Detection of Marker Chromosome in the Abortion Material; Does It Reflect the Karyotype of the Pregnancy Lost Tissue or the Maternal Decidual Tissue? Case Report

Düşük Materyalinde Marker Kromozomu Taramak: Anneye Ait Desidual Dokunun mu, Yoksa Gebeliğin Kayıp Dokusunun mu Karyotipini Yansıtmaktadır?

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ABSTRACT Determination of the origin of the marker chromosomes is very difficult and requires effort. Evaluation of the marker chromosomes of the pregnancy lost tissue could be the most difficult one as the contamination with maternal decidual cells complicates this process. Conventional cytogenetic techniques and fluorescence in situ hybridization technique or more advanced molecular techniques are preferred to determine the origin of these chromosomes. In this report, the study for identification of the origin of an abortion material derived from the primary tissue cell culture, with a 47,XX,+mar(15) karyotype was presented, by using an effective algorithmic approach which ended with genotyping. The result of the genotyping was informative, since the assumed abortion material actually belonged to the mother's decidual tissue. This brief study reminds the efficient algorithmic approaches to the determination process of the marker chromosomes of the abortion material.

Key Words: Abortion, missed; cytogenetic analysis; molecular diagnostic techniques

ÖZET Marker kromozomların orijinini belirlemek hem zor, hem de çok fazla çaba gerektiren bir durumdur. Gebelik tahliye materyalinde belirlenen marker kromozomlarda, maternal desidual hücre kontaminasyon sorunu olabileceği için bu süreç daha da zordur. Marker kromozomun hangi kromozoma ait olduğunu belirlemek için, konvansiyonel sitogenetik tekniklerin yanısıra, floresan in situ hibridizasyon tekniği veya daha ileri moleküler genetik teknikler kullanılmaktadır. Bu çalışmada, uzun dönem primer doku kültürü sonrası 47,XX,+mar(15) karyotipi saptanan düşük materyalinde, marker kromozomun orijinini belirlemek için izlenen, genotiplendirme ile sonuçlanan, etkin algoritma sunulmaktadır. Genotiplendirme sonucunda marker kromozom içeren dokunun, aslında fetüse değil, anneye ait olduğu belirlenmiştir. Bu kısa çalışma, gebelik tahliye materyallerinde görülen marker kromozomların orijinlerinin belirlenmesinde izlenmesi gereken doğru algoritmayı hatırlatması açısından anlamlıdır.

Anahtar Kelimeler: Düşük, fark edilmeyen; sitogenetik analiz; moleküler tanısal yöntemler

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Marker chromosomes appear at a frequency of 0.3-5/1000 in humans; they are frequently supernumerary and 40% are familial.[1-3] The supernumerary marker chromosome (SMC) 15 is the most common one, accounting for as much as 50-60% of all those observed.[1,4-6] They predominantly occur as small pseudodicentric chromosomes and referred as psu dic (15;15) or inv dup(15).[4,7] The majority of the small de novo SMC (15)s studied so far, has been maternally derived.[1,4,8] The incidence of an abnormality due to non-satellited and satellited marker chro-

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mosomes is 14.7% and 10.9%, respectively and approximately half of the pregnancies with de-novo marker chromosomes were electively terminated due to possible risk of abnormalities.[9]

Despite the well known adult phenotype of patients with SMC (15)s, the number of prenatally diagnosed (alive or ex fetuses) SMC(15) cases are limited and further reports are needed to clarify the fetal phenotype.[4,8,10] It has not yet been ascertained whether the association between a marker chromosome and the loss of a fetus is causal or coincidental.[10] Besides the difficulties of prenatal genetic counseling of marker chromosomes, abortion materials with SMC (particularly 47,XX,+mar) need special effort to discriminate the origin of the tissue because there is a probability of contamination with maternal cells especially if the mother is a carrier of a marker chromosome.

In this report, the identification of the origin of an abortion material which had been derived from tissue culture , with a 47,XX,+mar(15) karyotype and had been confirmed by fluorescence in situ hybridization (FISH) technique was presented via an effective algorithmic approach ending with maternal genotyping. The result of the genotyping was informative, since the assumed abortion material actually belonged to the maternal decidual tissue and the karyotype of the abortion material was still obscure as the abnormal cytogenetic result was reflecting the mother's karyotype. This brief study highlights for the first time in the literature, the necessity of the advanced molecular techniques to eliminate the contamination of the abnormal karyotyped abortion material with maternal decidual tissue.

