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Original Research Article

Correlation of Proteinase Inhibitory Activities at 11-14 Weeks with Second Trimester Fetal Growth Parameters and Birth Weight

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Abstract

Background: Proteinase inhibitors (Antitrypsin and Anti chymotrypsin) have been identified in trophoblasts and play an important role in placental implantation by modulating maternal immune response towards fetus. Studies have shown the association of antitrypsin and antichymotrypsin with preeclampsia and fetal growth restriction, hence we attempted to study the correlation of maternal serum markers with fetal growth restriction.

Objective: To find correlation between maternal serum proteinase inhibitory activities at 11-14 weeks with second trimester fetal growth parameters and birthweight.

Materials and Methods: Prospective observational study, from 2014 to 2016 done in a tertiary care hospital. 198 antenatal women were recruited at 11-14 weeks out of which 7 were excluded from the study, 191 women analysed. Maternal serum was collected at 11-14 weeks and tested for antitrypsin and antichymotrypsin levels, ultrasound growth parameters were measured at 18-20 weeks; birth weights of newborns of the patients delivered during the study period (134) were noted and analysed.

Results: The median values of antitrypsin and antichymotrypsin calculated in our study population were 71.2U/ml and 91.6U/ml respectively. No association was found between antitrypsin and biparietal diameter, head circumference, femur length and estimated fetal weight at 18-20 weeks. A statistically significant association was noted between antitrypsin levels and abdominal circumference. No association was noted between antichymotrypsin levels and any of the growth parameters. No association was noted between antitrypsin levels and birth weight. Though there was an apparent association between antichymotrypsin and birthweight, statistical significance (p- 0.059) was not proven.

Conclusion: Maternal serum antitrypsin and antichymotrypsin may have role in predicting early fetal growth restriction.

Keywords: Fetal growth restriction, Antitrypsin, Anti chymotrypsin, Small for Gestational age.

Introduction

Fetal growth restriction (FGR) is an important determinant and one of the leading causes of perinatal morbidity and mortality. The overall incidence of FGR is 3-7% in total population1. Fetal growth

restriction due to uteroplacental factors have better prognosis when diagnosed early and intervened appropriately. Timely diagnosis and management is one of the major interventions with which perinatal mortality could be reduced. Identifying fetal growth restriction is a real challenge. There are a large number of reports that addressed the association of gross and histopathologic findings in placentae with preeclampsia and fetal growth restriction. Efforts are on to identify markers to predict FGR. Although fetal growth restriction manifests in second half of pregnancy, the mechanisms that initiate FGR are present as early as first trimester of pregnancy. For screening of FGR, there are no accurate predictive tests. Useful screening tools are history, physical examination, use of customized growth curves, biochemical screening and ultrasound examination. Various studies have proven the association of serum markers (beta hCG, PAPP-A i.e. dual test) for prediction of FGR. Proteinase inhibitors have been identified in trophoblasts and they play an important role in placental implantation by modulating maternal immune response towards fetus. Studies have shown the association of antitrypsin and antichymotrypsin with pre-eclampsia and FGR.

Alpha-1 Antitrypsin Deficiency (A1AD) is a hereditary condition characterized by low levels of circulating alpha-1 antitrypsin (AAT) in plasma. It is the best understood genetic risk factor for the development of chronic obstructive pulmonary disease (COPD) [1,2]. It is often under-recognized as there is significant heterogeneity in disease presentation in relation to the severity of symptoms and prognosis. It is not uncommon for young individuals and women of child bearing potential to already have moderate to advanced lung disease at the time of initial diagnosis [3]. We attempted to study the correlation of maternal serum antitrypsin and antichymotrypsin at 11-14 weeks and fetal growth thus validating their use in early prediction of growth restriction.

