


Research Article

Association of Serum Protein C and Protein S Level in Unexplained Recurrent Pregnancy Loss

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Abstract

Background: Recurrent pregnancy loss (RPL) is an intense personal calamity to the couples and an arduous clinical challenge to the obstetricians. One of the greatest challenges is not being able to identify the baseline etiology of RPL when all the routine and recommended investigations return normal or negative. RPL affects about 1-3% of women of reproductive age trying to conceive. Thrombophilia has been identified as one of main causes of RPL. Inherited thrombophilia includes Protein C deficiency, Protein S deficiency are linked to RPL and could be associated with severe obstetric complications such as preeclampsia, intrauterine growth restriction, or stillbirth which are further associated with microvascular thrombosis in placental blood vessels. Protein C and Protein S deficiencies are associated with a variably increased risk of thrombosis and are inherited independently in an autosomal dominant trait.

Objective: The aim of the study was to determine the association of serum protein C and protein S level in patients with unexplained recurrent pregnancy loss.

Methods: A case control study was carried out in the outpatient Department of Feto-maternal Medicine, Bangabandhu Sheikh Mujib Medical University, Dhaka. After obtaining the ethical clearance from the Institutional Review Board, a convenient sampling technique was applied for data collection. According to the availability of the patients as per inclusion and exclusion criteria, 30 patients with RPL and 30 ages and BMI matched control was taken and necessary information was recorded in a structured questionnaire. With all aseptic precaution 2 ml of venous blood was collected from antecubital vein of each participant by trained blood collector of Department of Hematology, BSMMU, in a supplied coagulation vial. After centrifuging, supernatant plasma was used for mixing with protein C and protein S reagent. By clotting method using a fully automated coagulation analyzer Sysmex CS-1600 machine results were obtained. The results were noted in the questionnaire and data was analyzed using SPSS software.

Result: Mean age and BMI of the RPL patients was 26.85±4.17 years and 25.03±2.42 Kg/m², which was matched with the control groups. The study found that RPL patients experienced an average of 4 pregnancy losses and about 70% pregnancy loss occurred during first trimester. A total of 3(10%) protein C deficient, 5(16.7%) protein S deficient and 2(6.7%) both protein C and S deficient patient were found in the case group. The mean of Protein C and S level among RPL patients was 78.46±13.18 and 81.69±14.06 respectively, which was significantly lower than the control

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group level. Protein C and S level did not vary for patients experienced 1st trimester loss or 2nd trimester loss. The odds ratio for RPL group due to protein C and protein S deficiency was considerably higher [3.22, 95% CI: 0.32-32.89 for Protein C and 5.80, 95% CI: 0.64-53.01 for PS] and a significant association of protein C and protein S level with RPL was found with adjusted binary logistic regression.

Conclusion: The present study found higher incidence of protein C and S deficiency among RPL patients with most of the miscarriage occurring in the first trimester. Hence, pregnant women should be tested for protein C and S deficiencies after excluding common causes of RPL and treated accordingly.

Keywords: Recurrent Pregnancy Loss; Protein C; Protein S; Thrombophilia

Introduction

Recurrent pregnancy loss (RPL) is a devastating & challenging condition for couples as well as clinicians which needs sensitive and reassuring care as well as optimum management. One of the greatest challenges is not being able to identify the baseline etiology of RPL when all the routine and recommended investigations return normal or negative. RPL may be defined as the loss of three or more consecutive pregnancies (RCOG, 2011)[1]. According to ASRM, 2012[2] and ESHRE, 2017 RPL[3] may be defined as two or more clinical and consecutive pregnancy losses with either ultrasound or histopathological documentation, with exclusion of molar and ectopic pregnancies. Thus, the couples should be evaluated after two losses. RPL is a common disorder that affects 3-5% of pregnant women. Identification of causes of RPL is the most challenging issue for the Fetomaternal specialists. Several causes may lead to RPL, such as chromosomal anomalies, congenital or acquired uterine and cervical anatomical abnormalities, ovarian dysfunction, endocrinological problems, maternal thrombophilia, infection, autoimmune disorders[4]. However, in about 50% cases, the reason of miscarriage is unknown[5]. Unfortunately, rest half never have a cause identified even after extensive investigations. These cases are referred to as unexplained recurrent pregnancy loss and serve as the submerged portion of iceberg for the researchers[6]. Thrombophilia has been identified as one of the main causes of RPL [7]. After chromosomal abnormality, thrombophilic disorders have generated considerable interest in the field of RPL of genetic origin, especially in the unexplained cases[8]. Thrombophilia is a blood coagulation disorder that increases the risk of venous thromboembolism. It also causes recurrent miscarriages in early weeks of pregnancy[9], and development of preeclampsia, intrauterine growth restriction,

