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Thrombophilia in Pregnant Females with Recurrent Early Pregnancy Loss: A Systematic Review

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ABSTRACT

Recurrent pregnancy loss is a complex health challenge. It is estimated that around 5% of females could experience two or more consecutive miscarriages. The exact etiology of recurrent pregnancy loss remains questionable. In clinical practice, determine the role of Protein C and Protein S Levels in pregnant females with recurrent early pregnancy loss. From a public health perspective, even a low molecular weight heparin and progesterone in this high-risk group of women might be worthwhile. The initial screening using search strategy and keywords on PubMed, Medline, Cochrane database, and Google Scholar identified 792 articles. After the screening, 584 articles were excluded based on titles, repeated records, the different language used (other than English), unavailable full texts, etc. 208 abstract were examined and based on abstarct dissimillar containt 169 articles were excluded. 39 full articles were examined for enrollment in study and 29 articles were excluded based on dissimilarity study design, methodology and containt. So in present review article, 10 articles up-to-date articles were included. This review aimed to summarize and critically analyze assembled knowledge on the etiology, risk factors i.e. protein C and protein S levels and management approach to recurrent early pregnancy loss and also determine the role of Protein C and Protein S Levels in pregnant females with recurrent early pregnancy loss.

KEYWORDS: Protein C, Protein S, Pregnant Females, Recurrent pregnancy.

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INTRODUCTION

In 1976, Stenflo designated a bovine plasma vitamin-K-dependent protein that eluted in the third peak (peak C) from an anion exchange column as bovine “protein C.” Protein C was found to be identical to a previously identified anticoagulant factor, auto prothrombin II-A [1]. Biochemical studies showed that protein C is a zymogen that can be isolated from either bovine or human plasma and that can be converted to an anti coagulantly active serine protease by the action of thrombin. Protein C is *incorporated* in the liver. It is a vitamin K dependent zymogen of a serine protease (activated protein C). The molecular weight of Protein C is 62,000 daltons. Protein C functions as an anticoagulant by using protein S as a cofactor to degrade activated factors V and VIII. Protein C must first be converted to activated protein C by interacting with a thrombin-thrombomodulin composite on the surface of endothelial cells [2]. APC also has a profibrinolytic effect. This effect is because of its effect on TFAI. TFAI is activated by the a-thrombin– thrombomodulin complex and acts to prolong clot lysis. APC cleavage of factor Va inhibits a-thrombin generation, thus reducing a-thrombin– thrombomodulin–mediated TFAI activation. Protein C has a relatively short half-life of 6-8 hours. In the course of liver dysfunction it is one of the first hepatic coagulation proteins to decrease in quantity. Purified protein C concentrates have been successfully used to treat patients with thrombotic episodes [3].

In the year 1979, DiScipio and colleagues first purified Protein S from plasma andthey named it (protein S) in the honor of city of its discovery“Seattle, Washington”.The action of activated protein C (APC) on activated factor 5 (F5a) and the activated factor 8 (F8a) is facilitated by Protein C.The inability to control coagulation, resulting in the excessive formation of blood clots (thrombophilia) and venous thromboembolism (VTE) is characterised by the the deficiency of protein S [4]. It is a vitamin-K-dependent glycoprotein that is synthesized by hepatocytes, neuroblastoma cells, kidney cells, testis, megakaryocytes, and endothelial cells and is found in platelet α granules. Protein S is inducible by IL-4 in T cells.Protein S deficiency may be acquired or it may be hereditary. In human plasma, about 30% of protein S circulates as free protein; 70% is bound to the complement regulatory protein C4b-binding protein. Protein S in the free form functions as a co-factor to activated protein C [5].

Etiology of protein S and C:

Protein S deficiency can be acquired or it may be congenital. The mutations in the PROS1 gene may cause the Congenital protein S deficiency [6].

