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PROTEIN C AND PROTEIN S ABNORMALITIES IN PATIENTS WITH PLACENTAL ABRUPTION AND ITS ASSOCIATION WITH ADVERSE FETO-MATERNAL OUTCOME

Dr. Shivangi[1], Dr. Harsha S Gaikwad[2]

VMMC And Safdarjung Hospital, New Delhi, India

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Corresponding author: Dr. Shivangi
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Abstract

Placental abruption is an important cause of maternal and fetal morbidity and mortality. Inherited thrombophilia, which includes Protein C and Protein S deficiency, is an important risk factor for abruption.

Objective: To find the prevalence of Protein C and Protein S abnormalities in patients with placental abruption and its association with adverse feto-maternal outcome.

Methods: A prospective observational study conducted in a tertiary hospital India from 2018 to 2020 for a period of 18 months. All pregnant women with abruption (n=254) were recruited. Peripheral blood samples of patients were collected in 3.2% sodium citrate vacutainers. All these coagulation investigations were performed on compact ceveron Alpha Automated Coagulation Analyzer(Compact bio-sciences Ltd),based on clotting, chromogenic and turbidimetric analysis. Adverse feto-maternal outcome was assessed by finding out the proportion of Prematurity, small for gestation age(SGA), Neonatal intensive care (NICU) admissions, Intra Uterine Death (IUD), number of maternal death, vaginal deliveries, caesarean section, blood transfusion

Result: 222 out of 254 cases had protein C in the normal range i.e 67-135 (87.40%). 1 patient had (0.39%) had protein C deficiency and rest 12.20% had protein C level greater than normal range. Out of 254 subjects no case had protein S deficiency. About 43.70% had normal protein S value(33-101).56.30% subjects had value greater than normal range .Range was between 53-148 and mean value was 103 ± 16.51.

Adverse fetal outcome was assessed in the patients having abnormal protein C and protein S values, here, mainly those having values greater than the normal range.

In our study 1.40% had NND, 6.99% had IUD, 8.39% had perinatal mortality, 39.16% had blood transfusion,26.57 % had LSCS, 40.56% had SGA, 29.32% NICU admission in subjects with abnormal protein S value. For those with normal protein S value 4.50% had NND, 6.31% had IUD, 10.81% had perinatal mortality, 39.64% had blood transfusion, 39.64 % had LSCS, 40.56% had SGA , 29.81% had NICU admission.

3.13% of those with abnormal protein C had IUD, 6.25% had perinatal mortality,3.13% had NND,34.38% had blood transfusion,43.75% had SGA babies,22.58% had NICU admission,25 % had LSCS. For those with normal values, 2.70% had NND, 7.21% had IUD, 9.91% had perinatal mortality, 40.09 % had blood transfusion, 40.54% had SGA, 30.58% had NICU admission,33.33 % had LSCS.

Conclusion: Protein C and Protein S deficiency was not found in our patients with abruption rather the values were greater than the normal range. No significant adverse feto-maternal outcome was found in patients with values of Protein C and Protein S greater than normal.

Keywords: NICU, IUD, LSCS, SGA

Introduction

Placental abruption refers to separation of placenta partially or totally from its implantation site before delivery. It can be either revealed, with bleeding typically insinuating between membranes and uterus ultimately escaping through the cervix to cause external haemorrhage, or it can be concealed, with blood retained between detached placenta and uterus .[1]

Placental abruption is initiated by haemorrhage into the decidualis. The decidua then splits, leaving a thin layer adhered to myometrium. placental abruption occurs when the maternal vessels tear away from the placenta and bleeding occurs between the uterine lining and maternal side of the placenta.[2,3] Rarely bleeding originates from fetal placental level. The blood which keeps on collecting splits the decidua, separating the

deciduous from its placental attachment. Sometimes complete or near complete separation of the placenta occurs at the centre due to bleeding from an artery. It leads to development of potential life-threatening conditions like DIC and FHR abnormalities. Low pressure venous bleeding occurs at the periphery and its effects takes time to occur like intermittent bleeding, oligohydramnios, growth restriction.

Placental abruption is a significant cause of maternal morbidity and perinatal mortality. Maternal adverse outcomes include haemorrhage, need for blood transfusions, emergency hysterectomy, disseminated intravascular coagulopathy, renal failure and maternal death. Fetal adverse outcomes include IUGR ,low birthweight, preterm delivery, asphyxia, stillbirth and perinatal death.[4-7]

Among various causes of abruption, coagulation disorders like thrombophilia inherited or acquired leading to venous thromboembolism and bleeding. Placenta of thrombophilic women are characterised by increased rate of vascular damage, placental infarcts and fibrinoid necrosis.