abruptio placentae, stillbirth in later part of gestation which are associated with microvascular thrombosis in placental blood vessels[10]. Inherited thrombophilia includes Antithrombin-III, protein C deficiency, protein S deficiency, Factor V Leiden mutation, Prothrombin gene mutation (20210A)[11]. Normal pregnancy is associated with increased procoagulants, decreased fibrinolysis & decreased anticoagulants to maintain placental hemostasis during pregnancy. The hemostatic system plays an important role in the success of pregnancy and the process of implantation and placentation. Implantation of the fertilized egg into the uterine decidua establishes a contact between the fetus, the placenta and the maternal circulation. This contact is crucial for the success of pregnancy. However, thrombophilia might be a risk factor with these changes especially in women with deficiency of any of natural anticoagulant factors. Protein C and protein S are natural anticoagulants and their deficiency was found to be associated with placental thrombosis, hypoperfusion, fetal loss and fetal death[12]. Relationship between RPL and thrombophilia is a much-debated topic with well entrenched expert opinion on both sides. Potential association between two is based on the theory of thrombosis in decidual vessels and inhibition of trophoblast differentiation causing fetal loss[13]. Protein C is a vitamin K-dependent plasma glycoprotein activated by thrombin-thrombomodulin complex on the surface of endothelial cells[14]. Activation of protein C into the serine protease-like enzyme, activated protein C[15] is catalyzed by thrombin when it is bound to the endothelial proteoglycan thrombomodulin. Activated protein C exerts its anticoagulant activity primarily through inactivation of coagulation factors Va and VIIIa, which are required for factor X activation & thrombin generation. Protein S is a cofactor for APC. In the presence of protein S, phospholipids and calcium, APC inactivates membrane bound F Va and F VIIIa so results in attenuation of thrombin generation which leads to inhibit clot formation[16]. Protein C deficiency is generally subdivided into two types: type I decreased levels of protein C and type II decreased functional activity of protein C. Protein C deficiency is inherited as autosomal disorders and, in most cases, derived from heterozygous mutations[17]. There are two main types of assays: activity assay which is either clotting time-based assay or chromogenic by spectrophotometer. Quantitative assay for protein C antigen which are immunoassays generally done by using ELISA; it is considered to measure the quantity of protein C irrespective of its function. Genetic testing by DNA sequencing is indicated if the results of functional and antigenic assays do not approve the diagnosis clearly[18]. Acquired protein C deficiency can develop with vitamin K deficiency, liver disease, treatment with vitamin K antagonists, autoimmune syndromes, nephritic syndrome, disseminated intravascular coagulation [19]. A recent study stated an observation of a higher rate of late fetal loss in patients with protein C deficiency compared to non-deficient

patients[20]. Protein S is a vitamin K-dependent glycoprotein which acts as natural anticoagulant. Protein S exists in plasma both free (40%) and bound to the complement C4b binding protein (60%)[21]. Protein S has both APC-dependent and independent anticoagulant properties and consequently is an important protector in controlling thrombin generation and fibrinolysis[22]. The protein S deficiency is identified more than protein C deficiency and its prevalence has been assessed with about 0.5% in the healthy population and 2% to 12% of thrombophilia patients[23]. Regarding pregnancy complications, in a meta-analysis showed that protein S deficiency consulted an overall 15-fold increased risk of recurrent pregnancy loss and a 7-fold higher risk of late fetal loss[24]. Hereditary protein S deficiency is an autosomal dominant disorder. Acquired deficiency of protein S is detected in several pathological conditions including nephrotic syndrome, DIC, liver disease, oral anticoagulant drugs and could be associated with an increased risk of thrombosis[25]. There are two types of PS assays: Immunoassays for the determination of total and free PS levels and clotting assays to measure APC cofactor activity. Immunoassays for free and total PS are preferred for screening pregnancy loss. This study was designed to determine the association of serum protein C and protein S level in unexplained recurrent pregnancy loss.

Objectives

General objective

The general objective of the study to evaluate the association of serum protein C and protein S level in patients with unexplained recurrent pregnancy loss.

Specific objectives

- To estimate the serum protein C and protein S level in RPL patients
- To estimate the serum protein C and protein S level in age and BMI matched control
- To compare serum protein C and protein S level in RPL group with the women with normal reproductive history
- To figure out the association of serum protein C and protein S deficiency with recurrent pregnancy loss.