Most of the PROS mutations which are point mutations, like transversion mutations which produces a premature stop codon resulting in a

truncated molecule of protein S [7]. The literature describes more than 200 PROS mutations which may cause three different forms of deficiency of protein S mentioned as below:

- Type 1: Quantitative defect showing total protein S (TPS) at lower level along with free protein S (FPS) and reduced activity of protein S level.
- Type 2 (also called as 2b type): Results in decreased activity of protein S, along with normal TPS levels and FPS antigens.
- Type 3 (also called as2a type): It is described as quantitative defect which presents the normal TPS levels, along with the reduced FPS levels and activity of protein S.

Protein S deficiency is described as an autosomal foremost pathology. The mild protein S deficiency in may be a observed among individuals with mutations in a single copy in heterozygous individuals. Whereas severe protein deficiency might be observed in individuals with homozygous mutations

The acquired variations in protein S levels may be present due to due to following causes:

- Therapy of Vitamin K-antagonist
- Chronic infections
- Disease of Severe hepatic
- Systemic lupus erythematosus
- Myeloproliferative disorders
- Nephritic syndrome
- Disseminated intravascular coagulation (DIC)
- The increased risk of VTE is observed among pregnant patients and patients using oral contraceptives [7,8].

Pathophysiology of protein C and S :

Protein S is a vitamin K-dependent protease. It circulates in blood plasma at lower concentrations and it also serves a vitalcharacter in the regulation of coagulation. In regularconditions, the anticoagulant protein helps to keep the blood in a liquid non-thrombotic state. In circulation, around 40% of protein S is in free form, and approximately 60% of protein S is in a high-affinity complex along with the complement regulatory factor C4b-binding protein (C4BP) [4]. Following are the two ways that describe the anticoagulant activity of protein S:

1. Protein S inactivates coagulation factors 5a and 8a and it operates as a cofactor for activated protein C (APC).With the process of switching off the cofactor proteins F5a and F8a, the clotting of blood is stopped.FVa is activated by Protein S and APC.Factor 5 is needed in order to inactivate factor 8a, APC and protein S.
2. In order to inactivate factor 10a and tissue factor (TF)/factor 7a, Protein S acts as a cofactor for the tissue factor pathway inhibitor (TFPI) protein [9,10].

Protein S has multiple structural moieties and it is a complex protein and it is a single-chain glycoprotein, which depends on vitamin K action for post-translational alterations of the protein to a normal functional state. The 3-dimensional structure of protein S is yet to be resolved and it is expected to improve therecognition of the complex functional nature of mutations of PROS1.

Epidemiology:

Congenital protein S deficiency has variable penetrance and is autosomal dominant in nature. Among half of the heterozygous protein S deficient patients develop VTE. Among healthy population, the Protein S deficiency is rarely observed.

Table 2: Incidence of pregnancy-associated VTE with inherited thrombophilia. [19]

Thrombophilia Pregnancy	In pregnancy (%/ year)	Overall (%/year)
Factor V Leiden heterozygous	2.1 (0.7–4.9)	0.5 (0.1–1.3)
Prothrombin gene mutation heterozygous	2.3 (0.8–5.3)	0.4 (0.1–1.1)
Protein S or ATIII, protein C deficiency	4.1 (1.7–8.3)	1.5 (0.7–2.8)

In clinical practice, determine the role of Protein C and Protein S Levels in Pregnant females with recurrent early pregnancy loss. From a public health perspective, even a Low Molecular Weight Heparin and progesterone in this high-risk group of women might be worthwhile.whether there is evidence for this is the objective of this review.

Objectives:
The objective of this review was to determine the role of Protein C and Protein S Levels in Pregnant females with Early Pregnancy Loss.

METHODS:

A systematic literature review was conducted by the authors via searching exciting sources on *role of protein C and protein S levels in pregnant females with recurrent early pregnancy loss*. The literature search was performed in PubMed, Google Scholar, Scopus, Medline and chochran databases up to December 2023. The keywords and combinations of keywords used for the literature search are reported.

