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**Original Research Article**

# Association of antithrombin (antithrombin III) gene mutation with unexplained recurrent pregnancy loss

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## ABSTRACT

**Background:** Recurrent pregnancy loss (RPL) is an emotionally painful occurrence for couples and presents Obstetricians with a difficult clinical problem. Because a primary etiology cannot be determined in roughly half of the instances, it is irritating for both patients and obstetricians. The present study aimed to determine the association of the antithrombin III gene (SERPINC1) mutation with unexplained RPL.
**Methods:** This case-control observational study was conducted at the out-patient department of feto-maternal medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, with a total sample size of was 68, with 34 in the control group and 34 in the case/RPL group.
**Results:** The mean±standard deviation (SD) age of the RPL group was 28.44±5.25, and in the control group it was 29.15±4.72. The mean±SD body mass index (BMI) was 24.95±3.48 and 23.69±4.07 in RPL and control groups respectively. Among the RPL group patients, 68% (23) had the primary RPL, and 32% (11) had a second pregnancy loss.
**Conclusions:** 5.88% of the cases have a heterozygous mutation which might be the cause of their RPL. There was no homozygous mutation was found for G878A in the case group. The allele for G878A was also higher in the case group. But these differences were statistically non-significant. So, to clarify this association with unexplained RPL, further research is necessary including multi-centre and large sample sizes.

**Keywords:** Mutation, Pregnancy, Recurrent, Antithrombin

## INTRODUCTION

Recurrent pregnancy loss (RPL) is an emotionally traumatic experience for couples and poses a strenuous clinical challenge to obstetricians. RPL not only represents the loss of a future child or motherhood but also engenders doubts regarding her ability to procreate.[1] The definition of RPL has long been debated and differs among international societies. American society of reproductive medicine (ASRM) and the European society for human reproduction and embryology (ESHRE) has defined RPL as two (2) or more clinical (documented by ultrasonography or histopathologic examination) and consecutive pregnancy losses along with the exclusion of ectopic and molar pregnancies ASRM, 2012; GDG, ESHRE, 2017). Royal college of obstetricians and gynecologists (RCOG, 2011) has defined "RPL" as the loss of three (3) or more consecutive pregnancies.[2] The incidence of RPL varies widely among reports because of the differences in definitions and criteria used. Approximately 1% of prospective couples experience recurrent miscarriage (when RPL is considered as 3 or more losses).[3,4] On the contrary, RPL affects 5% of

couples, further magnifying the scale of the problem, if the working definition is altered to two or more losses.[5] Identification of causes of RPL is the most challenging issue for the Fetomaternal specialists. Etiologies are so far determined in approximately 50% of couples with RPL. Unfortunately, remain without an identified cause, even after extensive investigations. These cases are referred to as unexplained RPL and serve as the submerged portion of the iceberg for the researchers. Being multifactorial in origin, various genetic and non-genetic factors are attributable to RPL. Chromosomal abnormalities (aneuploidy, rearrangement), congenital or acquired uterine and cervical anatomical abnormalities, ovarian dysfunction, endocrine problems like thyroid dysfunction, polycystic ovary syndrome (PCOS), immunologic abnormalities, and acquired or inherited thrombophilia act heterogeneously for the causation of RPL in different trimesters.[6] After chromosomal abnormality, thrombophilic disorders have generated considerable interest in the field of RPL of genetic origin, especially in unexplained cases. Physiologically pregnancy inclines more towards a prothrombotic state and pathological exaggeration of this hypercoagulability has been increasingly linked to pregnancy loss and placenta-mediated complications.[7,8] Hereditary thrombophilia is a group of genetic disorders characterized by the presence of mutated and functionally altered blood coagulation factors that predispose to thrombosis in blood vessels. During pregnancy, thrombophilic persons may show a propensity for thrombosis of placental vasculature that in turn results in spontaneous pregnancy loss in the early weeks, and development of preeclampsia, intrauterine growth restriction, placental abruption, and stillbirth in the latter part of gestation.[4,9-11] The relationship between RPL and thrombophilia is a much-debated topic with well-entrenched expert opinion on both sides. The potential association between the two is based on the theory of thrombosis in decidual vessels and inhibition of trophoblast differentiation causing fetal loss.[12,13] Antithrombin (AT) is a tiny protein molecule that inhibits various coagulation system enzymes. It is a glycoprotein made by the liver that has 432 amino acids. Antithrombin III belongs to the superfamily of serine proteinase inhibitors (SERPIN).[14] Antithrombin III deficiency is a risk factor for RPL. Inherited thrombophilia due to mutations of the gene SERPINC1 leads to antithrombin III deficiency. The present study was conducted to observe any form of association between antithrombin III gene (SERPINC1) mutations with unexplained RPL.