Materials and Methods

Objective: To find correlation of maternal serum proteinase inhibitors activities at 11-14 weeks with second trimester growth parameters and birth weight

Study Design: This is a prospective observational study done from October 2014 to August 2016. Permission for the study was obtained from Institutional ethical committee (IEC 467/2014)

Inclusion criteria: All singleton pregnancies with excellent dating who had nuchal translucency scan and dual test at 11-14 weeks at our hospital and were willing for follow up for second trimester scan and planning to deliver at our hospital were recruited after obtaining informed consent. Women who had spontaneous abortions, multiple pregnancy, diagnosed fetal anomalies, wrong dates and women who were lost for follow up were excluded from the study.

11-14 weeks scans were done using Voluson P8 machine, when crown rump length was noted along with nuchal translucency and gross anatomical survey. Blood samples were collected for dual test, antitrypsin and antichymotrypsin levels in the same sitting. At 18-20 weeks, during ultrasound for detailed anatomical survey, all growth parameters i.e. biparietal diameter (BPD), head circumference (HC), femur length (FL) and estimated fetal weight (EFW) were measured and plotted on a growth chart as percentiles. All parameters which were less than or equal to 25th centile were considered low growth profile and more than 25th centile as normal growth. Patients were followed up till delivery and birth weights of the babies were noted in those who delivered at our hospital. According to the birth weight for the gestational age of delivery, they were

divided into Small for Gestational age and non-small for gestational age.

Median value was calculated for antitrypsin and antichymotrypsin from the patients who had normal birth weight babies which was considered as standard reference level in our study.

The analysis was carried out using Scientific Package for Social Sciences (SPSS version 16).

For statistical analysis, Chi square test was used. Statistical significance was accepted at $p < 0.05$

