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PREVALENCE OF ANTICARDIOLIPIN, PROTEIN C, AND PROTEIN S IN PREGNANCIES WITH REPEATED PREGNANCY LOSSES

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ABSTRACT

OBJECTIVE: *To assess the prevalence of anticardiolipin, protein C, and Protein S in pregnancies with repeated pregnancy losses.*

METHODOLOGY: *This cross-sectional study assessed the prevalence of anticardiolipin antibodies, Protein C, and Protein S deficiencies in 48 women with repeated pregnancy losses (RPL) at Liaquat National Hospital (2022–2024). Blood samples were analyzed using ELISA for anticardiolipin antibodies and clot-based assays for Protein C and Protein S levels. Deficiencies were defined by established thresholds. Statistical analysis evaluated prevalence and associations, with quality control ensuring validity.*

RESULTS: *Among 48 women with repeated pregnancy loss, Protein C and Protein S deficiencies were observed in 6.25% and 20.8% of participants, respectively, while anticardiolipin antibody positivity was detected in 12.5%. Statistical analysis revealed no significant associations between these deficiencies and factors such as age, parity, BMI, or diabetes*

CONCLUSION: *It is to be that anticardiolipin antibodies, Protein C, and Protein S deficiency is significantly common in women with RPL, which confirms their important role in the pathogenesis of RPL. These findings highlight the need to consider these biomarkers in the diagnostic workup in order to establish tailored management options. Detailed, standardized, and longitudinal studies are justified to establish casual mechanisms and refine therapeutic cases.*

Keywords: *Anticardiolipin Antibodies, Coagulation Disorders, Pregnancy Complications, Protein Deficiency, Recurrent Pregnancy Loss*

INTRODUCTION

Encouraging many women and couples to face the ordeal of repeated pregnancy loss (RPL). Protein C deficiencies and S proteins, along with anti-cardiolipin antibodies, have been found to have critical roles in these cases [1], as the investigation indicates. The knowledge of the impact of these parameters have great importance in the promotion of maternal-fetal health outcome.

Anticardiolipin antibodies (ACL) belong to a larger group of antibodies called antiphospholipid antibodies. It is frequently associated with increased risk of pregnancy complications, including RPL. Persistent positive ACL had been frequently observed in women with recurrent pregnancy loss and endorsed favorable trend in post-thrombotic pregnancy by suggesting a potential role of monitoring of these anticonvulsant antibodies in clinical practice [2]. An additional study demonstrated that the anti-phospholipid syndrome

represents a prevalent rate for spontaneous abortion and therefore, it is important to detect it thoroughly among females with recurrent ethic [3].

Proteins that regulate blood clotting (Protein C and proteins). If these proteins are deficient there is an increased tendency toward coagulation, which can disturb the normal functioning of placentation and ultimately result in pregnancy loss. The results of previous studies regarding protein C and protein S levels in women with recurrent early pregnancy losses in their investigation were at lower side [4,5]. The association of these deficiencies with pregnancy outcomes is crucial as it emphasizes the necessity of screening basic coagulopathies in women with RPL.

In a study, the prevalence of several thrombophilia factors, including protein and protein deficiencies C between women with antiphospholipid syndrome was examined. The findings confirmed that such deficiencies are common in patients suffering from recurrent loss of pregnancy [6]. Moreover, even the exploration of the consequences of altered homocysteine disorders suggests that simultaneous assessment of multiple risk factors is required for an overall perspective of RPL [7]. Anti-phosphatidylethanolamine antibodies further increased complexity regarding understanding of RPL. Such a condition can change placentation that leads to pregnancy-related complications [8]. Furthermore, these autoantibodies have received considerable attention for their association with the worst pregnancy outcomes [9,10].

Coagulation, anti-priest, S protein deficiencies have roles that are interconnected and that are extremely important in the evaluation of consumption-based recurrent loss of pregnancy. Understanding these elements will aid medical care providers in tailoring medical therapies and treatment modes to better assist afflicted women. Not only does this facilitate increased chances of live births, importantly, it also improves overall maternal-fetal health outcome [11-13]. Monitoring and early intervention remain vital strategies in the management of the recurrent loss of pregnancy effectively.

