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Original Research Article

Association of Factor V Leiden Mutation with Unexplained Recurrent Pregnancy Loss

Sanjukta Chowdhury[1*], Masuda Sultana[2], Surayea Bulbul[3], Ferdous Ara Banu[4], Prof. Nahreen Akhtar[5], Prof. Firoza Begum[6]

[1]Junior Consultant (Obs & Gynae), FCPS Subspeciality Course, Department of Fetomaternal medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU)
[2]Junior Consultant (Obs & Gynae), FCPS Subspeciality course, Department of Fetomaternal Medicine (BSMMU), Bangladesh
[3]Junior Consultant (Obs & Gynae), FCPS Subspeciality course, Department of Fetomaternal Medicine (BSMMU), Bangladesh
[4]Junior Consultant (Obs & Gynae), FCPS Subspeciality course, Department of Fetomaternal Medicine (BSMMU), Bangladesh
[5]Chairman, Department of Fetomaternal Medicine, BSMMU, Bangladesh
[6]Founding Chairman, Department of Fetomaternal Medicine, BSMMU, Bangladesh

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***Corresponding author:** Dr. Sanjukta Chowdhury
Junior Consultant (Obs & Gynae), FCPS Subspeciality Course, Department of Fetomaternal Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Bangladesh

Abstract

Introduction: Recurrent pregnancy loss (RPL) is considered as a significant public health problem. In many studies, Factor V Leiden mutation is considered to have significant relationship with unexplained recurrent pregnancy loss. ***Aim of the study:*** The aim of this study was to determine the association of Factor V Leiden mutation with unexplained recurrent pregnancy loss. ***Methods:*** This case-control study was conducted in the out-patient Department of Fetomaternal Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, from November 2020 to April 2021 (6 months). Sample size was taken as 40 for each case and healthy control group. ***Result:*** Mean (±SD) age was found 28.2±4.9 years in RPL group and 27.1±5.24 years in non-RPL group. Maximum number of patients fell into the BMI category of 23.0-26.9 kg/m2 (BMI for Asian women) in both groups. Among the RPL cases, 30% had experienced consecutive 2 pregnancy losses with mean (±SD) number of losses 3.07±1.14. About more than half percentages (n=23, 57.5%) shared the primary RPL group. In this study, normal homozygous FVL mutation was equally distributed among RPL patients and control individuals. Only 2 cases (5%) were found positive for Factor V heterozygous mutation (GA) in the RPL group. G allele occurred in most of the cases (97.5%) of RPL. Two cases aged 25 years and 35 years respectively were found positive for heterozygous mutation of Factor V Leiden. Both of them exhibited 3 consecutive recurrent second trimester pregnancy losses. Factor V Leiden was found in higher prevalence (100%) in 2nd trimester recurrent pregnancy loss sub-group of cases and revealed significant association ($p < 0.001$) between two variables. ***Conclusion:*** The impact of Factor V Leiden mutation has not stated any causal association with unexplained recurrent pregnancy loss. The results do not support Factor V mutation screening as an initial approach in Bangladeshi women suffering from recurrent pregnancy loss.

Keywords: Association, Factor V Leiden Mutation and Unexplained Recurrent Pregnancy Loss.

INTRODUCTION

Recurrent pregnancy loss (RPL) is an emotionally traumatic experience for the couples and poses a strenuous clinical challenge to the High risk Pregnancy Specialists. Female partner assumes recurrent pregnancy loss as deprivation from a future child or from motherhood, and sometimes questions her ability to procreate [1]. Definition of RPL has long been debated and differs among International Societies. First consensus evolved from Royal College of Obstetricians and Gynaecologists (RCOG)[2]. This prestigious body has defined "Recurrent pregnancy loss" as the loss of three (3) or more consecutive pregnancies from the time of conception up to 24 weeks' gestation [2]. ASRM defined consecutive two pregnancy losses as RPL and emphasized for evaluation of the bereaved couples after 2 losses. This definition has been adopted for the

