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Original Research Article

Association of prothrombin gene mutation (G20210A) with recurrent pregnancy loss

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ABSTRACT

Background: Recurrent pregnancy loss (RPL) is a significant reproductive health concern, often with multifactorial etiologies. Among the possible causes, thrombophilic gene mutations, such as the Prothrombin G20210A mutation, has the most potential role. This study aimed to investigate the association between Prothrombin G20210A gene mutation and RPL in a selected group of Bangladeshi women.
Methods: This case-control study was carried out in the outpatient Department of Fetomaternal Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, from December 2020 to May 2021. Total 35 women with history of recurrent pregnancy losses were selected as cases. The control group consisted of 35 women with at least one successful pregnancy and no history of recurrent pregnancy loss.
Results: Out of 35 cases two patients have Prothrombin gene mutation, one in 1st trimester and another in 2nd trimester. One was primary RPL and another one was secondary RPL. Normal homozygous (GG) were 94.3% and mutant heterozygous (GA) were 5.7%, mutant homozygous (AA) were 0.0% in case group. In control group there were no mutation of prothrombin gene (G20210A). The difference was statistically not significant (p=0.421) between two groups. Fisher exact test was done. This test was done to see the results were statistically significant or not. It is usually employed when sample sizes are small but it is valid for all sample sizes.
Conclusions: This study found no statistically significant association between the Prothrombin G20210A gene mutation and recurrent pregnancy loss. Although 5.7% of cases had heterozygous mutations, none were observed in controls.

Keywords: Association, Prothrombin gene mutation (G20210A), Recurrent pregnancy loss

INTRODUCTION

RPL is defined as the loss of two or more pregnancies.[1] Primary RPL is described as RPL without a previous ongoing pregnancy (viable pregnancy) beyond 24 weeks' gestation, while secondary RPL is defined as an episode of RPL after one or more previous pregnancies progressing beyond 24 weeks' gestation.[1] RPL is a common clinical

problem that occurs in approximately 5% of reproductive-aged women who had two or more losses of pregnancy. Among the known causes of RPL chromosomal and uterine anatomic abnormalities, endometrial infections, endocrine abnormalities, antiphospholipid syndrome, inherited thrombophilia, alloimmune causes, genetic factors and exposure to environmental factors are common.

The evidence is conflicting regarding the association with pregnancy complications. A delicate balance between coagulant and anticoagulant factors is needed to achieve a successful pregnancy. A balanced system maintains the blood flow to the feto-maternal exchange unit and promotes trophoblastic proliferation. The hypercoagulable state that occurs during pregnancy makes it tempting to postulate that pregnancy association with a thrombophilic condition may be detrimental through either RPL, intrauterine fetal death (IUFD) and/or other complications such as placental abruption, fetal growth restriction (FGR) and early onset of preeclampsia. Researchers have been working on inherited thrombophilia to explain RPL, especially that 30–50% of RPL cases remain enigmatic.[2]

Among the inherited thrombophilia prothrombin gene mutation is the most common genetic marker.[3] Prothrombin G20210A mutation was first described by Poort S.R. and his colleagues in 1996. The prothrombin gene has been localized to chromosome 11 (11p11–q12).[4] It represents the nucleotide replacement of guanine (G) with adenine (A) in the 3-untranslated region of the gene (G20210), which leads to an increase in the prothrombin level in blood plasma by 1.5–2 relative to the normal range. Prothrombin or factor II, a vitamin K-dependent glycoprotein zymogen, is known to be a precursor of thrombin, which turns into thrombin under the influence of activated Factor X coagulation. Prevalence of prothrombin G20210A mutation depends on ethnicity and ranges from 0.7 to 6.7%.[5]

Sequence variation of a G-A transposition in position 20210 of the prothrombin gene recently was identified as a genetic risk factor for prothrombin gene mutations in Egyptian cases with recurrent pregnancy loss.[4] This mutation is associated with a 20% to 50% increase in prothrombin plasma levels and affected women have a three-fold increased risk of venous thrombosis. Studies of G20210A polymorphism have also shown a strong association between the polymorphism and a recurrent abortion.[6]

The association between thrombophilia and RPL has become an undisputed fact. Clinical studies suggest that hypercoagulation is the main underlying pathophysiological mechanism which leads to uteroplacental insufficiency and, subsequently, pregnancy loss. It is believed that inherited thrombophilia (IT) impairs the placental function by causing arterial and/or venous thrombosis at the maternal-fetal interface.