Results

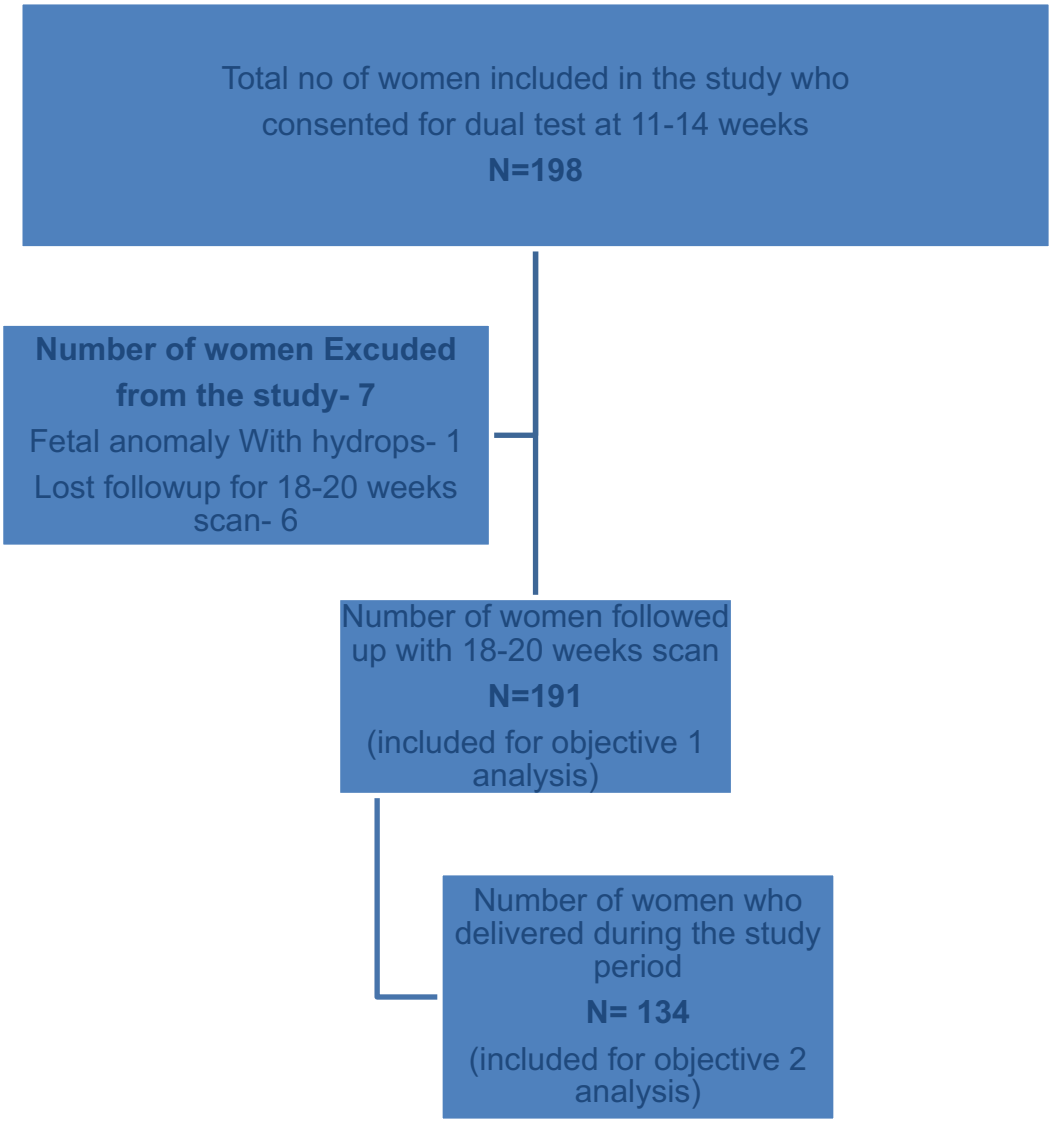

Figure 1: Consort statement

A total of 198 cases booked at 11-14 weeks who had ultrasound and dual test were included in the study. Out of them, 7 were excluded from the study in view of diagnosed fetal hydrops(1) and 6 women were lost to followup. Remaining 191 had second trimester scan at 18-20 weeks; 134 delivered in the study period where birth weights were correlated with the biochemical factors.

Table 1: Dempgraphic details in present study

Characteristic		Total number N=191
Age (in years)		28.7 ± 4.09
Parity	Primi n (%)	134 (70.15)
	Multi n (%)	57 (29.85)
BMI (in kg/m^2)		23.5 ± 2.3
Bad Obstetric history n (%)		12 (6.28)

Among 191 women finally included in the study, 134 were primigravidae and 57 were multigravidae. The mean maternal age was 28.7 ± 4.09 years and mean BMI was 23.5 ± 2.3 kg/m^2. Bad obstetric history was noted in 12 women (6.28%). All these patients had routine anomaly scan at 18-20 weeks during which growth parameters were noted. The pregnancies were followed up till delivery (134 women delivered in our study period).

Table 2: Complications in pregnancy

Complication	Total number N=191 (%)
Gestational Hypertension	8 (4.2)
Chronic Hypertension	3 (1.6)
Pre-eclampsia	2 (1.04)
Eclampsia	1 (0.52)
GDM	6 (3.14)
Overt diabetes	1 (0.52)
SLE	1 (0.52)
Chronic kidney disease	1 (0.52)
Cardiac disease	2 (1.04)
Uncomplicated	167 (88.48)

Preexisting medical disorders seen in 8/191 (4.18%). Out of them 3 had chronic hypertension, 2 had cardiac disease and one each had overt diabetes and gestational hypertension, SLE and chronic kidney disease. Seventeen patients developed complications later in pregnancy. Two women with preeclampsia and one with eclampsia had FGR and SGA babies.

Table 3: Association of β hCG with USG findings

	β hCG ≤ 0.5MoM (N=36) N (%)	β hCG>0.5MoM (N=155), N (%)	p VALUE
Association of β hCG with Estimated fetal weight, Biparietal diameter and Head circumference			
Estimated fetal weight			
≤ 25th	5 (13.88)	23 (14.83)	0.88
> 25th	31 (86.12)	132 (85.17)	
Biparietal Diameter			
≤ 25th	4 (11.1)	11 (7.09)	0.419
> 25th	32 (88.9)	144 (92.91)	
Head circumference			
≤ 25th	8 (22.2)	30 (19.3)	0.765
> 25th	28(77.8)	125(80.6)	
Association of β hCG with Abdominal circumference, Femur length			
≤ 25th	9(25)	17(10.9)	0.06*
> 25th	27(75)	138(89.03)	

