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ESTIMATES OF SPERM SEX CHROMOSOME ANEUPLOIDY RATES BY FLUORESCENCE *IN SITU* HYBRIDIZATION IN LOW LEVEL 47, XXY MOSAICISM

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Klinefelter syndrome was the first human sex chromosomal abnormality to be reported, and close to 15% are mosaic cases, usually with two cell lines (47,XXY and 46,XY) and rarely with further cell lines. In this report, an infertile patient with low level 47,XXY and 46,XY mosaicism (4% by GTG banding) is presented. This mosaicism was confirmed by fluorescence *in situ* hybridization in lymphocyte nuclei and in sperm nuclei, 4.2% and 6.9%, respectively. In conclusion, for accurate genetic counseling concerning probands' fertility and estimated karyotypes of their offspring, fluorescence in situ hybridization is recommended in addition to conventional techniques in mosaic Klinefelter syndrome patients.

Key Words: Aneuploidy, Mosaic Klinefelter Syndrome, Sperm FISH.

DÜŞÜK ORANDA 47,XXY MOZAİSİZMİNDE, FLORESAN *İN SİTU* HİBRİDİZASYON TEKNİĞİ KULLANILARAK SPERM CİNSİYET KROMOZOMU ANÖPLOİDİ ORANININ TAHMİNİ

İnsanda ilk tanımlanan cinsiyet kromozomu anomalisi olan Klinefelter sendromunun yaklaşık % 15'i mozaik olgulardır ki bunlar nadiren diğer formlarda olmakla beraber, sıklıkla 47, XXY ve 46, XY hücre dizilerine sahiptir.

Bu çalışmada, düşük oranda 47,XXY/46,XY mozaik (GTG ile %4 oranında) infertil bir olgu sunumu yapılmaktadır. Belirlenen mozaisizm lenfosit hücrelerinde (%4.2) ve sperm nükleuslarında (%6.9) floresan *in situ* tekniği ile doğrulanmıştır. Sonuç olarak, mozaik Klinefelter Sendromlu hastalarda, hastanın ne oranda fertil olabileceği ve sonraki kuşakların tahmini karyotiplerini genetik danışmada doğru olarak yorumlayabilmek için, konvansiyonel tekniklerin yanısıra floresan *in situ* hibridizasyon tekniği de önerilmektedir.

Anahtar Kelimeler: Anöploidi, Mozaik Klinefelter Sendromu, Sperm FISH.

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INTRODUCTION

Klinefelter syndrome is a numeric sex chromosome aberration, occurring 1 in 500 male births. About 15% of patients are mosaic and mostly present a 47, XXY and 46, XY karyotype (1). In mosaic forms, the severity of the syndrome is supposed to increase in parallel with the proportion of the abnormal cell population (2). Most individuals with mosaic Klinefelter syndrome are infertile (2).

In this report, we describe a secondary infertile patient with a low level mosaic Klinefelter syndrome. Besides the use of conventional cytogenetic and fluorescence *in situ* hybridization (FISH) in the lymphocytes, mature sperms from a semen sample were examined by two color FISH.

CASE REPORT

The patient was a 38-year-old married man with a 4-year history of secondary infertility. His mother was 26 years old and his father was 45 years old when he was born. A clinical investigation revealed a normal-sized penis and testes, with normal masculine pubic and axillary hair, no gynecomastia, average height (178 cm) and weight (78 kg), and no clinical signs of osteoporosis. His phenotypically healthy wife was 30 years old and her first pregnancy (4 years before) terminated with spontaneous abortion in the 10th week of gestation. There were no further analyses performed after the termination of this pregnancy. Her gynecological examination was normal.

The seminal fluid volume was 3.0 ml with a concentration of 17.0 M/ml. Semen analysis showing < $1x10^6$ sperm/ml with overall motility of 42% was concordant with oligospermia. Endocrine studies showed normal free testosterone (17.1 pg/ml, range; 8.69-54.09 pg/ml), FSH (5.37 mIU/ml, range 1.37-13.58 mIU/ml), LH (2.57 mIU/ml, range 1.26-10.05 mIU/ml) and PRL (11.7 ng/ml, range 0.00-18.00 ng/ml).

