Evaluation of chromosomal abnormalities and common trombophilic mutations in cases with recurrent miscarriage

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Abstract

Background: Recurrent miscarriage (RM) is a frequent obstetric problem. Its' pathophysiology is poorly understood. Infections, genetic, endocrine, anatomic and immunologic problems have been suggested as causes for RM.

Objective: To evaluate the frequency of chromosomal abnormalities and 3 common thrombophilic mutations in couples with RM.

Methods: A retrospective data collection was performed for the results of the cytogenetic analysis of the couples and Methylenetetrahydrofolate Reductase (MTHFR) C677T, Factor V Leiden (FVL) G1691A and Prothrombin (PTm) G20210A mutations of the mother in 142 couples suffering from RM.

Results: Prevalence of FVL, MTHFR, and PTm gene mutations were similar between cases shaving 2 or ≥3 abortions (P=0.528; P=0.233; P=0.375). In patients with FVL, MTHFR and PTm gene mutations, the OR's of having ≥3 abortions when compared to having 2 abortions were 1.515 (95% CI: 0.414-5.552), 0.573 (95% CI: 0.228-1.441), and 2.848 (95% CI: 0.355-22.871). All cases with PTm mutation had ≥3 abortions and all abortions occurred between 6-8 gestational weeks.

Conclusion: Chromosomal abnormalities and thrombophilic mutations (especially PTm) seem to have an important role in RM. Additional larger studies involving investigation of more genes that may have a role in pregnancy are needed to assess this association.

Key Words: Recurrent miscarriage, chromosomal abnormalities, inherited thrombophilic polymorphisms

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Introduction

Recurrent miscarriage (RM) is defined as the occurrence of 3 consecutive first trimester pregnancy losses. It affects 1% of all women (1). The ratio increases from 1% to 5%, if it is defined as ≥2 losses (2). It is a frustrating condition both for the couple and the clinician. Among women with RM, recurrencerisk appears to be higher in those with prior fetal death in contrast to early first trimester losses (3). Historically, RM has been attributed to chromosomal abnormalities, throm bophilic, endocrinologic, immunologic, microbiologic, metabolic, anatomic or iatrogenic factors. Maternal age and obesity at conception have both been shown to be associated with RM (4,5). However, a great proportion of the causes remain un-explained, despite detailed investigation (6).

In approximately 4% of couples with RM, one of the partners carries either a balanced reciprocal translocation, in which there is an exchange of two terminal segments from different chromosomes, or, a Robertsonian translocation, in which there is a centric fusion of two acrocentric chromosomes (7,8).

Pregnancy is a hypercoaguable state (9,10) and thrombophilic disorders are a diverse group of coagulation disorders associated with a predisposition for thrombotic events. Three common thrombophilic mutations were identified: Factor V Leiden (FVL) G1691A; factor II prothrombin (PTm) G20210A and methylene tetrahydrofolate reductase (MTHFR) C677T. There are conflicting studies in the literature about the role of these thrombophilic mutations in RM, and results of some of thesis were in favor of the possible association between RM and thrombophilic mutations (11,12).

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In this study, we wanted to evaluate the frequency of chromosomal abnormalities and these 3 common thrombophilic mutations in couples with RM.

Methods

This retrospective study was carried out by the Obstetrics & Gynecology and Medical Genetics Departments of Duzce University School of Medicine. The study was approved by Non-Invasive Human Research Ethics Committee of Duzce University. 178 couples suffering from RM (≥2 pregnancy losses that occurred before the 20th gestational week) and admitted to the obstetrics and gynecology outpatient clinic, between January 2010 and August 2012 were retrospectively evaluated. Demographic characteristics, results of the maternal and paternal chromosomal analysis as well as the results of FVL G1691A; PTm G20210A and MTHFR C677T polymorphisms in the mother were obtained from patients' files. Patients with incomplete records were excluded. Patients were evaluated in two groups as those having 2 abortions and those having ≥3 abortions.

Cytogenetic Analysis

Metaphase chromosome preparations from the peripheral blood cultures were made according to standard cytogenetic protocols. Cytogenetic analysis was performed by G-bands by trypsin using Giemsa (GTG) banding at approximately 400-450 band level. For each cases, 20 metaphases were analyzed. Chromosomal abnormalities were detected according to the present international standard nomenclature (ISCN).

