Original Article

Impaired Fibrinolysis in Pregnancy Failure

Samina Mohyuddin, Rabab Zehra, Samar Ekram, Zareen Naz, Shahida Kashif, & Badar Jahan

ABSTRACT

Objective: To explore the association between plasma plasminogen activator inhibitor 1 (PAI-1) levels and women experiencing recurrent miscarriages with no live births to those who have had one or more live births.

Methodology*:* This cross-sectional study included healthy women aged 18–35 years with a history of two or more consecutive miscarriages before 20 weeks of gestation, after obtaining ethical approval and informed consent. The study was conducted in a local private hospital in Karachi, Pakistan. Women with medical and reproductive disorders were excluded. Seventy-five females were selected for the collection of blood samples. Plasma Plasminogen Activator Inhibitor-1 (PAI-1) levels were measured using a Human PAI-1 ELISA kit.

Results*:* Among women with recurrent pregnancy loss, 18.7% had elevated PAI-1 levels, which was significantly correlated with fewer live births ($P < 0.05$). Notably, 87.5% of those with increased PAI-1 had no live children, while only 12.5% had two or more live children.

Conclusion: Elevated plasma levels of Plasminogen Activator Inhibitor-1 can disrupt fetomaternal circulation and may contribute to recurrent pregnancy loss.

KEYWORDS: Fibrinolysis, Miscarriage, Plasminogen Activator Inhibitor-1

INTRODUCTION

Fibrinolysis refers to the breakdown of the fibrin within blood clots and is crucial for restoring proper blood flow after a thrombotic event. The process is regulated by plasminogen activators, such as tissue-type and urokinase-type, which convert plasminogen to plasmin, initiating fibrin breakdown. Conversely, inhibitors like plasminogen activator inhibitor 1 (PAI-1) regulate this process by inhibiting fibrinolysis.[1]

PAI-1 plays a significant role in regulating fibrinolysis during pregnancy. Detection of PAI-1 polymorphism is not routinely recommended, as nearly half of individuals have this polymorphism which is linked to increased tendency for clotting. Its association with implantation failure has been observed in many studies.[2]

During pregnancy, PAI-1 is present in the placenta, particularly within invading trophoblasts. PAI-1 has a significance in preventing excessive invasion of the trophoblast, linked to recurrent pregnancy loss (RPL), preeclampsia, and intrauterine growth restriction (IUGR). Elevated Plasma PAI-1 is associated with recurrent miscarriages, intrauterine growth restriction, gestational diabetes and polycystic ovary syndrome (PCOS).[3]

Several factors have been linked to recurrent miscarriages, such as parental chromosomal abnormalities, maternal thrombophilic disorders,

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infections, endocrine abnormalities, and fetal anomalies. The majority of miscarriages occur within the first trimester and are often associated with thrombophilia.[4]

Thrombophilia represents abnormalities in blood clotting that predisposes individuals to thrombotic events. It may be either inherited or acquired. Hereditary thrombophilia, like factor V Leiden, prothrombin G20210A gene mutation, as well as deficiencies in protein C, protein S, and anti-thrombin III, are associated with pregnancy loss.[5]

The 4G/4G homozygous genotype of the PAI-1 gene is associated with increased levels of PAI-1, which leads to a state of hypofibrinolysis, thereby increasing the risk of venous thromboembolism.[6]

Thrombophilia acts as a risk factor for recurrent pregnancy loss, infertility, and obstetrical complications.[7,8] Despite limited evidence, anti-coagulation is sometimes employed to safeguard women with hereditary thrombophilia during pregnancy.[9]

Up to 40% of pregnancy losses globally remain unexplained despite routine gynecological, endocrine, and cytogenetic diagnostics. Recent studies highlight the -675 ID, 4G/5G endothelial plasminogen activator inhibitor 1 gene (Serpine 1, PAI-1) polymorphism as a potential thrombophilic factor contributing to increased pregnancy loss. Understanding its variants may enhance reproductive outcomes. Individuals homozygous for 4G exhibit higher PAI-1 concentrations than homozygous 5G, while heterozygous 4G/5G show intermediate levels. Elevated PAI-1 serum levels have been linked to a proclivity for clotting. Additionally, PAI-1 plays a pivotal role in fibrinolysis, and alterations in its concentrations or activity might contribute to thrombotic changes in the utero-placental unit.[10]