current study purpose [3]. In the guideline of European Society for Human Reproduction and Embryology (ESHRE), definition of RPL has been liberalized as two (2) or more consecutive or non-consecutive pregnancy losses up to 24 weeks' following spontaneous conception or artificial reproductive technology along with exclusion of ectopic and molar pregnancies [4]. 1% of prospective couples' experience recurrent pregnancy loss (when RPL is considered as 3 or more losses), on the contrary, RPL affects 5% of couples, if working definition is altered to 'two or more losses [5]'. Identification of causes of RPL is the most challenging issue for the Fetomaternal specialists. Various genetic and non-genetic factors are attributable to the causation. It was established that majority of first trimester pregnancy losses occur due to embryonic chromosomal abnormalities like aneuploidies and parental chromosomal rearrangement. Apart from that, maternal anatomical defect, such as congenital (septate, bicornuate, unicornuate, arcuate uterus) or acquired (submucous myoma, endometrial polyp and uterine adhesions) uterine anomalies and cervical insufficiency, may also contribute to recurrent pregnancy loss, particularly in second trimester [4, 6]. The role of endocrine factors like thyroid dysfunction and polycystic ovarian syndrome, infections, immunological dysfunction and hereditary thrombophilia in the aetiology remain contentious [5]. Cases of recurrent pregnancy loss in which no established causes identified, are referred to as unexplained recurrent pregnancy loss and serve as the submerged portion of iceberg for the researchers [7]. Hereditary thrombophilia like Factor V Leiden mutation, Antithrombin deficiency, Protein C or S deficiency, Prothrombin gene mutation are included in the battery of list of unexplained RPL. Like other thrombophilia, Factor V Leiden (FVL) has been studied extensively to find out the causation with RPL. Most carriers of Factor V mutation remain asymptomatic. Presence of risk factors, such as pregnancy, use of oral contraceptive pill, surgery, immobilization; synergistically amplifies the risk of thrombosis in carrier persons and occasionally leads to life-threatening thrombotic events [8]. Persons with mutated Factor V sometimes present with deep vein thrombosis and abnormal vascular thrombosis like in placental vessels, representing clinical scenario of RPL and early preeclampsia [9]. Different studies have reported 3 to 42% prevalence of Factor V mutation among women with recurrent miscarriage. In general, its prevalence in Caucasian Population is 4 to 7% and it is extremely rare in indigenous populations from Africa, Southeast Asia and Australia [5]. Factor V Leiden mutation can be presented as homozygous-AA or heterozygous-GA state with the clinical implication of more thrombosis in homozygous form [10]. Existing data on the association between Factor V Leiden and recurrent pregnancy loss is weak. To further link up the association, this study was designed to determine the association of Factor V Leiden gene mutation with unexplained recurrent pregnancy loss. Not only that treatment with low molecular weight heparin would improve the live birth rate in these group of women suffering from RPL and factor V Leiden mutation.

OBJECTIVES

General objective

- To determine the association between Factor V Leiden mutation and unexplained recurrent pregnancy loss.

Specific objectives

- To document the frequency and heterozygous distribution of Factor V Leiden mutation in cases and controls.
- To determine the frequency of Factor V Leiden mutation in cases with primary and secondary RPL.
- To determine the frequency of Factor V Leiden mutation in context with first and second trimester RPL.
- To determine the frequency of Factor V Leiden mutation in context with number of RPL.

MATERIALS AND METHODS

Study group

This case-control study was conducted in the out-patient Department of Fetomaternal Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh from November 2020 to April 2021 (6 months). Considering the short study period, expensive gene mutation test and scarcity of patients due to COVID 19 situation, sample size was taken as 40 for each case and healthy control group through purposive and convenient sampling technique.

Inclusion Criteria

- Case: Women of age group 18-40 years, seeking preconception counselling due to consecutive two or more RPL.
- Control: Age and BMI (Body Mass Index)-matched healthy control (non-gravid) subjects with no history of fetal loss and had at least 1 healthy child.