Isolation of Genomic DNA

Blood samples were collected in tubes containing ethylene diamine tetra acetic acid (EDTA) for DNA isolation. Genomic DNA was isolated from individuals via fenol-chloroform extraction methods.

Determination of MTHFR C677T, Factor V Leiden G1691A and Factor II Prothrombin G20210A polymorphisms

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) by using appropriate primers was used to detect these single nucleotide polymorphisms. MTHFR C677T polymorphism was detected by using the previously described couples of primers and PCR products were digested by Hinf I restriction endonuclease enzyme (13). FVL polymorphism was detected with by using to primers FV1, FV2 and the restriction enzyme Mnl 1(14). PTm was detected by using 2 primers, PT1 and PT2 followed by digestion of the product with the restriction enzyme Hind III (15).

Statistical Analysis

All analyses were performed using the Statistical Package for Social Sciences (SPSS version 15.0) and the data were given as mean ± SD and percentages. Pearson $\chi 2$ and Mann–Whitney U tests were used. The odds ratios (OR's) with 95% confidence intervals (CI's) were calculated. The statistical significance level was defined as $P < 0.05$.

Results

A total of 142 couples were included in the study. Mean age of the women was 30.3±5.5 years (20 - 44), and 32 of them were ≥ 35 years. Mean age of the men was 33.1±5.5 years (23 - 46). Parity, abortion and the number of living children were similar in patients < and ≥35 years of age (p=0.167; p=0.358; p=0.153, respectively).

Chromosomal analysis of the couples revealed 128 normal results, 9 polymorphisms, 3 translocations and 2 trisomy X for women; 123 normal results and 19 polymorphisms for men (Table 1).

Table 1. Chromosom analysis results of women and men with recurrent miscarriage.

Chromosomal analysis results of women	n	Chromosomal analysis results of men	n
46,XX,1qh+	5	46,XY,1qh+	4
47,XXX	2	46,XYqh-	2
46,XX,16qh+	3	46,XYqh+	5
46,XX,13pstk+ps+	1	46,XY,15ps+	1
46,XX,t(13;16) (q34,q12)	3	46,XY,9qh+	4
46,XX	128	46,XYqh+, 9qh+	1
		46,XY,21pss	1
		46,XY,21cenh+	1
		46,XY	123
Total	142		142

95% of the abortions were in the first trimester and 5% of them were in the second trimester. When results of the chromosomal analysis were investigated according to the number of abortions; there were 23 normal results and 1 polymorphism in couples with 2 abortions; 84 normal results, 8 polymorphisms, 2 trisomy X and 2 translocations in couples with 3 abortions and 21 normal results and 1 translocation in couples with >3 abortions.

FVL status was normal, showed a heterozygous mutation and showed a homozygous mutation in 118, 19 and 5 of the cases, respectively. MTHFR results were normal in 63 cases, and there was a heterozygous or homozygous mutation in 67 and 12 of the cases, respectively. The analysis for PTm were normal in 128 cases and showed a heterozygous mutation in 14 cases. Distribution of the FVL, MTHFR, and PTm results of women according to the number of abortions were shown in table 2 (p=0.560; p=0.266; p=0.558, respectively).

Table 2.Thrombophilia analysis of women according to abortus number.

Abortus number		**N=142, (%)**	**2 24**	**3 96**	**>3 22**	**P**
FVL	Normal	118 (83%)	21 (87.5%)	80 (83.3%)	17 (77.2%)	0.560
	Heterozygotes	19 (13.3%)	3 (12.5%)	13 (13.5%)	3 (13.6%)	
	Homozygotes	5 (3.5%)	-	3 (3.2%)	2 (9.2%)	
MTHFR	Normal	63 (44.4%)	8 (33.3%)	47 (49%)	8 (36.4%)	0.266
	Heterozygotes	67 (47.2%)	14 (58.3%)	43 (44.8%)	10 (40.1%)	
	Homozygotes	12 (8.4%)	2 (8.4%)	6 (6.2%)	4 (18.2 %)	
PTm	Normal	128 (90.1%)	23 (95.8%)	85 (88.5%)	20 (90.9%)	0.558
	Heterozygotes	14 (9.9 %)	1 (4.2%)	11 (11.5%)	2 (9.1%)	