The American Society of Reproductive Medicine (ASRM) defines recurrent pregnancy loss (RPL) as two or more miscarriages before the 20th week of pregnancy.[11] RPL can be categorized as primary (occurring without prior successful viable pregnancies) or secondary (when one or more viable pregnancies were experienced previously). Miscarriage is a prevalent pregnancy-related complication, accounting for approximately 15% of clinically recognized pregnancies.[12]

Early pregnancy losses occur 12 to 15 percent globally whereas miscarriages occur in 10-15 percent of pregnancies in Pakistan. The prevalence of spontaneous miscarriage in Pakistan is 8 percent, with a higher rate of unreported pregnancy loss.[13]

METHODOLOGY

Our cross-sectional study was approved by the Institutional Review Board (IRB) of the Liaquat College of Medicine and Dentistry (Ref. No. IRB/M-000105/24). The study started in 2024 and ended in early 2025. Written informed consent was obtained from seventy-five (75) healthy females aged 18-35 years with a history of two or more consecutive miscarriages before 20 weeks of pregnancy**.** The sample size was calculated using the Sample Size Calculation in Health Sciences software**.** considering a 95% confidence level and a 5% margin of error, the calculated sample size was 75. Purposive sampling was employed. A detailed history was taken and, a clinical examination was performed. Women with hypertension, obesity, liver function abnormalities, inflammatory pelvic disease, history of ectopic or molar pregnancy, polycystic ovary syndrome (PCO), genital anomalies, endocrine dysfunction, irregularities in menstrual cycle, autoimmune disease, diabetes mellitus, were excluded from the study.

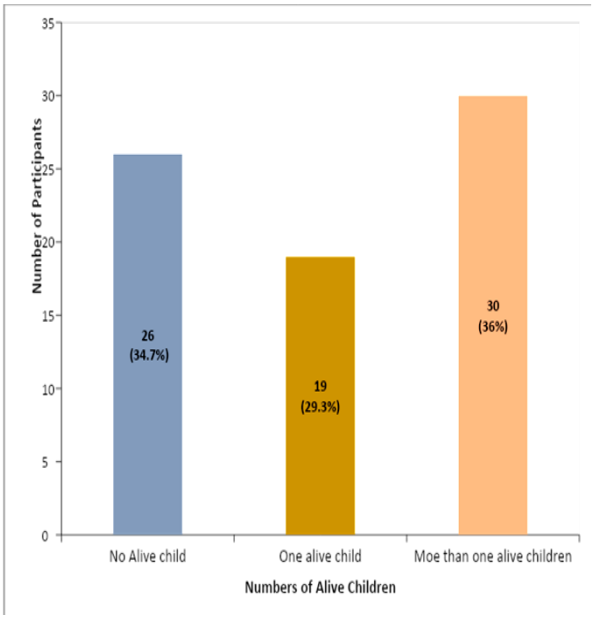
Complete medical history was obtained from all the subjects. Blood samples were collected in 8.5 ml tubes, containing an anticoagulant. The plasma was frozen at -30°C. Frozen plasma samples were thawed and the protein PAI-1 was measured by plasminogen activator inhibitor -1 ELISA kit. This enzyme-linked immunosorbent assay (ELIZA) measures the PAI-1protein in plasma. It uses a technique called a sandwich enzyme immunoassay

to quantify the amount of PAI-1 present. By analyzing the color intensity, the test provides a quantitative measurement of PAI-1 levels in blood. Information was recorded on SPSS version 20 for analysis. Mean ± SD, median, minimum, and maximum were calculated for numerical data (i.e., age and PAI-1). For qualitative data, frequencies and percentages were used to represent descriptive analysis. The association between categorical variables was assessed using the chi square test. A p-value of < 0.05 was considered as significant.