## METHODS

This case-control observational study was conducted at the out-patient department of feto-maternal medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. The study duration was 6 months, starting from May 2021 to October 2021. The sample size for this study was determined by using the formula for case-control. So, both the case and the control group had 34. 34 patients among those who attended the fetomaternal OPD for preconception counselling for RPL who had a history of consecutive two or more failed clinical pregnancies were selected for the case group, and age and BMI matched 34 women with at least one successful pregnancy and no history of pregnancy loss were selected from the inpatient and outpatient department of the study hospital for the control group. A convenient sampling technique was applied, according to the availability of the patients as per inclusion and exclusion criteria. A structured questionnaire was prepared including all the variables of interest in the study, which was used to collect and record all data. Detailed history and clinical information were obtained by using the preformed structured questionnaire after acquiring the informed written consent of the participants. Informed written consent was obtained from the participants of both the case and the control group, and the study was also approved by the ethical review committee (IRB) of the study hospital. All patients underwent a complete diagnostic workup for RPL including relevant history, clinical examination, and investigations to exclude chronic hypertension, diabetes mellitus, autoimmune disorders, chronic renal disease, thyroid disorders, PCOS, chromosomal analysis of both partners, and uterine anomalies.

Inclusion criteria of the study woman in the case group having reproductive age, Patients who had given consent to participate in the study, and history of ≥2 failed consecutive clinical pregnancies. In the control group age-matched women with at least one successful pregnancy and no history of pregnancy loss. Exclusion criteria were mentally ill, unable to answer the criteria question, women diagnosed to have a known cause for RPL (e.g. chromosomal abnormalities, anatomical defects of the uterus, and thrombophilic disorders) and exclude those affected with other chronic diseases.

## RESULTS

A maximum of 64.7% of patients in the RPL group and 67.7% in the control group, were between 25 to 34 years of age. Only 5 (14.7%) patients in the RPL group were from 35-40 years of age. An independent sample t-test was done to compare the mean BMI between RPL and control groups. The mean difference was not statistically significant (p=0.208). So it can be said that the BMI of the RPL group (24.95±3.48) was matched with the control group (23.69±4.07). The number of previous conceptions of the study sample was compared for RPL and control groups. In the RPL group, the number of pregnancies ranged from 2 to 7 and the majority were pregnant thrice. In the control group, the number of pregnancies ranged from 1 to 4 where most of the participants in the group had been pregnant once or twice with no history of spontaneous abortion. It appears from the figure that 59% (20) of the RPL cases had 3 pregnancy losses and 14% (5) had 2 pregnancy losses. It appears from the figure that 68% (23) had the primary RPL, and 32% (11) had a second pregnancy loss. It appears from Figure 4 that 55% (31) patients of the RPL group experienced pregnancy loss in

the first trimester (includes only first-trimester loss and combined first and second-trimester loss) and 24% (14) cases patients in 2nd trimester (includes only second-trimester loss and combined first and second trimester losses). 20% (11) of patients had pregnancy losses in both the first and second trimesters. According to the frequency of SERPINC1 G878A genotypes, wild type (no mutation) GG and homozygous mutation AA in cases were 32 (94.12%), 0 (0.00%) respectively. Heterozygous mutations GA was 2 (5.88%) found among in case group. While it controls the frequencies were wild type GG and genotype AA in cases was 34 (100.00%), 0 (0.00%) respectively. No mutant heterozygous GA was found among in control group. Heterozygous mutant GA genotype was found more frequent in cases than controls (5.88% versus 0.00%). Mutant A allele was also found more frequent in cases compared to the controls (5.88% versus 0.00%). But both the differences were not statistically significant (p=0.4752 and 0.0927 respectively).

