

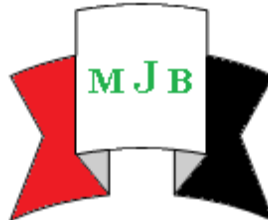
Deficiency of Protein C and Protein S in Recurrent Pregnancy Loss

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Abstract

Recurrent pregnancy loss (RPL) is a common disorder that affects around 3-5% of pregnant women. It has different causes and in about 50% it is of unknown etiology. Normal pregnancy is associated with increased procoagulants, decreased fibrinolysis and decreased anticoagulants to maintain placental hemostasis during pregnancy. However, hypercoagulability or 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, hypo perfusion, fetal death and fetal loss.

The aim of the study is to identify protein C and protein S deficiency in women with recurrent pregnancy loss. 90 women were involved in the study, 45 of them have three or more miscarriages in the first and second trimester considered as patient group, 45 healthy women at time of full term delivery with at least one alive child as control group. Full history was taken from patients and controls. Blood investigations were done for protein C and protein S levels by using ELISA (enzyme linked immune sorbent assay). Platelets count was performed by auto analyzer (Ruby).

The results showed a significant relation of low protein S with recurrent miscarriage ($P = 0.002$) $OR = 2.250$ (95% C.I. 1.764-2.870), while the relation of low protein C with recurrent miscarriage was not significant ($P > 0.05$). There was a significant association of low protein S and low protein C with the abortion occurred in the second trimester ($P < 0.05$). Positive family history for thrombosis was significantly associated with recurrent abortion ($p < 0.05$). Platelets count has non-significant association with recurrent miscarriage.

There is a significant association of protein S deficiency with recurrent miscarriage especially in the miscarriages occurring in the second trimester. The positive family history of thrombosis is considered a risk factor for recurrent miscarriage. Protein C deficiency had no statistical significance.

Keywords: Protein C, protein S, recurrent pregnancy loss.

الخلاصة

الاجهاض المتكرر هو حالة شائعة يصيب حوالي ٣-٥% من الحوامل. هذه الحالة متعددة الاسباب وتبقى في معظمها غير معروفة. يرافق الحمل الطبيعي تغيرات فسلجية في الية التخثر لغرض الحفاظ على استقرار الدورة الدموية في المشيمة خلال الحمل، حيث تزداد العوامل المولدة للتخثر، وتقل تراكيز العوامل المضادة للتخثر ولكن في بعض الحالات قد تؤدي هذه التغيرات الى فرط التخثر، خصوصا في حالة وجود خلل في بعض عوامل موانع التخثر الطبيعية. بروتين C و بروتين S احد اهم موانع التخثر الطبيعية التي قد يسبب نقصها لحالة فرط التخثر في الاوعية الدموية للمشيمة واحتشاء المشيمة وبالتالي موت الجنين وفقدانه.

كان الهدف تمييز نقص بروتين C و بروتين S في الاجهاض المتكرر. اجريت الدراسة على تسعين امرأة، خمس وأربعين منهن لديهن ثلاثة أو أكثر من حالات الإجهاض في الأشهر الثلاثة الأولى والثانية كمجموعة المرضى، خمس وأربعين امرأة سليمة في وقت الولادة

التامة ولديها على الأقل طفلا واحدا على قيد الحياة كمجموعة السيطرة. فحوصات الدم تشمل قياس مستوى بروتين C وبروتين S باستخدام الانزيم المرتبط بالتحليل المناعي، الصفائح الدموية باستخدام المحلل الآلي (Ruby). أظهرت النتائج وجود علاقة ذات دلالة بين انخفاض بروتين S مع النساء اللاتي لديهن إجهاض متكرر ($P=0.002$). بينما نقص بروتين C لم تكن له علاقة ذات دلالة ($P>0.05$). وأظهرت النتائج وجود علاقة ذات دلالة بين انخفاض مستوى بروتين S وانخفاض مستوى بروتين C مع الإجهاض الحاصل في الثلث الثاني من الحمل ($P<0.05$). بالنسبة للتاريخ الإيجابي العائلي للتخثر كانت له علاقة ذات دلالة مع الإجهاض المتكرر ($P<0.05$) وكان هناك ارتباط غير مهم بين الصفائح الدموية والإجهاض المتكرر. وجود ارتباط ملحوظ بين نقص بروتين S والإجهاض المتكرر والإصابة هي أكثر في الإجهاض الذي يحدث في الثلث الثاني من الحمل. التاريخ الإيجابي العائلي للتخثر هو عامل خطر للإجهاض المتكرر. نقص بروتين C ليس له علاقة ذات دلالة احصائية.

