Original Article

Thrombogenic indices in an evaluation of pregnant Nigerian women with pregnancy loss

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ABSTRACT

Background: Pregnancy losses (PLs) are usually a source of pain and psychological stress to the expectant couples. The association between ABO blood groups and some thrombogenic markers with PL among Nigerian women is mostly unknown.

Aim: This study investigates the association between ABO blood group, deficiencies of protein C (PC), and protein S (PS) and PL.

Patients and Methods: A cross-sectional study involving 170 pregnant women grouped into two, those with or without a history of clinically- or ultrasonographically recognizable PL. ABO blood groups using the tile method, plasma concentrations of free protein S (fPS) antigen, protein C antigen (PCAg) by the enzyme-linked immunosorbent assay-based method, and PC activity (PCAc) by PROTAC method was determined.

Results: There was no difference in mean values between the two groups for PCAg, PCAc, FPS, and blood group ($P > 0.05$). The chances of PL were; non-O blood group (AOR 1.29; 95% CI 0.65--2.54), deficient PCAg (AOR 1.75; 95% CI 0.87--3.54), and deficient PCAc (AOR 1.05;95%CI 0.25--4.13). There was a very poor correlation of miscarriage with FPS (rho 0.04), PCAg (rho 0.09), and PCAc (rho = 0.05).

Conclusion: There was no significant association between PLs and ABO blood group phenotypes, PCAg, PCAc, fPS.

Key words: Free protein S; Nigerian women; pregnancy loss; protein C antigen; thrombogenic markers.

Introduction

Unplanned miscarriages or pregnancy losses (PLs) are usually a source of pain and psychological stress to the expectant couple. It becomes recurrent when the woman experiences three or more consecutive PLs[1] with the incidence of about 5%, which varies with the time and cause of PL.[2] The rate is higher among blacks who are known to have over the three-fold increased risk of miscarriages and fetal deaths than their white counterparts.[3] There are different identifiable possible causes that have been identified including genetic abnormalities, endocrine derangements, anatomical defects, hematological disorders, and infections.[4]

Controversies exist as to the importance of thrombophilia in PL.[5] Thrombophilia may account for up to 40--55% of recurrent PL.[6] However, Parand *et al*. reported that

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PL is mostly not associated with all thrombophilia.[6] Pregnancy being a prothrombotic state, deficiency of the natural anticoagulants, protein S (PS), and protein C (PC), heightens the likelihood of thrombotic events in pregnancy. Deficiencies of PC, PS, and presence of non-O blood group are possible risk factors for adverse pregnancy outcomes including PL and thromboembolism.[6,7] For PL, the pathogenetic mechanism is presumed to be because of thrombosis in the decidual vessels or sites of implantation with resultant infarction and placental insufficiency which impair blood flow to the fetus, thereby causing intrauterine growth restriction or death.[3]

Indeed the contributions of non-O blood group, PC, and PS to poor pregnancy outcome have been previously described.[8] Most of these studies were on Caucasians or non-blacks with a paucity of data among Nigerian women who are thought to have a different prevalence of thrombophilia.[5,6] Furthermore, recurrent PL may be the first presentation of a hematological problem,[2] thus the need for complete hematological workup is critical to identify possible risk factors in such patients who present with unknown etiology. This study investigates the association of ABO blood group, deficiencies of PC and PS with PLs.

Patients and Methods

The study center was the Obstetrics and Gynecology unit of the University of Port Harcourt Teaching Hospital. The choice of study subjects was by probability sampling method. A systematic sampling method, the second of every three-clinic attendee was chosen. A total of 170 healthy consenting pregnant women were interviewed with the aid of a pre-tested, pre-validated structured questionnaire. Sociodemographics, anthropometric data, medical data, history of loss of clinically-/ultrasonographically recognized pregnancies, gynecologic and parities were obtained.

Women with evidence of liver, renal, or sickle cell diseases or thrombotic disorder (acute phase) and receiving vitamin K and anticoagulant/antifibrinolytic therapies were excluded from the study. The institutional ethical committee approved this study, and informed consent was obtained from the participants. There were two groups of study subjects: group A comprising of 60 women with a history of one or more previous PLs, group B which included 110 women who had no history of PL at the time of recruitment. Participants were included in group A if there was a positive pregnancy test confirmation of index pregnancy as well as the ultrasonographical recognizable pregnancy of 24 weeks or less.[9] Women who were unsure about the ultrasound diagnosis were excluded from the study.