Searching resources:
Based on the authors evaluation, the most pertinent to the subject of the investigation sources published in English have been read and used for the review. The results of the literature search have been presented logically to illustrate what has been reported on the topic of the discussion. Due to the nature of the findings, a narrative synthesis of the results from selected articles has been opted for. This work has some limitations: (1) only English language papers were included; (2) due to the heterogeneous nature of the studies included in this review (a diverse quality, study design, and outcomes assessed), only a narrative synthesis was possible.

Criteria for considering studies for this review:
Types of studies Randomised controlled trials and quasi-randomised controlled trials that assessed role of protein C and protein S levels in pregnant females with recurrent early pregnancy loss. Search methods for identification of studies.

Study Selection:
The initial screening using search strategy and keywords on PubMed, Medline, Cochrane database, and Google Scholar identified 792 articles. After the screening, 584 articles were excluded based on titles, repeated records, the different language used (other than English), unavailable full texts, etc. 208 abstract were examined and based on abstarct dissimillar containt 169 articles were excluded. 39 full articles were examined for enrollment in study and 29 articles were excluded based on dissimilarity study design, methodology and containt. So in present review article, 10 articles up-to-date articles were included.

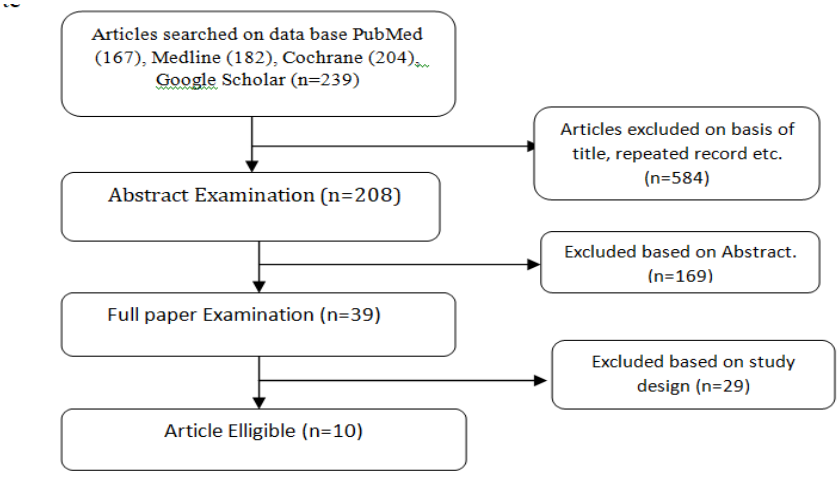

Figure 1: Flow chart of Article selection process

Protein C and protein S in pregancy:
Faught W, et al [11] [1995] conducted a cross-sectional study that included a random sample of 91 normal pregnant women having measured plasma concentrations of protein S and protein C during the I'st, II'nd and III'rd trimesters. There was no statistically significant change in antigenic or functional protein C levels during normal pregnancy. Total protein S levels also remained unchanged. Free protein S levels fell significantly from first to second trimesters (0.45 U/ml mean to 0.26 U/ml mean, $p < 0.001$), but no further fall occurred during the third trimester. The second-trimester which fall in free protein S levels is a physiological pregnancy adaptation. In order to avoid the misdiagnosis and treatment, the women appearing for the first time during pregnancy with a thromboembolic event should have done investigations for protein S deficiency delayed until the postpartum period.