≤ 25th	6 (16.6)	18 (11.6)	0.408
> 25th	30(83.4)	137 (88.4)	
Association of PAPP-A with Estimated fetal weight, Biparietal Diameter and Head circumference			
≤ 25th	5(53.8)	23 (11.7)	0.0029*
> 25th	6(46.1)	157 (88.2)	
Biparietal diameter			
≤ 25th	1(9.09)	14(7.7)	0.875
> 25th	10(90.91)	166(92.3)	
Head circumference			
≤ 25th	5(45.4)	33(18.33)	0.028*
> 25th	6(54.6)	147(91.67)	

P <0.05 considered significant, Chi square test

There was significant association found between PAPP-A and Head circumference; higher PAPP-A values were associated with better growth.

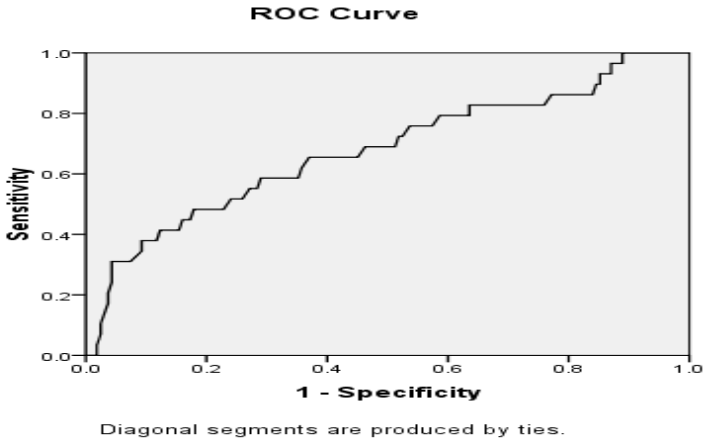

Figure 2: Association between PAPP-A and Estimated fetal weight.

Using ROC analysis, the cut off value of PAPP-A was determined as ≤ 0.99 to detect estimated fetal weight ≤ 25th percentile with a sensitivity of 67.5% and specificity of 63%.

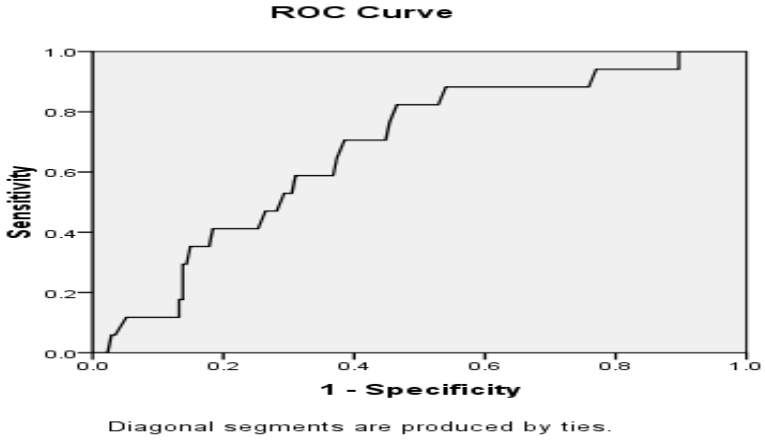

Figure 3: Association between PAPP-A and Biparietal diameter

Using ROC analysis, a cut off value for PAPP-A to detect low growth profile from BPD was ≤ 0.95MoM with a sensitivity of 64.5% and specificity of 63%.