Blood samples were collected from the participants for ABO blood group phenotyping and determination of PC and PS levels. ABO blood group was determined using the tile method as previously described.[10] PC and FPS antigen levels were determined using the enzyme-linked immunosorbent assay (ELISA)-based methods outlined in Technozym® and Zymutest Hyphen BioMed® test kits, respectively,[11,12] whereas the activity level of PC was evaluated with Technochrom® using the PROTAC method by Technoclone.[10] For this study, the ABO blood types were grouped as "O blood group" and "non-O (A, B, AB) blood groups," the subject was regarded as being deficient if PCAg, PCAc, and FPS levels were <70%, <70%, and <20%, respectively.

Data were analyzed using IBM Statistical Package for Social Sciences version 21 (Chicago, Illinois, USA). Findings were presented in tables using frequencies and proportion for categorical variables as well as a mean and standard deviation for quantitative variables. Associations of PL with blood group, PCAg, PCAc, and FPS levels were determined using Chi-square test. Spearman rho correlation coefficient was equally used for the relationship between variables including age, parity, PCAg, PCAc, and FPS levels. Level of significance was at $P \leq 0.05$.

Results

Sociodemographic characteristic

Of the 170 pregnant women recruited for the study, 110 (65%) of Group A had no previous miscarriages, whereas 60 (35%) reported the previous history of one or more PL. The mean age and SD of women in group A and B were 30.2 (4.4) and 30.7 (5.1), respectively. Most in group A and B were of the O blood group, 69 (63%) and 41 (68%), respectively [Table 1].

Comparison of mean levels of PCAg, PCAc, and fPS and other variables between Group A and B

The mean and SD levels of PCAg, FPS, and PCAc in group A were 80.1 (25.2), 51.5 (16.6), and 112.4 (29.1), whereas those of group B were 77.8 (28.8), 52.1 (16.6), and 112.1 (30.2),

Table 1: Characteristics of participants

Variables	A (Miscarriage) $n=60$		B (No Miscarriage) $n=110$		*t* test	*P*
	Mean	Std dev	Mean	Std dev		
Age	30.2	4.4	30.7	5.1	0.64	0.520
Parity	1.2	1.4	1.4	1.5	0.74	0.460
Protein_C_antigen	80.1	25.2	77.8	28.8	0.51	0.612
Protein_C_activity	112.4	29.1	112.1	30.2	0.06	0.955
Free_protein_S	51.5	16.6	52.1	16.6	0.19	0.848
Blood group	**Number**	**Percent**	**Number**	**Percent**	χ^2	*P*
Non-O	19	31.7	41	37.3	0.53	0.465
O	41	68.3	69	62.7		

respectively. It was observed that 20 (29.4%) and 3 (27.3%) of participants with history of PL were deficient in PCAg and PCAc, respectively. Comparison of these mean levels of these variables between the group using t-test was nonsignificant as $P > 0.05$; for age ($P = 0.520$), parity ($P = 0.460$), PCAg ($P = 0.612$), PCAc ($P = 0.955$), fPS ($P = 0.848$), and blood group ($P = 0.465$) [Table 1].

The relationship between levels of PCAg, PCAc, fPS, and PL

Table 2 shows that there were no statistically significant associations of PL with blood group ($\chi^2 = 0.53$, $P = 0.465$), fPS (FT, $P = 0.123$), PCAg ($\chi^2 = 1.72$, $P = 0.190$), and PCAc (0.33, $P = 0.749$). It showed that non-O blood group was 1.3 times more likely to experience PL than those of blood group O (AOR 1.29; 95% CI 0.65--2.54) although this was not statistically significant. Those women with deficient PCAg were 1.8 times more likely to experience PL than those of normal PCAg (AOR 1.75; 95% CI 0.87--3.54), whereas those with deficient PCAc were about 1.1 times nonstatistically significant likely to have PL than those of normal PCAc (AOR 1.05; 95% CI 0.25--4.13).

There was a nonsignificant poor correlation of PL with age (rho = 0.08), Parity (rho = 0.05), PS (rho = 0.04), PC antigen (rho = 0.09), and PC activity (rho = 0.05) [Table 3].