Methodology

This was a case-control study; a convenient sampling technique was applied for this study. This case-control study was conducted in the outpatient Department of Fetomaternal Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), and Dhaka, Bangladesh from December 2020 to November 2021. A total of 60 participants were included in this study, 30 for RPL and 30 for control participants group. The participant's age and BMI matched women (Patient's attendants) from both outpatient and in patients of BSMMU.

Inclusion criteria

The case groups

Women aged 18-40 years attending Feto-maternal Medicine OPD for preconceptional counseling for RPL who

- Had H/O consecutive two or more failed clinical pregnancies
- Had given consent to participate in the study.

Control groups

Age and BMI matched women who had at least one successful pregnancy with no history of spontaneous pregnancy loss from Outpatient and inpatient Department of Fetomaternal Medicine, Bangabandhu Sheikh Mujib Medical University, Dhaka.

Exclusion criteria:

Women whom cause for RPL was identified such as:

- Parental chromosomal abnormalities
- Anatomical defect of cervix and uterus
- Type I and type II diabetes mellitus
- Thyroid disorders
- Chronic hypertension
- Diagnosed case of chronic renal disease
- Autoimmune disorders
- Polycystic ovarian disease

Study procedure

All patients underwent a complete diagnostic work up for RPL including relevant history, clinical examination and investigation to exclude chronic hypertension, diabetes mellitus, thyroid disorders, chronic renal disease, polycystic ovarian disease, chromosomal analysis of both partners, and uterine anomalies. Regarding investigation CBC, blood sugar, serum TSH, TPO antibody, ANA, antids DNA, Lupas anticoagulant, anticardiolipin antibody, TVS, chromosomal analysis of each partner was done to exclude exclusion criteria. Then 30 participants were ultimately regarded as cases after scrutiny. Age and BMI matched 30 healthy participants who had no history of recurrent pregnancy loss and having at least one healthy child were selected as controls. A convenient sampling technique was used. Then physical examination findings and relevant laboratory investigation findings were recorded. After taking weight and height for each patient, BMI was calculated by the following formula: weight in kg/ height in meter²(kg/m²), and recorded in individual data sheet. Determination of the functional activity of protein C and protein S was performed in the Department of Hematology, BSMMU. After collection of specimens from antecubital vein in a supplied coagulation vial to obtain plasma, carefully

1 part of sodium citrate solution was mixed with 9 parts of venous blood, avoiding the formation of foam then was centrifuged at no less than 1500 rpm for at least 15 minutes at room temperature. During removal of plasma, carefulness was maintained regarding withdrawn of no platelets. Plasma sample was stored at -180c then thawed within 10 minutes at +370c after which the assay was to be performed by clotting method within 4 hours by using protein S Ac and protein C reagent in Sysmex CS-1600 Auto analyzer Machine. Internal quality control test was ensured. The results were obtained using a reference curve prepared beforehand by serially diluting standard Human plasma with protein S and protein C deficient plasma. Results were usually presented in term of percentage. Expected value of protein C range from 70-140% of normal activity. Expected value of protein S range from 70-125% of normal activity. In this study protein C and protein S level <70% was considered as deficiency. All information and investigation result were recorded in a predesigned data collection sheet.

Data Analysis of the Study

Statistical analysis was performed using the SPSS version 23.0 software for windows. The results of the study were presented in tables, figures and diagrams. The descriptive statistics of the study was presented in tables, figures, mean as per the requirement of qualitative and quantitative variables. Mean comparison between two groups was done by independent sample t-test or Mann-Whitney U test. Mean comparison between more than two groups was done by ANOVA test or Kruskal-Wallis test. Chi-square test was conducted to find out any association between categorical variables. Binary logistic regression analysis was conducted to figure out the association of protein C and protein S with RPL P<0.05 was considered as statistically significant.

Ethical consideration

Ethical clearance for the study was taken from the Institutional Review Board of BSMMU. Permission for the study was taken from the concerned departments. The entire study population was thoroughly appraised about the nature, purpose and implications of the study, as well as entire spectrum of benefits and risks of the study. Written consent of all the study participants.

Results

Table 1 showed it appears that maximum (53.3 %) of the patients in RPL group and 67.7% in control group were

from 25 to 34 years of age. Only 6(20%) patients in RPL group were from 35-40 years of age. The mean of age of the RPL patients was (26.85±4.17), whereas the mean of age of the Controls was (29.30 ±4.63). There was no significant difference between these two groups in terms of age.

Table 2 showed A Mann Whitney test was used to compare the mean BMI between RPL group and Control patient group. The test was not statistically significant. So, it can be said that BMI of RPL patient (25.03±2.42) was not significantly different from Control patient (24.43±1.96).