CASE REPORT

A 30-year-old, multigravid woman was referred to our clinic at the sixth week of gestation to investigate chromosomal abnormalities in her missed abortion material on parents' request. Informed consent was obtained for all procedures. In her obstetric history, the mother had one earlier first-trimester elective termination and two normal pregnancies, which resulted in live births. The children (seven years old female, one year old male) were phenotypically normal. The parents were healthy and nonconsanguienous, and the medical history of the family was unremarkable.

Abortion materials were cultured in two different tissue cultures by using the slightly modified procedure of Verma and Babu.[11] The methods of chromosome harvesting and G banding of metaphase chromosomes have been described in detail elsewhere.[11,12] Twenty-three metaphases analyzed from two separate primary cultures which were cultivated in two weeks' time, revealed 47,XX,+mar karyotype (Figure 1a, b). Because of the inadequate cell count, we could not carry on our search using C-banding and NOR banding. Owing to the insufficient material, we performed FISH technique by using the commercial D15Z1 α-satellite

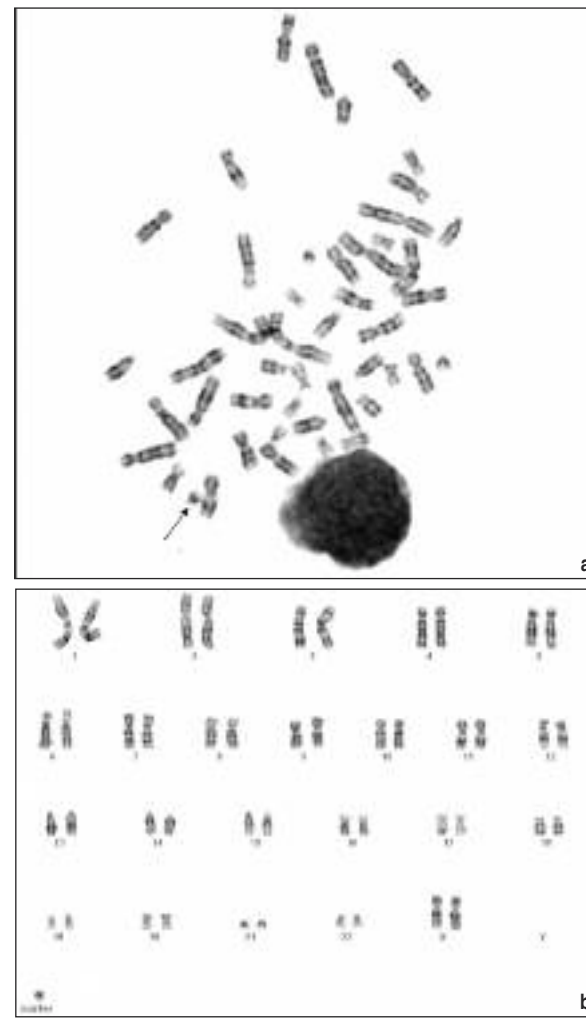

FIGURE 1: Spread metaphase **(a)** and karyotype **(b)** of the abortion material showing the marker chromosome detected by GTG banding.