V. Sephton et al [12] [2003] concludeded that continuation of Anticoagulation for VTE for at least 3–6 months from the development of VTE. The authors observed that anticoagulation should be continued through delivery if VTE is found early in gestation and it should be continued for at least 4–6 weeks postpartum depending on underlying thrombophilic conditions and recovery from birth. Either bridging to warfarin is acceptable options or continuation of low molecular weight heparin are the acceptable options in the postpartum period. For pregnant women, no optimal prophylactic dose of heparin or LMWH been determined. Higher doses of UFH are required for pregnant women in order to achieve prophylactic and therapeutic levels of anticoagulation. The activated protein C resistance was significantly associated with early recurrent fetal loss (OR= 3.48, 95% CI 1.58-7.69), and prothrombin G20210A mutation with early recurrent (OR =2.56, 95% CI 1.04-.29) and late non-recurrentfetal

loss (OR = 2.30, 95% CI 1.09-4.87). Protein S deficiency was observed to be significantly associated with recurrent fetal loss (OR =14.72, 95% CI 0.99-218.01) and late non-recurrent fetal loss (OR = 7.39, 95% CI 1.28-42.63). No significant association of fetal loss was observed with protein C, antithrombin deficiencies and methylenetetrahydrofolate mutation (p>.05). According to the type of thrombophilia and type of fetal loss, the strength of association between thrombophilia and fetal loss varies E. Lindhoff-Last [13] [2004] conducted a case-control study which included 97 consecutive women having pregnancies complicated by unexplained small for gestational age infants. Control group included 97 women who had delivered infants with a birth weight G10th percentile w61x. Among women with infants small for their gestational age, significantly higher frequency of lupus anticoagulants or anticardiolipin antibodies and antinuclear antibodies were observed as compared to the women in control group (ps 0.02 and ps 0.004, respectively). Further it is observed that the prevalence of inherited thrombophilia including factor V Leiden mutation ,the homozygous MTHFR-mutation,deficiencies of protein S, protein C, the prothrombin gene mutation and antithrombin were comparable among cases and controls. Hence, the aetiology of infants small for their gestational age remains unidentified in most of the cases and may be not associated with inherited thrombophilia.

Vora S, et al [32] [2008] studied, 381 women with pregnancy loss, 183 had 2 and 198 had ≥3 pregnancy losses. Early pregnancy loss occurred in 136 patients, late pregnancy loss in 119, and both early and late pregnancy losses in 126. The strongest association was observed with ACA (OR 32.5, 95% CI: 8.6–21.8, p<0.001) followedby annexin V (OR 17.1, 95% CI: 2.9–99.4, p<0.001), LA(OR 8.2, 95% CI: 1.4–47.7, p=0.01) and anti-β2GP1 (OR5.8, 95% CI: 1.6–22.1, p=0.007). No association ofantiphospholipid antibodies with the time of pregnancy loss wasfound except LA which was significantly associated with earlypregnancy loss compared with late pregnancy loss (p<0.05). Therisk of pregnancy loss with PS deficiency (OR 17.8, 95% CI: 3.1–102.9, p<0.001) was the highest observed for any heritable thrombophilia followed by PC deficiency (OR 5.8, 95% CI: 1–34, p=0.06). Joanne M. Said et al [14] [2010] found that there is a growing tendency for obstetricians and haematologists to order antithrombin, protein C and protein S tests in early pregnancy among patients who have history of adverse pregnancy outcomes. Our data confirms that comparison of these results to reference ranges established from "non-pregnant" populations is not valid even during the early phase of pregnancy. Present study finding of significant reductions in functional proteins S activity in early pregnancy, suggest that this assay may be of limited value in testing for protein S deficiency during early pregnancy if results are evaluated using non-pregnant reference ranges. These data would suggest that a diagnosis of protein S deficiency would be unlikely in women with protein S activity above 9% during the first half of pregnancy. In addition, the important corollary of our observed changes in protein C is that our large cross-sec tional study has also generated significant changes in the lower limit of the population reference range, which is used to define protein C deficiency.

P Jyotsna et al [15] [2011] conducted a study among Indian women on Coagulation inhibitors and activated protein C resistance in repeated pregnancy losses. The results of the study concluded that thrombophilic defects were seen in a significant number of Indian patients with recurrent pregnancy loss(RPL). Hence, thrombophilic defects should be investigated for all patients with unexplained RPL. Further prospective research needs to be condudedin order to ascertain the causal link between adverse pregnancy outcome and thrombophilia. This will help in studying the adverse pregnancy outcome and assess the efficiency of thromboprophylaxis in pregnant women with recurrent pregnancy loss.