Table 3 showed among the RPL patients, 18(60%) had pregnancy loss for 3 times. Moreover, 7(23%) of the RPL patients had pregnancy loss for 5 times or more. In addition, 80% of the cases were primary RPL. It also appeared that 28(93.3%) RPL patient experienced pregnancy loss in 1st trimester.

Figure II showed, gravidity of the study sample was seen for both RPL and control group. In RPL group, all patients had been pregnant at least thrice. In addition, pregnancy-time ranges from 3 to 7 for RPL patients and majority were pregnant thrice. In control group, pregnancy-time ranges from 1 to 3 where most of the sample in this group had been pregnant ones or twice.

Figure IV showed, among the RPL patient, 80% (24/30) were not able to give birth to any child. Only 6.7% RPL patient was multiparous. In contrast, 50% of the control patient was primiparous and 50% multiparous.

Figure V showed among RPL patients, 80% of the cases were primary RPL.

Figure VI showed, that 28 (93.3%) RPL patient experienced pregnancy loss in 1st trimester.

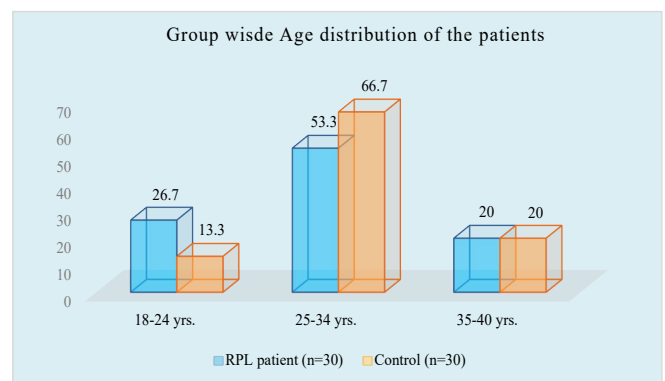


Figure I: Column chart showed age wise patients (N=60)

Table 1: Age of the patients (N=60)

Age groups	RPL patient		Control		P value
	(n=30)	%	(n=30)	%	
18-24 yrs.	8	26.7	4	13.3	0.462
25-34 yrs.	16	53.3	20	66.7	
35-40 yrs.	6	20	6	20	
Mean ± SD	26.85 ± 4.17		29.30 ± 4.63		

Table 2: Body mass index (BMI) (N=60)

BMI (kg/m ²)	RPL (n=30)		Control (n=30)		P value
	n	%	n	%	
18.5-24.9	16	52.9	19	62.9	0.367
25-29.9	9	35.3	7	24.3	
30 ±	5	11.8	4	12.8	
Mean ± SD	25.03±2.42		24.43±1.96		
Range	19-34.4		18.1-31.4		

Table 3: Obstetric history among RPL Patients (N=30)

Parameters	Frequency (n)	Percentage (%)
Pregnancy loss		
3 losses	18	60
4 losses	5	17
5 loss or more	7	23
RPL type		
Primary	24	80
Secondary	6	20
Pregnancy loss as per trimester*		
1 st trimester		28(93.3)
2 nd trimester		15(50)
Combined (1 st + 2 nd)		13(43.3)

*Multiple responses considered

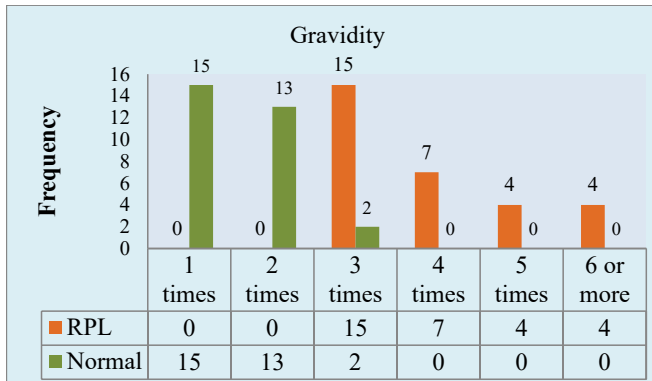


Figure II: Gravidity of the study population. (N=60)

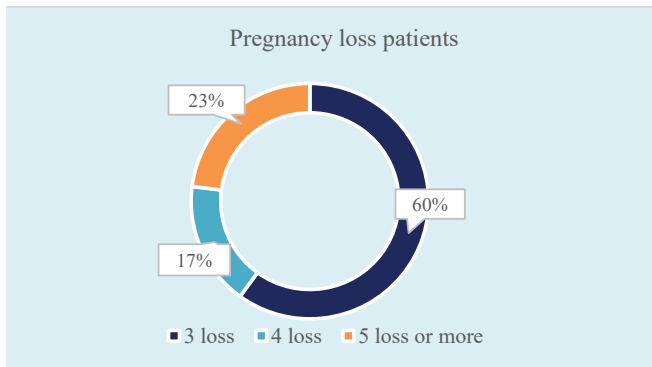


Figure III: Ring chart showed pregnancy loss in RPL patients. (N=60)