Cytogenetic Analysis

Cytogenetic studies were performed on peripheral blood cells, after 72-hour culture with phytohemaglutinin stimulation in the proband and in his wife. Chromosomal analysis of 75 metaphase spreads using GTG-banding revealed low level 47,XXY mosaicism (representing 3 metaphases or 4% of the total cell population) in the proband (Fig. 1a, 1b). Seventy-two metaphase spreads revealed a 46,XY karyotype. His wife's karyotype was normal.

FISH analysis

FISH was performed as an adjunct to conventional cytogenetics using the X/Y dual-color probe (Cytocell Product Catelogie 2001-2002) by the slighty modified procedue of verma and Babu (3). FISH analysis of 542 lymphocytes nuclei revealed that 23

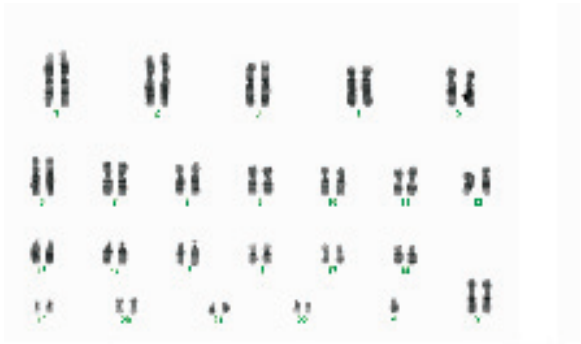

Fig 1(a): GTG-banded karyotype of the patient, illustrating a 47,XXY karyotype.

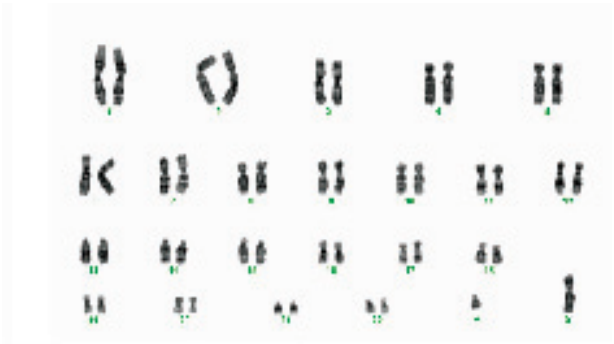

Fig 1(b): GTG-banded karyotype of the patient, illustrating a 46,XY karyotype.

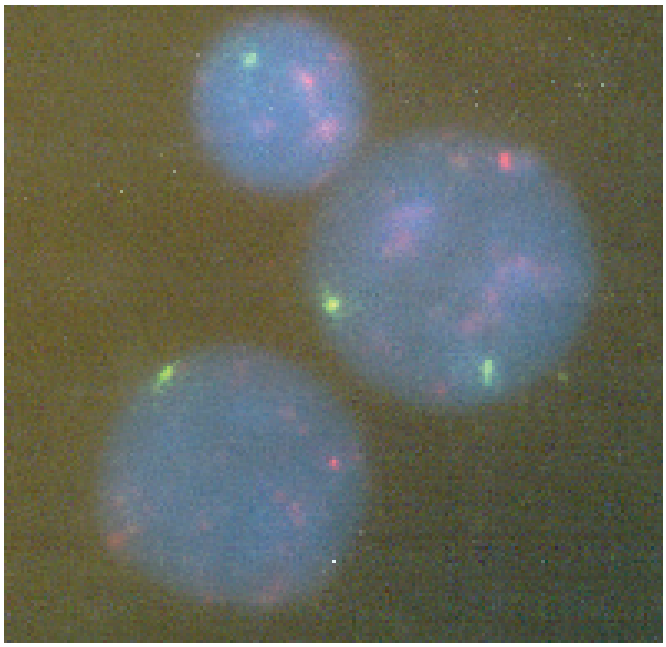

Fig 2: Two-color fluorescence in situ hybridization with X-specific (green) and Y-specific (red) DNA probes: XXY in one interphase nucleus and XY in two interphase nuclei.