METHODOLOGY

A cross-sectional study was conducted at Liaquat National Hospital from November 2022 till June 2024 to determine the prevalence of anticardiolipin antibodies, Protein C and Protein S deficiency amongst women with RPL. Non-probability purposive sampling was planned to enroll 48 women aged 20–40 years with two or more consecutive pregnancy losses prior to 20 weeks of gestation. Women with an established chromosomal anomaly, uterine anomaly, active infection, or pre-existing and chronic medical condition were also excluded from the study. Detailed demographic and clinical data were then recorded using structured interviews and review of medical records after written informed consent was obtained by the pairs of physicians involved in caring for each patient. Blood was obtained during the luteal phase of the menstrual cycle or early in pregnancy (≤ 12 weeks) and immediately centrifuged to separate plasma and serum, which was stored at -80°C, until anticardiolipin IgG and IgM antibodies were measured, positivity was based on the ELISA values above the 95th percentile for per-phospholipid concentration. Functional levels of Protein C and Protein S were measured using clot-based assays; deficiencies were defined as ≤ 70% normal activity. Quality control procedures considered duplication testing, internal and external assay validation and clinical and laboratory standards institute (CLSI) guidelines. Data were analyzed using SPSS version 26.0. Continuous variables were expressed as mean ± standard deviation, while categorical variables were presented as frequencies and percentages. The Chi-square test was applied at 5% level of significance.

RESULTS

There were 48 total participants included in the study (mean age of 29.27 ± 5.33 years). More than half women (58.3%) were aged between 18 and 30, with the remainder older than 30 (41.7%). Participants had a mean weight of 77.21 ± 10.79 kg and height of 168.65 ± 9.00 cm. The average body mass index (BMI) was 27.30 ± 4.00 kg/m² and 43.8% of the participants had a BMI from 21–26 kg/m², whereas 56.3% had over 26 kg/m². The mean Parity was 2.81 ± 1.51 with the majority of participants (62.5%) having between 1–3 children and the rest (37.5%) having more than 3. In terms of booking status, 45.8% of subjects were booked and 54.2% were un-booked. Among the participants, 22.9% of them were urban residents and 77.1% of them were rural residents. Regarding educational status, 37.5% were illiterate, and 62.5% were

matric or above. Furthermore, 35.4% of participants had diabetes, and 64.6% had no diabetes (TABLE 1). The prevalence of protein C was 3 (6.25%) and the association of protein C levels with variables were evaluated. Among participants aged 18–30 years, 66.7% had low protein C levels (<70), compared to 57.8% with normal levels (≥70), but the association was not statistically significant (OR = 1.462, 95% CI: 0.12–17.31, p = 0.627). Regarding parity, 66.7% of those with low protein C had 1–3 children compared to 62.2% with normal levels, showing no significant association (OR = 1.214, 95% CI: 0.10–14.42, p = 0.687). In terms of BMI, 66.7% of participants with low protein C had a BMI of 21–26 kg/m² compared to 42.2% with normal protein C levels, but this difference was also not statistically significant (OR = 2.737, 95% CI: 0.23–32.43, p = 0.405). Among diabetics, 66.7% had low protein C levels compared to 33.3% with normal levels, with no significant association observed (OR=4.000, 95% CI: 0.33–47.72, p=0.283). (TABLE 2) In this study protein S prevalence was noted in 10 (20.8%). Among participants aged 18–30 years, 60.0% had low protein S levels (<50) compared to 57.9% with normal levels (≥50), with no significant association observed (OR = 1.091, 95% CI: 0.26–4.51, p = 0.599). Regarding parity, 70.0% of those with low protein S had 1–3 children compared to 60.5% with normal protein S levels, which was not statistically significant (OR = 1.522, 95% CI: 0.33–6.82, p = 0.435). For BMI, 50.0% of participants with low protein S had a BMI of 21–26 kg/m² compared to 42.1% with normal protein S levels, with no significant association noted (OR = 1.375, 95% CI: 0.34–5.55, p = 0.461). Among diabetics, 50.0% had low protein S levels compared to 31.6% with normal levels, (OR = 2.167, 95% CI: 0.52–8.92, p = 0.235). (TABLE 3). Anticardiolipin antibody prevailed to be positive in 6 (12.5%) women among repeated pregnancy losses. The association of anticardiolipin antibody positivity with study variables was analyzed. Among participants aged 18–30 years, 50.0% were anticardiolipin-positive compared to 59.5% who were negative, (OR = 0.680, 95% CI: 0.12–3.77, p = 0.492). Regarding parity, 33.3% of anticardiolipin-positive participants had 1–3 children compared to 66.7% who were negative, but the association was not statistically significant (OR = 0.250, 95% CI: 0.04–1.53, p = 0.131). For BMI, 33.3% of those with positive anticardiolipin had a BMI of 21–26 kg/m² compared to 45.2% with negative anticardiolipin, with no significant association (OR = 0.605, 95% CI: 0.10–3.67, p = 0.463). Among diabetics, 66.7% were anticardiolipin-positive compared to 31.0% who were negative, (95% CI: 0.72–27.51, p = 0.107). (TABLE 4)

