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ORIGINAL ARTICLE

Can Factor V Leiden and Prothrombin G20210A Testing in Women with Recurrent pregnancy Loss Result in Improved Pregnancy Outcomes Results from a Targeted Evidence-Based Review

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Abstract

Background: ***Recurrent pregnancy loss (RPL) remains a challenging concern for women of childbearing age. Genetic mutations, particularly prothrombin G20210A and factor V Leiden, have contributed to RPL. Understanding their impact on pregnancy outcomes is crucial for tailored interventions.***

Aim: ***To determine the possible mutation of Factor V Leiden and the Prothrombin G20210A mutation by reverse hybridization technique encountering RPL.***

Methods: ***This case-control research was executed on 100 patients (50 experiencing RPL and 50 controls). Patients underwent thorough clinical evaluations and genetic analyses to detect thrombophilia gene variants, including PCR and sequencing techniques. Laboratory assessments encompassed various coagulation factors and protein levels.***

Results: ***Significantly higher occurrences of stillbirth/neonatal death were observed in the patient group (71.4%) in contrast to controls (98.0%). The patient group showed a median of three successful pregnancies (vs. 0.5 in controls) and a higher median number of abortions (20 vs. 3 in controls). Additionally, gestational age at abortions was notably higher in the patient group (9.22 weeks vs. 8.08 weeks in controls). The patient group exhibited significant differences in protein S free antigen, protein C antigen, antithrombin III activity, and protein S activity compared to controls. Factor V Leiden heterozygosity was found in 68.0% of patients, while Prothrombin G20210A heterozygosity was present in 24.0%.***

Conclusions: ***It is not comparable to compare pregnancy loss and live birth rates, stillbirths and neonatal deaths in females with or without mutations in Prothrombin or Factor V Leiden.***

Keywords: Recurrent Pregnancy Loss; Factor V Leiden; Prothrombin G20210A; Genetic Mutations; Pregnancy Outcomes

1. Introduction

In the United States, two or more consecutive clinically unsuccessful pregnancies confirmed by histopathology or ultrasound are considered recurrent pregnancy loss (RPL). It is explained in the UK as losing three or more early pregnancies in a row. Pregnant women who experience two consecutive miscarriages make up just approximately 2% of the population. The aetiology of up to 50% of RPL patients is unclear.[1] There are two types of RPL: primary and secondary. Primary RPL is defined by pregnancy loss among women without a history of childbirth. On the other hand, pregnancy loss in mothers who have previously given birth alive is known as secondary RPL.

RPL may be linked to the development of microthrombosis induced by maternal thrombophilia in the placental blood vessels and placental infarcts. Under these circumstances, maternal thrombophilia-related poor placental perfusion may result in foetal mortality. [2]

When evaluating RPLs, tests for underlying maternal conditions, identification of maternal exposures, and detection of parental chromosome abnormalities are frequently performed. In some instances, however, surgical interference, such as the correction of uterine anomalies and aspirin and heparin treatment, can be carried out. It should be noted that numerous reports have established a connection between coagulation abnormalities and RPLs. [3]

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Mutations in FVL G1691A, PT G20210A, and C677T in methylenetetrahydrofolate reductase are among the genetic risk factors connected to thrombophilia. [4]

The G20210A mutation impacts the prothrombin gene's three-untranslated region at position 20210, resulting in higher prothrombin levels. The most frequent causes of inherited thrombophilia have been identified as prothrombin G20210A and FVL mutations. 5

Although there have been many studies on the connection between prothrombin G20210A and FVL G1691A mutations and RPL, it is still being determined whether or not these two mutations result in RPL, and data from various populations show differing outcomes. 6

This study's main objective was to determine the possible mutation of the Factor V Leiden and the Prothrombin G20210A mutation, which is suffering RPL through reverse hybridization.

2. Patients and methods

This case-control research was performed on 100 patients, 50 of whom were in the control group and 50 of whom experienced RPL for further evaluation at Al-Hussein University Hospital and Banha Teaching Hospital between August 2021 and January 2023. After explaining the purpose of the study, all patient acceptances were confirmed and documented.

Inclusion criteria were women aged 20 to 35 with two or more miscarriages in a row before week twenty.

Exclusion criteria were infections, chromosomal abnormalities, uterine structural abnormalities, induced abortions and systemic diseases, including lupus, diabetes, and thyroid issues.

Sample Size Calculation

Utilizing G. Power 3.1.9.2 (Universität Kiel, Germany), the sample size was determined in light of earlier research showing a 0.9% (P = 0.025) frequency of heterozygous mutation prothrombin in the control group and a 6 per cent frequency in the case group. 7 Considering a 0.05 α error, 80% study power, and a 1:1 allocation ratio, eight additional cases were included for potential dropouts, totalling 100 patients.

