

The PAPP-A holds promise as an early marker of placental dysfunction. Our study contributes further information in this regard for establishing reference ranges, cut off values, positive predictive value and specificity as an ideal predictive marker for miscarriage, especially in Indian scenario.

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ANNEXURE 1

Kuppuswamy Socio-economic Classification for 2017

Characteristics	Variables	Scores
Education of head of family	Profession or honours	7
	Graduate or postgraduate	6
	Intermediate or post high school diploma	5
	High school certificate	4
	Middle school certificate	3
	Primary school certificate	2
	Literate	1
Occupation of head of family	Profession	10
	Semi-profession	6
	Clerical, Shop-owner	5
	Skilled worker	4
	Semi-skilled worker	3
	Unskilled worker	2
	Unemployed	1
Monthly income of family	>41430	12
	20715-41429	10
	15536-20714	6
	10357-15535	4
	6214-10356	3
	2092-6213	2
	<2091	1
Socio-economic class		Total score
I	Upper	26-29
II	Upper middle	16-25
III	Lower middle	11-15
IV	Upper lower	5-10
V	Lower	<5