Exclusion Criteria

- Congenital or acquired anatomic defects of uterus and/or cervix
- Antiphospholipid syndrome (APS)
- Parental chromosomal abnormalities
- Type-I and type-II Diabetes Mellitus (WHO 2020 criteria)
- PCOS (Rotterdam criteria 2003)
- Hypertension

Additionally, the following factors were excluded in control group -

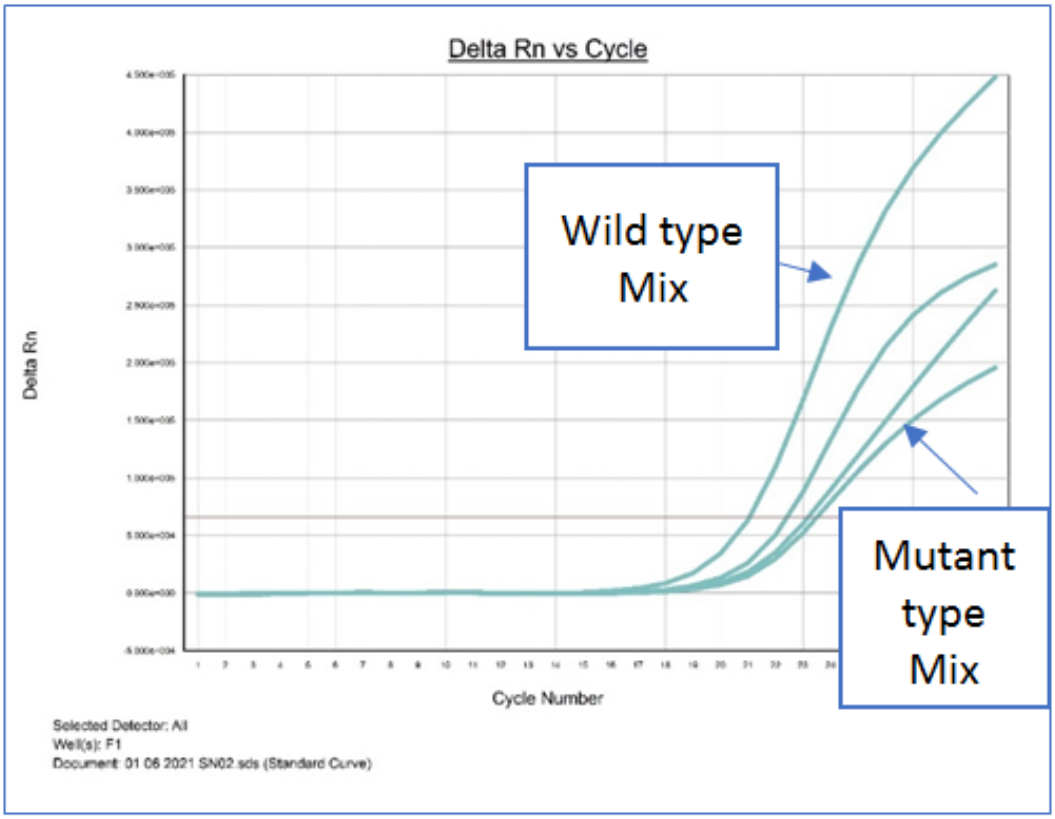
- History of venous thromboembolism (VTE) manifest as deep vein thrombosis or pulmonary

embolism during pregnancy and/or during use of oral contraceptive pill

- Personal or family history of recurrent thrombosis

DNA mutation analysis procedure: Genomic DNA, extracted from the anticoagulated blood according to manufacturer's instructions, was assayed to see the factor V Leiden mutation by "Thrombophilia Real-Time PCR kit multiplexes (SNP Biotechnology R&D Ltd. Hacettepe Technopolis – Ankara/Turkey, Cat. No: 10R-20-09). It provided reagents in a ready-to-use mastermix format which had been specifically adapted for 5´ nuclease PCR. Mastermixes were mixed with DNA and the tubes were placed in validated 7500 Fast Dx Real-time PCR Instrument (Applied Biosystems) for PCR programme. During polymerase chain reaction, DNA polymerase cleaved the probe at 5´ end and separated reporter dye from quencher dye and resulted in fluorescent signal. An increase in the fluorescent signal (CT) was proportional to amount of specific PCR product.