Introduction

Recurrent pregnancy loss or miscarriage can be defined as the loss of three or more successive pregnancies before viability and includes all pregnancy losses from the time of conception until 24 weeks of gestation [1, 2]. Pregnancy loss is divided into biochemical and clinical loss. The biochemical loss is a transient positive pregnancy test without ultrasonic visualization of the pregnancy [3]. The term clinical miscarriage is used when ultrasound examination or histological evidence has confirmed that an intrauterine pregnancy has existed. Clinical miscarriages can be subdivided into early clinical pregnancy losses (which is most common) that occur before the twelfth week of gestation, and late clinical pregnancy losses (which constitute small proportion of pregnancy losses) that occur in the twelfth week to twenty first week of gestation [4,5]. Despite a wide range of investigations, the cause of recurrent miscarriage remains unknown (idiopathic) in more than 50% of cases [6, 7], but several hypotheses have been proposed [8] including the following: genetic disorder, uterine anatomic malformation, immunological risk factors, infection, endocrine disorders and thrombophilia. Thrombophilia is a term which describes the increased tendency of excessive blood clotting due to inherited or acquired causes. It may occur during

pregnancy, where there is an increase in most clotting factors and decreased levels of anticoagulant factors with reduced fibrinolytic activity. This can result in placental insufficiency and abortion [9,10]. Factors associated with thrombophilia include Factor V Leiden (FVL) mutation associated activated Protein C Resistance (APCR), prothrombin G20210A gene mutation, anti-thrombin III deficiency, protein S deficiency, protein C deficiency and hyperhomocysteinaemia (methylenetetrahydrofolate reductase mutation, MTHFR) stasis and endothelial cell dysfunction[11].

Protein C (PC) is a vitamin K-dependent glycoprotein activated by thrombin-thrombomodulin complex on the surface of endothelial cells [12]. Protein C is a precursor of the serine protease, activated protein C (APC) [13]. In the presence of protein S which is a cofactor for APC, phospholipids and calcium, APC inactivates membrane bound FVa and FVIIIa so results in attenuation of thrombin generation which leads to inhibit clot formation [14]. Protein C deficiency is generally subdivided into two types: type I (quantitative deficiency) decreased levels of protein C. and type II (qualitative deficiency) decreased functional activity of protein C. Most patients with PC deficiency have type I deficiency, while Type II deficiency is observed in 10-15% of the cases [15, 16]. Protein C

deficiency is inherited as autosomal dominant disorders and, in most cases, derived from heterozygous mutations [17]. Acquired PC deficiency can develop with vitamin K deficiency, liver disease, treatment with vitamin K antagonists, severe and chronic inflammation, autoimmune syndromes, nephritic syndrome, or disseminated intravascular coagulation (DIC) [18]. 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 [19]. There are two main types of assays: activity assay (qualitative) which is either clotting time-based assay or chromogenic by spectrophotometer [20]. 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 [18]. Genetic testing by DNA sequencing is indicated if the results of functional and antigenic assays do not approve the diagnosis clearly [21].

Protein S (PS) is a vitamin K-dependent glycoprotein which acts as natural anticoagulant [22]. Protein S exists in plasma both free (40%) and bound to the complement C4b binding protein (60%) [23]. Protein S has both APC-dependent and independent anticoagulant properties and consequently is an important protector in controlling thrombin generation and fibrinolysis [24, 25]. The PS deficiency is identified more than PC deficiency and its prevalence has been assessed with about 0.5% in the healthy population and 2% to 12% of thrombophilic patients [25, 17]. Regarding pregnancy complications, a meta-analysis designated 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 [26].

Hereditary PS deficiency is an autosomal dominant disorder. Acquired deficiency of protein S is detected in several pathological conditions and could be associated with an increased risk of thrombosis. These include nephrotic syndrome, disseminated intravascular coagulation (DIC), liver disease, and the use of oral anticoagulants drugs [27, 28]. 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 [29]. If the results of functional and antigenic assays do not confirm the diagnosis, genetic testing is indicated [21].

