De Novo Balanced (X;14) Translocation in a Patient with Recurrent Miscarriages: Case Report

Tekrarlayan Düşükler Yapan Bir Hastada Doğumsal Dengeli (X;14) Translokasyonu

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ABSTRACT We report a 23-year-old phenotypically normal female patient who had previously suffered from recurrent spontaneous abortion (RSA) who found to have an X;14 trans location and a Methylene- Tetrahdrofolate-Reductase (MTHFR) C677T heterozygote mutation. G-banding cytogenetic analysis was cultured from the peripheral blood lymphocy tes. MTHFR, factor V Leiden and prothrombin gene mutations were studied from DNA obtained from peripheral blood lymphocytes with stripassay. DNA for X inactivation pattern study was also obtained with the method described above. G-banding cytogentic analysis from cultured peripheral blood lymphocytes of the patient revealed 46,XderX,t(X;14)(q13;q32) and found to be heterozygous for C677T MTHFR mutation. An X inactivation pattern study revealed a complete inactivated nor mal X chromosome, asexpected. The possible causes of recurrent miscarriages in our patient were unbalanced gametes, skewed X inactivation and MTHFR C677T heterozygote mutation.

Key Words: Abortion, habitual; heterozygote detection; X chromosome inactivation; translocation, genetic

ÖZET Bu çalışmada, 23 yaşında, fenotipik olarak normal ancak daha önceki tekrarlayan düşükleri nedeniyle X;14 translokasyonu ve Metilen-Tetrahidrofolat Reduktaz (MTHFR) C677T heterozigot mutasyonu taşıyıcılığı saptanan bir olgunun sunulması amaçlandı. Periferik kan lenfosit kültüründen Standart G bantlama ile sitogenetik analiz, yine periferik kan lenfositlerinden izole edilen DNA'dan strip yöntemi ile MTHFR, faktör V, protrombin gen mutasyonu ve X inaktivasyon paterni çalışıldı. Olguda dengeli X-otozom translokasyonu 46,XderX, t(X;14)(q13;q32), MTHFR C677T heterozigot mutasyonu ve X inaktivasyon paterninde tamamen normal X'in inaktif olduğu saptandı.Olgunun anne-babası, eşi ve son gebeliği sonucu doğan kızının ise sitogenetik analiz sonucunda karyotipleri normaldi. Olgumuzda tekrarlayan düşüklerin muhtemel nedenlerinin dengesiz gametler, sapmış X inaktivasyonu ve MTHFR C677T heterozigot mutasyonu olabileceği düşünüldü.

Anahtar Kelimeler: Düşük, tekrarlayan; heterozigot saptamak; X kromozomu inaktivasyonu; translokasyon, genetik

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Recurrent spontaneous abortion (RSA) is a common condition in 1-5% of pregnant women. The structural chromosomal abnormalities and thrombophilia are well-documented etiologic factors in RSA.[1,2] X autosome translocations are rare and are estimated as 1/30 000 in live births.[3] Females with balanced X; autosome translocations constitute a clinically heterogeneous group of patients ranging from healthy individuals to severely disabled patients depending on the X break-

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point position and replication behavior.[4] In this paper, we report a healthy women with X;14 translocation who previously had recurrent miscarriages and gave birth to a healthy girl.