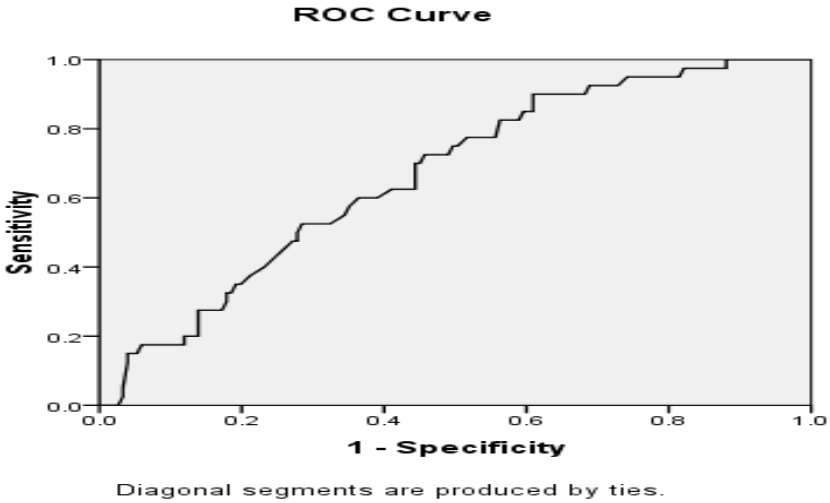

Figure 4: Association between PAPP-A and Head circumference

From the above ROC curve, the cut off value of PAPP-A to detect patients with low head circumference was ≤ 1.03 with sensitivity of 60% and specificity of 61%.

Table 4: Association of PAPP-A with Abdominal circumference,Femur length

	PAPP-A ≤ 0.5MoM (N=11) N (%)	PAPP-A >0.5MoM (N=180) N (%)	p VALUE
Abdominal Circumference (in percentile)			
≤ 25th	6(54.54)	20(11.1)	0.000045
> 25th	5(46.46)	160(88.9)	
Femur length(in percentile)			
≤ 25th	5(45.45)	19(10.55)	0.006
> 25th	6(54.55)	161(89.45)	

P <0.05 considered significant, Chi square test

Though there was no clear association found between low PAPP-A and AC, FL ≤ 25th centile, PAPP-A >0.5 MoM was significantly associated with normal growth.

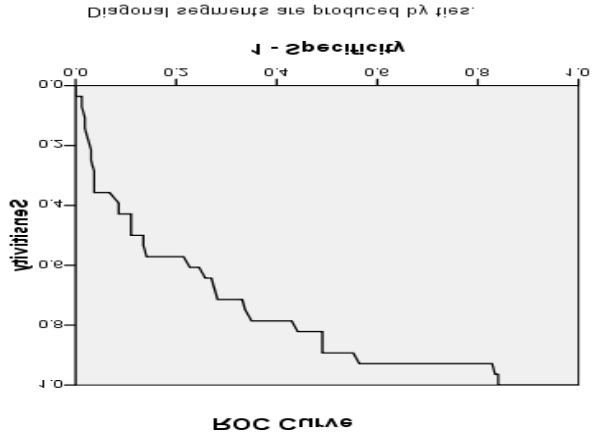

Figure 5: Association of PAPP-A and Abdominal circumference

From the above ROC analysis, the cut off value of PAPP-A was ≤ 0.92 with a sensitivity of 71.4% and specificity of 71.6% to predict abdominal circumference ≤ 25th centile.

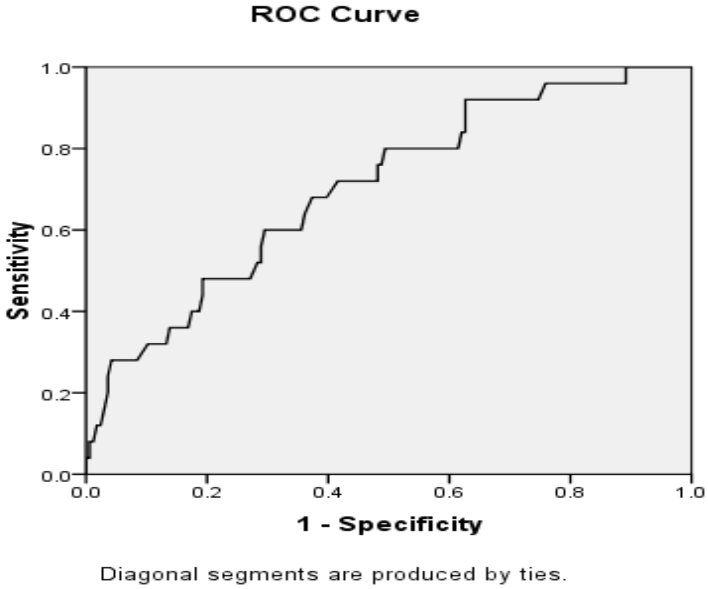

Figure 6. Association between PAPP-A and Femur length

Using ROC analysis for predicting femur length ≤ 25th centile. The cutoff value for PAPP-A was ≤ 1.03 with a sensitivity of 68% and specificity of 61%.