Materials and Methods

90 women were involved in the present study, 45 of them have three or more miscarriages in the first trimester (32 women) and second trimester (13 women) considered as patient group, 45 healthy women at time of full term delivery with at least one alive child as the control group. The study is carried out through a period from November 2013 to August 2014 at two hospitals; Babylon Teaching Hospital for gynecology and Pediatric and Al-Hilla Teaching Hospital. Complete history was taken including medical history, surgical history, drug history and family history of thrombosis for patient and control groups with exclusion criteria for other causes of pregnancy loss. The blood samples that obtained from the patient and control groups consist of 2ml of blood drawn in a tube contain ethylene diamine tetra acetate (EDTA) as anti-coagulants to prevent clotting of blood to be used for platelets count by auto analyzer (CELL-DYN Ruby, Abbotts). 1.8mL of blood drawn in tube contain 0.2mL of 3.8% trisodium citrate solution,

mixed gently and centrifuging for 15 minutes at 1500-2000 g for plasma preparation and then take the plasma into plane tube and preserved in -20°C for testing the levels of protein C and protein S by ELISA reader (BioTek, U.S.A) using kit of Aeskulisa Diagnostics. Statistical analysis was carried out using SPSS version 20. Categorical variables were presented as frequencies and percentages. Pearson's chi square (χ^2) test and fisher exact test were used to find the association between the

categorical variables. A p -value of ≤ 0.05 was considered as significant.

Results

The results showed a significant relation of low protein S with recurrent miscarriages ($p=0.002$) OR=2.250 (95% C.I. 1.764-2.870) as shown in table1. Patients with low protein S were 2 times more likely to have recurrent abortion. The relation of protein C and platelets were not significant with recurrent miscarriage ($P>0.05$).

Table 1: Association of protein C, S and platelets with patients of recurrent abortion and control groups

Variable	Recurrent Abortion		χ^2	<i>P</i> values	Odds Ratio 95% C.I
	Patients (%)	Control (%)			
Protein C**					
Low < 70	3 (6.7%)	0 (0.0%)	3.103	0.242 ^a	2.071 (1.667-2.575)
Normal ≥ 70	42 (93.3%)	45(100.0)			
Protein S**					
Low < 50	9 (20.0%)	0 (0.0%)	10.000	0.002*	2.250 (1.764-2.870)
Normal ≥ 50	36 (80.0%)	45(100.0)			
Protein C and S					
Low	2 (4.4%)	0 (0.0%)	2.045	0.494 ^a	2.047 (1.65-2.534)
Normal	43 (95.6%)	45(100.0)			
Platelet					
Low < 150	0 (0.0%)	1 (2.2%)	1.011	0.315 ^a	2.023 (1.639-2.496)
150-400	45 (100.0%)	44(97.8%)			

*p value ≤ 0.05 is significant. ^a: Fisher Exact test

**According to the AESKULISA Diagnostics kit using in the study, normal rang for protein C is (70_140%), and for protein S is (50_130%).

There were 71.1% (32/45) 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 (≥ 13 weeks of gestation). There was significant association of low protein S

and low protein C with the abortion occurred in the second trimester ($P<0.05$, $P=0.016$ respectively), however, no significant association was seen in combined proteins(C and S) deficiencies with gestational age. (Table 2)