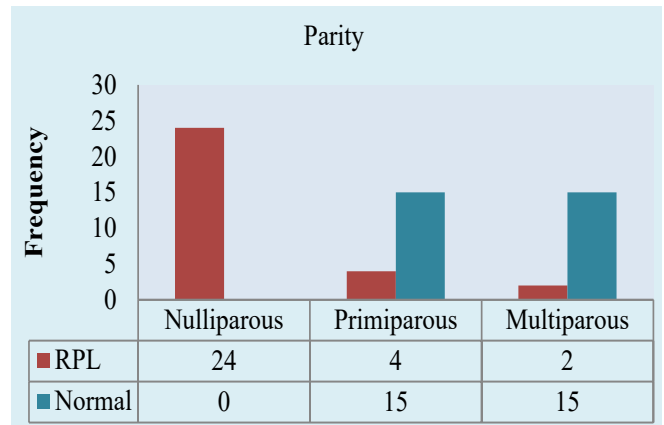


Figure IV: Parity of two groups of the study population (N=60)

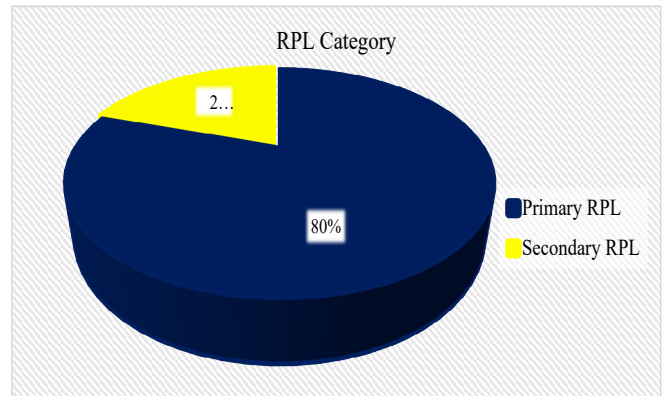


Figure V: Pie chart showed RPL category of the study population (N=60)

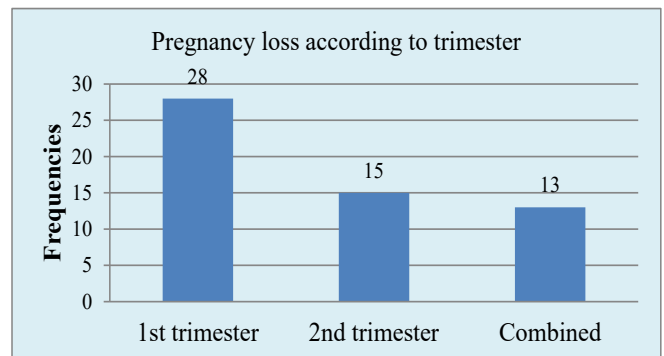


Figure VI: Bar chart showed Pregnancy loss according to trimester (N=60)

Table 4 showed most (69.4%) of the pregnancy loss occurred during first trimester. About 30.6% (34/111) pregnancy loss occurred during 2nd trimester.

Table 5 showed among the RPL patients, a total of 111 pregnancy loss was found. That is on average 3.7~4 losses occurred among RPL subjects.

Table 6 showed, normal distribution of the variables was checked first before comparing the means between RPL and control patients. Then according to the result parametric and non-parametric test was selected for comparing means of the two groups.

Parametric test

Parametric (independent sample t test) test was run to determine a significance difference of protein S between RPL and Control group since these data were normally distributed.

Non parametric test

Non parametric (Mann Whitney test) test was run to determine a significance difference of Protein C and BMI between RPL and Control patient group since these data were not normally distributed.

Table 7 showed among the RPL patients a total of 3(10%) protein C deficient, 5(16.7%) protein S deficient and 2(6.7%) both protein C and S deficient patient were found in the study.