Participants were divided into two groups:

Group (A) (Control): women in the same age group who have at least one living kid and have never experienced miscarriage or other pregnancy-related issues. Group (B) (Patients): women who have previously suffered two or more unfavourable, recurrent miscarriages.

Methods:

All participants underwent Full history taking, general examinations, prothrombin G20210 Mutation and factor V Leiden.

Laboratory Evaluation: Plasma was obtained by centrifuging venous blood twice at 2500 cycles for 15 minutes at room temperature in 0.109 M trisodium citrate. Using the ELISA method, the amounts of Protein S and Protein C were estimated for plasma.

For mutation analysis, 3 ml of venous blood with EDTA sampling tubes was collected and kept at −70 °C until analysis.

PCR analysis was executed after incubation and centrifugation of blood samples.

The equipment used was DTlite Real-Time PCR instrument TS 9443-003-96301278-2010.

For both control and study groups, tests included fibrinogen, Prothrombin time (PT), activated activated protein C resistance (APCR), partial thromboplastin time (APTT), and prothrombin. The study group experienced additional tests: Antithrombin (AT), Protein S (PS), and Protein C (PC).

Genotyping Analysis:

Extract genomic DNA from peripheral blood leucocytes. Utilizing PCR amplification and Sanger sequencing according to an improved technique, the variations of the thrombophilia gene, including MTHFR (677 C > T and 1298 A > C), F2, and F5, were identified.

Statistical analysis:

Data was gathered, edited, coded, and then imported into IBM SPSS, the statistical package for social science. When it was determined that the distribution of the quantitative data was parametric, it was represented as mean, standard deviations, and ranges; for qualitative data, it was stated as numbers and percentages. The chi-square test was utilized to contrast groups for qualitative data; in cases where any cell's predicted count fell below 5, Fisher's exact test was employed rather than the Chi-square test. An Independent t-test was employed to contrast two independent groups with quantitative data that showed a parametric distribution. At the same time, the Mann-Whitney Test was utilized to contrast groups with quantitative data that exhibited a non-parametric distribution. A confidence interval of 95% was set with an accepted margin of error of 5%. The significance level (p-value) was interpreted as follows: $P > 0.05$ indicated non-significance (NS), $P < 0.05$ indicated significance (S), and $P < 0.001$ was considered highly significant (HS).

3. Results

Age and BMI did not vary statistically significantly between Control and Cases groups.

Table 1. Comparison between Control group (no. =50) and Cases Group (no. =50) regarding Age and BMI.

		CONTROL GROUP	PATIENT GROUP	TEST VALUE	P-VALUE	SIG.
		No.= 50	No.= 50			
AGE	Mean ± SD	27.02 ± 1.93	27.84 ± 2.05	2.056•	0.053	NS
	Range	22 – 30	23 – 32			
BMI	Mean ± SD	21.38 ± 1.76	21.04 ± 2.06	0.900•	0.370	NS
	RANGE	17.42 – 25.44	16.8 – 26.4			

P-value > 0.05: Non-significant (NS); P-value < 0.05: Significant (S); P-value < 0.01: highly significant (HS), *: Chi-square test, •: Independent t-test.

Regarding stillbirths and neonatal deaths, amount of abortions and successful pregnancies, a statistically significant variation was seen between Control and Cases groups. Additionally, a statistically significant variation in the gestational age at abortion (weeks) was seen between Control and Cases groups.

Table 2. Comparison between Control group (no. =50) and Cases Group (no. =50) regarding Stillbirth/neonatal Death, Number of successful pregnancies, Number of abortions, Gestational age at abortions (weeks.

		CONTROL GROUP	PATIENT GROUP	TEST VALUE	P-VALUE	SIG.
		No.= 50	No.= 50			
STILLBIRTH/NEONATAL DEATH	No	49 (98.0%)	35 (71.4%)	1.895*	0.04	S
	Yes	1 (2.0%)	15 (30.6%)			
NUMBER OF SUCCESSFUL PREGNANCIES	Median (IQR)	0.5 (0 – 1)	3 (2 – 3)	-8.700¥	0.000	HS
	Range	0 – 1	1 – 4			
NUMBER OF ABORTIONS	Median (IQR)	3 (3 – 4)	20(0 – 1)	-8.683¥	0.000	HS
	Range	1 – 5	9– 11			
GESTATIONAL AGE AT ABORTIONS (WEEKS)	Mean ± SD	8.08 ± 2.35	9.22 ± 2.47	-2.363•	0.020	S
	Range	3 – 13	2 – 14			
	RANGE	44 – 163	56 – 151			

P-value > 0.05: Non-significant (NS); P-value < 0.05: Significant (S); P-value < 0.01: highly significant (HS), *: Chi-square test, •: Independent t-test, ¥: Mann- Whitney test.

