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Role of Protein C, Protein S and Antithrombin III Levels in High-risk Pregnant Women in Karachi's Gadap Region for Obstetric Complications

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MR and SSN designed the model and the computational framework and analysed the data. Authors IA and UA carried out the implementation. Author NI performed the calculations. Authors MR and SFK wrote the manuscript with input from all authors. All authors read and approved the final manuscript.

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ABSTRACT

Objectives: Thrombophilia has been connected to both pregnancy difficulties and recurrent miscarriage. The purpose of this project is to establish a baseline of protein C, protein S, and AT III levels in pregnant females with various pregnancy-related problems, assess the prevalence of acquired deficiency in these parameters, and compare the results to a control group.
Method: In conjunction with Muhammadi Blood Bank, Baqai Medical University conducted a cross-sectional study. There were 150 pregnant and non-pregnant women in the study. The immunoturbidity approach was used to assess the free protein S level, and the bichrome method was utilised to determine ATIII activity, while the clot-based methodology was used to determine

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PT, APTT, and protein C activity. All parameters were examined using the CA-650 automated coagulation analyzer.
Results: The mean age range of the participant was 18-45 years. Most of participants were Sindhi. The participants ranged in age from 18 to 45 years old. Sindhis made up the majority of the attendees. When all three were compared, there was a significant difference in Protein C and Protein S levels (p-value=0.001), while AT III levels were insignificant. Protein S levels were found to be lower in all four pregnancy problems (PIH, IUD, Miscarriages, and Multiple Pregnancies), however only Protein C levels were lower in IUD.
Conclusion: Our findings showed that protein S levels were low in both healthy pregnant women and those in the high-risk group, with no significant association with distinct problem groups. Reduced protein C levels, on the other hand, were found to have a statistically significant link to IUD instances. Antithrombin III (ATIII) levels were found to be normal in all of the groups studied.

Keywords: Protein C; Protein S; Antithrombin III; Hemoglobin; Platelets Count; PT; aPTT and Pregnant.

1. INTRODUCTION

Hemostasis is a basic physiological response in the human body that is designed to stop bleeding at the site of a vessel wall lesion, whether internal or external [1]. The vascular system, platelets, pro-coagulant factors, coagulation inhibitors, and fibrinolytic system are the primary components in this finely coordinated event and work under strict regulation by virtue of inhibitory and activating factors [2]. Any disturbance in the mechanism can either lead to hemorrhage or thrombosis [1,3].

Pregnancy, the most prevalent cause of hemostatic system changes, is a hypercoagulable state caused by increased procoagulant levels, reduced anticoagulant levels, and impaired fibrinolysis [4]. All of these changes save the pregnant woman from bleeding after delivery and keep the placental vasculature functioning, but at the same time these changes increase the risk of venous thrombo-embolism (VTE) or thrombophilia [5].

Thrombophilia is now defined as "an increased propensity toward thrombosis and associated morbidity" by Emanuel Favaloro in Semin Thromb Hemost 2019 [6]. It can be inherited or acquired, and it raises the risk of arterial and venous thrombosis in blood vessels while also predisposing it [7,8]. Inherited thrombophilia, first described by Egberg in 1965 [8,9] is a quantitative & qualitative problem of some proteins of hemostasis due to specific gene mutations. Protein C, protein S, antithrombin III (ATIII), factor FV Leidenand Factor II (prothrombin G20210A) genes are more prevalent and well-known, but hyperhomocysteinemia caused by a mutation in the methylenetetrahydrofolate reductase (MTHFR) gene is only recently discovered. All of these mutations have been proven to increase the risk of VTE by three times [10,11]. On the other hand, the acquired thrombophilia has been mainly associated with acquired deficiency of Protein C, protein S, antithrombin III (ATIII) and with the antiphospholipid syndrome (APS) which is characterized by the presence of phospholipid-binding antibodies, lupus Anticoagulant (LAC) and anti-cardiolipin antibodies (aCL) [12].