The exact mechanism by which IT causes implantation failure and subsequent RPL is unclear. It has been suggested that thrombophilia may lead to a syncytiotrophoblast invasion of the maternal blood vessels, which in turn leads to the formation of microthrombosis at the site of implantation, resulting in implantation failure and RPL.[7]

This study is designed to determine the frequency of prothrombin gene (G20210A) mutation with recurrent pregnancy loss.

Objectives

To determine prothrombin gene (G20210A) mutation among recurrent pregnancy loss.

METHODS

This case-control study was carried out in the outpatient department of Fetomaternal Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, from December 2020 to May 2021. Total 35 women aged 18–40 years with a history of two or more recurrent pregnancy losses were selected as cases through purposive and convenience sampling. The control group consisted of 35 age- and BMI-matched healthy non-gravid women with at least one successful pregnancy and no history of recurrent pregnancy loss or gestational complications. Inclusion and exclusion criteria were strictly applied to ensure valid results, excluding participants with known causes of RPL such as uterine anomalies, antiphospholipid syndrome, chromosomal abnormalities, diabetes, PCOS, chronic hypertension, thyroid disorders and systemic lupus erythematosus.

After obtaining informed written consent, data were collected through structured interviews and recorded on predesigned forms, covering socio-demographic characteristics, obstetric history and BMI. Clinical and laboratory evaluations were conducted to rule out other causes of RPL. Blood samples (3 ml) were drawn from each participant using aseptic techniques, stored in EDTA tubes and transferred to the PCR Lab of the Department of Microbiology and Immunology, BSMMU, where they were kept at 2–8°C until DNA extraction. Genetic testing for prothrombin gene mutation (G20210A) was conducted using PCR.

Data were analyzed using SPSS version 22. Categorical variables were expressed as frequencies and percentages, while continuous variables were reported as mean±SD. Associations were tested using Fisher's exact test and Chi-square test, considering $p<0.05$ as statistically significant. Ethical clearance was obtained from the Institutional Review Board (IRB) of BSMMU and participants were assured of confidentiality and provided with detailed information regarding the study's purpose, risks and benefits in comprehensible language.

RESULTS

A total of 70 patients participated in the study. RPL cases were n=35 and non-RPL (control) cases were n=35. Table 1 shows that the majority of patients were aged 26–33 years: 57.1% in the RPL group and 45.7% in the non-RPL group. Mean age was 28.2±5.26 in RPL and 27.2±5.37 in control group. The age difference was not statistically significant (p=0.421). Most patients were overweight in both groups: 54.3% (RPL) and 71.4% (non-RPL). Mean BMI was 24.1±2.25 in RPL and 24.72±1.95 in control group (p=0.230).

Consanguinity was reported in 11.4% of the cases and 2.9% of the controls, which also was not statistically significant (p=0.164). Table 2 presents trimester of pregnancy loss and number of abortions in case group. Most losses occurred in the first trimester (57.1%), followed by losses in both trimesters (34.3%) and only second trimester losses (8.6%). Regarding the number of abortions, 45.7% of the participants experienced three abortions, while 37.1% had two and 17.1% had more than three. The mean number of abortions was 3.0 with a standard deviation of±1.01 and the range varied from 2 to 6 abortions. Table 3 highlights nature of recurrent pregnancy loss in case group. Primary RPL was observed in 57.1% of the participants, while 42.9% had secondary RPL. A Z-test showed that this difference was not statistically significant (Z=0.80, p=0.406). Table 4 demonstrates association of G20210A in case and control Group.

