

Case Report: Y Chromosome Microdeletion in an Infertile Patient with Mosaic Klinefelter Syndrome

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ABSTRACT Among genetic factors which contribute about 10-15 percent of male infertility, the most common genetic causes of male infertility are Klinefelter's Syndrome (KS) and Y chromosome microdeletions respectively. Most of the KS patients carry 47, XXY karyotype and almost 15 percent of them are mosaic with variable phenotype. These genetic abnormalities characterized by hypogonadism, azoospermia or oligospermia etc. A 41-year-old male presented with primary infertility with small hard testes and upper limit of FSH and LH. Total azoospermia was showed on semen analysis. 47,XXY/46,XY mosaicism was found in the karyotype analysis from the whole blood culture. Molecular investigation revealed a single deletion of AZFa region (M259 STS in DDX3Y locus). This case illustrates a rare deletion of AZFa region and is differ from previously reported in literature.

INTRODUCTION

Infertility is not a rare disease accounting for approximately 10-15 percent of all married couples in their reproductive years. Male factor is responsible close to half of the cases. Although many factors are responsible in the etiology, genetic factors play a primary key role in male infertility. Chromosomal anomalies, including microdeletions of the Y chromosome, are the most frequently related genetic factors with male infertility. Among chromosomal anomalies, Klinefelter's syndrome (KS) is the most common sex chromosomal abnormality in male infertility (Bar et al. 2014). KS males have the karyotype 47,XXY and, of these 20 percent are classified as mosaic 46,XY/47,XXY or mosaic variant cases with additional cell lines 48,XXYY and 48,XXXYY. The

second most common genetic factor in male infertility after the Klinefelter's syndrome is microdeletions of Y chromosome. (Krausz et al. 2014). Microdeletions demonstrate in the azospermia factor genes (AZF) located in Y chromosome long arm locus 11 (Yq11) region required for spermatogenesis (Krausz et al. 2014). Most common deleted region in infertile men is Deleted in Azospermia (DAZ) Gene Family. Complete or partial loss of this gene is clearly associated with azoospermia, or oligospermia, unrelated to the testicular phenotypes (Reijo et al. 1995).

In this study, we present an infertile patient with a Y chromosome AZFa region microdeletion with mosaic Klinefelter's syndrome (46,XY/47,XXY).

CASE REPORT

The patient was a 41-year old male with primary infertility. The patient was initially assessed by a urologist. A detailed patient history was taken and a genital examination was done. The patient denied any childhood disease, environmental exposure, or medication that may cause infertility. Hormone, semen, and genetic analysis were performed in the diagnostic work-up

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according to the World Health Organization guidelines (WHO 1999).

Cytogenetic Analysis

Peripheral blood lymphocytes were harvested just as the original method of Moorhead et al. (1960). The 72-hour cultured cells from peripheral blood were stored. 34 metaphases in the trypsin GTG banded chromosomes were tested as stated in the International System for Human Cytogenetic Nomenclature (ISCN 2005).

Investigation of Y-Chromosome Microdeletions

Microdeletions were detected with multiplex polymerase chain reaction (PCR) method during the molecular genetic analysis. In case 14 sets of Y specific sequence tagged sites (STSs) spanning the euchromatic region of Y-chromosome from centromere to interval 7, with particular interest in interval 6 (AZF) were tested: the Zinc finger Y-chromosomal protein (ZFY), sex-determining region Y (SRY), sY84, sY86 (AZFa); sY127, sY134 (AZFb); sY254, sY255 (AZFc).

RESULTS

On physical examination, the testes were palpated as small and hard. Total azoospermia with low volume (0,7ml) was detected in the semen analysis. Hormonal tests showed FSH 13,85 mIU/ml (1,37-13,58), LH 9,91 mIU/ml (1,26-10,05) and testosterone 3,36 ng/mL (1,56-8,77), with all other tests being normal. Chromosomal analysis of 34 peripheral blood lymphocytes using GTG-banding revealed a 47,XXY karyotype in 30 metaphases and a 46,XY in 4 metaphases. Molecular investigation revealed a single deletion of AZFa region (M259 STS in DDX3Y locus). The result of the multiplex PCR was shown in Figure 1, and a deletion in the M259 STS DDX3Y locus was found. Micro TESE was not performed in this patient due to genetic analysis results.

DISCUSSION

The relation between male infertility and Y chromosome deletions was firstly found by Tiepolo et al. (1976). De novo deletions of Yq are believed to arise from intra-chromosomal recom-

bination events between large homolog repetitive DNA sequences during meiosis or early pre-implantation development (Edwards et al. 1997). Deletions in the AZF region of the Y chromosome directly damage genes in this region that is responsible for the proper course of spermatogenesis (Krausz et al. 2014). The incidence of microdeletions in the AZF region has been found from 3 to 55 percent frequently in patients with azoospermia. Although the frequency of microdeletions in azoospermic patients were different in the literature, (possible due to ethnic or geographic factors), the most frequent place of the deletions is in the AZFc sub-region (Dabaja et al. 2013). Behulova et al. (2011) evaluated six STS in 226 azoospermic patients from Slovenia and found the microdeletions in the AZFc region in 3.35 percent of cases. In contrast, Malekasgar et al. (2008) found microdeletions in AZFc sub-region in 51.16 percent of azoospermic patients from Iran (total number of evaluated patients was 31).