After the run time, data were analysed using the software with HEX (JOE), TEXAS RED, CY5, and FAM dyes. Homozygous wild type sample was given amplification signal only with wild type mastermix and heterozygote sample was given amplification signal both with wild type and mutant mastermix. Collected data were processed and analyzed using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 22 software for Windows. Data presented on categorical or qualitative variables like age group, educational status, monthly income, parity, nature and trimester of RPL, number of pregnancy loss were summarized in the form of frequencies and percentages. For comparison of mean of quantitative variables between two groups, an unpaired Student's t-test was performed. Association of Factor V Leiden mutation with RPL was tested using Fisher's exact test (as observations lesser than five per cell). $P < 0.05$ was considered statistically significant. To compare the categorical variables between two sub-groups divided on primary or secondary RPL and first trimester and second trimester RPL, Chi-square test ($\chi 2$) was used. Results of the study were presented in tables, figures and diagrams as appropriate.



Picture-1: Curve of Factor V Leiden Heterozygote

RESULTS

Table 1 show that mean (±SD) age was 28.2±4.9 years in RPL group and 27.1±5.24 years in non-RPL group. Maximum number of patients fell into the age group of 26-33 years [n=26 (65%) and n=20 (50%) in RPL and non RPL group respectively] and occupied the overweight (BMI 23.0-26.9 kg/m2) category in both groups [n=21 (52.5%) and n=27 (67.5%) in RPL and non RPL group]. Mean (±SD) BMI was 24.50±2.62 kg/m2 in RPL group and 24.87±2.19 kg/m2 in non-RPL group. Mean age and BMI differences were not statistically significant (p=0.397

and p=0.492) between two groups. Education, occupation and monthly income of the study populations consisting of two groups showed no significant statistical association (p value <0.05). Table 2 shows that among the RPL cases, 30% had experienced 2 pregnancy losses with mean (±SD) number 3.07±1.14. About more than half percentages (n=23, 57.5%) shared the primary RPL group. In context with trimester of pregnancy losses, 30% (n=12) cases suffered from pregnancy losses in both 1st and 2nd trimesters. Figure 1 shows that in parity distribution among non-RPL control group shows 65% (n=26) had 1-2 parity with rest (35%) had >2 parities. Table 3 shows that 12.5% (n=5) and 2.5% (n=1) of study subjects in RPL and non-RPL group respectively had history of consanguinity. The association of consanguinity with RPL was not statistically significant (p=0.090). In this study, normal homozygous for the FVL mutation were equally distributed among RPL patients and control individuals (Normal homozygous for FVL: 95% in case versus 100% in control). Only 2 cases (5%) were found positive for Factor V heterozygous mutation (GA) in the RPL group. We did not encounter a homozygous mutation of this gene in either group. As frequency of heterozygous mutation was 5%, Fisher exact test was done to find out the association between RPL and FVL mutation. No significant association (p=0.152) was found between the two variables. Table 4 presents that G allele occurred with a frequency of 97.5% among cases and 100.0% in controls while mutant A allele was seen only in 2.5% of the cases. The difference was not statistically significant (p=0.152). Table 5 shows that two cases aged 25 years and 35 years respectively were found positive for heterozygous mutation of Factor V Leiden. Both of them exhibited 3 consecutive recurrent second trimester pregnancy losses. They were categorized as primary RPL. Table 6 shows that Factor V Leiden was found in higher prevalence (100%) in 2nd trimester recurrent pregnancy loss sub-group of cases and revealed significant association (p <0.001) between recurrent 2nd trimester RPL and FVL mutation. Table 7 shows association of 'nature of RPL' with different variables was not statistically significant. Table 8 shows that 3 recurrent pregnancy loss group had 100% frequency of heterozygous Factor V Leiden. The association between Factor V Leiden and number of RPL was not statistically (p value =0.286) significant.