FVL: Factor V leiden, **MTHFR:** Methylenetetrahydrofolate Reductase**,** **PTm:**FactorII Prothrombin

When mutant allels (homozygotes and heterozygotes) were taken into consideration, the prevalence of FVL, MTHFR, and PTm gene mutations were similar between the cases having 2 and ≥3 abortions (P=0.528; P=0.233; P=0.375). In patients with FVL, MTHFR and PTm gene mutations, the OR's of having ≥3 abortions when compared to having 2 abortions were 1.515 (95% CI: 0.414-5.552), 0.573 (95% CI: 0.228-1.441), and 2.848 (95% CI: 0.355-2.871**)** (Table 3).

Table 3. Evaluation of FVL, MTHFR, and PTm gene mutations in cases with 2 and ≥3 abortions.

Thrombophilic mutations		**2 abortion (n:24)**	**≥3 abortion (n:118)**	**P**	**OR**	**95% CI**
FVL	Negative	21 (87.5%)	97 (82.2%)	0.528	1.515	0.414-5.552
	Positive	3 (12.5%)	21 (17.8%)			
MTHFR	Negative	8 (33.3%)	55 (46.6%)	0.233	0.573	0.228-1.441
	Positive	16 (66.7%)	63 (53.4%)			
PTm	Negative	23 (95.8%)	105 (89%)	0.375	2.848	0.355-2.871
	Positive	1 (4.2%)	13 (11%)			

FVL: Factor V leiden, **MTHFR:** Methylenetetrahydrofolate Reductase**,** **PTm:**Factor II Prthrombin
OR: Odds Ratio **CI:** Confidence Interval

The prevalence of combined mutations of FVL + MTHFR, FVL + PTm, MTHFR + PTm and combination of 3 mutations in patients with 2 and with ≥3 abortions were similar (P=0.634; P=0.189; P=0.259; P=0.430, respectively) (Table 4).

Table4. Evaluation of two and three thrombophilic factor combinations (FVL, MTHFR, and PTm gene) in cases with 2 and ≥ 3 abortions.

Thrombophilic factors		2 abortion (n:24)	≥3 abortion (n:118)	P	OR	95% CI
FVL+ MTHFR	Negative	21 (87.5%)	107 (90.7%)	0.634	0.720	0.185-2.803
	Positive	3 (12.5%)	11 (9.3%)			
FVL+ PTm	Negative	24 (100%)	110 (93.2%)	0.189	-	-
	Positive	0	8 (6.8%)			
MTHFR+PTm	Negative	24 (100%)	112 (94.9%)	0.259	-	-
	Positive	0	6 (5.1%)			
Two factor positive	Negative	21 (87.5%)	99 (83.9%)	0.657	1.343	0.364-4.957
	Positive	3 (12.5%)	19 (16.1%)			
Three factors positive	Negative	24 (100%)	115 (97.5%)	0.430	-	-
	Positive	0	3 (2.5%)			

FVL: Factor V leiden, **MTHFR:** Methylenetetrahydrofolate Reductase**,** **PTm:**FactorII Prothrombin
OR: Odds Ratio, **CI:** Confidence Interval

Chromosomal abnormalities and thrombophilic mutations (especially PTm) seem to have an important role in RM.

Discussion

The rate of karyotypically abnormal abortion specimens increases with maternal age, affected mainly by the increase in the rate of trisomy (16). Chromosomal abnormalities are detected in about one-third of RM's (17). It was reported that normal polymorphic variants contribute to chromosomal instability during meiosis with a tendency towards an increased risk of aneuploidy which can cause RM or sub-fertility (18). Aberrations in the heterochromatin region of the Y chromosome were identified as the most frequent polymorphism in infertile men (19). The long (Yqh+) and short (Yqh-) chromosome were reported in chromosomal studies of human. (20). While Verp et al. (21) reported Yqh+ to be related with an increased risk of miscarriage, De Braekeleer et al. (20) did not report a relation between Y chromosome size and increased risk of pregnancy loss.

In the present study, there were 14 chromosomal abnormalities in women (8 polymorphism, 3 translocations, 2 trisomy X in women <35 years, and only 1 polymorphism ≥ 35 years of age). In addition to this, there were 19 polymorphisms in men, and the rest were normal. Five were long (Yqh+) and 2 were short (Yqh-) polymorphisms (Table 1). Therefore Yqh+ and/or Yqh- may be related with an increased risk of RM.

