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Rania Judi Taref[1] ✉, Maysoon Sharief[2]
[1] Maternal and Child Hospital, Basrah, Iraq
[2] College of Medicine, University of Basrah, Basrah, Iraq

The Association Between Serum Level of Protein C and Protein S and Antithrombin III Level in Women with Recurrent Miscarriage

Conflict of interest: nothing to declare.
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Contacts: maysoonsharief60@yahoo.com

Abstract

Introduction. Several studies have reported that thrombophilia is responsible for recurrent pregnancy loss (RPL).
Purpose. The aim of this study was to evaluate the prevalence and role of inherited thrombophilia in early pregnancy loss, specifically in the first trimester.
Materials and methods. A total of 104 women (patients) with a history of 2 or more miscarriages during the 1st trimester of pregnancy and 110 women (controls) who had experienced 2 or more births without a miscarriage were included in this study. In both groups, we determined the biological activities of antithrombin III (ATIII) and protein C (PC) using the chromogenic method and the biological activity of protein S (PS) were examined using a clotting method.
Results. In the patient group, deficiencies of ATIII, PC, and PS were detected in 3 (2.88%), 4 (3.85%), and 6 (5.77%) cases, respectively. In the control group, ATIII (0%) deficiencies were not detected, and deficiencies for PC (0.9%) and PS (0.9%) were each detected in 1 patient. APCR was detected in 9 patients (8.65%) and 4 control subjects (3.63%).
Conclusion. Based on our results, we can conclude that thrombophilia is a causal factor for miscarriages in the 1st trimester of pregnancy, although there are the conflicting data in the literature.
Keywords: antithrombin III, miscarriage, protein C, protein S, thrombophilia

■ INTRODUCTION

Based on a global assessment, it is estimated that there are around 23 million cases of spontaneous pregnancy loss on an annual basis [1]. One to two percent of pregnancies in the mid trimester are ended before the 24-week stage [2]. The significant importance for women is of concern and therefore, it is essential to conduct a thorough examination for every instance of pregnancy loss in order to ascertain the precise etiological reasons and associated risk factors [3].

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Mutations occurring in the genes associated with (also known as the prothrombin gene), and genetic conditions that contribute to hereditary thrombophilia and spontaneous miscarriages [4]. Furthermore, deficiencies in antithrombin III (ATIII), protein C (PC), or protein S (PS) are significant factors contributing to thrombophilia, a condition characterized by an increased susceptibility to venous thromboembolisms during pregnancy [5].

Thrombophilia is a group of conditions that contribute to an increased susceptibility to blood hypercoagulability and subsequently lead to the occurrence of thrombosis especially the uteroplacental circulation. Thrombophilia may arise for several reasons, including the presence of the prothrombin mutation deficiencies [6]. The hereditary factor V Leiden is a prevalent form of thrombophilia that is often linked to recurrent pregnancy loss (RPL) [7].

Antithrombin (AT) is a small protein molecule that serves as an inhibitor for a range of enzymes within the coagulation cascade. The presence of an antithrombin III deficiency is a potential risk factor for recurrent pregnancy loss (RPL). Protein C and S deficiency are uncommon, hereditary conditions that have a higher risk of developing thrombophilia and eventually placental insufficiency and miscarriage. However, this hypercoagulation may result in problems for both the mother and the foetus, including pre-eclampsia, placental abruption, foetal growth restrictions, and recurrent pregnancy loss (RPL) [8].

So, relationship between blood disease and repeated abortion remains questionable. Therefore, the aim of this study is to evaluate the prevalence and the role of inherited thrombophilia in recurrent pregnancy loss.

■ MATERIALS AND METHODS

This is case control study which was done in Maternity and Child Hospital and include 150 women with history of 2 or more miscarriages in the 1st trimester as patient group. Second group include females had one or more live births. Both groups were matched by age. Females with history of spontaneous abortions in the first trimester of pregnancy should had the following investigations like chromosomal study, TORCH infections, immunological test, and endocrine assessment like TSH, T3, random blood sugars and hyperprolactinemia. The exclusion criterion for both groups was current pregnancy. In both groups, we determined the biological activities of antithrombin III (ATIII) and protein C (PC) and Factor V Leiden (FVL), using the chromogenic method and the biological activity of protein S (PS) will be examined using a clotting method.