Association of Proteinase inhibitors with second trimester growth

Antitrypsin and Antichymotrypsin are the proteinase inhibitors included in our study. As there were no standard values of proteinase inhibitors for normal growth, we have calculated the standard value for our study by calculating median of the values of women who had normal birth weight babies. Antitrypsin and Antichymotrypsin values were measured at 11-14 weeks in the sample along with β hCG and PAPP-A and values were given in Units/milliliter. Values more than median value were taken as higher values of Antitrypsin and Antichymotrypsin and less than median value as lower values. These were compared with second trimester growth parameters. The median values derived for Antitrypsin was 71.2U/ml and Anti chymotrypsin was 91.6 U/ml.

Table 5: Association of Anti trypsin with Estimated fetal weight, Biparietal diameter and Head circumference

	Antitrypsin ≤ 71.2U/ml (N=112) N(%)	**Antitrypsin >71.2 U/ml (N=79) N(%)**	**p value**
Association of Anti trypsin with Estimated fetal weight, Biparietal diameter and Head circumference			
Estimated fetal weight			
≤ 25th	13 (11.6)	15(18.98)	0.155
>25th	99(88.4)	64(81.02)	
Biparietal diameter			
≤ 25th	8(7.14)	7(8.9)	0.309
>25th	104(92.86)	72(91.1)	
Head circumference			

≤ 25th	19(16.96)	19(24.05)	0.107
>25th	93(83.04)	60(75.95)	
Association between Antitrypsin and Abdominal circumference and femur length			
Abdominal circumference			
≤ 25th	10(8.9)	16(20.2)	0.024*
>25th	102(91.1)	63(79.8)	
Femur length			
≤ 25th	13(11.6)	11(13.92)	0.634
>25th	99(88.4)	68(86.08)	
Association of Antichymotrypsin with Estimated fetal weight, Biparietal diameter and Head circumference			
Estimated fetal weight			
≤ 25th	18(17.1)	10(11.6)	0.28
>25th	87(82.9)	76(88.4)	
Biparietal diameter			
≤ 25th	10(9.5)	5(5.81)	0.34
> 25th	95(90.5)	81(94.19)	
HeadCircumference			
≤25th	22(20.9)	16(18.6)	0.68
>25th	83(79.1)	70(81.4)	
Association of Antichymotrypsin with Abdominal circumference, Femur length			
Abdominal circumference			
≤ 25th	17(16.19)	9(10.46)	0.25
>25th	88(83.81)	77(89.54)	
Femur length			
≤ 25th	16(15.23)	8(9.3)	0.218
>25th	89(84.77)	78(90.7)	

P <0.05 considered significant, Chi square test

A statistically significant association was noted between Antitrypsin and abdominal circumference which indicates that higher levels (20.2%) are associated with low growth profile with abdominal circumference ≤ 25thcentle when compared to lower levels (8.9%).

Second trimester Ultrasound and Birth weight

Out of 191 women included in the study, 134 women delivered in the study. Out of 134 babies, 17 were small for gestational age and 117 were not small for gestational age. Small for gestational age (SGA) babies were defined as birth weight<10th percentile for the corresponding gestational age.

Table 6: Association of second trimester EFW and birth weight

Birth weight	EFW ≤ 10thcentile N= 7(%)	EFW >10th centile N=127(%)	p value
SGA	4(24)	15(8.9)	0.0008
Non SGA	3(76)	112(91.1)	

P <0.05 considered significant, Chi square test

Above table shows association of second trimester ultrasound and Birth weight. 24% of women who had EFW ≤ 10th centile had SGA babies and 8.9% of women with EFW>10th centile had SGA babies. The association was found to be statistically significant. Small for gestational age was defined as birth weight < 10th percentile for the corresponding gestational age at delive.

