

RESEARCH ARTICLE

Study the predictable correlation between recurrent abortion with the level of proteins S, C, age and blood groups in women in AL-Najaf governorate, Iraq

Maysoon Khudair. AL-Hadrawi¹, Ahmed Abdul Hasan Mohsin², Kais K.AL- Hadrawai³, Noor Ismeal Nasser⁴, Ali k. Metiab

¹Asisst. Prof, Department of Medical Laboratory Techniques, Kufa, Technical Institute, Al-Furat Al-Awsat Technical University 31001, Kufa, Al-Najaf, Iraq.

²Asisst. Lecturer Department of Community Health, Kufa, Technical Institute, Al-Furat Al-Awsat Technical University, Kufa, Al-Najaf, Iraq.

³Asisst. Lecturer Master of Bacteriology, Radiology Techniques Department, College of Medical Technology. The Islamic University, Najaf, Iraq.

⁴Assist. Prof. Department of Medical Laboratory Techniques, Kufa Technical Institute, Al-Furat Al-Awsat Technical University, Kufa, Al-Najaf, Iraq.

⁵Department of medical Laboratory Techniques, Kufa Technical Institute, Al-Furat Al-Awsat Technical University, Kufa, Al-Najaf, Iraq.

*Corresponding Author E-mail: kin.msn@atu.edu.iq

ABSTRACT:

Context: Repeated pregnancy loss is causing great distress to couples and undermining their confidence in achieving reproductive success in the future. A doctor can usually diagnose the cause and manage it directly, but in some cases, the cause is not known. **Aim:** This study aims to investigate whether there is a correlation between recurrent miscarriage and the level of protein S, C, age and blood groups in women who have experienced recurrent miscarriage without identifying a cause for this condition. **Material and methods:** one hundred twenty from infected women by recurrent abortion for unknown causes were classified according to age groups. Diagnosed blood group by using a specific kit for Biorex diagnostic Limited (the United Kingdom, 2016). The concentration of protein S and C were calculated by using the equipment produced by the AESKU.DIAGNOSTIC (Germany) **Statistical analysis:** the statistical analyses of the data were performed by using the (SPSS) 20.0 software package. We used the following analyses, frequencies, percentage, mean, stander diversion and Pearson correlation to identify the potential correlation between the miscarriage and the level of protein S, C, age and blood groups, the p-value of less than 0.05 was considered significant. **Results:** The study showed that out of a total of 120 women, who suffer from recurrent abortion, 82/120 have a decrease in the level of C protein, 96/120 have a decrease in the level of S protein and 74/120 have low level of both proteins C&S. The results were statistically significant (P=0.000, P=0.000 and p=0.000), There was an intermediate direct correlation between recurrent abortion and the level of proteins C and S ($r_p = 0.545$, $r_p = 0.643$), also there was correlation between the level of protein S and the level of protein C ($r_p = 0.713$). It was also found that there was no correlation between age and blood groups with cases of recurrent abortion in women the results was statistically non-significant ($r_p = -0.084$, $p = 0.279$; $r_p = 0.108$, $p = 0.159$). **Conclusion:** There are several reasons for the occurrence of recurrent abortion, including what is caused by microbial infections, or it may be caused by certain abnormalities in the reproductive system, etc., also appear to the results of this research, the low level of one of the proteins S, C or both may be a cause in the case of recurrent abortion.

KEYWORDS: Protein S, protein C, recurrent abortion, Blood group.

INTRODUCTION:

Unprompted pregnancy loss is a common occurrence. Whereas approximately 15% of all clinically documented pregnancies product in unprompted loss, there are many more pregnancies that fail before being clinically recognized¹.

Unprompted pregnancy loss can be physically and emotionally strenuous for couples, particularly when faced with recurrent losses. it referred to as *recurrent abortion*, is traditionally defined as 3 repeated pregnancy losses before to 20 weeks from the last menstrual period. Though, epidemiologic studies have revealed that (1 – 2) % of women suffer recurrent pregnancy loss².

There are many causes of repeated miscarriages, including viral and parasitic causes, as well as physiological causes. Common causes are the imbalance in the concentration of protein C and S. Proteins C and S are two vitamin K-dependent plasma proteins that work in concert as a natural anticoagulant system. Activated protein C is the proteolytic component of the complex and protein S serves as an activated protein C binding protein that is essential for assembly of the anticoagulant complex on cell surfaces The anticoagulant activity is expressed through the selective inactivation of Factors Va and VIIa³.