RESULTS

Among females with a history of recurrent pregnancy loss (n=75), 18.7% (n=14) exhibited increased levels of PAI-1, while 81.3% (n=61) exhibited PAI-1 within the normal range. Additionally, 34.7% (n=26) had no live births, 25.3% (n=19) had one live birth, and 36% (n=30) had two or more live births. (Figure 1).

Figure:1. Distribution of number of children among females of recurrent pregnancy loss



An association of PAI-1 level is shown with number of alive/no alive child in RPL women (n=75). A significant association (P= 0.019) was found between elevated PAI-1 and poor reproductive outcome (Table 1). A comparison was done among females having no alive child with females having two or more alive children. (Table 2). In the group with normal PAI-1 levels, 19 (40%) had no alive child, whereas 29 females (60%) had two or more alive children. On the other hand, in the group with increased PAI-1 levels, 7 (87.5%) females did not have an alive child and only 1 (12.5%) had two or more alive children. This indicates that women with elevated PAI-1 levels were more likely to have no alive children compared to those with normal levels. This shows that women with normal PAI-1 levels were far more likely to have multiple alive children compared to those with elevated levels. The P-value of (<0.05) strengthens the association between the levels of plasminogen activator inhibitor-1 and the number of alive children. Specifically, a P-value of 0.01 underscores a strong relationship, with elevated PAI-1 levels being linked to worse pregnancy outcomes, including a higher likelihood of having no alive children.

Table 1. Association of PAI-1 level with number of alive children in RPL women (n=75)

Outcome	Normal PAI-1 (n=61)	%	Elevated PAI-1 (n=14)	%	Total (n=75)	%	P-Value
No alive child	19	31.1	7	50.0	26	34.7	
One alive child	13	21.3	6	42.9	19	25.3	
Two or more alive children	29	47.5	1	7.1	30	40.0	0.019
Total	**61**	**100**	**14**	**100**	**75**	**100**	

Significant p value: < 0.05 ↕ Pearson Chi square test.

Elevated PAI-1 is significantly associated with poorer reproductive outcomes, mainly due to fewer women having ≥2 alive children.

Women with elevated PAI-1 are much more likely to have no alive child compared with women having 2 or more alive children.

Furthermore, when women with no live child were compared to those with a single live child in relation to elevated PAI-1 levels, no significant association was observed (P > 0.05) (Table 3) There was no significant difference between .

Table:2 Pairwise comparison: No alive child vs. Two or more alive children (n=56)					
TOTAL n=56	No alive child		Two or more alive children		P-Value
	n=26	(%)	n=30	(%)	<0.05
PAI-1Normal (n=48)	19	40	29	60.41	0.01
PAI-1Greater than normal (n=8)	7	87.5	1	12.5	

Significant p value: < 0.05 ↕ Pearson Chi square test.

elevated and normal PAI-1 when comparing women with no alive child and those with one alive child (P > 0.05).

Table: 3 Pairwise comparison: women with no alive child vs. women with a single alive child (n=45)			
PAI-1 Levels (n=45)	No alive child (n=26) (%)	Single alive child (n=19) (%)	P Value
PAI-1 Normal (n=32)	19 (59.37)	13 (40.62)	0.056
PAI-1 Greater than normal (n=13)	7 (53.84)	6 (46.15)	

Significant p value: < 0.05 ↕ Pearson Chi square test.

A statistically significant correlation (P < 0.05) emerged between women with a single alive child and those with two or more alive children (Table 4). Specifically, 85.7% of females with elevated PAI-1 levels had only one alive child, and only 14.28% had two or more live children. In contrast, 31% of females with normal PAI-1 levels have single alive child and 69% have two or more alive children. This indicates that women with normal PAI-1 levels are more likely to have multiple alive children than females with higher PAI-1 levels (P-value < 0.05) (Table 4).

Table:4 Women with a single alive child vs woman with two or more alive children (n=49)			
PAI-1 Levels (n=49)	Single alive child (n=19) (%)	Two or more alive children (n=30) (%)	P-Value
PAI-1 Normal (n=42)	13 (31)	29 (69)	0.04
PAI-1 Greater than normal (n=7)	6 (85.7)	1 (14.28)	

Significant p value: < 0.05 ↕ Pearson Chi square test.