probe (Vysis, Downers Grove, Illinois, USA) (Figure 2). All of the interphase cells had three signals specific to *the* centromere of chromosome 15 due to SMC(15) and analyses of the metaphases also confirmed the presence of isodicentric structure of SMC(15) (Figure 2). The final karyotype was; 47,XX,+mar.ish der(15)(q11.1) (D15Z1++). To exclude the familial transmission of the marker chromosome, parental cytogenetic analyses were performed, and mother was found to be the carrier of the marker chromosome. C and NOR banding of mother's metaphase spreads showed the isodicentric bisatellited marker chromosome (Figure 3a, b). The centromere specific staining of chromosome 15 by FISH technique was informative as in the abortion material but the probes specific for SNRPN locus, 15qter regions and whole chromosome painting for chromosome15 (Cytocell, Cambridge, United Kingdom), determined the absence of the signals on supernumerary marker chromosome of the mother (Figure 4). The final karyotype of the mother was; 47,XX,+mar.ish der(15)(q11.1)(D15Z1++,SNRPN, WCP-,qter-). To confirm the possible contamination with maternal tissue, we performed genotyping of the parents and the abortion material by using D15S1032, DXS987 microsatellite markers (Figure 5). Fetal DNAs were prepared from cultured abortion materials while the paternal and maternal DNAs were obtained from their peripheral venous blood cells according to manufacturer's protocols. The genotyping results verified that the abnormal karyotype which was assumed to be derived from abortion material actually reflected the cytogenetic result of maternal deciduas (Figure 5). Parents were informed about this conflicting results and genetic counseling was given.

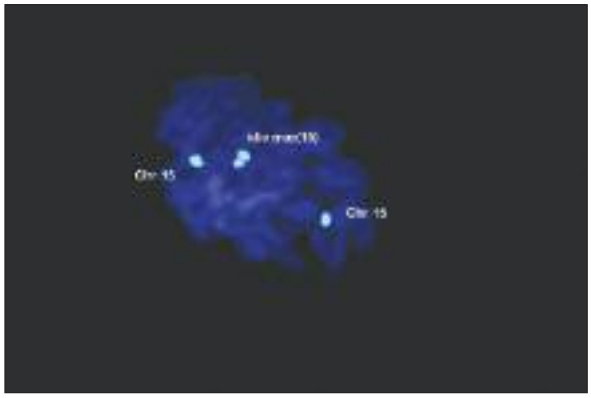

FIGURE 2: The α-satellite probe (D15Z1) of chromosome 15 by FISH technique; the isodicentric marker chromosome 15 in spread metaphase of abortion material is labelled.

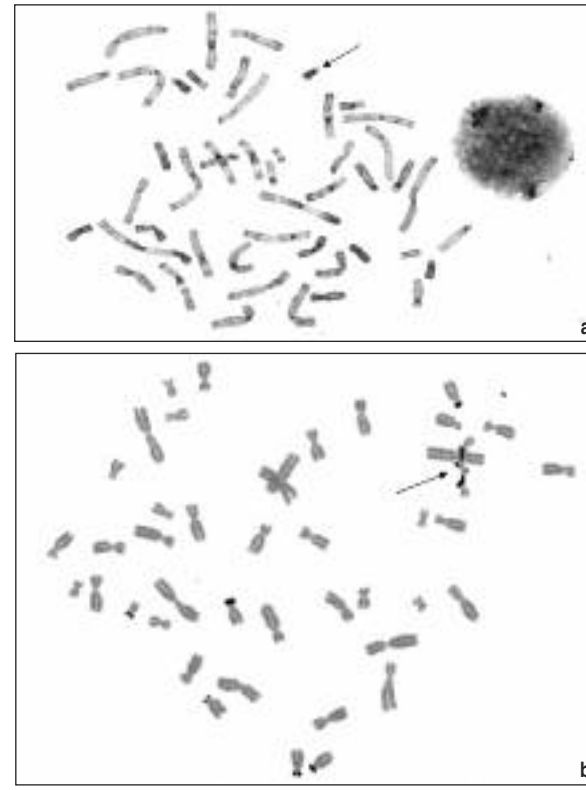

FIGURE 3: The spread metaphase of the mother's lymphocytes showing isodicentric marker chromosome by C-banding **(a)**, and showing biosatellite marker chromosome by NOR-banding **(b)**.

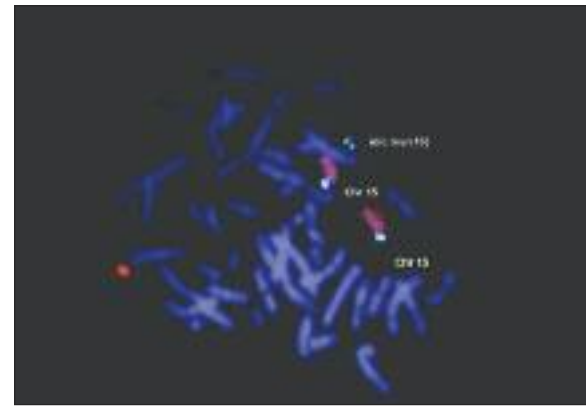

FIGURE 4: Whole chromosome painting (WCP) of the mother's metaphase spread by FISH technique: Normal chromosome 15s are stained by WCP probes, the marker chromosome is not stained by this probe but isodicentric nature of SMC(15) is clearly seen by the hybridisation of the α-satellite probe of chromosome 15 (DZ151).