60% of protein S in the plasma is known active and remains bound to a binding protein. Deficiency of Protein S is binding with an increased risk of thrombosis. Both quantitative and qualitative abnormalities of protein S have been identified. also, vitamin K deficiency can be caused by Protein S deficiency, protein C (PC), or PS lacks are vital causes of thrombophilia, which are linked with a high risk of venous thromboembolism through pregnancy⁵. Women should be testing for thrombophilia because mysterious first abortion occurs in 10% of pregnancies^{5,6}, natural pregnancy consequence needs a system uteroplacental vascular system^{7,8}, and thrombosis in a decidual vessel may cause late growth retardation, death of fetal, and possibly abortion⁹, treatment with warfarin, liver disease, systemic sex hormone therapy, pregnancy, and infections by HIV. Mild protein S deficiency may occur in pregnancy and treatment needs consideration regarding bleeding complication¹⁰. The current study aimed to know if a relationship between the incidence of recurrent abortion of unknown cause and the level of protein C and S and if the factor of age and blood type has a relationship with that.

MATERIALS AND METHODS:

The place and period of study:

Al –Saader Medical city /infertility Unite and AL Hayat center in Najaf governorate, from March 2017 to April 2018

The study population:

Women infected by recurrent abortion attending to Al – Saader Medical city/infertility Unite and AL Hayat center in Najaf governorate, in addition to 50 healthy women as controls.

Inclusion criteria:

Women infected with recurrent abortion with unknown causes.

Exclusion criteria:

Women infected by recurrent abortion with known causes.

Study steps:

Clinical examination and some question after that taken sample for measure protein S and C and diagnosis of blood group.

Blood collection:

The study includes 120 infected women with recurrent abortion come to AL Hayat center and Infertility center in Al Sadar Medical city at the period from October 2016 to April 2017 in Al-Najaf governorate. Five ml from each of the blood samples were drawn from each patient and put in clean, dry and empty tubes with anticoagulant 3.8% sodium citrate. The samples were centrifugation at 3000rpm for 5 minutes to separate the plasma and collected in another sterile tubes, the sample of plasma should be used immediately, otherwise stored tightly closed at 2-8°C up to eight hours, or frozen at (-20°C) for a longer period until measure protein S and C. In same time, determent blood group for each patient.

Determent of blood group:

Diagnostic of blood group by used specific kit by Biorex diagnostic Limited (United Kingdom, 2016) with used slide and microplate test is based on the principle of agglutination between Red blood cells and antibodies (Anti –A, Anti-B, Anti-D)

Measurement of protein S:

The concentration of proteinS was calculated by using the equipment produced by the AESKU.DIAGNOSTIC (Germany). Before, we were starting in the work steps. The materials and samples should be prepared according to the dilution required. Steps procedure include.

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Control and sample:

Pipette 100µl of each patients diluted plasma into the designated microwells and pipette 100µl of each working dilution of the Reference plasma and the diluted controls into the designated wells. Then Incubate for 30 minutes at 20-26°C. after that wash 3x with 300 µl washing buffer (diluted 1:50)

Conjugate:

Pipette 100µl conjugate into each well then Incubate for 30 minutes at 20-26°C. After that, wash 3x with 300µl washing buffer (diluted 1:50).

Substrate:

pipette 100µl TMB substrate into each well, then Incubate for 30 minutes at 20-26°C protected from intense light.

Stop:

pipette 100µl stop solution into each well, using the same order as pipetting the substrate, then incubation 5 minutes minimum, after that Agitate plate carefully for 5 sec. Read absorbance at 450nm within 30 minutes.

Measurement of protein C:

The concentration of C protein was measured by using the same technique it's used to measure the protein S above.

Statistical Analysis:

Data analyzed by using SPSS version 18 software, the categorical changeable was given as percentage and frequencies, Pearson chi-square, P-value of ≤ 0.01 was reflected as statistically significant. Continuous changeable was given as mean, Pearson correlation, and coefficient standard deviations to determine the association between recurrent abortion and protein S, C, Age factors and Blood group.

RESULT:

The study included 120 women who suffer from recurrent abortion and different age groups ranging between (15-45) years compared with 50 healthy women. The Mean for study population was 3.45 and standard deviation 1.496. The study showed that the most affected groups are (30-34) years (34/120) women compared with the control group (11/ 50) women (Table 1).

Table (1): the distribution of study population according to age groups.

Study population	Age							Pearson correlation	Sig. (2-tailed)
	15-19	20-24	25-29	30-34	35-39	40-45	Total		
Patients	8	26	24	34	12	16	120	-.084-	.279
Controls	9	8	11	11	5	6	50		
Total	17	34	35	45	17	22	170		

Table (2): the distribution of the study population according to blood groups.