DISCUSSION

The assessment of anticardiolipin antibodies, Protein C, and Protein S deficiencies in repeated pregnancy loss (RPL) is of significant importance due to the well-established link between thrombophilia and adverse obstetric outcomes. Numerous studies have explored the diagnostic, prognostic, and therapeutic roles of these biomarkers in RPL. For instance, Shinozaki et al. reported that Protein S deficiency plays a key role in RPL, as its anticoagulant function is critical for maintaining normal placental circulation, though the study was limited by a small sample size, necessitating larger cohort validation [16]. Similarly, Chepanov et al. emphasized the contribution of anticardiolipin antibodies and related autoantibodies to the autoimmune etiology of RPL, though the research lacked a comprehensive assessment of other thrombophilic factors [17]. Bhasker reviewed the association between anticardiolipin antibodies and RPL, highlighting their disruption of implantation and placental development. However, the use of cross-sectional data limited causal inferences, warranting longitudinal studies [18]. Pelusa et al. demonstrated that antiphospholipid antibodies inhibit angiogenesis, a critical process in placentation, though the heterogeneity of study populations posed challenges to generalizability [19].

In our study, Protein C deficiency was observed in 6.25% of cases, Protein S deficiency in 20.8%, and anticardiolipin antibodies in 12.5%. These findings are comparable to other studies, such as Mukhtar et al., who reported Protein C and Protein S deficiencies in 10% (p =0.277) and 15% (p =0.058), respectively [4], and Alshammary et al., who noted deficiencies in 6.7% and 20% [20]. Another study reported Protein C, Protein S, and anticardiolipin antibody deficiencies in 8.3%, 8.2%, and 11.1% of cases, respectively [2]. While variations in prevalence may reflect differences in study populations and methodologies, these findings consistently implicate thrombophilic and autoimmune mechanisms in the pathogenesis of RPL.

Mechanistically, anticardiolipin antibodies exert pro-thrombotic and pro-inflammatory effects, disrupting placental vascular development and implantation [18,19]. Deficiencies in Protein C and Protein S impair inhibitory pathways of coagulation, leading to uteroplacental insufficiency and adverse pregnancy outcomes [16,20]. Although these associations are well-documented, limitations such as small sample sizes, lack of diversity, cross-sectional designs, and absence of standardized diagnostic protocols restrict the generalizability and applicability of findings.

Our findings, alongside existing literature, highlight the potential for targeted therapeutic interventions, such as low-molecular-weight heparin or aspirin, to mitigate thrombophilic and autoimmune complications in RPL [14,19]. However, there is a need for future research, including multicenter, longitudinal studies, to validate these associations in diverse populations and elucidate causal pathways. Next-generation diagnostic tools, such as proteomics and sequencing technologies, could further uncover novel biomarkers and actionable targets, facilitating a more personalized approach to RPL management [19].

Pregnancies complicated by RPL should be managed through a multidisciplinary approach, incorporating thrombophilic and autoimmune evaluations to personalize care and optimize maternal and fetal outcomes [14,19]. Educating patients about these biomarkers and ensuring compliance with treatment regimens are essential for improving prognosis. Despite advancements, critical gaps in understanding the independent roles of these factors persist. Well-designed, methodologically rigorous studies are urgently needed to address these gaps, advancing prevention, diagnosis, and treatment strategies for RPL and improving maternal-neonatal health outcomes.