Inherited thrombophilia has been linked to an array of adverse pregnancy

outcomes, including pre-eclampsia (severe form), foetal growth restriction, stillbirth, and placental abruption. The varied defects being factor V leidin mutation, protein c deficiency, protein S deficiency, antithrombin deficiency, increased prothrombin levels, hyperhomocysteinemia (inhibits activation of protein c). protein s and activated protein c in combination are necessary for the activation of factors V and factor VIII, therefore a deficiency in either of these may result in a hypercoagulable state. Antithrombin is a small protein that inactivates both factor Xa and thrombin and serves as a regulator of clot formation. Deficiency in this protein result in severe coagulopathy and these women do have an increased risk of embryonic demise and fetal death.[8-11]

in our study normal values for protein C and protein S was as follow in three trimesters.[12-15]

Serial No.	name	First trimester	Second trimester	Third trimester
1	Protein C, functional (%))	78-121	83-133	67-135
2	Protein S total (%)	39-105	27-101	33-101

Methodology

This study was prospective observational study, conducted in the department of obstetrics and gynaecology department of a tertiary care hospital in collaboration with Department of haematology and Department of Community medicine for a period of 18 months.

Study Population

All pregnant women diagnosed as abruptio placentae within the study period were eligible to participate.

Inclusion Criteria

All women in whom-:

- There was confirmed sonographic diagnosis of placental abruption>28 weeks before or during delivery.
- All women in whom after delivery, the delivered placenta showed retroplacental bleeding or retroplacental haematoma/ clots >5cm on the placental surface or disc.

Exclusion Criteria

Women with-:

- Diagnosed placenta previa

- Premature rupture of membranes
- Multiple pregnancy
- History of thromboembolism
- Uterine leiomyomas
- Genital tract lesions
- Medical co-morbid condition
- On any anti coagulants

Consent- informed and written consent was obtained from all participants in a language which they understood.

Sample Size

The Normal Anticoagulant System and Risk of Placental Abruption: Protein C, Protein S and Resistance to Activated Protein C was observed by Cande V. Ananth, et al.(14)

The study observed mean (± standard deviation) protein C (114.2 ± 25.6 vs 121.4 ± 27.6; P=0.009) and protein S (39.9 ± 18.4 vs 35.7 ± 15.2; P=0.043) were different between cases and controls. Taking these values as reference, the minimum required sample size with 80% power of study and 5% level of significance is 254 patients in each study group. So total sample size taken is 254

Formula used is :-

For comparing mean of two groups

$N>=2(\underline{\text{standard deviation}})^2 *(Z_\alpha + Z_\beta)^2$

$(\text{mean difference})^2$

Where Z_α is value of Z at two sided alpha error of 5% and Z_β is value of Z at power of 80% and mean difference is difference in mean values of two groups.

Calculations:-

1) Protein C

Pooled standard deviation=Square root((25.6*25.6+27.6*27.6)/2)=26.62

$n>=(2*26.62*26.62*(1.96+.84)^2)/(121.4-114.2)^2=214.34=215$(approx.)

2) Protein S

Pooled standard deviation=Square root((18.4*18.4+15.2*15.2)/2)=16.88

$n>=(2*16.88*16.88*(1.96+.84)^2)/(39.9-35.7)^2=253.27=254$(approx.) Eligible pregnant women were recruited from labour room and wards. Informed consent was taken, thorough clinical examination was done, history was taken, prior investigations like ultrasound were studied, all patients were investigated to rule out coagulation defect.

Peripheral blood samples of patients were collected in 3.2% sodium citrate vacutainers. All these coagulation investigations were performed on compact ceveron Alpha Automated Coagulation Analyzer (Compact bio-sciences Ltd), based on clotting, chromogenic and turbidimetric analysis.