Among the cases, 94.3% were normal homozygous (GG) and 5.7% were heterozygous mutants (GA), while no homozygous mutants (AA) were detected. In contrast, all individuals in the control group were normal homozygous (100%). Fisher's exact test showed that the difference was not statistically significant (p=0.493). Table V shows descriptive characteristics of cases with mutation. The first patient, aged 32, experienced a first-trimester loss with a secondary RPL history and had three abortions. The second patient, aged 25, had a second-trimester loss with primary RPL, also with three abortions. Both were identified as having the GA mutation.

Table 1: Baseline characteristics of the study groups (n=70).

Characteristics	Case (n=35) No. (%)	Control (n=35) No. (%)	P value
Age group (in years)			
18–25	10 (28.6)	15 (42.9)	0.421^{ns}*
26–33	20 (57.1)	16 (45.7)	
34–40	5 (14.3)	4 (11.4)	
Mean±SD	28.2±5.26	27.2±5.37	
Range (min–max)	18–40	19–38	
BMI (kg/m²)			
Normal weight	13 (37.1)	5 (14.3)	0.230^{ns}*
Overweight	19 (54.3)	25 (71.4)	
Obese (≥ 27)	3 (8.6)	5 (14.3)	
Mean±SD	24.1±2.25	24.72±1.95	
Range (min–max)	20.0–28.6	21.9–29.80	
Consanguinity			
Yes	4 (11.4)	1 (2.9)	0.164^{ns}**
No	31 (88.6)	34 (97.1)	

*=Unpaired Student t-test was done, ns=not significant, **=Chi-square test was done, ns=not significant.

Table 2: Trimester of pregnancy loss and number of abortions in case group (n=35).

Parameter	Frequency	(%)
Trimester of pregnancy loss		
1st Trimester	20	57.10
2nd Trimester	3	8.60
Both Trimesters	12	34.30
Number of abortions		
2 Abortions	13	37.10
3 Abortions	16	45.70
>3 Abortions	6	17.10
Mean±SD	3.0±1.01	
Range	(2–6)	

Table 3: Nature of recurrent pregnancy loss in case group (n=35).

Nature of pregnancy loss	Frequency	(%)	Z test
Primary RPL	20	57.10	Z=0.80
Secondary RPL	15	42.90	p=0.406^{ns}

p-value obtained by Z-proportion test, ns=not significant.

Table 4: Association of G20210A in case and control group (n=70).

Prothrombin gene mutation	Case (n=35) No. (%)	Control (n=35) No. (%)	P value
GG (normal homozygous)	33 (94.3)	35 (100.0)	0.493^{ns}
GA (mutant heterozygous)	2 (5.7)	0 (0.0)	
AA (mutant homozygous)	0 (0.0)	0 (0.0)	

Fisher exact test was done, ns=not significant.

Table 5: Descriptive characteristics of cases with mutation (n=2).

Case no.	Age	Trimester	Nature of RPL	No. of abortions	Mutation
30	32	1st	Secondary	3	GA
33	25	2nd	Primary	3	GA

DISCUSSION

This case-control study was investigated for possible association of Prothrombin Gene Mutation (G20210A) with RPL. The cases were n=35 and non-RPL (control) cases were n=35. The summary of demographic data of the study subjects showed no significant differences regarding maternal age, BMI and consanguinity between the analyzed groups. The mean (±SD) age was 28.2±5.26 years in RPL group and 27.2±5.37 in the non-RPL group.

Bigdeli et al similar to this showed the mean age of women in the case group was 23.0±3.8 years, regarding the control group the mean age was 25.1±4.4 years in control group, which were similar to current study.[8] The risk of RPL was significantly higher in women older than 29 years (OR: 1.91, 95% CI: 1.61–6.11) and a positive relationship was observed between prothrombin G20210A mutation and fetal loss.[9]

Nassour-Mokhtari et al in his case-control study found a significant correlation between age and pregnancy loss.[10] In the present study maximum patients were overweight both in RPL 54.3% and non-RPL group 71.4%. The mean (±SD) BMI was 24.1±2.25 in RPL group and 24.72±1.95 in non-RPL group. The difference regarding BMI was statistically not significant (p=0.230) between two groups. Nikolaevia, et al also found non-significant BMI between both groups (p=0.6).[11] It was observed in this study that, primary RPL were 57.1% and secondary RPL were 42.9%.