**Table 1: Age distribution of patients.**

| Age group (years) | RPL patients (n=34) | | Control (n=34) | | P value |
|---|---|---|---|---|---|
| | N | % | N | % | |
| 18-24 | 7 | 20.6 | 5 | 14.7 | |
| 25-34 | 22 | 64.7 | 23 | 67.7 | 0.562$^{NS}$ |
| 35-40 | 5 | 14.7 | 6 | 17.6 | |
| Mean±SD | 28.44±5.25 | | 29.15±4.72 | | |

**Table 2: Body mass index distribution of the study sample.**

| BMI (kg/m$^{2)}$ | RPL (n=34) | | Control (n=34) | | P value |
|---|---|---|---|---|---|
| | N | % | N | % | |
| Less than 18.5 | 0 | 0 | 1 | 2.9 | |
| 18.5 to 24.9 | 18 | 52.9 | 21 | 61.8 | |
| 25 to 29.9 | 12 | 35.3 | 9 | 26.5 | 0.208$^{ns}$ |
| 30 and above | 4 | 11.8 | 3 | 8.8 | |
| Mean±SD | 24.95±3.48 | | 23.69±4.07 | | |
| Range | 20-32.4 | | 18-31.2 | | |

**Table 3: Distribution of SERPINC1 G878A genotypes.**

| SERPIN C1 | Genotype frequency (%) | | | | | | Allele (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| G878A | GG | % | GA | % | AA | % | G | % | A | % |
| Cases | 32 | 94.12 | 2 | 5.88 | 0 | 0 | 64 | 94.12 | 4 | 5.88 |
| Controls | 34 | 100 | 0 | 0 | 0 | 0 | 68 | 100 | 0 | 0 |
| P value | 0.4752$^{NS}$ | | | | | | 0.0927$^{NS}$ | | | |