Regarding small for gestational age < 5th percentile and SGA < 10th percentile, live birth rate during the first pregnancy, early and late pregnancy losses, and pregnancy loss, there was no statistically significant variation observed between Control and Cases groups.

Table 3. Comparison between Control group (no. =50) and Cases Group (no. =50) regarding Pregnancy loss, Early Pregnancy loss, Late Pregnancy loss, Live birth rate first pregnancy, SGA < 5th percentile and SGA < 10th percentile.

		CONTROL GROUP		PATIENT GROUP		TEST VALUE*	P-VALUE	SIG.
		No.	%	No.	%			
PREGNANCY LOSS (N, %)	No	44	88.0%	30	60.0%	2.439	0.118	NS
	Yes	6	12.0%	20	40.0%			
EARLY PREGNANCY LOSS		4	66.7%	12	60.0%	0.138	0.710	NS
LATE PREGNANCY LOSS		2	33.3%	8	40.0%			
LIVE BIRTH RATE FIRST PREGNANCY	No	3	6.0%	8	16.0%	2.554	0.110	NS
	Yes	47	94.0%	42	84.0%			
SGA < 5TH PERCENTILE	NO	43	86.0%	41	82.0%	0.298	0.585	NS

Control and Cases groups did not exhibit any statistically significant variations in relation to Protein C activity or Protein S total antigen and a statistically significant difference in antithrombin III activity was observed between the Control group and the Cases group. Protein S activity was significantly different between the Control group and the Cases group in relation to both the Protein C antigen and the Protein S free antigen.

Table 4. Comparison between Control group (no. =50) and Cases Group (no. =50) regarding Antithrombin III activity, Protein C activity, Protein C antigen, Protein S activity, Protein S total antigen and Protein S free antigen.

		CONTROL GROUP	PATIENT GROUP	TEST VALUE	P-VALUE	SIG.
		No.= 50	No.= 50			
ANTITHROMBIN III ACTIVITY	Mean ± SD	100.30 ± 7.04	103.58 ± 7.63	-2.233	0.028	S
	Range	85.61 – 119.2	86.9 – 117.93			
PROTEIN C ACTIVITY	Mean ± SD	109.71 ± 22.63	117.21 ± 19.24	-1.784	0.077	NS
	Range	53.6 – 164.4	70.7 – 154.8			
PROTEIN C ANTIGEN	Mean ± SD	107.34 ± 17.08	114.03 ± 13.35	-2.180	0.032	S
	Range	67.61 – 148.22	91.23 – 152.93			
PROTEIN S ACTIVITY	Mean ± SD	50.38 ± 13.24	44.02 ± 8.91	2.814	0.006	HS
	Range	16.77 – 80.01	18.93 – 78.18			
PROTEIN S TOTAL ANTIGEN	Mean ± SD	97.23 ± 13.68	97.35 ± 9.12	-0.053	0.958	NS
	Range	59.88 – 126.06	77.15 – 116.63			

PROTEIN S FREE ANTIGEN	Mean ± SD RANGE	60.74 ± 10.87 36.2 – 84.66	55.66 ± 9.52 33.9 – 81.77	2.484	0.015	S

P-value > 0.05: Non-significant (NS); P-value < 0.05: Significant (S); P-value < 0.01: highly significant (HS), *: Chi-square test, •: Independent t-test, ¥: Mann- Whitney test.

Regarding the Factor V Leiden, 68.0% were Heterozygous, and 2.0% were Homozygous. For the Prothrombin G20210A, 24.0% were Heterozygous and 2.0% were Homozygous, and there were 4 (8.0%) patients with Compound heterozygous.

Table 5. Distribution of the studied cases according to Factor V Leiden, Prothrombin G20210A and Compound heterozygous.

		CASES GROUP	
		No.	%
FACTOR V LEIDEN	Heterozygous	34	68.0%
	Homozygous	1	2.0%
PROTHROMBIN G20210A	Heterozygous	12	24.0%
	Homozygous	1	2.0%
COMPOUND HETEROZYGOUS	No	46	92.0%
	YES	4	8.0%

4. Discussion

The study revealed a significant disparity between Control and case groups regarding Stillbirth/neonatal Death. Additionally, retrospective findings demonstrated substantially higher odds ratios for stillbirth in the female population with mixed thrombophilic abnormalities as opposed to those with unique deficiencies.[8] These results emphasize the impactful relationship between thrombophilic conditions and adverse pregnancy outcomes like stillbirth, suggesting a marked increase in risk for those with multiple thrombophilic defects.