The difference was not statistically significant (p=0.406). Lund, et al in his study also found statistically not significant in primary and secondary RPL group (p<0.01).[12] Nassour–Mokhtari et al in his case-control study showed no significant difference (p=0.01) among type of pregnancy loss.[10] Present study showed normal homozygous (GG) were 94.3%, mutant heterozygous (GA) were (5.7%) and no mutant homozygous were (0.0%) in case group. In control group there were no mutation of Prothrombin gene (G20210A). As my sample size is small and no mutation was found in control groups, no hypothesis testing can't be done and odds ratio cannot be calculated. The difference was statistically not significant (p=0.421) between case and control groups. This result is consistent with Bigdeli et al the genotype frequencies among prothrombin gene mutations (G20210A) were not observed to be different among RPL and non RPL groups (p=0.0579).[8] Gao, et al, in a systemic review and meta-analysis found a combined odds ratio (OR) of 1.81 (95% confidence interval (CI): 1.26–2.60).[9]

However, the risks differed in the subgroup analyses, categorized by study sites, maternal age and type of miscarriages. The pooled OR remained significant in European studies because white races are more prone to Prothrombin gene mutation (OR: 1.80, 95% CI: 1.35–2.41), whereas in the Middle-Eastern studies, it was not significant (OR: 2.39, 95% CI: 0.96–5.92). Yenicesu et al in a case-control study found heterozygous mutations of prothrombin G20210A were associated with RPL in Turkish couples.[13]

Present study showed heterozygous mutations in two patients, one in 1st trimester and another one in 2nd trimester, the 1st trimester one is secondary RPL and the 2nd trimester one is primary RPL. The meta-analysis of Gao et al suggests that the G20210A prothrombin mutation increases the risk of RPL (fetal loss, primary RPL or secondary RPL), particularly in Europeans.[9] Badaway et al in their study in Northern area of Saudi Arabia found inherited thrombophilic mutations have been reported as one of the main causes of RPL.[5] In his study, the

heterozygous mutant GA genotype of Prothrombin Gene were significantly associated with RPL compared to the controls ($p<0.0001$). These results support the relative high incidence of thrombophilic mutations in the Northern area of Saudi Arabia. In their study, the mutations have been implicated as common genetic variants that predispose to early (within 1st trimester) and/or late (after 1st trimester) RPL in Egyptian and Palestinian women.

However, several studies did not report a significant association between these mutations and RPL.[2,4,14] The association with Prothrombin gene mutation in recurrent pregnancy loss susceptibility has been widely researched, with contradictory results. In this current study, we could not test the association as the sample size was very small but out of 35 cases only 2 were positive that means if we did large scale study, association could be found out.

On the basis of the reasons of an association with placental thrombosis, preliminary case control studies suggest that low-dose Aspirin plus LMWH therapies are effective in preventing subsequent pregnancy loss in RPL women with thrombophilia.[15] RPL workup does not include thrombophilia screen till now.

In our study, there was small sample size and absence of control for comparison. Study population was selected from one center in Dhaka city, so may not represent wider population. The study was conducted at a short period of time. Scarcity of genetic laboratory facility.

CONCLUSION

This study concludes that about 5.7% of the recurrent pregnancy loss cases have heterozygous mutation (GA), there is no homozygous mutation and in the control group there is no mutation of prothrombin gene either heterozygous or homozygous. The difference was statistically not significant. Further research on this subject including large sample size and multicenter should be conducted. Thrombophilia screening policy for recurrent pregnancy loss group and to institute appropriate antithrombotic treatment is recommended. Laboratory facilities should be made available and kits should be cost effective.

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