Maternal outcome was assessed in terms of

1. Mode of delivery
2. Haemodynamic instability/ shock
3. Number of blood transfusion
4. Number of fresh frozen plasma (FFP) transfused
5. Number of women who went into disseminated intravascular coagulation (DIC)
6. Maternal deaths

Foetal outcome was assessed as-:

1. Prematurity
2. Fetal Growth Restriction (FGR)
3. Neonatal intensive care (NICU) admissions
4. Intra Uterine Death (IUD)

Investigation

- Blood group, Rh typing, complete blood count
- Liver function test, renal function test, blood sugar, thyroid function test
- Prothrombin time (PT)

- Activated partial thromboplastin time (APTT)
- Internalised Normalised Ratio (INR)
- Thrombin time (TT)
- Protein C
- Protein S

Outcome Measures

PRIMARY OUTCOME OF THE STUDY- Proportion of women with abruptio placentae having abnormal Protein C and and Protein S and adverse feto maternal outcome in those patients.

Result

Table 1: Distribution of protein C functional(%) of study subjects.

Protein C functional (%)	**Frequency**	**Percentage**
<67	1	0.39%
67-135	222	87.40%
>135	31	12.20%
Protein C functional(%)(Abnormal/normal)		
Abnormal	32	12.60%
Normal	222	87.40%
Mean ± Stdev	110.52 ± 19.94	
Median(IQR)	106.5(97-128)	
Range	10-154	

Most of the study subjects had protein C in the normal range i.e 67-135 (87.40%). 0.39% had protein C deficiency and 12.20% had protein C level greater than normal range.

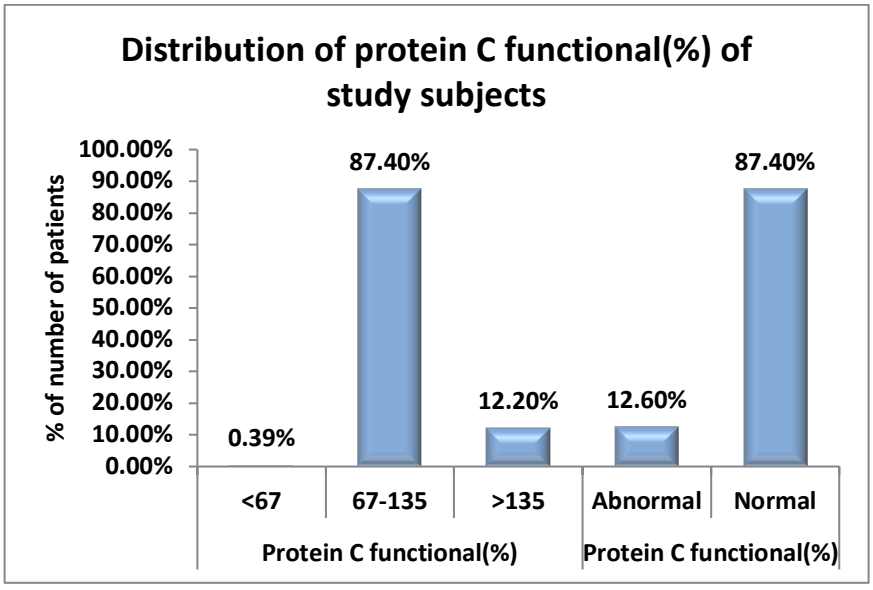

Figure 1: Bar diagram showing distribution of protein C functional (%) of study subjects.

222 out of 254 cases had protein C in the normal range i.e 67-135 (87.40%). 1 patient had (0.39%) had protein C deficiency and rest 12.20% had protein C level greater than normal range.

Table 2: Distribution of protein S total (%) of study subjects.

Protein S total (%)	Frequency	Percentage
33-101	111	43.70%
>101	143	56.30%
Protein S total(%) (Abnormal/normal)		
Abnormal	143	56.30%
Normal	111	43.70%
Mean ± Stdev	103 ± 16.51	
Median (IQR)	104(92-112.75)	
Range	53-148	

Out of 254 subjects no case had protein S deficiency. About 43.70% had normal protein S value (33-101).56.30% subjects had value greater than normal range . Range was between 53-148 and mean value was 103 ± 16.51.

