

Mosaic Status of Lymphocytes in Infertile Men with Klinefelter Syndrome

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ABSTRACT Chromosomal aneuploidies such as Klinefelter syndrome (47,XXY) are the most frequent chromosomal aberrations in infertile men. Normally the chromosomal status of patients is detected by karyotyping of up to 20 metaphase spreads, where low-grade mosaicism may be missed. This study was carried out to test whether Klinefelter patients with 47, XXY karyotype with few characteristic features of KFS may harbour normal 46,XY cell line. We analysed 150 well-spread G-banded metaphases in each Klinefelter patient (n=20). **RESULTS:** Of 20 patients, 4 patients had (5-12%) normal 46,XY cell line. This had not been identified on analyzing 20 metaphase spreads. **CONCLUSIONS:** These results suggest that 47,XXY patients with presence of XY cell line may have a higher probability of finding germ cells and isolated foci of spermatogenesis in the seminiferous tubules. This is a good diagnostic and prognostic marker for ART. Therefore infertile Klinefelter patients with low-grade mosaicism with 46, XY normal cell line, are good candidates for ART/ICSI but in such cases if fertilization occurs it should be followed by preimplantation genetic diagnosis (PGD).

INTRODUCTION

In its classical form Klinefelter syndrome is characterized by gynaecomastia, small, firm testis with hyalinization seminiferous tubules, hypergonadotrophic hypogonadism and azoospermia (Klinefelter et al. 1942). In the general population its prevalence is 0.1-0.2 % (Nielsen and Wohler 1991; Lanfranco et al. 2004) and among infertile patients up to 11% azoospermic and 0.7 % oligospermic men are reported to have 47, XXY karyotype (De Braekeleer and Dao 1991; Yoshida et al. 1996). It is the commonest cause of testicular failures which results in impairment in both testosterone production and spermatogenesis. Though this is the commonest sex chromosomal aneuploidy however such cases may remain undetected or diagnosis may be delayed because of marked variation in clinical phenotype (Dada et al. 2006). This may be due to presence of normal cell line or due to supernumerary X and Y chromosome. Though 47,XXY cases are azoospermic but in the present study we found that previously reported 47,XXY cases with few features/lack of features of hypogonadism (20 cases of 52 cases with no

characteristic feature of KFS and hypogonadism) harboured 46,XY cell line which was not detected on initial cytogenetic analysis by counting 20 metaphase spreads. Therefore this study was planned with the aim to detect presence of other cell lines (46,XY), other than 47,XXY in cases with few characteristic features of KFS as such candidates are ideal candidates for ICSI/ART. ART/ICSI has revolutionized management of severe male factor infertility and has offered hope to millions of infertile couples. Karyotyping 15-20 well spread G-banded metaphase in human genetic departments, a procedure considered the gold standard defines the chromosomal status of a patient (Zang 1984; Kamischke et al. 2003). The relatively low number of metaphases investigated by karyotyping may miss low grade aneuploidy rates (Westlander et al. 2001). This study was designed to analyze low level mosaicism in infertile patients with Klinefelter syndrome. Patients diagnosed were included after conventional cytogenetics was performed.

SUBJECTS AND METHODS

Subjects: For this study, patients were referred to our laboratory of Molecular Reproduction and Genetics from the infertility clinic of Urology Department of AIIMS. Twenty infertile men with lack of characteristic features of KFS and cytogenetically confirmed (47,XXY) Klinefelter

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syndrome cases were selected after the conventional karyotyping and 20 age matched normal fertile men were selected as controls. Heparinized peripheral blood samples were obtained from patients and control subjects after informed consent.

Lymphocyte Preparation and Fixation: 5 ml heparinized peripheral blood was collected from all the patients and controls. Chromosome analysis was done to identify any numerical or structural chromosomal aberrations, by using standard protocol by Rooney and Czepulkowski (1994). G-banding was done by using standard protocol by Sumner (1982). Metaphases were analyzed using cytovision software (ZEISS microscope) classified according to ISCN 1995. At least 150 metaphases were counted to analyze gonosomal mosaicism in each case.

Semen Analysis: Semen samples were analyzed according to the current WHO laboratory manual (World Health Organization 1999) for volume, pH, concentration, morphology and viability of sperms. In the cases of extremely low sperm counts or azoospermia, the ejaculates were centrifuged and analysis was performed on the sediment. Azoospermia was defined as no sperm found even after centrifugation and analysis of pellet. The patients were requested to abstain from sexual activity for 5 to 7 days prior to semen analysis.

RESULTS

Karyotype: Out of 20 patients, four patients showed 4-12% normal 46, XY cell line. This had not been identified on analyzing 20 metaphases spreads. Sixteen patients with Klinefelter syndrome showed only 47, XXY cell line. All control subject showed 46, XY cell-line only.