In this study the researchers found a single deletion of AZFa region (M259 STS in DDX3Y locus). Samli et al. (2006) also found a single deletion of AZFa region but in different locus sY84 in a patient diagnosed with Klinefelter's Syndrome from Turkey. These findings are different from those previously reported in the literature, where their results do not show microdeletion of Y chromosomes in patients with Klinefelter syndrome (Tateno et al. 1999; Lee et al. 2000; Choe et al. 2007; Balkan et al. 2008).

CONCLUSION

Y chromosome microdeletion screening is an appropriate diagnostic method for patients with Klinefelter syndrome who need assisted reproduction techniques. Genetic testing for Y chromosome microdeletion is of prognostic and diagnostic significance for micro-TESE procedure. For men with AZFc deletions alone are recommended proceeding with TESE because of successfully retrieval of spermatozoa, but for men with complete deletion of AZFa or AZFb region are not recommended.

RECOMMENDATIONS

Since the incidence of chromosomal abnormalities is high among infertile men, cytogenetic analysis and detection of Y chromosome mi-

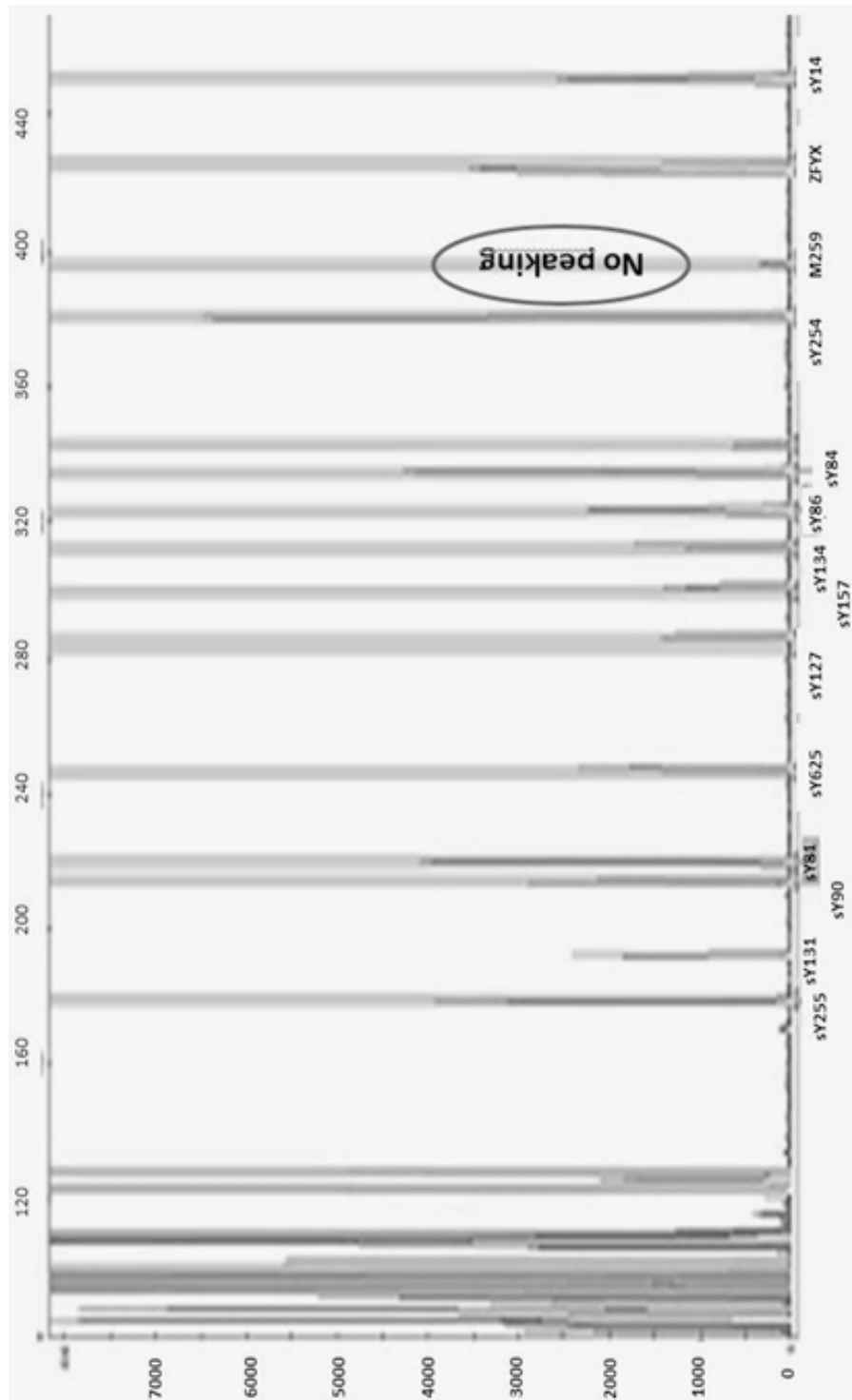


Fig. 1. Multiplex PCR showing microdeletion (M259 STS in DDX3Y locus)

crodeletions should be done prior to the application of assisted reproductive techniques.

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