When thrombophilia occurs, it is likely to have a negative impact on placental circulation due to the formation of thrombi within the placental blood vessels, which can result in severe placenta-mediated pregnancy complications such as Pre-eclampsia, PE/HELLP syndrome, Intrauterine foetal death (IUFD), Intrauterine Growth Restriction (IUGR), Placental abruption, early or late pregnancy loss, recurrent miscarriages, pregnancy-induced hypertension (PIH) and Stillbirth [13,14]. The existence of various other factors, such as family history, surgery, immobility, obesity, smoking, estrogen medication, and so on, increases the risk of thrombophilia [15,16]. In a developing country like Pakistan, where access to tertiary care is limited, pregnancy-related difficulties are common, and many pregnancies end in miscarriage [17]. Pregnant women are routinely encouraged to undertake thrombophilia marker screening during the second and third trimesters, but on the other hand, no testing is done at all in this regard even when it is recurrent with a history of difficult pregnancy and previous adverse obstetrical outcome [18]. The augmenting factors of VTE (as mentioned above) are usually present in our population [19].

Due to lack of literacy &qualified medical staff, understanding of the cause does not occur,

further fueling the problem is lack of proper investigations which leads to recurrence of pregnancy loss. In addition, the previously published available studies have conflicting data regarding the protein C, protein S and AT III levels in healthy pregnant and high risk pregnant women [20-22]. In Pakistani population very few studies are carried out and analyzed only one parameter with one or two risk factors, none used the normal baseline values of our population [17,23].

The current study focuses on three markers (protein C, protein S, and AT III), as well as normal baseline values, in women who had multiple pregnancy-related complications. This bleak situation necessitates various investigations in this area.

Our study's goal was to establish a baseline of protein C, protein S, and AT III levels, determine the prevalence of acquired deficiency in these parameters in pregnant females with various pregnancy-related complications, and compare the results to a control group in order to determine the role of these factors in pregnancy loss issues.

2. MATERIALS AND METHODS

The case control study was undertaken between August 2019 and August 2020, through collaboration between Baqai Medical University and Muhammadi Blood Bank in Karachi. Samples were taken from pregnant and non-pregnant women of the same age range who presented to the Gynae/Obs dept., Fatima Hospital, BMU, in the Gadap region, on the outskirts of Karachi. A total of 150 blood samples were taken based on inclusion and exclusion criteria from non-pregnant, healthy pregnant, and high-risk pregnant women between the ages of 18 and 45. There were 50 women who were not pregnant. Pregnant women were divided into two groups: 50 healthy pregnant women with no history of pregnancy-related complications, and 50 high-risk pregnant women with a current or previous history of pregnancy-related complications, such as pre-eclampsia, IUGR (intrauterine growth restriction), IUFD (intrauterine foetal death), placental abruption, recurrent abortion, and stillbirth. A serial number was assigned to each sample.

A detailed personal history was gathered, which included her name, residence, phone number, ethnic group, primary diagnosis, gestational age in weeks, gravidity/parity, and any current or past history of pregnancy-related complications.

2.1 Inclusion Criteria

- **Non-pregnant women (Group A)**

  Non - pregnant women included in this study as a control.

- **Healthy Pregnant women (Group B)**

  Healthy pregnant women without any previous history of adverse pregnancy outcomes.
  No one of them had history of thrombosis.

- **High risk pregnant women (Group C)**

  Pregnant women having current and previous history of :

  a. Pre-eclampsia.
  b. IUGR (Intrauterine Growth Restriction)
  c. IUFD (Intrauterine Fetal Demise)

2.2 Exclusion Criteria

Females not fulfilling the inclusion criteria.

2.3 Sample Collection, Plasma Separation and Storage

- Freshly drawn 5ml whole blood was collected into (3.2%) blue top citrated tube and in EDTA tube both tubes were labeled for serial number.
- Blue top sodium citrated tube was centrifuged at 3000 RPM for 15 min at room temperature within 2 h of sample collection.
- Platelet poor plasma (PPP) was separated and stored at -80^0 C until analysis.
- EDTA tube for determination of platelet count and citrated tube for screening of coagulation and of thrombophilia markers (PT, APTT, Protein C, protein S and ATIII).
- The clot-based technique was used to determine PT, APTT, and protein C activity, while the immunoturbidity method was used to determine the free protein S level and the bichrome method was used to determine ATIII activity. The CA-650 automated coagulation analyzer was used to examine all parameters.