Table-1: Demographic characteristics of the study populations (n=80)

Variable		RPL (Case) n=40		Non-RPL (Control) n=40		p-value
		N	%	N	%	
Age group (years)	18-25	9	22.5	16	40	
	26-33	26	65	20	50	
	34-40	5	12.5	4	10	
	Total	40	100	40	100	
	Range	21-40		19-38		
	Mean±SD (years)	28.2±4.9		27.1±5.24		[a]0.397[ns]
BMI (kg/m^2)	*18.5-22.9	13	32.5	6	15	
	23.0-26.9	21	52.5	27	67.5	
	≥27.0	6	15	7	17.5	
	Total	40	100	40	100	
	Range	20.0-29.8		21.9-29.8		
	Mean±SD (kg/m^2)	24.50±2.62		24.87±2.19		[b]0.492[ns]
Educational status	Primary	6	15.00%	7	17.50%	
	SSC	10	25.00%	9	22.50%	[c]0.801[ns]
	HSC & above	15	37.50%	18	45.00%	
	Others	9	22.50%	6	15.00%	
Occupation	Housewife	32	80.00%	28	70.00%	
	Student	2	5.00%	0	0%	[d]0.118[ns]
	Service holder	6	15.00%	12	30.00%	
Monthly income (BDT)	<10,000	4	10.00%	0	0%	
	10,000-25,000	19	47.50%	16	40%	[e]0.065[ns]
	≥25,000	17	42.50%	24	60%	

***** BMI for Asian women was taken into account

ns= not significant

[a,b] p value reached from Unpaired student's t-test

[c,d,e] Chi-square test was done.

Table-2: Distribution of the RPL cases by number of pregnancy loss, nature of RPL and trimester of RPL (n=40)

Characteristics		Frequency	Percentage (%)
Number of pregnancy loss	2 pregnancy losses	12	30
	3 pregnancy losses	21	52.5
	>3 pregnancy losses	7	17.5
	Range (min-max)	2-7	
	Mean±SD	3.07±1.14	
Nature of pregnancy loss	Primary RPL	23	57.5
	Secondary RPL	17	42.5
Trimester of pregnancy loss	1st Trimester	25	62.5
	2nd Trimester	3	7.5
	Both trimester	12	30

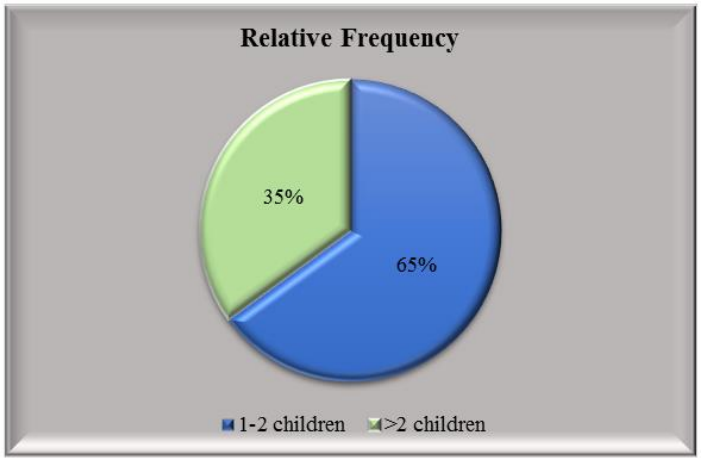


Fig-5: Pie Chart showing distribution of the controls by parity (n=40)

Table-3: Distribution of consanguinity and factor V mutation (R506Q) between two groups (n=80)

Characteristics		RPL Case (n=40)		Non-RPL Control (n=40)		p-value
		N	%	N	%	
Consanguinity	Present	5	12.5	1	2.5	[a]0.090[ns]
	Absent	35	87.5	39	97.5	
Factor V Leiden gene mutation	GG (normal homozygous)	38	95	40	100	[a]0.152[ns]
	GA (mutant heterozygous)	2	5	0	0	
	AA (mutant homozygous)	0	0	0	0	