Table 8 showed (Mann Whitney test) to compare the

Table 4: Total Pregnancy loss as per trimester

Gestational age	Frequency (n=111)	Percentage (%)
1st trimester (1-12 weeks)	77	69.4
2nd trimester (13-24 weeks)	34	30.6

Table 5: Average pregnancy loss in RPL patient

Total pregnancy loss	111
Number of RPL patients	30
Average	3.7~4

Table 6: Normality test of continuous variable (N=60)

Tests of Normality	Kolmogorov-Smirnov		
	Statistics	df	P value
Protein C	0.134	60	0.009
Protein S	0.097	60	0.2
BMI	0.304	60	<0.001

Table 7: Protein C and S deficiency among RPL patients (n=10)

Deficiency	Frequency (n)	Percentage (%)
Protein C deficiency	3	10
Protein S deficiency	5	16.7
Both Protein C & S deficiency	2	6.7

Table 8: Protein C level difference between the two groups

Parameter	RPL (Mean ± SD)	Control Mean ± SD	P value
Protein C level	78.46±13.18	112.84 ± 17.57	<0.001

Table 9: Comparison of protein C level in different trimester (n=30)

Parameter	RPL = 30			P value
	1 st Trimester loss	2 nd Trimester loss	(1 st + 2 nd) Trimester loss (Combined)	
	Mean ±SD	Mean ±SD	Mean ±SD	
Protein C	78.20±13.49	79.60 ±10.37	79.20 ±10.77	0.929

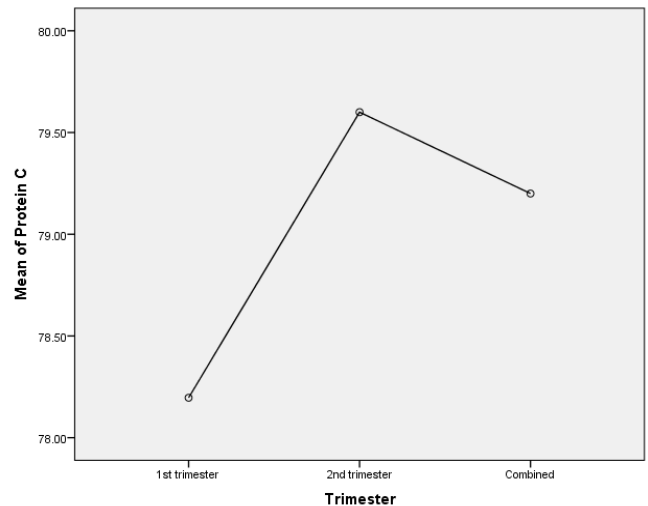


Figure IX: Comparison of protein C among groups.

mean Protein C level between RPL group and Control group. The test result was statistically significant (U= 831.500; P <0.001). So, it can be said that Protein C level of RPL patient (78.46 ± 13.18) was significantly lower than Control patient (112.84 ± 17.57).

Table 9 showed, that there were no significant differences in protein C level between patients who had pregnancy loss in 1st Trimester, 2nd Trimester or together.

Table 10 showed, that the mean Protein S level between RPL group and Control group. The test result was statistically significant (P<0.001). So, it can be said that Protein S level of RPL patient (81.69±14.06) was significantly lower than Control patient (104.23±15.13).

Table 11 showed, that there were no significant differences in protein S level between patients who had pregnancy loss in 1st Trimester, 2nd Trimester or together.

According to table 12, it appeared that both in protein C and S deficient group, most of the pregnancy loss occurred during 1st trimester. Moreover, even for both deficient patient, 66.7% had loss in first trimester.

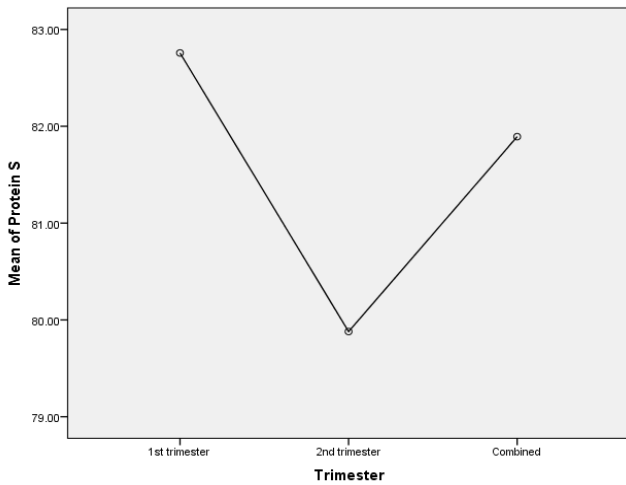


Figure X: Comparison of protein S among groups

Table 10: Protein S level difference between the two groups (N=60)

Parameter	RPL (Mean ± SD)	Control Mean ± SD	P value
Protein S level	81.69±14.06	104.23±15.13	<0.001

Table 11: Comparison of protein S level in different trimester (n=30)