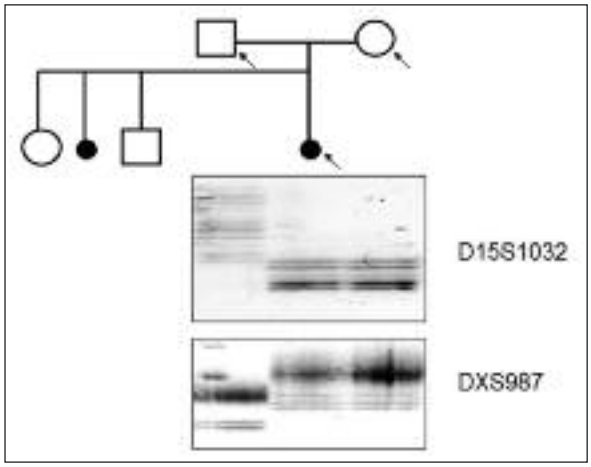

FIGURE 5: Genotypings of parents and abortion material by D15S1032 and DXS987 microsatellite markers on silver stained polyacrylamide gel showing that the cultured abortion material actually belonged to the mother, not to the fetus.

DISCUSSION

Supernumerary marker chromosomes are big challenges of genetic counselling, since the clinical outcomes are still obscure. The challenge is even more pronounced in prenatally reported markers detected in the fetuses or in the abortion materials. To overcome this obstacle, extensive attempts have been made to identify the content of the marker chromosomes, and the in situ hybridization (by fluorescence or by non-isotopic probes) is generally the technique of choice to determine the nature of the markers.[6,10,13] However, due to the restrictions of in situ hybridization in revealing the constituents of markers and the origin of the marker chromosome, further studies are needed for counseling. The risk for contamination of fetal materials with maternal decidua also complicates the issue.[14-19]

Contamination with maternal decidua is reported to be 6-89.7% in 46,XX karyotyped abortion materials which is quite a common situation.[14-18] In most abortion cases, the cells derived from cultured material are regarded as throphoblasts when there is a chromosomal anomaly. Although the suggested approaches are satisfying for the common clinical conditions, there may be some special situations as in our case. Advanced molecular techniques, like genotyping, may be necessary to eliminate the contamination of cultured abortion materials with maternal decidua (Figure 5).

To our knowledge, only one pregnancy loss having a mosaic marker chromosome (46,XX/47, XX, +mar) in abortion material has been reported.[10] The reported patient had recurrent pregnancy losses; maternal karyotyping was performed and it was found that she also had the same chromosomal configuration. The authors performed FISH technique by using probes for chromosomes 16, 18, 21 and X; quite uncommon chromosomes were observed in the markers and finally they could not detect the origin of the marker chromosome. As there was no attempt to verify the possibility or the improbability of contamination with maternal tissue, it seems difficult to suggest that the reported marker chromosome was really reflecting the fetal lost tissue.[10] We had insufficient material for the detection of the possible satellite and centromere content of the presented marker chromosome by C and NOR banding. Hybridisation of the remaining material with the most probable candidate marker chromosome FISH probe -α-satellite probe of chromosome 15- was performed, and the origin was detected (Figure 2). The mother was also a carrier for the SMC (15) and the informative microsatellite markers were used in the process of genotyping to discriminate contamination with maternal decidual and maternal uniparental disomy 15 (Figure 3-5). Finally, the contamination of the abortion material with maternal decidua was confirmed (Figure 5).

This brief study highlights the importance of the algorithmic approach with cytogenetic techniques and the molecular procedures for evaluating the karyotypes of the abortion materials. It also reminds the researchers the probability of contamination with maternal decidua in abortion material which was reported to have the marker chromosome.

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