[a]Fisher exact test was done, ns= not significant

Table-4: Individual Allele frequency of Factor V among cases of RPL compared to control (n=80)

Individual Allele frequency	Case (no. of Allele = 80)		Control (no. of Allele = 80)		p-value
	No.	Percentage (%)	No.	Percentage (%)	
G Allele	78	97.5	80	100	[a]0.152[ns]
A Allele	2	2.5	0	0	

[a] Fisher exact test was done, ns= not significant

Table-5: Descriptive characteristics of the two cases who found positive for heterozygous Factor V (Leiden) mutation

Age (year)	No. of RPL	Trimester of pregnancy loss	Nature of RPL	Consanguinity	Mutation
25	3	2nd	Primary	No	*GA
35	3	2nd	Primary	Yes	GA

*GA = Mutant heterozygous form of Factor V

Table-6: Association of FVL Genotypic variants with trimester of RPL (n=40)

Variables	Genotypes				p-value
	GG (n=38)		GA (n=2)		
	N	%	N	%	
1st Trimester RPL	25	100	0	0	*<0.001[s]
2nd Trimester RPL	1	33.33	2	66.67	
Both trimester RPL	12	100	0	0	

*Chi-square test was done, s= significant

Table-7: Association of variables with the nature of RPL (n=40)

Variables		Nature of RPL				p-value
		Primary (n=23)		Secondary (n=17)		
		N	%	N	%	
Age group (years)	18-25	6	26.1	3	17.6	0.378[ns]
	26-33	13	56.5	13	76.5	
	34-40	4	17.4	1	5.9	
Educational level	Primary	3	13.0	3	17.6	0.869[ns]
	SSC	5	21.7	5	29.4	
	HSC and above	9	39.1	6	35.3	
	Others	6	26.1	3	17.6	
Occupation	Housewife	19	82.6	13	76.5	0.891[ns]
	Student	1	4.3	1	5.9	
	Service	3	13.0	3	17.6	
Monthly income (BDT)	<10,000	1	4.3	3	17.6	0.370[ns]
	10,000-25,000	12	52.2	7	41.2	
	>25,000	10	43.5	7	41.2	
BMI (kg/m^2)	18.5-22.9	9	39.1	4	23.5	0.338[ns]
	23.0-26.9	12	52.2	9	52.9	
	≥27	2	8.7	4	23.5	
Trimester of RPL	1st	13	56.5	12	70.6	0.282 [ns]
	2nd	3	13.0	0	0.0	
	Both	7	30.4	5	29.4	
Consanguinity	Yes	2	8.7	3	17.6	0.397[ns]
	No	21	91.3	14	82.4	
FVL gene mutation	GG	21	91.3	17	100.0	0.212[ns]
	GA	2	8.7	0	0.0	

p-value was obtained from Chi-square test, ns= not significant

Table-8: Association of FVL Genotypic variants with number of RPL (n=40)

Variables	Genotypes				p-value
	GG (n=38)		GA (n=2)		
	N	%	N	%	
2 recurrent losses	12	31.6	0	0.0	0.286[ns]
3 recurrent losses	19	50.0	2	100.0	
>3 recurrent losses	7	18.4	0	0.0	

Chi-square test was done, ns= not significant

DISCUSSION

This case-control study was carried out in the Fetomaternal Medicine Department, BSMMU, Dhaka among 40 cases who had history of at least 2 consecutive 1st or 2nd trimester or both trimester pregnancy losses and 40 age and BMI matched controls. The present study investigated the association of Factor V Leiden gene mutation with unexplained recurrent pregnancy loss. The Mean±SD age of RPL cases were 28.2±4.9 years and non-RPL control group were 27.1±5.24 years. Kashif *et al*. [11] included 56 cases from Punjabi women and showed the mean age of their cases was 28.55±4.69 years and that of controls was 28.61±4.38 years (p=0.950, not significant) which