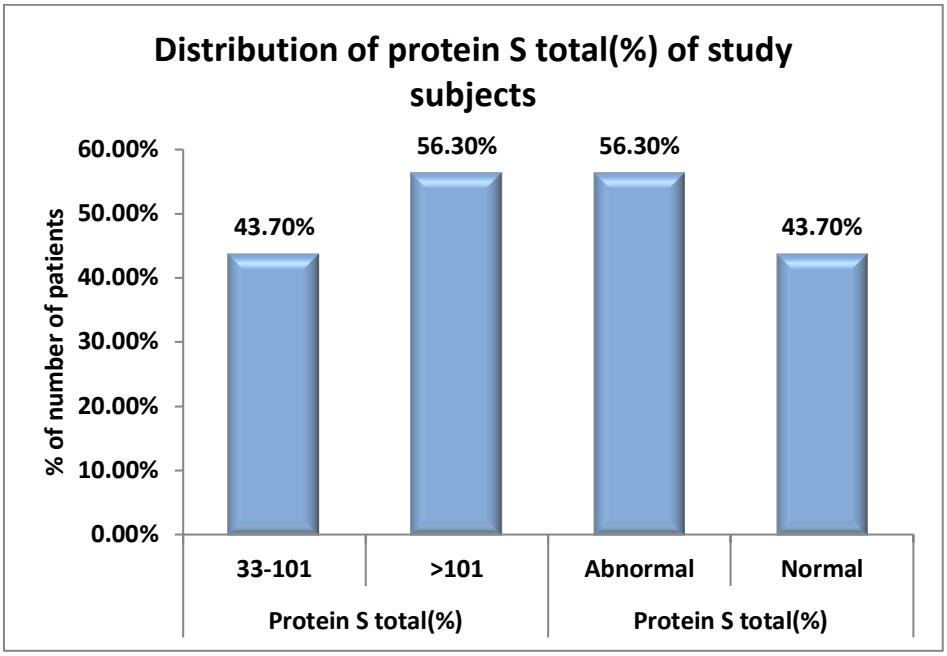

Figure 2: Bar chart showing distribution of protein S total(%) of study subjects.

Out of 254 subjects no case had protein S deficiency. About 43.70% had normal protein S value (33-101).56.30% subjects had value greater than normal range .Range was between 53-148 and mean value was 103 ± 16.51.

Adverse fetal outcome was assessed in the patients having abnormal protein C and protein S values, here, mainly those having values greater than the normal range.

Table 3: Association of feto-maternal outcome with protein S.

Feto-maternal outcome	Abnormal (n=143)	Normal (n=111)	Total	P value	Test performed
Neonatal death	2 (1.40%)	5 (4.50%)	7 (2.76%)	0.245	Fisher Exact test
Intrauterine death	10 (6.99%)	7 (6.31%)	17 (6.69%)	0.828	Chi square test,0.047
Perinatal mortality	12 (8.39%)	12 (10.81%)	24 (9.45%)	0.513	Chi square test,0.427
Blood transfusion	56 (39.16%)	44 (39.64%)	100 (39.37%)	0.938	Chi square test,0.006
Bleeding per vaginum	62 (43.36%)	55 (49.55%)	117 (46.06%)	0.513	Fisher Exact test
Bleeding per vaginum + blood stained liquor	17 (11.89%)	12 (10.81%)	29 (11.42%)		
Bleeding per vaginum + meconium + blood stained liquor	0 (0%)	1 (0.90%)	1 (0.39%)		
Bleeding per vaginum + retroplacental clots	21 (14.69%)	18 (16.22%)	39 (15.35%)		
Blood stained liquor	38 (26.57%)	20 (18.02%)	58 (22.83%)		
Meconium + blood stained liquor	1 (0.70%)	0 (0%)	1 (0.39%)		
Retroplacental clots	4 (2.80%)	5 (4.50%)	9 (3.54%)		
SGA	58 (40.56%)	46 (41.44%)	104 (40.94%)	0.887	Chi square test,0.02
NICU admission	39 (29.32%)	31 (29.81%)	70 (29.54%)	0.935	Chi square test,0.007
LSCS	38 (26.57%)	44 (39.64%)	82 (32.28%)	0.027	Chi square test,4.88
Normal vaginal delivery	105 (73.43%)	67 (60.36%)	172 (67.72%)		

Figure 3:- Bar chart showing association of feto-maternal outcome with Protein S total(%).

In our study 1.40% had NND, 6.99% had IUD, 8.39% had perinatal mortality, 39.16% had blood transfusion,26.57 % had LSCS, 40.56% had SGA, 29.32% NICU admission in subjects with abnormal protein S value. For those with normal protein S value 4.50% had NND, 6.31% had IUD, 10.81% had perinatal mortality, 39.64% had blood transfusion, 39.64 % had LSCS, 40.56% had SGA, 29.81% had NICU admission.

Table 4: Association of feto-maternal outcome with protein C.

