

## A 45,X/47,XYY/46,XY KARYOTYPE AND Y CHROMOSOME MICRODELETION IN AN INFERTILE MALE

Bulakbasi T<sup>1</sup>, Sahin Fİ<sup>1,\*</sup>, Yil maz Z<sup>1</sup>, Zeyneloglu HB<sup>2</sup>

**Corresponding Author:** Professor Dr. Feride Iffet Sahin, Department of Medical Genetics, Faculty of Medicine, Baskent University, Kubilay Sokak No:36, Maltepe 06570, Ankara, Turkey; Tel : +90-312-2324400; Fax : +90-312-2319134; E-mail: feridesahin@hotmail.com

### ABSTRACT

Infertility affects 10-15% of all couples and the male factor is responsible in approximately half of them. The advances in assisted reproduction techniques and genetic diagnoses have increased the percentage of couples who are able to conceive. We report on an infertile male patient with azoospermia, referred to our laboratory before intracytoplasmic sperm injection (ICSI), whose peripheral lymphocyte karyotype showed a 45,X/46,XY mosaicism. A Y chromosome (Yq) microdeletion was detected in the azoospermia factor (AZF) regions, AZFb and AZFc. The karyotype from testicular tissue cultures was mosaic 45,X[13]/47,XYY[12]/46,XY[25]. Fluorescence *in situ* hybridization (FISH) performed on blood lymphocytes and testicular cells showed a 47,XYY cell line in both, in 8 and 23% of cells, respectively. Histological examination of the testicular biopsy revealed very few seminiferous tubules, which were hyalinized and composed only of Sertoli cells. Intracytoplasmic sperm injection was not suggested to the patient due to the sex chromosome mosaicism, Yq microdeletion and biopsy findings.

**Key words:** Male infertility; Sex chromosome mosaicism; Y Microdeletion (Yq)

### INTRODUCTION

Infertility is an important health problem that affects about 10-15% of all couples attempting pregnancy [1,2]. Sex chromosome abnormalities are an important cause of male infertility [1,2]. Intracytoplasmic sperm injection (ICSI) is an effective therapeutic method, especially in couples with male infertility and/or unsuccessful *in vitro* fertilization (IVF) experiences [1]. Cytogenetic evaluation of couples prior to ICSI shows that both genders are equally at risk for sex chromosome abnormalities. An increased incidence of the 47,XYY karyotype in infertile males has been reported [2], although the majority of individuals with this karyotype are fertile and show normal spermatogenesis [2,3]. In those males who have spermatogenetic failure, abnormalities of azoospermia factor genes in the euchromatic region on the long arm of the Y chromosome (Yq) have been reported [2]. The Yq microdeletions of azoospermia factor (AZF) regions are major causes of infertility associated with severe oligospermia and azoospermia [4] and may also be associated with somatic and germinal gonosomal mosaicism [5-7].

### CASE REPORT

A 42-year-old infertile male patient with non obstructive azoospermia was referred to our genetics laboratory for diagnostic studies before ICSI treatment. In prior IVF attempts no mature spermatozoa

<sup>1</sup> Department of Medical Genetics, Faculty of Medicine, Baskent University, Ankara, Turkey

<sup>2</sup> Department of Gynecology and Obstetrics, Faculty of Medicine, Baskent University, Ankara, Turkey