Parameter	RPL = 30			P value
	1 st Trimester loss	2 nd Trimester loss	(1 st + 2 nd) Trimester loss (Combined)	
	Mean ±SD	Mean ±SD	Mean ±SD	
Protein S	82.76 ± 13.61	79.88 ± 12.99	81.89 ± 11.96	0.79

Table 12: Total pregnancy loss of Protein C and Protein S deficient patient as per trimester

Gestational age	Number of losses (n)	Percentage (%)
Protein C deficient		
1st trimester (1-12 weeks)	10	71.4
2nd trimester (13-24 weeks)	4	28.6
Protein S deficient		
1st trimester (1-12 weeks)	15	75
2nd trimester (13-24 weeks)	5	25
Both deficient		
1st trimester (1-12 weeks)	6	66.7
2nd trimester (13-24 weeks)	3	33.3

Table 13: Association of protein C deficiency with RPL

	RPL	Control	OR	95% CI	P value
Protein C Deficiency (<70%)	3	1	3.22	0.32 - 32.89	0.612
Protein C Normal (70-140%)	27	29			
Total	30	30			

Table 14: Association of protein S deficiency with RPL

	RPL	Control	OR	95% CI	P value
Protein S Deficiency (<70%)	5	1	5.8	0.64 - 53.01	0.195
Protein S Normal (70-125%)	25	29			
Total	30	30			

Table 15: Association of Protein C and Protein S with RPL

	OR	95% C.I. for OR		P value
		Lower	Upper	
Protein C	1.196	1.064	1.344	0.003
Protein S	1.201	1.037	1.39	0.014

According to table 13, it appeared that no significant association was found between Protein C deficiency (<70) and RPL (P>0.05). However, the odds for RPL due to protein C deficiency (<70) was 3.22 [95% CI: 0.32-32.89].

Table 14 stated, no significant association was found between Protein S deficiency (<70) and RPL (P>0.05). However, the odds for RPL due to protein S deficiency (<70) was 5.80 [95% CI: 0.64 - 53.01].

Table 15 stated, binary logistic regression analysis was conducted to figure out the association of protein C and protein S with RPL [χ^2 (2, N=60) = 63.270; P<0.001]. The odds for RPL with 1-unit decrease of Protein C level was 1.196 [95% CI: 1.064-1.344] and the odds for RPL with 1-unit decrease of Protein S level was 1.201 [95% CI: 1.037-1.390]. Both variables had significant association with RPL (P<0.05).

Discussion

For both patients and obstetricians, recurrent pregnancy loss is heartbreaking and disappointing because a causative etiology cannot be found in around half of the cases. Maternal thrombophilia has recently been identified as a major cause of adverse pregnancy outcome, including recurrent pregnancy loss, still-birth, severe pre-eclampsia, placental abruption, and intrauterine growth restriction[26,27]. However, deficiencies of the natural anticoagulant like protein C, S and antithrombin occur much less than 1% to 2% in RPL patients[27]. The present study found 3patients had protein C deficiency, 5patients had protein S deficiency and 2patients had both protein C and protein S deficiency. On the other hand, only three patients with protein C deficiency and no patients with Antithrombin III deficiency were found among 93 cases[28]. The prevalence of protein C, and protein S deficiencies are higher in patients with thromboembolic events compared with those in the healthy population[29]. On the other hand, the present study found limited number of protein C (10%) and protein S deficient (16.7%) patient which contradict with the above-mentioned literature. Similarly, Yamada et al.