CASE REPORT

A phenotypically normal 23 years old female patient had suffered from two miscarriages in previous three years (at 7th and 14th weeks of pregnancy) but no cytogenetic analyses were performed for these fetuses. Obstetrical work-up included ultrasonography (USG) and hysterosalpingography (HSG) which were found to be normal. In addition, there were no systemic, endocrine, anatomic and environmental risk factors**.** Cytogenetic analysis of the patient was performed and she received genetic counseling. G-banding cytogenetic analysis was performed on cells cultured from peripheral blood lymphocytes. The MTHFR, factor V Leiden and prothrombin gene mutations work-up was done from DNA obtained from peripheral blood lymphocytes with strip assay (Vienna Labs).[5] DNA was extracted from 10 ml venous blood samples with NucleoSpin Blood kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol. DNA for X inactivation pattern study as well as PCR and electrophoretic analyses were performed with the method described by Allen et al.[6] Then, gel products were visualized by ethidium bromide staining, and densitometric analysis of the alleles was performed at least twice using the MultiAnalyst version 1.1 software. A corrected ratio (CrR) was calculated by dividing the ratio of the predigested sample (upper/ lower allele) by the ratio of the non-predigested sample for normalization of the ratios that were obtained from the densitometric analyses. The use of CrR compensates for preferential amplification of the shorter allele when the number of PCR cycles increases. After consultation, she decided for a third pregnancy but did not accept amniocentesis. The karyotyping of her mother, father and husband were all-normal. A clinical risk of miscarriage was evident at the 6th week of the pregnancy and low molecular weight heparin was used until the 20th week of pregnancy because of the high fetal loss risk. All over, she had a healthy baby girl at the 40th week of her pregnancy.

RESULTS

G-banding cytogenetic analysis from cultured peripheral blood lymphocytes of the mother revealed 46,XderX,t(X;14)(q13;q32) (Figure 1a, b, c). The karyotype of the baby was normal (46,XX). The mother was also found heterozygote for C677T MTHFR mutation. The factor V Leiden (G1691A) and the G20210A mutations in the prothrombin gene were found to be negative.

X inactivation pattern study revealed a complete inactivated normal X chromosome as expected (Figure 2). A skewed population is defined as a cell population with greater than 80% expression of one of the *AR* alleles. This corresponds to CrR values of <0.33 or >3. As a result of this analysis, we

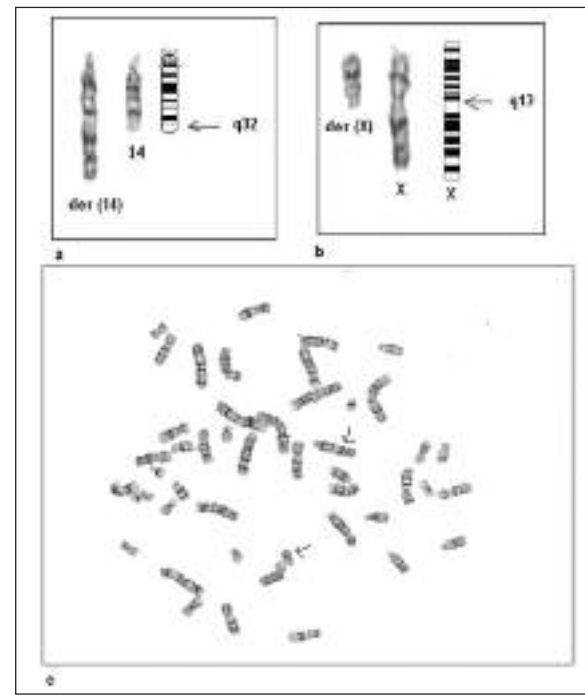

FIGURE 1a, b, c: G-banding cytogenetic analysis from cultured peripheral blood lymphocytes of the mother.
a) der (14) (14pter→14q32::Xq13→Xqter) chromosome, chromosome 14, and chromosome 14 ideogram.
b) der (X) (Xpter→Xq13::14q32→14qter) chromosome, chromosome X, and chromosome X ideogram.
c)Metaphase image of the patient:
Upper arrow: der (14) chromosome.
Lower arrow: der (X) chromosome.

observed complete skewing of the X-chromosome inactivation in our patient.

DISCUSSION

In females with balanced X; autosome translocations, X breakpoint position and replication behaviour can influence phenotypic outcome. Female carriers of balanced X; autosome translocations are generally phenotypically normal but may suffer from multiple congenital abnormalities and/or developmental delay, premature ovarian failure, recurrent miscarriages and X-linked syndrome. Moreover, offspring of the patients with balanced translocation may carry an unbalanced translocation and may have various abnormalities.[4] Therefore, we suggest that one of the possibilities which caused recurrent miscarriages in our patient might be the unbalanced gametes.

