Cytogenetic Analysis and Thrombophilia-Associated Gene Mutations of Couples with Recurrent Miscarriage

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Introduction

As the loss of three or more pregnancies before 20 weeks of gestation is generally defined recurrent miscarriage [1,2] And that problem affects 1–5% of the reproductive age population and is costly for patients/health care systems since the etiology generally remains unknown [3]. In human recurrent spontaneous abortion is a general phenomenon and cytogenetical rearrangements are important reason for this phenomenon [4]. Parental chromosomal translocations, thrombophilic gene polymorphisms, autoimmune factors, uterine, endocrine factors associated with recurrent miscarriage (RM). Factor V Leiden, prothrombin gene mutation G20210A, protein S/Protein C/antithrombin deficiency and MTHFR mutations responsible for hereditary thrombophilia and has been included to keep a very common practice of RM pathogenesis. This study aimed to determine the incidence of these factors believed to be the effects of RM.

Materials and methods: All patients were took a full genetic analysis; full genetic examination and pedigree drawing was done to exclude known nonchromosomal causes of the anomaly. Cytogenetic analysis was performed for 635 patients. The study included peripheral lymphocyte culture by a standard method using Leishmann-banding technique, centromerebanding (C-banding), and nucleolar organizing region staining was done when needed. Pyrosequencing was used to genotype the 392 individual.

Results: Male/female in patients with an inherited thrombophilia tested four types of changes, including; MTHFR C677T/1298, FV Leiden G1691A and prothrombin G20210A. Among them, 152 men and woman did not carry any mutation.

Keywords: Cytogenetics; Pyrosequencing; Recurrent miscarriage

Abbreviations: RM: Recurrent Miscarriage; MTHFR: Methylene tetrahydrofolate reductase; C-banding: Centromeric Banding; FISH: Fluorescent In Situ Hybridization; CMA: Chromosomal Microarray Analysis; CNVs: Copy Number Variants; G-banding: Giemsa Banding; SNP: Single Nucleotide Polymorphism; PCR: Polymerase chain Reaction; KCL: Potassium Chloride

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Abstract

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Half of the sporadic miscarriages are caused by fetal chromosome abnormalities. Cytogenetic analysis has been shown that a large portion of numerical chromosomal abnormalities anomalies (86%) and structural chromosomal abnormalities in a small proportion of cases (6%) and in addition to mosaic chromosomal abnormalities (8%) [6]. Protein S/Protein C, Factor V Leiden, prothrombin G20210A and antithrombin deficiency and methylenetetrahydrofolate reductase (MTHFR) gene changes in the pathogenesis of RM has been a very common practice. RM etiologies of inherited thrombophilia in the literature there are conflicting studies about the possible role. There was a statistically significant correlation between the cytogenetic abnormalities with recurrent miscarriages reported in a study by Choi et al. [7].

Materials and Method

Chromosomal abnormalities in patients with two consecutive spontaneous abortions reported a high rate for this reason we have included the study of this group patients. Couples were included in the study with a loss of 2 or 3 pregnancies before 20 weeks of gestation. This study evaluated the relationship between parental chromosomes and four types of hereditary thrombophilia frequency; in male and female patients. All patients were took a full genetic analysis; full genetic examination and pedigree drawing was done to exclude known nonchromosomal causes of the anomaly. Cytogenetic analysis was done for 635 patients and 392 persons included for thrombophilia panel (MTHFR C677T/1298, FV Leiden G1691A and prothrombin G20210A). The study included peripheral lymphocyte culture by a standard method using Leishmann-banding technique, centromerebanding (C-banding) and nucleolar organizing region staining was done when needed. It was incubated with peripheral blood lymphocytes cultured for 72 hours at about 0.5-1 ml. Lymphocyte cultures were treated with hypotonic KCl solution to obtain metaphase then were harvested by addition of colcemid for 45 min (0.075 M) treatment for 4 min and fixation using standard fixative prepared with methanol and acetic acid (3:1) [8,9]. At least 25 metaphases were scored for each patient. The best metaphases were karyotyped and the total chromosome count was usually determined in 25 cells. If mosaicism was suspected, then 100 or more cell counts were carried out for documentation for abnormal cases. Fluorescent in situ hybridization (FISH) was used if needed and the international system for human cytogenetic

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nomenclature were used for the nomenclature of human chromosomes. We used pyrosequencing to genotype 392 individuals. Pure genomic DNA obtained from peripheral blood which received EDTA tubes were performed by MagNA Pure LC DNA Isolation Kit (Roche) or manually. We chose pyrosequencing for thrombophilia panel analysis. This method, compared with conventional sequencing, including all reagents and PCR is a fast and cost-effective. SNP genotyping is also due to the high efficiency more effectively to today’s challenges [10].