Blood samples were collected with 3.2% sodium citrate in a 9:1 blood to citrate ratio. They were centrifuged at 2000 rpm for 15 min to separate the plasma, which was quick-frozen and maintained at –35 °C until testing. The study was approved by the Ethical Committee of the Iraqi Board for Medical Specialization. Written informed consent before inclusion in the study were provided for all patients as well as the control groups.

Full screening tests for hemostasis: The Prothrombin Time, Activated Partial Thromboplastin Time, and Thrombin Time (ATIII) were performed for all subjects.

Statistical analysis

The odds ratio (OR) and 95% confidence interval were estimated separately for each inhibitor, and APCR was analyzed with the Chi-square test in Instate 3.0 software (GraphPad Software, San Diego, CA, USA). Data were compared by using the t-test and the difference was significant if $P<0.05$.

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RESULTS

In this study 60 women (the patient group) had 2 abortions before 12 weeks pregnancy and in 70 females (the control) with high parity. Mean age for patients was 30.3±23 years with a range of 20–42, in comparison to the mean age of the control women which was 31.5±21 years with a range of 21–40. Among patients' group, 34 (56%) females had 2 abortions, 15 (31.60%) had 3 abortions and 11 (10.53%) with more than 3 abortions (Table 1).

Table 1
Rates for abortions

No.	Group (n=60)	%
2	34	56
3	15	31.6
>3	11	10.53
1st trimester abortion	39	
2nd trimester abortion	21	

The estimation for antithrombin, protein c, protein S, deficiencies showed in Table 2. Among patients, the estimation of antithrombin, protein C, protein S decrease and showed as: 2.88% (3/60), 3.85% (4/60), 5.77% (6/60), and 2.88% (3/60) respectively. While among the control females, antithrombin decreased to 0% (0/104) in addition to 0.9% in both protein C and protein S (1/60). Values reported for antithrombin, protein C, and protein S decrease were different between patients and control women (Table 2).

Table 2
The decreased values for antithrombin III, protein C and protein S in the women with disease and in the non-disease

Variables	Disease females (n=60)	Non diseases (n=70)	Odds ratio (95% CI)	P value
ATIII	3	0	6.67 (0.78–55)	0.05
Protein C	4	1	4.36 (0.48–39.7)	0.04
Protein S	9	1	6.67 (0.78–56)	0.04
Factor V Leiden (FVL)	6	2	2.51 (0.27–8.41)	0.05

There were 71.1% (39) of patients had abortions occurred in the first trimester (<13 weeks of gestation), and 13 patients (28.9%) had abortions occurred in the second trimester (≥13weeks of gestation). There was significant association of low protein C with the abortion in the second trimester (P<0.01 respectively) (Table 3).

Table 3
The association of gestational age with protein C & protein S

Variable	Gestational age <13	Gestational age >13	P value
Protein C	0	3	0.01
Protein S	1	5	0.01
Protein S&C	4	3	0.1

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■ 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 one of the causes of adverse pregnancy outcomes, including recurrent pregnancy loss, still-birth, severe pre-eclampsia, placental abruption, and intrauterine growth restriction [9]. However, deficiencies of the natural anticoagulant like protein C, S and antithrombin occur much less than 1% to 2% in RPL patients. The incidence of hereditary hemophilia in the community is low in comparison with women with RPL [10].

In this study, most of the pregnancy loss cases who had Protein C and protein S deficiency occurred during 1st trimmest. In contrast, Alshammary et al. [11] has found Protein C and protein S deficiency had a significant association with 2nd-trimester pregnancy loss.

The present study as well as Jyotsna et al. [12] have found a significant association in the low mean value of protein C in the patient group compared with the control group. They believed that protein C deficiency may increase the risk of pregnancy loss but the incidence of miscarriage and stillbirth in women with severe protein C deficiency is unknown. In contrast, other studies have reported that protein C had no significant difference between patients and controls [13, 14].