Kelly McNamee et al [16] [2012] conducted clinical trial on thrombo-modulin deficient mice, having deficiency of the anticoagulant protein C pathway, observed pregnancy demise prior to 9 weeks. Rare studies has been carried involving Protein S deficiency and recurrent miscarriage. In a case–control study involving 52 women who having recurrent miscarriage history were studied for inherited thrombophilia. The result indicated almost twice incidence of protein S deficiency was observed among the recurrent miscarriage cohort compared to the controls. However, the difference was not statistically significant.

Hussein Naji Alshammary et al [17] [2015] observed a significant association between low protein S and recurrent miscarriage (P =0.002) OR=2.250 (95%C.I. 1.764-2.870). The observed association between the low protein C with recurrent miscarriage was not statistically significant (P>0.05). A statistically significant association was seen between low protein S andlow protein C with the abortion occurred in the second trimester (P<0.05). The recurrent abortion and the positive family history for thrombosis was significantly associated (P<0.05). The association between recurrent miscarriage and the platelets count not statistically significant. A significant association was observed between the protein S deficiency and recurrent miscarriage (mostly among the miscarriages occurred in the second trimester). The positive family history of thrombosis is one of the important riskfactorfor recurrent miscarriage. Protein C deficiency had no statistically significant association with the recurrent miscarriage.

Alshammary H.et al [18] [2015] conducted a study at Babylon hospital in Iraq and studied the Protein C and Protein S deficiency among recurrent pregnancy loss in women. The results of the study indicated a statistically significant association between low protein S and the recurrent miscarriages. The chances of recurrent abortion are twice among the patients with low protein S. No significant association between protein C and platelets was observed with recurrent miscarriage (P>0.05). The proportion of patients in study group with positive family history of thrombosis was 17.8% whereas the proportion among controls was 6.7%. The proportion among study group is significantly higher as compared to the controls (p<.001).

Mousumi Saha et al [2023] [19] conducted a study to determine the association of serum protein C and protein S level in patients with unexplained recurrent pregnancy loss [RPL]. This study found that RPL patients experienced an average of 4 pregnancy losses and about 70% pregnancy loss occurred during first trimester. A total of 3(10%) protein C deficient, 5(16.7%) protein S deficient and 2(6.7%) both protein C and S deficient patient were found in the case group. The mean of Protein C and S level among RPL patients was 78.46±13.18 and 81.69±14.06 respectively, which was significantly lower than the control group level. Protein C and S level did not vary for patients experienced 1st trimester loss or 2nd trimester loss. The odds ratio for RPL group due to protein C and protein S deficiency was considerably higher [3.22, 95% CI: 0.32- 32.89 for Protein C and 5.80, 95% CI:

0.64-53.01 for PS] and a significant association of protein C and protein S level with RPL was found with adjusted binary logistic regression. They found higher incidence of protein C and S deficiency among RPL patients with most of the miscarriage occurring in the first trimester.

CONCLUSION:

From this review article we conclude that the protein C and protein S deficiency is one of the causative factors for recurrent pregnancy loss. Therefore, screening for protein C and S deficiency is mandatory in all cases of recurrent pregnancy loss. Low molecular weight heparin (LMWH) can be used to treat conditions related to protein C and protein S deficiencies. So, the treatment with Low Molecular Weight Heparin and progesterone should be initiated to ensure good fetal outcome and also to prevent post-partum/postoperative period catastrophic event. From a public health perspective, even a Low Molecular Weight Heparin and progesterone in this high-risk group of women might be worthwhile, and large studies of strong methodological quality are awaited to clarify the real risk-benefit of such an approach and conditions related to protein C and protein S deficiencie. Moreover, the inclusion of a placebo or no-treatment arm in these studies is necessary since it would provide an adequate control to the active treatment and allows assessing a risk-benefit ratio.

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