The Statistical Package for Social Sciences (SPSS) Version 21 was used for data entry and analysis. The mean±standard deviation of the data was calculated. The statistical significance of three groups was determined using the one-way ANOVA. The *p* value of (< 0.05) was taken as statistically significant.

3. RESULTS

The mean ages of the non-pregnant, healthy pregnant, and high-risk pregnant patients were 29.5 ± 17.8, 32.4 ± 16.4 and 31.7 ± 17.1 respectively. The age range in all the three groups was 18-45 years. Most of the participants were Sindhi (n = 74), followed by Pathan (n = 51) and Urdu Speaking (n =25) as mentioned in Table 1.

In our study, the statistical comparison of mean and SD values of haemoglobin, PT, APTT, and platelet count between the three groups was shown to be insignificant.

Table 2 shows the mean and SD values of PT, APTT, Protein C, Protein S, and ATIII among the study group's various problems. In all the four complications related to pregnancy (PIH, IUD, Miscarriages and Multiple Pregnancies) showed a decrease in Protein S levels whereas only IUD showed a decrease in Protein C level.

Table 1. Demographic Data of the Controls and Patients

	Non- Pregnant	Healthy Pregnant	High Risk Pregnant
Age, y, mean ± SD	29.5 ± 17.8	32.4 ± 16.4	31.7 ± 17.1
Age, y, range	18 - 45	18 - 45	18 – 45
Ethnicity	(n)	(n)	(n)
Pathan	17	19	15
Sindhi	26	21	27
Urdu Speaking	7	10	8

Table 2. Comparison of Hemoglobin, Platelet count, PT and aPPT levels among the groups

Parameter	Non Pregnant (Controls)	Healthy Pregnancy Mean± SD	High risk pregnancy Mean± SD	p-value
Hemoglobin (g/dl) (12 - 14 g/dl))	11.0 ± 1.60	10.27 ± 1.20	10.11 ±1.20	0.491
Platelets(10^3/µl (15,000 - 450,000)	267.13 ± 87.23	277.76 ± 73.25	253.25±65.24	0.077
PT (12.7 - 15.4 seconds)	12.8 ± 2.3	12.1 ± 1.9	11.51 ± 1.33	0.321
aPTT 30-40 seconds	31.9 ± 2.3	36.73 ± 1.9	37.68 ± 1.7	0.067

Table 3. Comparison of protein C, protein S and AT III among three group

	Participants	Normal	Abnormal	*p*-value
Protein C (%)	High risk	91.3712	19.47064	
	Non- pregnant	106.954	17.26861	0.001
	Healthy pregnant	104.28	16.55086	
Protein S (%)	High risk	52.5404	16.58445	
	Non- pregnant	92.328	13.60948	0.001
	Healthy pregnant	45.5642	9.36418	
ATIII (%)	High risk	104.4788	8.79446	
	Non- pregnant	105.168	11.07602	0.218
	Healthy pregnant	101.63	12.04355	

Table 4. Comparison of study parameters among different complications

Type Of Risk Factors	PT	aPTT	PROTEIN C	PROTEIN S	AT III
PIH (n= 10)	11.17±0.73	41.16±25.65	99.10±14.72	58.94±26.52	106.04±8.07
	Normal	Normal	Normal	Decreased	Normal
IUD (n= 15)	11.26±1.11	37.48±16.79	83.09±28.17	49.89±12.05	104.72±7.94
	Normal	Normal	Decreased	Decreased	Normal
Miscarriages (n=23)	11.26±1.29	37.57±14.36	94.30±14.07	51.45±14.97	103.10±9.29
	Normal	Normal	Normal	Decreased	Normal
Multiple pregnancy (n=2)	12.35±2.19	34.50±1.77	97.10±28.99	52.65±0.35	106.60±13.58
	Normal	Normal	Normal	Decreased	Normal

There was a significant difference in Protein C levels when all the three were compared (p-value=0.001). Protein S was shown to be statistically significant in all the three group i.e. non pregnant, healthy and high-risk pregnant women (p-value=0.001). When ATIII levels were compared, they were statistically insignificant (p-value=0.218) as shown in Table 3.