Table 2: Association of gestational age with protein C, S and combined

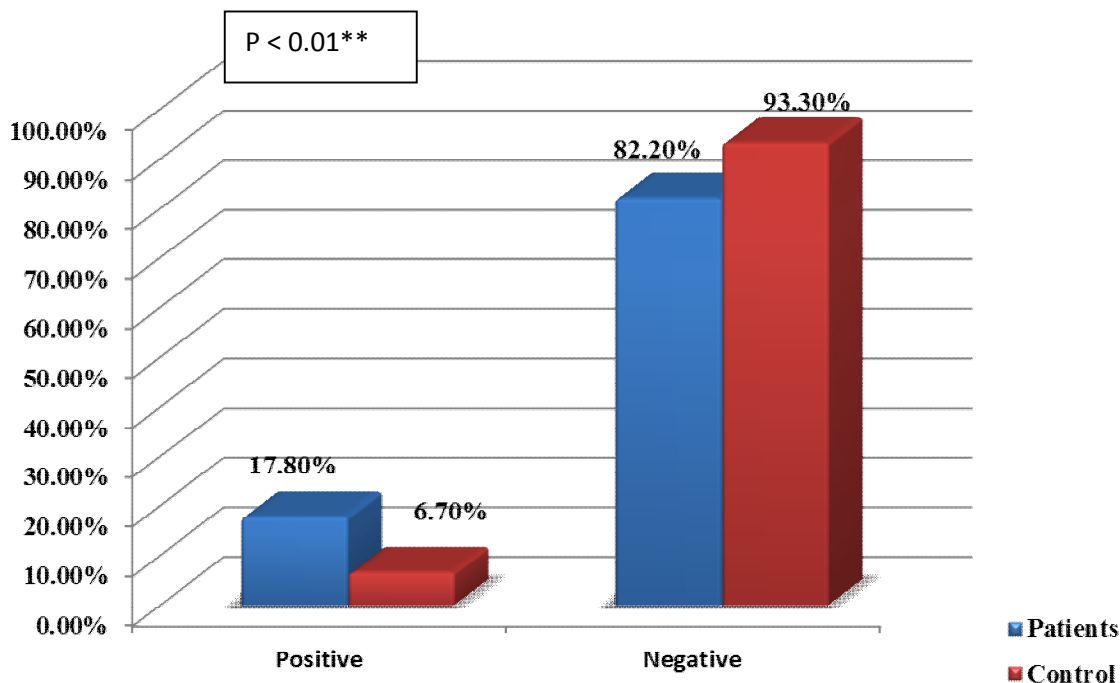
Variable	Gestational Age		χ^2	<i>P</i> values
	< 13 weeks N (%)	≥13 weeks N (%)		
Protein C				
Low < 70	0 (0.0)	3 (23.1%)	7.912	0.016^{*a}
Normal ≥ 70	32 (100.0%)	10 (76.9%)		
Protein S				
Low < 50	1 (3.1%)	8 (61.5%)	18.715	0.001[*]
Normal ≥ 50	31 (96.9%)	5 (38.5%)		
Protein C and S				
Low	0 (0.0)	2 (15.4)	5.756	0.067 ^a
Normal	32 (100.0%)	11 (84.6)		

*p value ≤ 0.05 is significant

^a: Fisher Exact test

This study demonstrated that 17.8% of the patients had positive family history of thrombosis while in the control group the percentage of positive family history of

thrombosis was 6.7 %. So there is significant relationship ($p < 0.01$) between positive family history of thrombosis and recurrent abortions (Figure 1).



**p value < 0.01 is significant

Figure (1): Positive family history of thrombosis in patients and control groups.

Discussion

In this study a significant association was found between RPL and protein S deficiency ($p=0.002$), this is in agreement with many previous studies [30, 31, 32, 33, 34, 35, 36]. Rey *et al.*, (2003) in meta-analysis consisting of 31 studies, established the association of PS deficiency with RPL (OR 14.72, 95% CI 0.99-218.01)[32].

Protein C had no significant difference between patients and controls, this is in agreement with Vora *et al.* (2008) [33] and Parand *et al.* (2013) [36]. Rey *et al.* (2003) [32] excluded PC and antithrombin (AT) deficiencies as thrombophilia risk factors for pregnancy loss. Yamada *et al.* (2001) [37] could not find an increased incidence of protein C deficiency in patient with RPL. While Jyotsna *et al.* (2011) [34] found a significant association in the mean value of protein C in patient group comparing with control group.

In the combined defect of both protein C and S which was documented in (4.4%) of the patients, there was no statistical significant comparing with control group. While the study of Cosmi B. *et al.* (2013) [38] and Yildiz *et al.* (2012) [39] 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 combined thrombophilia is not clear [9].

Protein C and protein S deficiency had a significant association with second trimester pregnancy loss. This is consistent with the meta-analysis of 31 retrospective study [32] which had shown that the relationship of thrombophilia with late pregnancy loss is stronger than early miscarriages. Alonso *et al.* (2002)[40] also stated that the prevalence of thrombophilia was more prominent in second trimester, also in

agreement with studies done by (Rey and Regan, 2000)[41] which established that protein C deficiency has been associated with an increased risk of second-trimester miscarriage and stillbirth. However, a systemic review of 25 studies by Robertson *et al.* (2005) [42] revealed a positive association between early pregnancy loss and thrombophilia, which disagree with our study.

Conclusions

There is a significant association of protein S deficiency with recurrent miscarriage and the incidence is more in the miscarriage occurring in the second trimester. Positive family history of thrombosis is considering a risk factor for recurrent miscarriage.

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