Elevated PAI-1 levels are strongly associated with having only one live child rather than multiple alive children (P < 0.05).

DISCUSSION

Several studies have shown a strong association between increased PAI-1 levels and recurrent miscarriages. PAI-1 inhibits fibrinolysis, and elevated levels can result in the formation of microthrombi in the placental vasculature, leading to impaired blood flow to the developing fetus. The reduced blood flow to the placenta results in oxygen and nutrient deprivation, which leads to early pregnancy loss.[14] Another study revealed that elevated plasminogen activator inhibitor-1 levels were significantly linked with placental insufficiency in recurrent miscarriage cases. This study highlighted that increased PAI-1 levels impair normal fibrinolysis within the uteroplacental circulation, leading to micro-thrombosis and subsequent placental damage. The formation of thrombi leads to local ischemia, compromising trophoblast invasion, a process critical for the formation of a placenta.[15,16] A meta-analysis emphasized the role of PAI-1 in fibrinolysis, embryo implantation, and placental processes.[17] Additionally, the trophoblast cells, responsible for invading the uterine wall to establish normal placental blood flow, can be affected by elevated PAI-1. This can lead to incomplete development of the placenta, impaired vascularization resulting in pregnancy loss. Furthermore, to maintain adequate blood flow to the placenta, an efficient fibrinolytic system is required. In addition, elevated PAI-1 levels can cause endothelial dysfunction resulting in impairment of placental blood flow (Zhao et al., 2021).[18, 19.]

Genetic and environmental factors can also influence PAI-1 levels. The plasminogen activator inhibitor-1 gene 4G/5G polymorphism has been linked with high PAI-1levels, predisposing individuals to thrombotic events, including recurrent miscarriage. Li et al. found that women with the 4G/4G genotype have considerably

increased PAI-1 levels, increasing their risk of pregnancy loss.[20] Another study by Xie et al**.** established that women with recurrent miscarriages had considerably elevated PAI-1 levels compared to those with normal pregnancies, suggesting a strong association of high PAI-1 level with reduced fibrinolysis in the uteroplacental circulation, resulting in micro thrombosis and impaired placental development.[21.] Baxter et al. further demonstrated that inhibition of plasminogen activation by PAI-1 leads to inadequate vascular remodeling, resulting in compromised placental perfusion and subsequent miscarriage.[22] Elevated PAI-1 levels have emerged as a significant factor in the pathophysiology of recurrent miscarriage, through mechanisms such as impaired fibrinolysis, endothelial dysfunction, and placental insufficiency, increasing the risk of miscarriage.[23.]Increased PAI-1 levels may promote spiral arterial or intervillous thrombosis that reduces placental perfusion. PAI-1 gene polymer-phism contributes to the development of recurrent miscarriages by altering thrombotic events. Over expression of PAI-1 leads to the increased deposition of fibrin resulting in diminished blood flow through the placenta, affecting the development and growth of the fetus.[24]

CONCLUSION

Increased plasma level of plasminogen activator inhibitor-1 interferes with fetomaternal circulation and may have role in recurrent pregnancy loss. Women with normal PAI-1 levels tend to have better pregnancy outcomes, with a higher likelihood of having two or more alive children. On the other hand, elevated PAI-1 levels are strongly associated with adverse outcomes. These findings suggest that elevated PAI-1 levels could serve as a potential risk factor for poor pregnancy outcomes in women with recurrent pregnancy loss (RPL).

***Limitations*:** The study was limited by budget constraints, which restricted the sample size and the use of advanced molecular or genetic testing to further explore the mechanisms underlying elevated PAI-1 levels.

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Samina Mohyuddin: Conceived the study designed, carried out the data collection and statistical analysis and drafted the manuscripts.

Rabab Zehra: Participated in its design and coordination. drafted, read and approved the final manuscript.

Samar Ekram: Participated in its design and coordination. Statistical analysis, drafted, read and approved the final manuscript.

Zareena Naz: Participated in its design and coordination. Statistical analysis, drafted, read and approved the final manuscript.

Shahida Kashif: Participated in its design and coordination. Statistical analysis, drafted, read and approved the final manuscript.

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