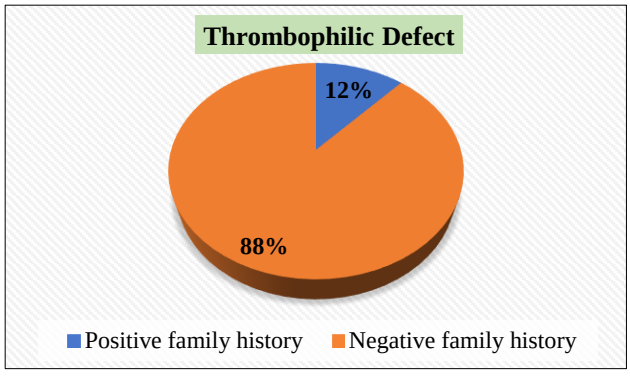


**Figure 1: Pie chart showing family history of thrombophilic defect among RPL cases.**

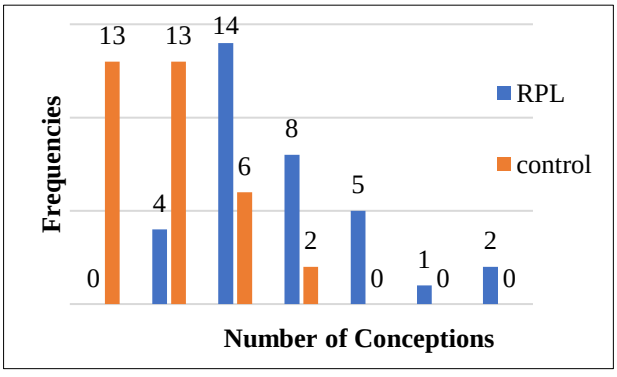


**Figure 2: Number of conceptions of the sample population.**

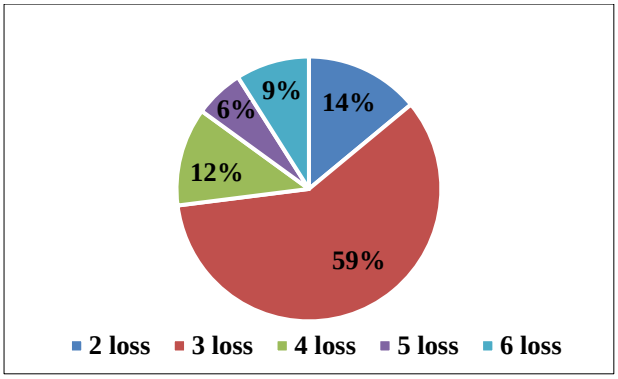


**Figure 3: Number of pregnancy loss in case group.**

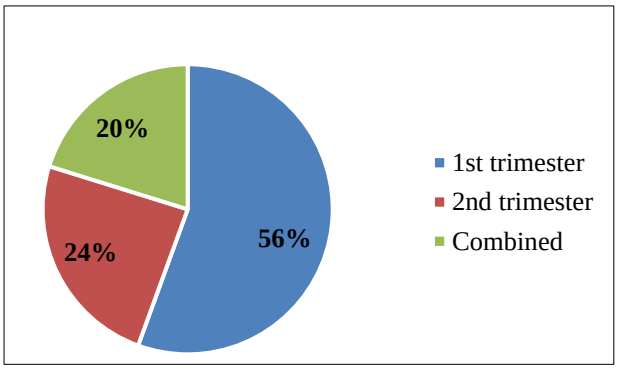


**Figure 4: Pie chart showing pregnancy loss according to trimester.**

## DISCUSSION

RPL has devastating consequences on the happiness of the couple and the maintenance of marital harmony. It is frustrating for both patients and obstetricians because a causative etiology cannot be identified in about 50% of cases. At present, one of the possible causes increasingly investigated in the literature is the thrombophilic status which may alter the placental circulation antithrombin III deficiency due to SERPINC1 gene mutation is part of these thrombotic risk factors and several studies have been investigated their potential association with RPL with inconclusive and controversial results.[15] In the present study, it was found that 64.7% of cases and 67.7% of controls belonged to the age group 25 to 34 years. The mean±SD was 28.44±5.25 years in the case group and 29.15±4.72 years in the control group. The difference was not statistically significant (p=0.562) between the two groups. The mean age of the present study was similar but somewhat lower than other studies.[16,17] There were no statistically significant differences between the case and control groups in terms of age in both of the studies, which were similar to the current study. In our study, the mean BMI of RPL patients was 24.95±3.48 kg/m² and that of the control was 23.69±4.07 kg/m² (p=0.208). So, the BMI of the RPL group was not significantly different from that of controls that reflects perfect matching of BMI between the two groups. The number of previous conceptions of the study sample was compared for RPL and control group in our study. In the RPL group, the number of pregnancies ranged from 2 to 7 and the majority were pregnant thrice. In the control group, the number of pregnancies ranged from 1 to 4 at evaluation where most of the participants had been pregnant once or twice with no history of spontaneous abortion. Several pregnancy losses in patients of the RPL group were shown in the Pie chart in Figure 2. It appeared that 59% (20) of the RPL patients had 3 pregnancy losses and 14% (5) had 2 pregnancy losses. The remaining had 4, 5, and 6 pregnancy losses of 12%, 6%, and 9% respectively. In this study, the average number of spontaneous pregnancy losses was 3, ranging from 2 to 7. In the present study, the pie chart in Figure 3, showed that 68% (23 in number) of patients had primary RPL and 32% (11 in number) had secondary RPL. A similar observation was also found in the study of Isaoglu et al where primary and secondary RPL was 68.33% and 31.67% respectively. Regarding the distribution of pregnancy loss according to trimester, this study showed that 55% of the RPL patients had the first-trimester loss, 23% had a second-trimester loss and 20% had combined first and second-trimester loss. The previously mentioned study showed in their findings that early and late RPL was 76.67% and 23.33% respectively. However, they did not calculate combined early and late loss in their study.