Feto-maternal outcome	Abnormal (n=32)	Normal (n=222)	Total	P value	Test performed
Neonatal death	1 (3.13%)	6 (2.70%)	7 (2.76%)	1	Fisher Exact test
Intrauterine death	1 (3.13%)	16 (7.21%)	17 (6.69%)	0.704	Fisher Exact test
Perinatal mortality	2 (6.25%)	22 (9.91%)	24 (9.45%)	0.749	Fisher Exact test
Blood transfusion	11 (34.38%)	89 (40.09%)	100 (39.37%)	0.536	Chi square test,0.383
Bleeding per vaginum	11 (34.38%)	106 (47.75%)	117 (46.06%)	0.499	Fisher Exact test
Bleeding per vaginum + blood stained liquor	4 (12.50%)	25 (11.26%)	29 (11.42%)		
Bleeding per vaginum + meconium + blood stained liquor	0 (0%)	1 (0.45%)	1 (0.39%)		
Bleeding per vaginum + retroplacental clots	7 (21.88%)	32 (14.41%)	39 (15.35%)		
Blood stained liquor	10 (31.25%)	48 (21.62%)	58 (22.83%)		
Meconium + blood stained liquor	0 (0%)	1 (0.45%)	1 (0.39%)		
Retroplacental clots	0 (0%)	9 (4.05%)	9 (3.54%)		
SGA	14 (43.75%)	90 (40.54%)	104 (40.94%)	0.73	Chi square test,0.119
NICU admission	7 (22.58%)	63 (30.58%)	70 (29.54%)	0.363	Chi square test,0.829
LSCS	8 (25%)	74 (33.33%)	82 (32.28%)		
Normal vaginal delivery	24 (75%)	148 (66.67%)	172 (67.72%)	0.346	Chi square test,0.888

