Original Research Article

Assessment of Protein C and Protein S in Pregnancy loss victims

ABSTRACT

Background: The haemostatic changes that result in hypercoagulation during the pregnant state has been linked to pregnancy loss. **Objective**: Assessment of Protein S and Protein C assays in pregnancy loss in Abia State, South East, Nigeria. **Materials and Methods**: This was a cross-sectional study involving women in their reproductive years. Study population was stratified into 3 groups and the Protein C and Protein S concentrations measured and compared among the three groups. **Results**: A total of 130 apparently healthy Nigerian women of child-bearing age were enrolled in the study. The study groups consisted of 70 women who had just lost a pregnancy, 30 women with normally progressing pregnancy and 30 nonpregnant women. The protein C concentration for the pregnancy-loss subjects was significantly lower than that of the normal pregnancy at $p \leq .01$, while that of Protein S showed non-significance ($p > 0.05$). **Conclusion:** Protein C deficiency is associated and can be incriminated with increase chance of pregnancy loss.

Key words: Pregnancy loss, Protein C, Protein S, haemostasis

1.0 INTRODUCTION

Human reproduction is accompanied by physiological changes that occur primarily to accomplish pregnancy as they ensure the proper development, growth and ultimate survival of the embryo/foetus. These changes include (but are not limited to) cardiovascular, respiratory, metabolic/endocrinal, renal, haematologic and immunologic changes (1). Some of these changes influence normal biochemical values while others may mimic symptoms of medical disease (1). The pregnant state is not failure-proof. Pregnancy loss (also termed miscarriage or abortion) is the spontaneous loss of an intra-uterine pregnancy without outside intervention before 20 weeks of gestation (2). Spontaneous pregnancy loss is a surprisingly common occurrence affecting approximately about 15% of all clinically recognized pregnancies (3; 4). Approximately 1-2% of couples have three or more consecutive losses (4), termed recurrent pregnancy loss (RPL). Pregnancy is associated with significant changes in the haemostatic profile (5). Women with a history of RPL are in a procoagulant state even when they are not pregnant (5). This hypercoagulable state has been attributed to alterations of the proteins that govern blood clotting (7), thus creating a thrombophilic state (8). Thrombophilia has been linked to pregnancy loss (9) and most especially to recurrent pregnancy loss (10). In this study, we investigated the relationship between pregnancy loss and Protein C deficiency as well as Protein S deficiency in women of Abia State, Nigeria.

2.0 MATERIALS AND METHODS

A total of 130 apparently healthy women of childbearing age (18-45 years) were enrolled in this study, using a cross-sectional study design stratified into three groups of study as follows:

pregnancy-loss (70 subjects), normal pregnancy (30 subjects) and non-pregnant control (30 subjects). Study lasted the period between December 2017 and March 2018.

2.1 Study Setting

The study took place at the following hospitals: Federal Medical Centre, Umuahia, Nazareth Specialist Hospital Aba, (a specialist gynaecological clinic), General Hospital, Aba and General Hospital, Ohafia, all in Abia state, Nigeria.

2.2 Blood collection

3mls of the blood was put into plain vacuum containers and the serum retracted after 30 minutes of clotting. The serum was used for the estimation of Protein C and Protein S.

2.3 Laboratory Analysis

The manufacturers' standard operation procedure (S.O.P.) for each investigation was used during each assay, and the operational instruction for each machine was strictly followed. The Protein C and Protein S estimations were done using the ELISA technique with kit procured from Melsin Laboratories, China.

3.0 Ethical Approval

Ethical approval was obtained from the Ethics Committee of the Federal Medical Centre, Umuahia, the Hospitals Management Board, Umuahia and the Nazareth Specialist Hospital, Aba.

4.0 Statistical Analysis

Statistical analysis was done using SPSS windows version 20 (IBM Corporation, 2011). Data was grouped into pregnancy-loss, normal pregnancy and non-pregnant control subjects. The Kolmogirov-Smirnov test was used to determine normality. Parametric data were expressed as +/- SD. Immunological and haematologic parameters were skewed. Hence, they were expressed

as median, 2.5th (p2.5) and 97.5th (p97.5) percentile, and were log-transformed prior to analysis. ANOVA and Games-Howell post hoc procedures were used to test for group differences. Data was considered significant at error probability *P*-level less than or equal to (≤) .05.

5.0 RESULT

Table 1: Comparison of Protein C and Protein S concentrations between Pregnancy-Loss, Normal Pregnancy, and Non-pregnant Subjects.

Parameter		Pregnancy Loss (n=70)	Normal Pregnancy (n=30)	Non-pregnant (n=30)	*F*	*P*
Protein S (µg/ml)	*Mdn*	9.00	9.30	8.60	0.27	0.77
	P2.5 – P97.5	6.04 – 11.50	4.0 – 9.30	2.60 – 8.60		
Protein C (µg/ml)	*Mdn*	0.90	1.20	1.20	11.74	.00**
	P2.5 – P97.5	0.70 – 3.89	0.80 – 1.20	0.70 – 1.20		

Key: n = number of subjects, *Mdn* = median, P2.5 = 2.5th percentile, P97.5 = 97.5th percentile, *F* = F-test statistic, *p* = error probability, **Significant difference observed at $p < .01$, using ANOVA.