is comparable with the current study. The mean age distribution was also in agreement with the results obtained by Goncalves *et al*. [12]. In the present study no significant difference was found regarding mean BMI between case (24.50±2.62 kg/m2) and control (24.87±2.19 kg/m2). Kashif *et al*. [11] stated that their cases had mean BMI of 22.86±2.95 kg/m2 whereas controls had 22.50±2.44 kg/m2 (p=0.487). In the present study, number of pregnancy loss among cases was ranged from 2 to 7 with average of 3.07±1.14. 1st trimester and 2nd trimester pregnancy losses were seen in 25 and 3 patients, respectively. In addition, 12 patients showed combined first and second trimester losses. The relative frequencies of the heterozygous FVL genotypes in the cases with RPL were 5%. The heterozygous mutant Factor V Leiden G1691A genotype in patients suffering from recurrent pregnancy loss was not significantly different from controls (P = 0.152). The present study did not reveal an association of Factor V Leiden mutation with RPL. Ayadurai *et al*. [13] stated that differences in the methodological aspects of the studies, such as inclusion of participants with other potential causes of RPL or the lack of stratification based on the ethnicity and gestational age of loss of patients may have some impact on the study quality. Dutra *et al*. [14] and Baumann *et al*. [15] had also reported no significant association of RPL and FVL, results similar to the present study. However, the study conducted by Settin *et al*. [16] on cases of Nile Delta region revealed a significant association of FVL GA mutation (heterozygous mutation) with unexplained recurrent pregnancy loss (OR=21·38, P<0·0001), not in agreement with the study. As recurrent pregnancy loss is a multifactorial entity; the variations in the strength of the association between various polymorphisms and RPL seen in different studies may be indicative of additional risk factors [17]. Therefore, this study was attempted to diminish these potential biases by selectively including patients with recurrent pregnancy loss that was unexplained during the first and second trimester. Frequency of mutant A allele was seen only in 2 (2.5%) of the cases. Also, no significant difference was found between the allelic variants of FVL with RPL group compared to controls. Serrano *et al*. [18] in their study showed the overall prevalence of FVL was similar in women with recurrent miscarriage (5%) and controls (5%) [OR 1.36 (CI 95% 0.45–4.08)]. In recurrent embryonic loss subgroup, FVL prevalence (2.6%) was inclusively lesser than that of controls. This study showed that the FVL mutation rate was high among cases with 2nd trimester recurrent pregnancy loss (100%) and absent in other two groups. Statistically significant (p<0.001) association was found between Factor V Leiden mutation and 2nd trimester pregnancy loss. In a study Farahmand *et al*. [19] found increased prevalence of FVL mutation in Iranian women with only second trimester abortions (p<0.0001) that was statistically significant, but stated insignificant association between FVL mutation with first trimester (p=0.33) and combined first and second trimesters (p=0.73) abortions. Kujovich [11] explained some evidence suggesting that women with Factor V Leiden have a higher risk for late pregnancy loss than early first-trimester loss, report consistent with the present study. Gawish *et al*. [20] revealed that cases carrying FVL mutation (heterozygous or homozygous) genotypes had slightly higher frequency among cases with no successful pregnancy (primary aborters) compared to those having successful pregnancy (secondary aborters) (26.9% vs. 20% respectively). This difference was statistically significant (p<0.05).

Limitations of the study

In our study, small sample was recruited. The study was conducted within a short period of time. As prevalence of Factor V Leiden is very low in South East Asian region, larger sample sizes had to be incorporated to find a significant Odds Ratio.

CONCLUSION & RECOMENDATION

The impact of Factor V Leiden mutation has not stated any causal association with unexplained recurrent pregnancy loss. These data reinforce the results of some previous researches and indicate that Factor V Leiden mutation is not associated with pregnancy wastage. The results do not support Factor V mutation screening as an initial approach in Bangladeshi women with recurrent pregnancy loss. As a challenging topic for High-risk Pregnancy Specialists, it is recommended to expand these data for future larger-scale studies to yield conclusive evidence about its impact on obstetric outcome. Establishing the prevalence of this mutation and finding the association with RPL, we can refute or recommend the FVL screening policy for unexplained recurrent pregnancy loss group and institute appropriate antithrombotic treatment.

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