(2011)[30] could not find an increased incidence of protein C deficiency in patient with RPL. In the present study, mean age and BMI of the RPL group was 26.85±4.17 years and 2.42kg/m², which was matched with the control group. Moreover, primary RPL was more frequent in the study. Other than that, in this study, most of the pregnancy loss for Protein C and protein S deficient women occurred during 1st trimester. However, Alshammery et al. (2015)[31] found that Protein C and protein S deficiency had a significant association with second trimester pregnancy loss. Jyotsna et al. (2011) [32] found a significant association in the mean value of protein C in patient group comparing with control group. Regarding the comparison of mean value of protein C level in RPL group and control group, the present study found a significant difference of protein C and protein S between RPL and controls. To exemplify, Protein C level of RPL patient (78.46±13.18) was significantly lower than Control patient (112.84± 17.57). However, protein C had no significant difference between patients and controls, found in Vora et al. (2018), Raziel et al. (2011)[33,34]. Moreover, according to a meta-analysis by Rey et al. (2013)[35], PC deficiencies are not linked with fetal loss. The prevalence of protein S deficiency in healthy individuals is 0.03-1.3%, [24] but the risks associated with PS deficiency are similar to those of PC deficiency[36]. A significant association of RPL with protein S deficiency was found in the present study, which supports the studies of Raziel et al. (2011) and Parand et al. (2013)[27,34]. Moreover, PS deficiency was also found associated with late-term fetal loss (Rey et al., 2013)[35]. However, Laghoff-Ross et al. (2016) reported that PS is not associated with recurrent early fetal loss. In the present study 2(6.7%) both protein C and S deficient patient in the RPL group was found. Other studies didn't find any statistically significant for both protein C and S deficiency in RPL patients comparing with control group[31,32] described a significant risk of RPL in pregnant Indian women with thrombophilia. Moreover, In the European Prospective Cohort on Thrombophilia study, which involved 1384 women, the authors reported that the risk of fetal loss was increased in women with thrombophilia compared with controls (168/571 vs. 93/395; OR: 1.35 [1.01-1.82]) [37]. In the same study, the authors reported increased fetal losses in women with familial thrombophilia and stillbirths [37]. A meta-analysis of 31 retrospective study which had shown that the relationship of thrombophilia with late pregnancy loss is stronger than early miscarriages[35]. Alonso et al. (2012) [38] also stated that the prevalence of thrombophilia was more prominent in second trimester, also in agreement with studies done by Rey and Regan (2011) [39] which established that protein C deficiency has been associated with an increased risk of second trimester miscarriage and stillbirth. Robertson et al. (2016)[40] conducted a systematic review of thrombophilia in pregnancy that included a total of 79 studies (three randomized controlled trials, eight prospective cohorts,

and 68 retrospective studies) to evaluate the risk of early pregnancy loss in thrombophilia patients. They found that women with thrombophilia were more likely to experience miscarriage than those without it. It was determined that there was an ORs for Antithrombin III, PC, and PS deficiency were 0.88 (0.17-4.48), 2.29 (0.20-26.43), and 3.35 (0.35-35.72) respectively. In the present study, the odds for RPL due to protein C and protein S deficiency (<70) were 3.22 [95% CI: 0.32-32.89] and 5.80 [95% CI: 0.64-53.01] respectively. Moreover, the odds for RPL with 1(one) unit decrease of Protein C and protein S level were 1.196 [95% CI: 1.064-1.344] and 1.201 [95% CI: 1.037-1.390] respectively. The protein C and protein S deficits that occur during pregnancy are key contributors to thrombophilia, which is related with an increased risk of venous thromboembolisms in the pregnant women[41]. When combined with thrombophilia (a broad spectrum of, the hypercoagulable state of pregnancy may increase the risk of thromboembolism during pregnancy or postpartum[42]. Among the top causes of maternal death in the United States, pulmonary embolism ranks first[41]. Since, the present study found a considerable number of protein C and protein S deficiency among RPL pregnant women, therefore, screening and subsequent therapy for thrombophilia is required among pregnant women coming for check-up in hospitals. There are contradictory hypotheses concerning the role of thrombophilia as a cause of abortions. According to many authors, abortions do not occur in all cases of thrombophilia[43]. While the study of Cosmi B. et al. (2013) and Yildizet al. (2012)[44,45] stated that the combination of more than one inherited thrombophilic gene defect has been recognized as a cause of early and late RPL. In spite of that, the incidence of thrombophilia is not clear[46]. Moreover, Sibai et al. (2017)[41] found that approximately 0.2-1% of patients with a combined deficiency of PC and PS have normal pregnancy outcomes. Nevertheless, to detect an association between thrombophilia and recurrent pregnancy loss, it would be necessary to increase considerably the sample size but with unlikely clinical significance.

Recommendation

Large population-based studies are required to assess the relationship of Protein C and protein S with the risk of RPL. Laboratory facilities should be made available and reagents should be cost-effective so that the affected patients can be evaluated. Routine thrombophilia screening test can be done for evaluation of RPL patients. Low Molecular Weight Heparin should be given when patient has been diagnosed with thrombophilia.

Conclusion

The present study found that patients with RPL had a higher incidence of protein C and S deficit when compared to healthy controls. It has been found that protein C and protein

S deficiency are associated with recurrent miscarriage, with the rate of miscarriage occurring in the first trimester being the most prevalent. Based on the findings of the current study, it is possible to conclude that inherited thrombophilia due to protein C and protein S deficiency may be a contributing factor of unexplained recurrent pregnancy loss. Limitations of the study Time and resources were limited. This study was carried out in a specialized tertiary care hospital which perhaps not the true representation of all Bangladeshi RPL patients. The study was conducted in a relatively small sample size.

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