Table-7: Association between β hCG, PAPP-A levels and SGA

βhCG (in MoM)	SGA (N=19) N(%)	Non SGA (N=115) N(%)	P value
≤ 0.5	5(26.31)	20(17.39)	0.35
> 0.5	14(73.69)	95(82.61)	
PAPP-A (in MoM)			
≤ 0.5	1(5.2)	3(2.6)	0.52
> 0.5	18(94.8)	112(97.4)	

P <0.05 considered significant, Chi square test

Above table shows no association between β hCG, PAPP-A levels and SGA. Though women with β hCG levels ≤ 0.5MoM were more distributed in SGA group, there was no significant correlation found.

Discussion

Antiproteinase activity expressed as antitryptic and antichymotryptic activities is higher in the serum of pregnant women compared to non-pregnant women. Implantation involves a delicate balance between proteinases and their inhibitors.[4,5] Miao et al reported that low anti trypsin levels were associated with increased oxidative stress and associated with FGR. [6]Similarly a study by Karowicz BA et al also showed that low levels of antitrypsin was associated fetal growth restriction due to oxidative stress which improved on treating with arginine, by increasing antitrypsin levels.[7]

The potential implications of reduced AAT levels in pregnancy are several-fold. Pre-eclampsia is the most studied obstetric complication that has been associated with reductions in AAT serum levels and inhibitory capacity [8,9,10,11]. In addition, reductions in serum levels and inhibitory capacity have also been associated with recurrent and sporadic pregnancy loss where the reduced AAT level and activity was also accompanied by elevated circulating pro-inflammatory cytokines [12]. Further small case reports have also. activity for at least half an hour per day identified severe AAT reductions in preterm premature rupture of membranes[13] These findings are of particular relevance as the majority of studies showed that AAT levels while reduced, remained above the putative pulmonary protective threshold of 80 mg/dL (11 μMol/L) [2,14].

Given the findings that higher circulating levels of AAT are seen in pregnancy and its function in mitigating inflammation and untoward immune activation [8], individuals with A1AD with preexisting chronic lung disease may be at increased risk for loss of control or clinical worsening of their lung disease and/or obstetric complications secondary to either relative or absolute deficiencies. Secondly, individuals with even with minor reductions in levels or alterations in the AAT protein, while not at increased risk for lung disease, may be at increased risk for potential obstetric complications, particularly with continued smoking exposures, as it is known to inactivate the protein [15]. In contrast, our study showed

higher antitrypsin levels were more associated with AC <25th centile (20.2%), when compared to lower levels (8.9%). Similarly higher antichymotrypsin was associated with SGA though couldn't be proven statistically significant as the number of patients was low.

Though we did not find direct association between antitrypsin and SGA, there was association between antitrypsin levels and second trimester AC and further second trimester AC along with other growth parameters had correlated with birth weight. Considering the reports of the studies, it appears that extremes of proteinase inhibitory activities may result in placental insufficiency and thus fetal growth restriction.[16] Alpha 2 macroglobulin was also found to be associated with pre-eclampsia and adverse outcomes in some studies. Hence future studies are needed with a larger sample size to prove the association of these serum markers with adverse fetal outcomes. These may help in the early diagnosis of fetal growth restriction providing scope to intervene early and thus improving the outcome.

Conclusion

Maternal PAPP-A levels were useful for prediction of early fetal growth restriction which can direct fetal growth monitoring and timely intervention. β hCG, antitrypsin and antichymotrypsin were not found to be useful in predicting early fetal growth restriction.

Limitations: Bigger sample size would have given more definitive results where we obtained clinically significant observations that were not proven statistically. No standard values were available for antitrypsin and antichymotypsin which were included as novel markers to predict early fetal growth restriction in this study. There was no adequate data available for these maternal serum markers with respect to correlation with fetal growth restriction.

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