The investigation displayed a highly significant variation in successful pregnancies between the Control and Cases Groups. This aligns with findings by Hussien et al. (2021), indicating a stark contrast in the number of births that succeed, favouring the control group significantly.[9]

The present study demonstrated that the number of abortions performed by Control and Cases groups differed in a highly statistically significant way.

This is consistent with Hussien et al., who stated that the number of abortions varied statistically significantly between the two groups (P value =< 0.0001). [10]

The present study demonstrated that the difference in gestational age at abortion between the Control and Case groups was statistically significant.

This disagrees with Hussien et al., who demonstrated that it was discovered that the case group's mean gestational week at abortion was 8.4 weeks. In contrast, the control group was 8.5 weeks, but no statistically significant difference was found. [10]

Regarding pregnancy loss and early and late pregnancy loss, respectively, the study found no statistically significant variation between the Control and Cases Groups. Coppens et al. (2007) reported a higher chance of miscarriage among women who have FVL or PTm mutations, especially in the first pregnancy, primarily attributed to differences in late losses.[11] However, this contrasts with previous meta-analyzed studies on this subject, as noted by Rey et al.[12]

Rai et al. highlighted the significance of thrombophilic abnormalities in unfavourable pregnancy outcomes by finding that women who experienced repeated miscarriages exhibited a markedly elevated risk in comparison to people who have a typical genotype of Factor V. [13] Additionally, Cardona Henry et al. established RPL as three or more first-trimester pregnancy losses, comprising various vascular pregnancy manifestations.[14] Studies like Pauer et al. observed FVL prevalence in case groups but did not find statistical significance between case and control groups. Variations in spontaneous miscarriage rates, ranging from 9.1% to 18.1%, were noted in different populations,

The research indicated no statistically significant variation between the Control and Cases Groups concerning Live birth rate first pregnancy, SGA < 5th percentile, and SGA < 10th percentile. Coppens et al. (2007) sought to evaluate the results of second pregnancies following a first loss in women carrying or not carrying FVL or PTm mutations.

The study found a reduced rate of live births during the following pregnancy following a first loss. However, no difference in the pregnancy outcomes was seen between carriers and non-carriers. Similarly, Jivraj et al. found no significantly lower live birth rate among females with Factor V Leiden compared to non-carriers, aligning with the notion that carrying the Factor V Leiden allele does not preclude successful pregnancies. [15]

Regarding the total antigen of Protein S and the activity of Protein C, the study found no statistically substantial differences between the Control and Cases Groups. However, Protein C antigen, Protein S free antigen, and Antithrombin III activity all showed significant variations between groups, with a highly significant variance in Protein S activity. Previous research by Jivraj

et al. indicated that multiple thrombophilias in either partner doubled the possibility of miscarrying again in the future, potentially indicating the influence of paternal and fetal thrombophilia genotypes on pregnancy outcomes.[16] Conversely, studies by Hussien et al.[10] found no significant differences in abnormal APC resistance test parameters between case and control groups. At the same time, Teremmahi Ardestani et al. were unable to create a distinct connection between hereditary APCR and recurrent pregnancy loss.[15] Animal model studies have suggested that fetal thrombophilia in maternal FVL carriers may raise the chance of miscarrying, emphasizing how crucial the protein C anticoagulant pathway is to preserving pregnancy. [14,16]

The recent study revealed that, regarding Factor V Leiden, 68.0% were Heterozygous, and 2.0% were Homozygous. For the Prothrombin G20210A, 24.0% were Heterozygous, 2.0% were Homozygous, and 4 (8.0%) patients were Compound heterozygous.

Published research examining women who experienced losses in the first trimester independently has shown that factor V Leiden allele frequency ranges from 0.3 to 5.4 %. [16]

According to Hussien et al., who used the less expensive and time-consuming APCR test to look at the frequency of FVL mutation in RPL-affected women. They stated that genetic analysis verified that both individuals carried the FVL heterozygosity. [10]

Research has indicated that women who experience repeated miscarriages have a similar allele frequency of factor V Leiden to the whole population. [12]

4. Conclusion

It is not comparable to compare pregnancy loss and live birth rates, stillbirths and neonatal deaths in females carrying either a prothrombin or Factor V Leiden mutation.

Disclosure

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