were found, so the procedure was carried out with spermatid microinjection and failed. The patient had no significant family history and had undergone varicocelectomy 5 years previously. In sperm analysis, the seminal volume was 2.7 mL and no motile or immotile sperm was seen. Chromosome analysis of cultured blood lymphocytes showed a mosaic karyotype 45,X[32]/46,XY[68] and the karyotype interpretation was made according to ISCN 2005 [8]. A Yq microdeletion analysis was performed, screening for two deletions in AZFa region, five in AZFb, four in AZFc and one in AZFd, and for ZFY and SRY genes. The sY277 and sY283 regions of the DAZ gene in AZFc, sY131 and sY143 regions in AZFb, were found to be deleted. Histological examination of the testicular biopsy revealed hyalinization in the few seminiferous tubules present and they were composed only of Sertoli cells. No germinal epithelium or spermatogenesis was detected. The cells from the testicular biopsy culture showed the karyotype 45,X[13]/47,XYY[12]/46,XY[25]. To further investigate the new cell line in blood lymphocytes and to evaluate larger number of cells, fluorescence *in situ* hybridization (FISH) was performed on both tissues using the centromeric probes, Y chromosome DYZ3 and X chromosome DXZ1 (chromosome X/Y cocktail probe; Qbiogene Inc., Carlsbad, CA, USA). Two hundred cells were analyzed from each tissue and the XYY signal was detected in 8% of the lymphocytes and in 23% of the testicular cells. The patient was informed about his condition during genetic counseling. Due to the sex chromosome mosaicism, Yq microdeletion and biopsy findings, ICSI was not suggested. Sperm extraction from testis was performed, but the procedure produced no spermatids.

## DISCUSSION

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The relationship between infertility and chromosomal abnormalities has been well documented over the past 25 years [2]. Since an increase in chromosomal abnormalities correlates with a decrease in sperm count, abnormalities in sperm count are the most important indications for chromosome analysis in infertile males [2]. Numerical sex chromosome aberrations constitute a small percentage of the anomalies; since only 3.32% were reported in a study of 2,196 infertile men; and they tend to

have a broad spectrum of phenotypic effects [2]. Our patient revealed a 45,X/46,XY mosaicism during routine blood analysis. This karyotype was found in seven cases in the same study [2]. Our patient was infertile because of azoospermia, but he also had a Yq microdeletion covering the AZFb and AZFc regions. It has been reported that numerical Y chromosomal defects may accompany Yq microdeletions, as deleted regions tend to be lost during cell division as a result of mitotic instability [6,7]. There is a close association between large Yq deletions and gonosomal mosaicism in both somatic and germinal cells [5,7]. As in our case, mosaicism in germinal cells may remain undetected unless a specific analysis is performed. We first detected the 47,XYY cell line cytogenetically in testicular tissue and confirmed its presence by FISH analysis in 23% of these cells. The FISH analysis of peripheral lymphocytes revealed the same finding in 8% of cells, which were not identified by conventional cytogenetics.

The cases of sex chromosome mosaicism reported in the literature show great diversity, from Turner Syndrome to patients with ambiguous genitalia [9]. The karyotypes with 45,X and 47,XYY cell lines could both present as males and females phenotypically and features like short stature or gonadal tumors have also been reported [9]. Our patient is an apparently normal male, except for the azoospermia which could be due to the coexistence of a Yq microdeletion. The dominant cell line present in different tissues correlate with the cases reported before. Our patient had different percentages of the 47,XYY cell line in different tissues, as expected in these mosaic states, and this variation may explain the diversity of the phenotypes seen in these patients [3,9].

The formation of 45,X/47,XYY/46,XY mosaicism may be caused by at least two mechanisms. The first is paternal non disjunction at meiosis II followed by loss of the chromosome in subsequent mitoses. The second is a post-zygotic mitotic error which explains the different amounts of mosaicism present in different tissues [3]. Either mechanism could be the cause of mosaicism in our patient, while the presence of Yq microdeletion makes him especially interesting.

When sex chromosomal aneuploidy is detected in infertile males, it should be remembered that Yq

microdeletions may accompany the clinical picture as they may induce mitotic instability of the Y chromosome, and cause mosaicism that may be undetected in blood cells, but make considerable contribution to the germinal tissue. Evaluation of infertile males with spermatogenetic defects for gonosomal mosaicism could lead to a better assessment for estimating the outcome of assisted reproduction techniques. This case demonstrates the use of testicular biopsy material as another tissue to be karyotyped. The second tissue in this case revealed a third line of cells with gonosomal aberration and contributed to predicting an outcome and shaping the genetic counseling for this patient.

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