Table 2: Associations of Pregnancy loss with Thrombogenic Markers

Variable	Pregnancy loss (PL)		Bivariate analysis		Multivariate analysis	
	No	Yes	χ^2	*P*	AOR	95% CI AOR
Blood group						
Non-O	41 (68.3)	19 (31.7)	0.53	0.465	1.29	0.65-2.54
O	69 (62.7)	41 (37.3)			1	
Protein S						
Deficit	0 (0.0)	2 (100.0)	FT	0.123	NA	
Normal	110 (65.5)	41 (34.5)				
Protein C Antigen						
Deficit	48 (70.6)	20 (29.4)	1.72	0.190	1.75	0.87-3.54
Normal	62 (60.8)	40 (39.2)			1	
Protein C Activity						
Deficit	8 (72.7)	3 (27.3)	0.33	0.749	1.05	0.25-4.13
Normal	102 (64.2)	57 (35.8)			1	

Bivariate analysis, Chi square test, Multivariate analysis, Binary logistic regression, AOR, Adjusted Odd Ratio, CI, Confidence Interval, FT, Fischer's Test, NA, Not applicable

Table 3: Relationship of Miscarriage with Thrombogenic Markers and other variables

Variables	Number	Mean	Std dev	Correlation coefficient	*P*
Miscarriage	170	0.8	1.1	−0.08	0.306
Age	170	30.5	4.8		
Miscarriage	170	0.8	1.1	0.05	0.518
Parity	170	1.3	1.1		
Miscarriage	170	0.8	1.1	0.04	0.627
Protein S	170	51.9	16.3		
Miscarriage	170	0.8	1.1	0.09	0.244
Protein C Antigen	170	78.6	27.5		
Miscarriage	170	0.8	1.1	0.05	0.491
Protein C Activity	170	112.2	29.7		

*rho, Spearman correlation coefficient

Discussion

PL is not uncommon, occurring in upto 5% of women[2] and more in blacks.[3] Its cause is unknown in 50% of cases.[13]

In this study, the mean levels of PCAg, PCAc, and FPS were all above reference value and showed no significant difference between both groups. In our study, there was no significant association between non-O blood group women in group A and PL. Possible explanations by the findings in work done by Spiezia *et al*.[14] observed a three-fold increase in the risk of a thrombotic event in non-O blood group individuals with pre-existing thrombophilia, suggesting an additive effect. This study recorded lower incidences of deficiency of natural anticoagulants unlike their research, this could have weakened the strength of the association in our study.

Deficiencies of these proteins worsen the prothrombotic state of pregnancy and increase the likelihood of adverse pregnancy outcomes like PL. However, this study observed no significant statistical difference between deficiencies of PS and PC among women with or without a history of PL and as such there was no significant association between deficiencies of PCAg, PCAc, and FPS with PL. This finding is in keeping with the work done in Colombia by Cardona *et al*.[15] on Colombian women where he concluded that the low prevalence of thrombophilia in non-Caucasian population could be a cause.

On the contrary, Mekaj *et al*.[16] reported that deficiency of PC and PS are associated with first trimester PL even though thrombophilia is thought to be more contributory to second trimester PL. It has also been documented that PC and PS deficiency had a significant association with second trimester PL. This finding is also consistent with the meta-analysis of 31 retrospective study,[17] which had shown that the relationship of thrombophilia with late PL is stronger than early PL. Participants with PL were grouped in our study, irrespective of the trimester the PL occurred; moreover, they might have contributed to the reported differences knowing that 75% of first-trimester PLs are because of chromosomal abnormalities.[18]

Our study showed there was no significant association between women with a history of PL and deficient in PCAg and PCAc, suggesting that low-antigen levels do not prevent generation of enough activity to carry out required anticoagulation function during pregnancy. This may be buttressed by the fact that the mean level of PCAc was 1.4 times higher than the PCAg mean levels in this study.

Deficiency of PS is reported to be the most prevalent thrombophilia among the blacks in the United States[19] and accounting more for PL among the study group. Irrespective of this, the only two subjects that were PS-deficient in this study were in the PL group. PS deficiency did not show significant association with PL across both groups. It is worthy to note that the study excluded individuals with conditions that could cause PS deficiency, for example, liver, renal, or sickle cell diseases, suggesting that there were some unknown factors which may have contributed to the higher prevalence of PS deficiency in the former study. Racial and geographical constructs could explain these differences.

There was a positive but weak correlation of PL with maternal age, PCAg, PCAc, and FPS in this study. Interestingly, the study showed PL was more common in older women. The rate of PL increases steadily so that by the age of 45 years, there is about one-in-two times the risk of PL.[20]

Conclusion

There is no significant association between PL and non-O blood group, deficiencies in PS and PC; hence, routine thrombophilia screening may not be recommended for all women presenting with PL. Other possible options should be explored to ascertain the etiology.