CONCLUSION

It is to be that anticardiolipin antibodies, Protein C, and Protein S deficiency is significantly common in women with RPL, which confirms their important role in the pathogenesis of RPL. These findings highlight the need to consider these biomarkers in the diagnostic workup in order to establish tailored management options. Detailed, standardized, and longitudinal studies are justified to establish casual mechanisms and refine therapeutic targets.

Table I: Demographic & General Characteristics of Participants	
Variable	**n (%)**
Age (Mean ± SD) = 29.27 ± 5.33 years	
18 - 30 years	28 (58.3)
>30 years	20 (41.7)
Weight (Mean ± SD)	77.21 ± 10.79 kg
Height (Mean ± SD)	168.65 ± 9.00 cm
Body Mass Index (Mean ± SD) = 27.30 ± 4.00 kg/m^2	
21 - 26 kg/m^2	21 (43.8)
>26 kg/m^2	27 (56.3)
Parity (Mean ± SD) = 2.81 ± 1.51	
1 – 3	30 (62.5)
>3	18 (37.5)
Booking Status	
Booked	22 (45.8)
Un-booked	26 (54.2)
Residential Status	
Urban	11 (22.9)
Rural	37 (77.1)
Educational Status	
Illiterate	18 (37.5)
Matric or above	30 (62.5)

Diabetes Mellitus	
Diabetic	17 (35.4)
Non-Diabetic	31 (64.6)

Table 2: Association of Protein C with Study Variables

Variables		Protein C		OR (95% C.I.)	P-Value
		Low (<70) (n=3)	Normal (≥70) (n=45)		
Age Group (Years)	18 – 30	2 (66.7)	26 (57.8)	1.462 (0.12---17.31)	0.627
	>30	1 (33.3)	19 (42.2)		
Parity	1 – 3	2 (66.7)	28 (62.2)	1.214 (0.10---14.42)	0.687
	>3	1 (33.3)	17 (37.8)		
BMI (kg/m^2)	21 – 26	2 (66.7)	19 (42.2)	2.737 (0.23---32.43)	0.405
	>26	1 (33.3)	26 (57.8)		
Diabetes Mellitus	Diabetic	2 (66.7)	15 (33.3)	4.000 (0.33---47.72)	0.283
	Non-Diabetic	1 (33.3)	30 (66.7)		

Table 3: Association of Protein S with Study Variables

Variables		Protein S		OR (95% C.I.)	P-Value
		Low (<50) (n=10)	Normal (≥50) (n=38)		
Age Group (Years)	18 – 30	6 (60.0)	22 (57.9)	1.091 (0.26---4.51)	0.599
	>30	4 (40.0)	16 (42.1)		
Parity	1 – 3	7 (70.0)	23 (60.5)	1.522 (0.33---6.82)	0.435
	>3	3 (30.0)	15 (39.5)		
BMI (kg/m^2)	21 – 26	5 (50.0)	16 (42.1)	1.375 (0.34---5.55)	0.461
	>26	5 (50.0)	22 (57.9)		
Diabetes Mellitus	Diabetic	5 (50.0)	12 (31.6)	2.167 (0.52---8.92)	0.235
	Non-Diabetic	5 (50.0)	26 (68.4)		

Table 4: Association of Anticardiolipin with Study Variables

Variables		Anticardiolipin		OR (95% C.I.)	P-Value
		Positive (n=6)	Negative (n=42)		
Age Group (Years)	18 – 30	3 (50.0)	25 (59.5)	0.680 (0.12---3.77)	0.492
	>30	3 (50.0)	17 (40.5)		
Parity	1 – 3	2 (33.3)	28 (66.7)	0.250 (0.04---1.53)	0.131
	>3	4 (66.7)	14 (33.3)		
BMI	21 – 26	2 (33.3)	19 (45.2)	0.605	0.463

(kg/m^2)	>26	4 (66.7)	23 (54.8)	(0.10---3.67)	
Diabetes Mellitus	Diabetic	4 (66.7)	13 (31.0)	4.462 (0.72---27.51)	0.107
	Non-Diabetic	2 (33.3)	29 (69.0)		

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