A significant association of RPL with protein S deficiency was found in the present study, which is in agreement with others [15, 16]. Moreover, PS deficiency was also found associated with late-term fetal loss [14]. Moreover, In the European Prospective Cohort on thrombophilia study, which involved 1384 women, authors explained that only the levels of free protein S fell significantly during the 1st and 2nd trimesters of pregnancy, but there is no further decrease during the third trimester. Protein S deficiency is more commonly identified than protein C deficiency, constituting an overall 15-fold increased risk of recurrent pregnancy loss. In addition, they have observed that there are 2 S proteins; type I protein S deficiency is a reduction in the level of free and total protein S. Type III deficiency is a reduction in the level of free protein S only. Type II deficiency is a reduction in the cofactor activity of protein S, with normal antigenic levels. Age affects total protein S but not free protein S levels [17, 18].

A meta-analysis studies have established that protein C deficiency has been associated with an increased risk of second-trimester miscarriage and stillbirth [18, 19].

The prevalence of protein C, and protein S deficiencies are higher in patients with thromboembolic events compared with those in the healthy population because deficiency of protein C and protein S results in the loss of these natural anticoagulant properties, resulting in unchecked thrombin generation and thromboembolism [20].

Since, the present study found a considerable number of protein C and protein S deficiencies among RPL pregnant women, therefore, screening and subsequent therapy for thrombophilia is required among pregnant women coming for check-ups 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 [21]. Other reports [22, 23] have stated that the combination of more than one inherited thrombophilia gene defect has been recognized as a cause of early and late RPL. Despite that, the incidence of thrombophilia is not clear [24]. Moreover, Sibai et al. [25] found that

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approximately 0.2–1% of patients with a combined deficiency of PC and PS have normal pregnancy outcomes.

It has been recorder in a study involved 10,000 blood donors, that the prevalence of type-1 antithrombin deficiency was 0.02% [20], whereas the prevalence of heterozygote PC deficiency in the general population is 0.3% (approximately 10 times more than the prevalence of AT) [14] which indicated that risk of pregnancy complication is more with protein C than with antithrombin III.

ATIII deficiency has long been associated with a significant thrombotic tendency throughout gestation. But it was demonstrated only recently that hereditary ATIII deficiency is associated with miscarriages and stillbirth. The risks are greatest for women with ATIII deficiency who have ATIII, PC, PS, and PC resistance [26]. Although we found that the overall percentages of cases with ATIII, PC, and PS deficiencies were higher in the patient group than in the control group, we are not certain whether the recurrent miscarriages in these patients were caused by a deficiency of ATIII, PC, or PS because approximately 70% of 1st-trimester spontaneous abortions (12 weeks) result from chromosomal disorders [27]. Additionally, a significant number of early pregnancy spontaneous abortions result from the presence of antiphospholipid antibodies, anatomical abnormalities, specific infections, and endocrine disorders [11, 12, 28]. There are contradictory hypotheses concerning the role of thrombophilia as a cause of abortions. According to many authors of RLP, abortions do not occur in all cases of thrombophilia [4].

There is a consensus regarding the association between thrombophilia and mid-trimester losses, but the link with 1st-trimester miscarriages remains controversial. When compared to patients without thromboembolic events, individuals with these conditions had higher rates of antithrombin, protein C, and protein S deficits. According to literature research, it has been shown that patients with thromboembolic events have, whereas 0.5–1% of these individuals have antithrombin (AT) deficiency. On the other hand, both protein C (PC) and protein S (PS) deficiencies have a comparable incidence of 3% among the same group of patients [4].

■ CONCLUSION

Even there is no clear causal relationship could be drawn, protein C and S deficiency could be one of the causative factors for recurrent pregnancy loss. Thus, defect in clotting factors is needed in the diagnosis of 1st and 2nd trimesters and should be done in every woman with unexplained pregnancy loss should be tested for defect in clotting factors.

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