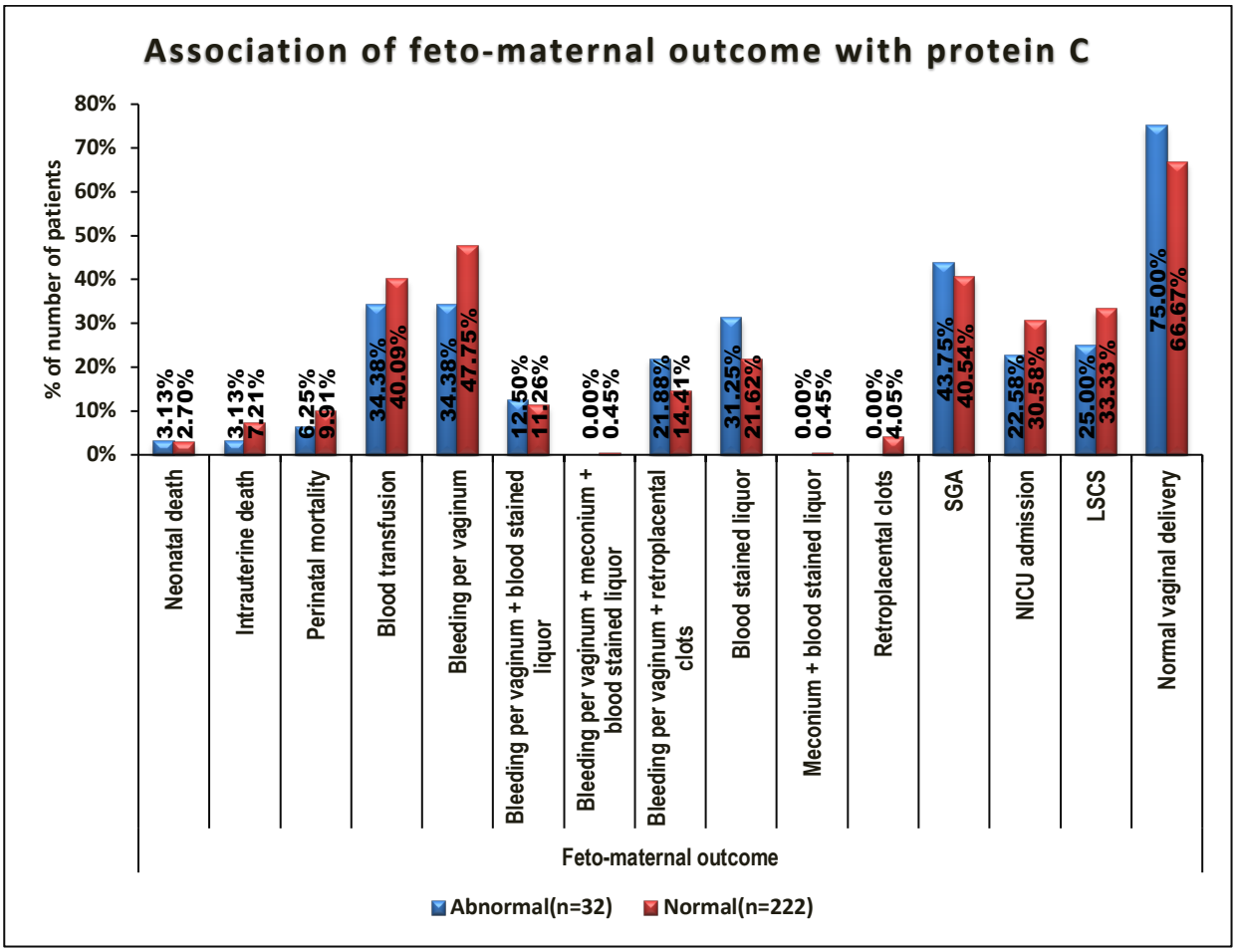

Figure 4: Bar chart showing association of feto-maternal outcome with Protein C functional(%).

In our study 3.13% of those with abnormal protein C had IUD, 6.25% had perinatal mortality,3.13% had NND,34.38% had blood transfusion,43.75% had SGA babies,22.58% had NICU admission,25 % had LSCS. For those with normal values, 2.70% had NND, 7.21% had IUD, 9.91% had perinatal mortality, 40.09 % had blood transfusion, 40.54% had SGA, 30.58% had NICU admission,33.33 % had LSCS

Discussion

Protein C and S deficiency has usually been linked to abruption and adverse feto maternal outcome. In our study there was no case of protein S deficiency and 1 case of protein c deficiency. Normal reference range for protein S was between 33 to 101 in third trimester in our study. Normal reference range for Protein C was between 67 to 135 in third trimester [19,20,2264,65].Protein S was found to be normal in 43.70% and greater than 101 in 56.30% of the cases whereas Protein C was found to be normal in 87.40% and greater than 135 in 12.2%. protein C deficiency in only 1 case that is 0.39%.

In a study done by C V Ananth et al to find the association between maternal thrombophilia and risk of placental abruption between 2002 to 2007.132 cases and 127 controls were taken in which 3 were heterozygous for factor V leidin mutation. Abruption cases were found to be associated with decreased protein C (<5th centile). Decrease in protein C and S and activated protein C resistance was not associated with abruption.[15]

In other observational case control study done by Hansda J et al to study the association between

thrombophilia and recurrent pregnancy loss protein S deficiency and elevated factor VIII level was found to be associated with recurrent pregnancy loss. A total of 34/53(64.15%) was found to be associated with RPL. Protein S deficiency was detected in 27/53 cases versus 1/47 (2.12%) subjects in control. Protein S was also found associated with other pregnancy complication like spontaneous abortion, IUGR, preterm birth.[16]

In a study done by Alfiveric et al in 2002 protein S and protein C deficiency was not found to be associated with abruption and Protein S was found to be associated with IUGR(OR 10.5)[17].similarly in our study no case of protein S deficiency was found and no case of Protein C deficiency was found. similarly in a case control study done by Larciprete in 2007 no case of protein S deficiency was found in abruption and Activated protein C resistance was found to be strongly related to abruption which wasn't evaluated in our study.[18]

Conclusion

No case of protein S deficiency was found. Protein S was found to be greater than normal range in 143(56.30%) cases and out of 143 cases 1.4%(p=0.245) had neonatal death,6.99%(p=0.828) had IUD,39.16%(p=0.938) had blood transfusion, 40.56%(p=40.94%) were SGA babies and 29.32%(0.935) had NICU admission,26.57 %(p=0.027) had LSCS

Protein C deficiency was found in one case .Protein C was found to be greater than normal range in 31(12.2%) cases. Out of 31, 3.13% had neonatal death, 3.13%(p=0.704) had IUD,34.38% (p=0.5360 had blood transfusion, 43.75%(p=0.73) had SGA babies and 22.58%(p=0.363) had NICU admission, 25%(p=0.346).

No significant adverse feto maternal outcome was found to be associated with abnormal protein C and Protein S (values greater than normal range).

So we do not recommend protein C and protein S estimation in patients with abruption.

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