cells (4.2%) were positive for two X chromosome signals and one Y chromosome signal, whereas 519 cells (95.8%) were positive for one X signal and one Y signal, consistent with the findings of the GTG-banding study (Fig. 2). In addition to FISH in lymphocytes, the FISH study in sperm nuclei demonstrated the presence of approximately the same level of XY disomy, manifested as 35 cells (6.9%; a total of 506 sperm nuclei were scored) with one X signal and one Y signal (Fig. 3a). XX disomy was not evaluated in this study, as only 2 mature sperm cells revealed two X signals. Moreover, 252 sperm nuclei were positive for only one X signal and 219 sperm nuclei were positive for only one Y signal, 49.8% and 43.3%, respectively (Fig. 3b).

DISCUSSION

In low level 47,XXY mosaicism, it is recommended in the first step to analyze at least 50 cells by G- or R-banding to avoid false positive results due to the missing of chromosomes at the time of spreading and false negative results due to a very low level mosaicism (4). Second, two-color or three-color FISH analysis of different materials of the proband should be applied to evaluate the correct level of mosaicism as an

Fig 3(a): Two-color fluorescence in situ hybridization with X-specific (green) and Y-specific (red) DNA probes on mature sperm of the patient: XY disomic sperm (one green signal, one red signal).

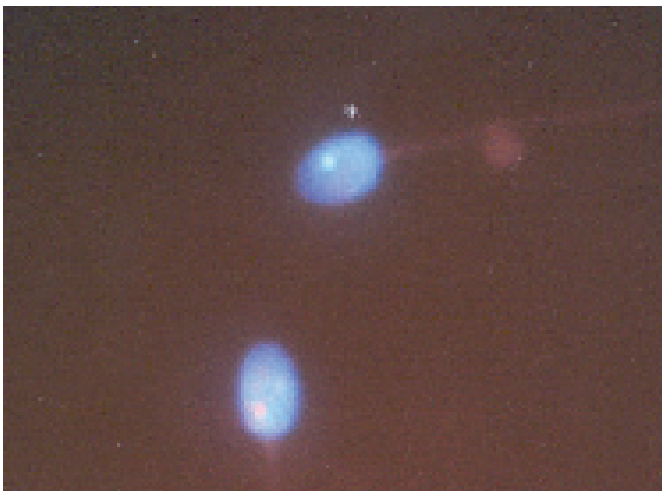

Fig 3(b): Two-color fluorescence in situ hybridization with X-specific (green) and Y-specific (red) DNA probes on mature sperm of the patient: one normal X-bearing sperm cell and one normal Y-bearing sperm cell.

adjunct to conventional cytogenetic analysis (2, 5, 6). In our case, 75 metaphase spreads by GTG-banding were analyzed and X/Y dual FISH was performed on lymphocyte and sperm nuclei. In most previous studies, FISH is considered a specific and sensitive technique to confirm aneuploidies (mosaic or not) (2, 4-6).

At the cytogenetic level, patients with mosaic Klinefelter's syndrome seem to have less features and may be fertile as the presence of spermatogenesis is related to the normal 46,XY germ cells and Sertoli cells proceeding through meiosis rather than 47,XXY cells (7, 8). The screening of ejaculated spermatozoa usually yields aneuploidy rates of 0.1%-1.0% in normal fertile men, with the frequency of sperm aneuploidy in the mosaic ones varying from 1.5% to 7%, which was somewhat concordant with ours (6.9%) (2, 3, 9, 10). In our case, the low level of aneuploidy in GTG and FISH in lymphocyte nuclei could be accompanying similar ratios of sperm nuclei, 4%, 4.2% and 6.9%, respectively.

In conclusion, performing different techniques on different tissues is important in every mosaic Klinefelter case (even in low or high level mosaic ones) in order to accurately estimate the frequency of abnormal cells and for accurate risk estimation and genetic counseling of the probands and their offspring.

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