Furthermore, X inactivation is a dosage compensation mechanism in women whose both X chromosomes are active in the early embryogenesis. Meanwhile, a random transcriptional silencing of a single X chromosome occurs in either paternally or maternally derived X chromosome.[7,8] Inactivation of the normal X chromosome in unbalanced translocation is a rare but possible event, to confer a survival advantage to the embryo.[3,9] In balanced female carriers of X chromosome; autosome translocations, the normal X chromosome is usually inactivated for allowing full expression of genes on the translocated segments.[10] Similarly, our patient's X inactivation pattern study showed a complete inactivation of the normal X chromosome, as expected. Since she has not demonstrated any X-linked disorder or phenotype alteration, the balanced translocation on the active X chromosome does not seem to harbor any genes. Contradictory reports exist about the association of recurrent spontaneous abortions and skewed X-chromosome inactivation pattern. Hogge et al. suggested that skewed X-inactivation was not associated with recurrent spontaneous abortions but it was associated with increasing maternal age.[1] However, Bagislar et al. reported that 17.7% of 62 patients with recurrent spontaneous abortions were extremely skewed with X-chromosome inactivation.[11]

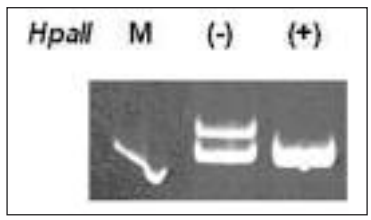

FIGURE 2: X-chromosome inactivation pattern of the patient. A single allele in the HpaII digested sample indicating non-random X-chromosome inactivation was observed. M represents the marker.

Inherited and acquired thrombophilia are responsible for recurrent pregnancy-loss of unknown cause in more than 50% of women. Thrombophilic risk factors are also frequent in women with other vascular placental pathologies such as placental abruption, preeclampsia, intrauterine growth retardation and late fetal loss. The greatest risk was documented in women with homozygous mutations for the factor V Leiden or prothrombin gene or combinations of these risk factors.[5] However, there is limited data in the literatue about the association of MTHFR C677T mutation and recurrent miscarriages. Baksu et al. reported two cases with deep venous thrombosis and fetal loss associated with FV Leiden and MTHFR C677T heterozygote mutations.[12] Recently, Xu et al. demonstrated that the genetic polymorphisms of MTHFR C677T were associated with unexplained recurrent early spontaneous abortion.[13] Although the evidence in the literature is limited, we think that the heterozygous MTHFR C677T mutation of our patient might be an additional risk factor for fetal loss. Thus, possible causes of recurrent miscarriages in our patient were unbalanced gametes, skewed X inactivation and MTHFR C677T heterozygous mutation.

Premature ovarian failure may be caused by some genetic disorders, which may induce skewed X-chromosome inactivation.[14] In addition, the importance of X-breakpoint position has been demonstrated in cytogenetic studies of females with gonadal dysfunction.[4] Since it has been shown that X inactivation center is localized at Xq13, and premature ovarian failure which has been usually confined to patients with breaks within a critical

region between Xq13 and Xq26,[3,15,16] we speculate that our patient might have a breakpoint at Xq13 along with skewed X-chromosome inactivation making her a possible candidate for early ovarian failure in the future.

CONCLUSION

As a result of 3:1 malsegregation, the offspring of female carriers of balanced X; autosome translocation may have normal karyotype as in our patient, or may have balanced X; autosome translocation with normal phenotype, but may also have unbalanced translocations resulting with recurrent miscarriages or multiple congenital abnormalities. Moreover, the skewed X-chromosome inactivation and MTHFR C677T mutations might be additional risk factors for recurrent miscarriages. Female carriers of balanced X; autosome translocation should receive genetic counseling for these possibilities as well as for premature ovarian failure. The newborn should be followed for the possibility of behavior abnormalities and checked for mosaicism.

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