Study population	Blood group					Pearson correlation	Sig. (2-tailed)
	A	B	AB	O	Total		
Patients	46	20	8	46	120	.108	.159
Controls	10	13	6	21	50		
Total	56	33	14	67	170		

Table (3): the distribution of the study population according to the level of protein C.

Study population	Protein C				Pearson correlation	Sig. (2-tailed)
	less than70	70-140	more than140	Total		
Patients	82	34	4	120	.545**	.000
Controls	0	50	0	50		
Total	82	84	4	170		

** . Correlation is significant at the 0.01 level (2-tailed).

Table (4): the distribution of the study population according to the level of protein S.

Study population	Protein S				Pearson correlation	Sig. (2-tailed)
	less than 60	60-160	more than 160	Total		
Patients	96	20	4	120	.643**	.000
Controls	0	50	0	50		
Total	96	70	4	170		

** . Correlation is significant at the 0.01 level (2-tailed).

Table (5): the correlation between the level of protein C and S.

Protein S	Protein C				Pearson correlation	Sig. (2-tailed)
	less than70	70-140	more than140	Total		
less than 60	74	22	0	96	.713**	.000
60-160	8	62	0	70		
more than 160	0	0	4	4		
Total	82	84	4	170		

** . Correlation is significant at the 0.01 level (2-tailed).

Table (6): the mean and stendered deviation for the variables.

Variables	Age	protein C	protein S	Blood group	Study population
Mean	3.45	1.54	1.46	2.54	1.29
Std. Deviation	1.496	.545	.545	1.306	.457
Std. Error of Mean	.115	.042	.042	.100	.035

when studied the relationship blood group with recurrent abortion the Mean 2.54 and standard deviation 1.306, the study showed that most women who suffer from recurrent abortion are of blood type (O and A), where the number of infected women (46/120) for both compared with control group 21, 10/50 respectively (Table 2).

The study explained that the majority of women in the studied sample had a decrease in the level of protein C and S (82, 96)/120 women respectively compared with the control group (0). The Mean for protein C&S was (1.54 1.46) respectively and standard deviation 5.45 for both (Table 3 and 4). also the result showed the relation between protein C and S, where most infected women had low levels from C and S protein (74/120). (Table 5)

DISCUSSION:

Recurrent abortion is a tragic event for both partners its etiology often remains unsolved and its effects on 2–5% of the population¹¹. This is not the first study regarding unexplained recurrent abortion so, this study about one causes of recurrent abortion. pathway of protein c anticoagulant affected by inherited thrombotic disorders results from specific defects in the genes for plasma coagulant. Resistance to activated protein C is almost always caused by a single point mutation in the factor V gene. This mutation knew factor V Leiden and is also inherited autosomal dominant¹². Has substantiated the association between familial thrombophilia and increased fetal loss, particularly stillbirth. Deficiency Protein S inherited the case, this means one altered copy of the PROS1 gene in each cell is enough to cause mild protein S deficiency. The person who inherits two altered copies of this gene in each cell has severe protein S deficiency¹³.

Pregnancy is bind with a change in the coagulation and fibrinolytic system to reduce blood loss. Such as factor I, II, VII, VIII, IX, XII increase and protein S decrease and inhibit fibrinolysis. after diagnosis of protein S deficiency, therefore, must be taken aspirin until 10th weeks then taken LMWH at 20th weeks of gestation¹⁴.

The study showed that the protein S, C had a significant effect on recurrent abortion, where it was found that women who suffer from recurrent abortion in this study, also have decrease in the level of protein C and S, Where (82/120) of infected women have low C protein level and (96/120) have low S protein level. The correlation between protein C and S was significant at the 0.01

level, This agrees with which demonstrated the relationship between deficiency of protein S and repeated miscarriage, and agreement with^{15,16,17,18,19,20,21}, also this study agree with²², who find the relation between recurrent pregnancy loss and protein S and C deficiency in women in Babylon city.

The study showed that the age group (30-34) years are the most vulnerable to infection, but when analyzing the results statistically it doesn't show any significance, the age factor had no significant effect on the prevalence of recurrent abortion. Although there was a study conducted on Norwegian women, proved that the age group (30-45) year are more likely to have recurrent miscarriages²³.

Also, the results of the study showed that women have blood group O and A more than infection, but when analyzing the result, the blood group have not a significant effect on the prevalence of repeated miscarriage, Studies on this factor are few, as most studies revolve around blood groups and recurrent miscarriages due to the incompatibility of the blood group for the mother and father.