As shown in Table 4 there was a decrease found in Protein S levels in different types of Pregnancy related complications. Moreover, a decrease in Protein S level was also observed in IUD Intrauterine Death. However, PT, aPTT, ATIII did not change in any of the pregnancy related complications.

4. DISCUSSION

Normal pregnancy causes significant changes to the coagulation and fibrinolytic systems. These physiological changes to the procoagulant and hemostatic systems are meant to minimise intrapartum blood loss [24]. This overall alteration prevents females from bleeding during birth and returns them to normal within 4-6 weeks after delivery, but it also increases the chance of pregnancy-related VTE or thrombophilia, especially if other risk factors are present [4]. During pregnancy thrombophilia or VTE is one of the major risks of maternal morbidity and mortality worldwide [25]. Pakistan, as one of those with a deficient health-care system and, in particular, ignored maternal and child health issues, requires more attention in this respect [26].

In the current investigation, all baseline indicators, such as haemoglobin, PT, APTT, and platelet count, were found to be within normal range and statistically insignificant in all three groups. The result was as expected and in line with prior investigation [27].

In present study, when compared to healthy pregnant and non-pregnant women, Protein C levels were shown to be lower exclusively in high-risk pregnant women, which is very significant (P =0.001). This finding is in line with previous studies [28-31], which reported significantly reduced protein C levels in both healthy and high-risk pregnancies. Protein C levels are hypothesised to be reduced during pregnancy due to an increase in procoagulant factors and consumption. Low levels of protein C have been found in 6/15 (40 percent) of IUD cases in high-risk pregnancies, despite other risk variables not showing a positive attitude toward the reduced levels of protein C, which could be related to a dependence on the severity of complications. In 5/15 cases, we discovered a combination of protein C and protein S insufficiency (33 percent). Pregnant women with a current or previous history of IUD were found to have a strong link to combined protein C and protein S deficiency in our study.

Moreover, Protein S levels in healthy, high-risk, and non-pregnant women were compared in this study, and the results were statistically significant (p-value=0.001). Both healthy pregnant women and high-risk pregnant women had lower levels, with the highest fall in the IUD percentage in the high-risk group. The current study's findings are highly correlated with those of other research conducted in various parts of the world which also showed significant reduction of protein S level in pregnancy [32-36]. According to studies, the dilutional impact, higher protein C resistance, and increased levels of coagulation factors may all contribute to lower free protein S levels in healthy and high-risk pregnancies [30].

Our findings revealed that the level of AT III in all three groups remained stable and normal, with no statistically significant results (p-value=0.218). This conclusion is consistent with the findings of previous studies [8,37] however some studies showing contrary results mentioned a significant decrease in ATII levels in both healthy and complicated pregnancies [38-42]. Normal ATIII levels seen in our study could be owing to a low prevalence of ATIII deficiency in the general population and a small sample size, which is why we were unable to detect ATIII deficiency in pregnancy. The majority of other authors believe that thrombophilia is a significant risk in early and late pregnancy losses, and that women who experience unexplained pregnancy loss should be tested for it [43-45]. Routine thrombophilia screening, on the other hand, is not suggested for all women who have an antenatal clinical examination [46].

5. CONCLUSION

Based on our findings, it is therefore concluded that a thorough thrombophilia workup for pregnant women in underdeveloped countries, even those with minimal problems, is unnecessary. Those who have had a previous IUD and/or have had a known instance of VTE should be evaluated, especially if they have thrombophilia boosting factors. Only PC levels can be evaluated if resources are limited.

6. FUTURE RECOMMENDATION

A population-based study should be carried out to establish a baseline value for thrombophilia parameters in healthy non-pregnant and pregnant females. A large-scale follow-up research may also be done to get a more accurate representation of the link between these markers and pregnancy-related problems.

LIMITATIONS

Although we tested healthy non-pregnant females for same parameters as control group but it would be better if same high-risk group females were tested before getting pregnant. Also this high risk group may be followed post-partum for levels of same parameters & to observe that how many, out of those having reduced PC & PS levels, develop thrombosis &/or suffer obstetrical complication.

CONSENT

Informed written consent was taken from all the women as per protocol.

ETHICAL APPROVAL

The Ethics Research Committee of Baqai Medical University Karachi gave its approval to the project.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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