Thrombophilias make individuals prone to thromboembolism and pregnancy complications including pregnancy loss. Although the mechanism is poorly understood, mutations in genes related with coagulation pathways may cause damage to chorionic vasculature, decreased trophoblast invasion, apoptosis, and impaired uteroplacental circulation due to placental thrombosis. The most common hereditary thrombophilia is the FVL gene mutation followed by the PTm mutation. Both are autosomal dominant conditions (22). In a meta-analysis, Rey et al. (12) had reported FVL mutation to be associated with early and late RM's and with late non-recurrent fetal loss. Some researchers found that there were no significant associations between RM and genetic thrombophilias (23, 24), others indicated that there may be a possible relationship between FVL polymorphism and late pregnancy loss (25). In our study, majority of women

(64.1%), suffering from RM had at least one of the 3 genetic thrombophilias including FVL, MTHFR or PTm polymorphism.

It was reported that heterozygous carriers for the PTm allele have a 2 to 8 fold increased risk for venous thrombosis (26). Homozygosity for this mutation has been described in very few cases (27). A significant association between PTm carrier-ship and recurrent abortion before 13 weeks of pregnancy was found (12). Similar to the literature findings, in our study, while 128 of cases had normal genotype, the others (14 cases) were heterozygous carriers for the PTm (Table 2). There was no homozygosity for this mutation.

Some researchers indicated that individuals carrying both FVL and PTm mutation have a 20-fold increased risk for venous thrombosis. This rate is higher than for heterozygous carriers of PTm or FVL alone. Thus DNA analysis of both mutations is highly recommended in cases with a personal or family history of thrombosis (28, 29). It was reported that a meaningful association between RM and PTm and FVL polymorphisms (12, 22, 30).

In the current study, there were 8 cases carrying both an FVL and a PTm mutation. All of them were heterozygous for PTm; 7 of them were heterozygous carriers for FVL, while the other case was a homozygous carrier for FVL (only 1 case had 4 abortions, others had 3). Although the differences were not meaningful for PTm and FVL analysis results of women, the abortion rate increased with the positivity for PTm (OR: 2.848 and 95% CI: 0.355-22.871) and FVL (OR: 1.515 and 95% CI: 0.414-5.552) as shown in table 3. This insignificant relation may be due to the small sample size.

It was reported that, although the association of pregnancy loss with certain thrombophilic states (such as antiphospholipid antibody syndromes, antithrombin III deficiency) is clear, its association with inherited thrombotic defects such as MTHFR C677T and FVL polymorphisms are slightly disputable (24). In fact, an estimated 10% of all clinical pregnancies are lost in the first and early second trimester (31), fewer than 3% of pregnancies are lost later in gestation. The recurrence risk for pregnancy loss appears to be higher in those with previous fetal death in contrast to early first trimester losses in women with RM (3). According to our findings, a great number of abortions (95%) were observed in first trimester, and (5%) in second trimester.

While a lot of studies showed the association of MTHFR C677T polymorphism and increased risk for RM (32-35), some studies reported a lack of relation between this polymorphism and RM (24, 36, 37). In this study, MTHFR gene mutations were associated with a 0.573 (95% CI: 0.228-1.441) fold risk of having ≥3 abortions when compared to having 2 abortions (Table 3).

The prevalence of FVL + MTHFR, FVL + PTm, MTHFR + PTm and combination of three mutations were similar in patients with 2 and with ≥3 abortions (Table 4). Interestingly, in the situations which included PTm mutation, all cases had≥3 abortions and all abortions occurred between 6-8th gestational weeks (Table 4). Therefore the presence of PTm gene mutation may be important and may cause early pregnancy losses. Therefore, evaluation of the PTm gene mutation in patients with RM may important.

Conclusion

Chromosomal abnormalities and abnormalities in the genes related to thrombophilia such as FVL, MTHFR and PTm mutations may be considered as risk factors for RM. Detection of these factors may be important to begin early and appropriate treatment for couples suffering from RM. Thus the physical, psychological discomfort for couples and financial loss for both of couples and governments might be decreased the minimum levels. Additional studies including many genes which have functionally significant variant alleles in pregnancy and a large case series should be performed to obtain more information about this issue.

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