CONCLUSION:

There are several reasons for the occurrence of recurrent abortion, including what is caused by microbial infections, or it may be caused by certain abnormalities in the reproductive system, etc., also appear to the results of this research, the low level of one of the proteins S, C or both may be a cause in the case of recurrent abortion.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

REFERENCES:

1. Macklon NS. Geraedts JPM. Fauser BCJM. Conception to ongoing pregnancy: the “black box” of early pregnancy loss. Hum Reprod Update. 2002;8:333–343.
2. Stephenson MD. Frequency of factors associated with habitual abortion in 197 couples. Fertil Steril. 1996;66:24–29.
3. Marlar RA. Neumann A. Neonatal purpura fulminans due to homozygous protein C or protein S deficiencies. Semin Thromb Hemost 1990;16: 299–309.
4. Lim W. Eikelboom JW. Ginsberg JS. Inherited thrombophilia and pregnancy associated venous thromboembolism. *BMJ*. 2007;334:1318–21
5. Brenner B. Thrombophilia and pregnancy loss in first intended pregnancy. *J Thromb Haemost*. 2005;3:2176–7.
6. Micco PD. Uva MD. Recurrent pregnancy loss and thrombophilia. *Open Atheroscler Thromb J*. 2009;2:33–5.
7. Kujovich JL. Thrombophilia and pregnancy complications. *Am J*

- Obstet Gynecol.* 2004;191:412–24.
8. Preston FE, Rosendaal FR, Walker ID, Briët E, Berntorp E, Conard J, et al. Increased fetal loss in women with heritable thrombophilia. *Lancet.* 1996;348:913–6.
 9. Carp H, Salomon O, Seidman D, Dardik R, Rosenberg N, Inbal A. Prevalence of genetic markers for thrombophilia in recurrent pregnancy loss. *Hum Reprod.* 2002;17:1633–7.
 10. Bowles L, Cohen H. Inherited thrombophilias and anticoagulation in pregnancy. *Best Pract Res Clin Obstet Gynaecol* 2003;17: 471–89.
 11. Coulam CB, Clark DA, Beer AE, et al. Current clinical options for diagnosis and treatment of recurrent spontaneous abortion. *Am. J. Reprod. Immunol.* 1997; 38:57–74
 12. Preston FE, Rosendaal FR, Walker ID, et al. Increased fetal loss in women with heritable thrombophilia. *Lancet.* 1996; 348: 913–916.
 13. Marlar RA, Gausman JN. Protein S abnormalities: a diagnostic nightmare. *Am J Hematol* 2011; 86(5):418-21.
 14. Lalan DM, Jassawalla MJ, Bhaleraonm SA. Successful Pregnancy Outcome in a Case of Protein S Deficiency. *J Obstet Gynaecol India* 2012; 62(1): 21–22.
 15. Shinozaki N, Ebina Y, Deguchi M, Tanimura K, Morizane M, Yamada H. Protein S deficiency complicated pregnancy in women with recurrent pregnancy loss. *Gynecological Endocrinology.* 2016; 32(8): 672-674.
 16. Preston FE, Rosendaal FR, Walker I, Briët E, Berntorp E, et al. Increased fetal loss in women with heritable thrombophilia. *Lancet* 1996; 348: 913-916.
 17. Raziel AK, ornberg Y, Friedler S, Schachter M, Sela BA, et al. Hypercoagulable thrombophilic defects and hyperhomocysteinemia in patients with recurrent pregnancy loss. *Am J ReprodImmunol* 2001; 45: 65-71.
 18. Rey E, Kahn SR, Davi M, Shrie, I. Thrombophilic disorders and fetal loss: a meta-analysis. *Lancet* 2003; 361 :901-908.
 19. Vora S, Shetty S, Salvi V, Satoskar, Ghosh K. Thrombophilia and unexplained pregnancy loss in Indian patients. *Natl Med J India* 2008; 21:116-9.
 20. Jyotsna, Sharma S, Trivedi SS. Coagulation inhibitors and activated protein C resistance in recurrent pregnancy losses in Indian women. 2011; 54(4): 752-755.
 21. Hansda J, Roychowdhury J. Study of Thrombophilia in Recurrent Pregnancy Loss. *J Obstet Gynaecol India* 2012; 62(5): 536–540.
 22. Hussein N, Hadi M, Almosawi F. Deficiency of Protein C and Protein S in Recurrent Pregnancy Loss. 2015. *Babylon Journal*, vol.12, No .2, page:348-356.
 23. Magnu MC, Wilcox A J, Morken NH, Weinber CR, Häberg, SE. Role of maternal age and pregnancy history in risk of miscarriage: prospective register based study. *bmj*, 2019; 364.