Results

Our patients group were calculated relative frequency; the percentage of patients with abnormal distribution were determined and the numerical and structural abnormalities (Tables 1-4). Frequencies of those values were compared with the parallel studies 392 males and females were tested for four types of hereditary thrombophilia’s, MTHFR C677T/1298 polymorphisms, FV Leiden G1691A mutation and Prothrombin G20210A mutation. Among these, 152 of the men or women did not carry any of these mutations (Table 5). The most common abnormality was heterozygous for the mutation MTHFR 1298 polymorphisms in this group (n=184).

<table>
<thead>
<tr>
<th>Authors</th>
<th>Total cases</th>
<th>Total abn.</th>
<th>Structural</th>
<th>Numerical</th>
<th>Variants</th>
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<tr>
<td>Dubey et al.</td>
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<td>Our cases</td>
<td>635</td>
<td>20</td>
<td>13</td>
<td>7</td>
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Table 3: Review of literature in reproductive disorders.

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<thead>
<tr>
<th>S. No.</th>
<th>Numerical chromosomal abnormalities identified in this study</th>
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<tr>
<td>1</td>
<td>47.XXY[3], 46.XY[47]</td>
</tr>
<tr>
<td>2</td>
<td>45.X[3]/47.XXX[2]/49.XXXX[1]/46.XX[94]</td>
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<tr>
<td>3</td>
<td>45.X[9]/46.XX[94]</td>
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<td>4</td>
<td>45.X[5]/46.XX[95]</td>
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Table 4: Numerical chromosomal abnormalities identified in this study.

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Table 5: The genotypes of polymorphisms in this study.

Discussion

Most of chromosomal abnormalities detected in embryos and fetuses will result in spontaneous abortion. Fetal chromosomal abnormalities associated with 50% of early gestation spontaneous abortion. The genetic etiology for multiple spontaneous pregnancy loss includes an unbalanced chromosome rearrangement, which may be the result of one parent being a carrier for a balanced chromosome rearrangement [4]. Sporadic abortion affects 25% women and can occur as a single isolated event. The relationship between with chromosomal abnormalities and early pregnancy loss have been established [11,12]. Other research groups all over the world apply these techniques in the late 1980s-1970s.

Chromosomal polymorphisms or variants of different sizes can be observed microscopically, are the areas showing the morphology and staining properties. It has no effect on the phenotype. A Mendelian inherited style and often found in highly variable regions chromosomes 1, 9, 16, away from the long arm of the Y chromosome and two-thirds acrocentric chromosome short arms and satellites [13,14]. In this study, the polymorphism of heterochromatic region 9qh+ was determined about 5%. This ratio is higher according to other polymorphisms. And total polymorphisms ratio is 13% in our study and total polymorphisms ratio is 635/13=2% (Table 3 and Figure 1). RM is a complex syndrome which seems to increase in frequency by thrombophilic gene aberrations. We carried out multiple studies to identified combinations of polymorphisms that could lead to an increased risk for RM [15]. Thrombophilia disorders develop because of irregular placental perfusion. For this reason pre-eclampsia is occurred. Consequently late fetal loss during pregnancy and pre-eclampsia can be seen. But the association of hereditary thrombophilia’s between early pregnancy losses is not identified yet. The roles of inherited thrombophilia have been studied mostly in RM and accumulated a large amount of contradictory literature about the subject. For example, Karata et al. recently, compared the prevalence of factor V G1691A Leiden, Prothrombin G20210A, and MTHFR C677T mutations in patients with...
recurrent miscarriages (RM) [16]. They found an association between MTHFR C677T homozygous and heterozygous mutations with RM.

As hemimethylated promoters and monoallelic expression of CGB5 has been found in patients with recurrent miscarriage (RM), aberrant DNA methylation levels at a certain gene locus might directly lead to this disease [18]. In addition, since the levels of histone methyltransferases G9a and its associated heterochromatin marker H3K9me1/2 are significantly reduced in some cases of RM, not only the genetic mutants but also the epigenetic mutants may contribute the occurrence of RM. Interestingly, since recent study has found that G9a also plays a role in the maintenance of DNA methylation at imprinted loci in mammals, RM might be an imprinted gene associated human disorders [19,20]. Therefore, for the diagnosis of RM, it is worth to investigate the expression patterns and levels of imprinted genes in the prospective patients.

Recently, chromosomal microarray analysis (CMA) has become more commonly used. Without the need for cell culture CMA provides genome analysis with a greater resolution than traditional karyotyping standards. CMA can detect small genetic gains and losses as well. This microduplications and microdeletions in general ‘copy number variants’ (CNVs) are named. So as a continuation of our work, we planned the chromosomal microarray analysis for detect genomic copy number variants in couples and products of conceptions [21].

References