[18] The event where the spontaneous loss of pregnancy occurs before the fetus reaches viability is termed miscarriage and RPL is defined as the consecutive loss of two or more pregnancies clinically documented. RPL has long been linked to thrombophilia predisposition. Several studies have found a link between protein S, protein C, antithrombin levels, and the FV Leiden mutation. However, the role of each component in RPL differs among studies, and no molecular research has been conducted in the Bangladeshi population with RPL. However, the presence of two patients with the G878A heterozygous condition and absence in the control population indicates that SERPINC1 in its homozygous mutant form may be contributory to the RPL resistance as previous studies have shown that this mutation is associated with mild RPL resistance and is a mild risk factor for thrombosis.[19] Although antithrombin III deficiency causes thrombophilia, more than 250 mutations, including large-scale deletions and insertions, are known to cause thrombosis. The SERPINC1 gene mutation was found in 2 patients (5.88 percent) of the study population in the RPL group, but not in the control group. As a result, the present investigation discovered that it might be a cause of unexplained RPL in a few cases. One of the two patients was 32 years old, had four spontaneous abortions between 8 and 11 weeks of pregnancy, and was heterozygous (GA) for the SERPINC1 A878A gene mutation. This patient had no healthy baby. Another was 34-years-old, who had five abortions, including both 1st and 2nd-trimester loss. The patient was also positive for heterozygous (GA) for SERPINC1 A878A gene mutation. She also had two late pregnancy complications. Regarding genotype distribution of SERPINC1 G878A, it was found that there was no homozygous mutation AA of SERPINC1 G878A either in the case or control group. Only two heterozygous

mutations of SERPINC1 G878A were found in the case group. From this we can assume that homozygous mutation of SERPINC1 is probably very rare in our country as already mentioned, there is a wide variation in the prevalence of SERPINC1 polymorphism. Demir et al, a state in their study that the frequency of the homozygous SERPINC1 G878A genotype among the general population in Europe varies according to the geographical area studied, being 6-10% in the Nordic countries and 13-18% in the Mediterranean area.[20] The frequency of heterozygous mutant GA of SERPINC1 G878A genotype in this study was 5.88% and 0.00% for the case group and control respectively. Mutant A allele was also found more frequent in cases compared to the controls (5.88% versus 0.00%). Both the differences were statistically non-significant. Though antithrombin III deficiency due to SERPINC1 gene mutation is a risk factor for unexplained RPL, in our study we have shown that the differences were statistically non-significant in the RPL case and control group. The association between SERPINC1 gene mutation and unexplained RPL has been widely researched, with contradictory results. However, treatment of women with RPL having SERPINC1 gene mutation and/or antithrombin III deficiency with aspirin and low molecular weight heparin showed a high rate of successful pregnancy in different studies.[21,22] RPL workup did not include gene mutation or thrombophilia screen till now. Considering the impact of RPL on a couple's mental health and maintaining a healthy family life, further research on this subject including a large sample size and multicenter should be conducted.

***Limitations***

The study was conducted in a single hospital with a small sample size. So, the results may not represent the whole community.

## CONCLUSION

The 5.88% of the cases have a heterozygous mutation which might be the cause of their RPL. There was no homozygous mutation was found for G878A in the case group. The allele for G878A was also higher in the case group. But these differences were statistically non-significant. So, to clarify this association with unexplained RPL, further research is necessary including multi-centre and large sample sizes.

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