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Conflicts of interest
There are no conflicts of interest.

References

1. Ford HB, Schust DJ. Recurrent pregnancy loss: Aetiology, diagnosis, and therapy. Rev Obstet Gynecol 2009;2:76-83.
2. Jeve YB, Davies W. Evidence-based management of recurrent miscarriages. J Hum Reprod Sci 2014;7:159-69.
3. Kocher O, Cirovic C, Malynn E, Rowland CM, Bare LA, Young BA, *et al*. Obstetric complications in patients with hereditary thrombophilia identified using the LCx microparticle enzyme immunoassay: A controlled study of 5,000 patients. Am J Clin Pathol 2007;127:68-75.
4. Meinardi JR, Middeldorp S, de Kam PJ, Koopman MMW, Van Pampus ECM, Hamulyák K, *et al*. Increased risk for fetal loss in carriers of the factor V Leiden mutation. Ann Intern Med 1999;130:736-9.
5. Roqué H, Paidas MJ, Funai EF, Kuczynski E, Lockwood CJ. Maternal thrombophilias are not associated with early pregnancy loss. Thromb Haemost 2004;91:290-5.
6. Parand A, Zolghadri J, Nezam M, Afrasiabi A, Haghpanah S, Karimi M. Inherited thrombophilia and recurrent pregnancy loss. Iran Red Crescent Med J 2013;15:e13708.
7. Okoye HC, Eweputanna LI, Okpani AO, Ejele OA. Associations between pre-eclampsia and protein C and protein S levels among pregnant Nigerian women. Int J Gynaecol Obstet 2017;137:26-30.
8. Condous G. Ultrasound diagnosis of miscarriage: New guidelines to prevent harm. Australas J Ultrasound Med 2011;14:2.
9. Rowley M, Cantwell C, Milkins C. Laboratory aspects of blood transfusion. In: Bain BJ, Bates I, Laffan MA, editors. Dacie and Lewis Practical Haematology, 12th ed, vol 12. Elsevier Limited; 2017. p. 470-96.
10. Technochrom protein C kit by Technoclone GmbH®. 67 Brunner Street, 67, 1230 Vienna Austria.
11. Technozyme protein C antigen assay by Technoclone GmbH®. 67 Brunner Street, 67, 1230 Vienna Austria.
12. Zymutest free protein S by HYPHEN BioMed®. 155 rue d'Eragny, 95000 Neuville-sur-Oise France.
13. Hussein NA, Hadi MA, Farah SH. Deficiency of protein C and protein S in recurrent pregnancy loss. Med J Babylon 2015;2:348-56.
14. Spiezia L, Campello E, Bon M, Tison T, Milan M, Simioni P, *et al*. ABO blood groups and the risk of venous thrombosis in patients with inherited thrombophilia. Blood Transfus 2013;11:250-3.
15. Cardona H, Castañeda SA, Maya CW, Alvarez L, Gomez J, *et al*. Lack of association between recurrent pregnancy loss and inherited thrombophilia in a group of Colombian patients. Thrombosis 2012;2012:367823.
16. Mekaj Y, Lulaj S, Daci F, Rafuna N, Miftari E, Hoxha H, *et al*. Prevalence and role of antithrombin III, protein C and protein S deficiencies and activated protein C resistance in KOSOR women with recurrent pregnancy loss during the 1st trimester of pregnancy. J Hum Reprod Sci 2015;8:224-9.
17. Rey E, Kahn SR, David M, Shrier I. Thrombophilic disorders and fetal loss: A meta-analysis. Lancet 2003;361:901-8.
18. Philipp CS, Faiz AS, Beckman MG, Grant A, Bockenstedt PL, Heit JA, *et al*. Differences in thrombotic risk factors in black and white women with adverse pregnancy outcome. Thromb Res 2014;133:108-11.
19. Hernandez W, Gamazon ER, Smithberger E, O'Brien TJ, Harralson, AF, Tuck M, *et al*. Novel genetic predictors of venous thromboembolism risk in African Americans. Blood 2016;127:1923-9.
20. Nybo Andersen AM, Wohlfahrt J, Christens P. Maternal age and fetal loss: Population based register linkage study. Br Med J 2000;320:1708-12.