Table 2: Post hoc Testing of Protein C and Protein S concentrations between Pregnancy-Loss, Normal Pregnancy, and Non-pregnant Subjects.

Immunological Parameter	*Post hoc* Pair	95% CI	*P*
Protein S (µg/ml)	PL vs Norm P.	-0.08 – 0.06	.91
	Norm P. vs Non-P.	-0.08 – 0.12	.86
	PL vs Non-P	-0.06 – 0.08	.95
Protein C (µg/ml)	PL vs Norm P.	-0.25 – -0.05	.00**
	Norm P. vs Non-P.	-0.17 – 0.12	.92
	PL vs Non-P	-0.30 – -0.05	.00**

Key: PL = Pregnancy Loss, Norm P. = Normal Pregnancy, Non-P. = Non-pregnant, *p* = error probability, 95% CI = 95% Confidence Interval of the Difference, **Significant difference observed at $p \leq .01$, *Significant difference observed at $p \leq .05$. All *post hoc* testing were done using Turkey HSD and Games-Howell methods as applicable.

6.0 DISCUSION

Heritable thrombotic states have been linked to pregnancy loss, most especially RPL (11,12,13). RPL is thought to have multiple aetiologies including chromosomal abnormalities, immune dysfunction, thrombophilic disorders and hormonal disturbances (14). Thrombophilia is genetical predisposition to thrombosis and describes a tendency of increased blood clotting (15, 16). The changes that occur during pregnancy regarding the haemostatic balance reflect a thrombophilic state and serves to arrest any potential haemostatic challenge that may arise during child birth (17). In addition, pregnant women carrying further thrombotic risk factors like inherited thrombophilia, logically have an increased risk towards thrombotic events during the course of gestation e.g. arterial and/or venous thrombosis at the site of implantation or in the placental blood vessels (18), thromboembolic phenomena and pregnancy loss (19). It has been reported that defects are noticed in the haemostatic variables just before miscarriages take place

(6). Protein C and Protein S are natural anticoagulants (20), and their deficiencies form part of inherited thrombophilias (16).

In this study, Protein S and Protein C assays were compared between pregnancy-loss, normal pregnancy, and non-pregnant control subjects as shown in Table1 and Table 2. Of these, Protein S showed non-significance ($p > .05$) when compared across all groups. But the Protein C concentration for the pregnancy-loss subjects (*Mdn*: 0.90 µg/ml, p2.5 – p97.5: 0.70 – 3.89 µg/ml) was significantly lower than that of the normal pregnancy (*Mdn*: 1.20 µg/ml, p2.5 – p97.5: 0.80 – 1.20 µg/ml) and non-pregnant control subjects (*Mdn*: 1.20 µg/ml, p2.5 – p97.5: 0.70 – 1.20 µg/ml) ($p< .01$ respectively). However Protein C assay showed no significant difference between the normal pregnancy and non-pregnant control groups ($p> .05$).

Several studies have reported a positive correlation between RPL and heritable thrombotic states (12; 13). However the existence of conflicting data in literature regarding any association between miscarriage on one hand and Protein C as well as Protein S on the other hand is acknowledged (9). In their work, Mekaj *et al*, in 2015 found that Protein C and Protein S are causal factors for miscarriage. In contrast with our work, Parand *et al*, in 2013, found a higher frequency of Protein S deficiency in patients with RPL compared with controls but found that the frequency of Protein C, was not significantly different between patients with RPL and healthy women. Singla and Jain in 2018, averred that while both Protein C and Protein S deficiencies are associated with increased tendency to recurrent pregnancy losses, the association between Protein S and RPL appears stronger than the association between recurrent pregnancy losses and Protein C. However our finding shows the reverse. Protein C and Protein S deficiencies are inherited independently (21). Combined deficiencies are rare and come with increased and earlier onset of risk of thrombosis (21). In their own work, Ogasarawa *et al,* (22) found no

differences in the subsequent miscarriage rates between patients with abnormal and normal values of Protein C and Protein S. In the majority of women with inherited thrombophilia, pregnancy is uneventful (20). However the risks for miscarriage and some other complications of pregnancy are increased in carriers of thrombophilia. Why certain women with thrombophilia present with gestational vascular complications is still unknown but may be related to a combined effect with another inherited or acquired prothrombotic risk either systemic or localised at the level of the placenta (23) which leads to compromised foetal viability and loss.

7.0 Conclusion

The physiological changes that occur in pregnancy tends to tilt the haemostatic balance towards thrombosis, making pregnancy a hypercoagulable state. Hypercoagulation has been linked to adverse pregnancy outcomes including pregnancy loss. The thrombotic state is worsened by genetically determined deficiencies in the concentration of circulating levels of anticoagulating agents, leading to significant increase in adverse pregnancy outcomes.

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