Semen and Hormonal Analysis: Of these 20 cases 19 cases were azoospermic and one case with 10% normal cell line was severely oligozoospermic with sperm count of 0.2 million/ml. The mean sperm count in controls was 44 million/ml.

Physical Examination: The most discriminating parameter in our study was testicular volume. The range of testicular volume was 5.5 ± 3.4 ml in the cases with KFS and in controls the volume was 34.9 ± 14.1 ml.

DISCUSSION

KFS with 47, XXY chromosomal complement

is a common genetic disorder resulting in testicular failure, androgen deficiency and infertility. It occurs due to chromosomal non disjunction in sperm or egg and in few cases occurs due to post zygotic mitotic errors. Advanced reproductive techniques have offered hope to many infertile couples. ART/ICSI has revolutionized the management of severe male factor infertility. Several successful pregnancies in Klinefelter patients have been reported (Vernaev et al. 2004; Lanfranco et al. 2004), therefore, it is important to divide those cases in which there are chances of *successful sperm retrieval*. This indicates that such cases may harbour a normal cell line and harbour foci of spermatogenesis. Therefore this study was planned to identify low level mosaicism which may be missed when we analyse 20 well spread G banded metaphases. This may help in providing comprehensive counseling to patients prior to enrolling in an ART program like ICSI. It is possible to distinguish between men with and without spermatogenesis based on their FSH values which is a direct indicator of testicular function. Men with spermatogenetic arrest have raised FSH values. But in Klinefelter cases, high FSH values indicate severe testiculopathy. KF cases which have normal cell line have been reported to have a higher chances of having isolated foci of spermatogenesis, which is a good prognostic marker for ART/ICSI. Sharara (1999) reported that mosaic XY/XXY KF cases produce variable number of spermatozoa ranging from severe oligozoospermia to normospermia. Klinefelter syndrome shows a variation in phenotype and thus diagnosis is often delayed or patients remain undiagnosed (Abramsky and Chapple 1997; Smyth and Bremner 1998; Amory et al. 2000; Wilkes 2000; Bojesen et al. 2003; lanfranco et al. 2004). Early diagnosis by karyotyping allows initiation of testosterone substitution therapy to avoid symptoms of androgen deficiency. It has been reported that >70% of Klinefelter cases benefited from testosterone therapy (Nielson et al. 1988). Diagnosis of Klinefelter is often made in adults, however it will be of great value to diagnose this condition as early as possible for example by measuring the size of testes in school going boys at the age of 11-15 years and by carrying out the chromosomal examination in boys with testicular volume less than 2ml. The best time to start testosterone therapy is around the age of 11-12

years when there is marked increase in FSH levels. (Nielson et al.1988). Such screening methods could be carried out readily as a part of prophylactic examination procedures by school physicians in most countries. This may allow future fertility to be preserved for young Klinefelter patients because germ cell depletion may progress with age.

Our hospital is a tertiary care hospital, and we get a large of KF cases for karyotyping. In our previous study we have reported that KFS cases exhibit marked variation in phenotype. Some cases have normal phenotype as compared to other cases. These cases may have a normal cell-line which may be missed by counting 15-20 well spread G-banded metaphases.

In our study testicular volume was the most discriminating factor between normal fertile men (24.9 ± 14.1 ml) and Klinefelter patients (5.5 ± 3.4 ml). Karyotyping of well spread G-banded metaphase of peripheral blood is still the Gold standard for the diagnosis of chromosomal abnormalities. But several studies have reported deficits of this technique (Okada et al. 1999; Westlander et al. 2001). Counting of 15-20 well spread G-banded metaphase may fail to identify low-level of gonosomal mosaicism in these cases. In our study we counted 150 metaphases in each case and controls to identify low-level mosaicism and we found mosaicism in four of the twenty cases, which was not identified by counting few metaphase spreads as in conventional cytogenetics. Such type of mosaicism may be present in the germ cell of these cases and such germ cells with normal 46,XY cell line could be used for Testicular Epididymal Sperm Aspiration in ART/ICSI. Such cases should be followed up by pre-implantation genetic diagnosis. Therefore analyzing a large number of metaphases in such cases with no/few features of hypogonadism may aid in identifying 46, XY cell line. However reports of successful, karyotypically normal pregnancies after ICSI obtained from testicular biopsy samples from Klinefelter patients have been published (Palermo et al. 1998). However analysis of these intra-testicular germ-cells has shown some sperm with an extra X-chromosome, which is a cause for concern (Foresta et al. 1999). This finding implies that Klinefelter syndrome may be transmissible by ART, and that sperm from testicular biopsy sample might require further selection and ART must be followed by pre-implantation genetic diagnosis. Gonosomal

mosaicism in lymphocytes is associated with increased sperm aneuploidies in men with idiopathic infertility (Rubes et al. 2002). It has been reported that proper control of the cell-cycle in mitosis and meiosis is a crucial component of the spermatogonia and the somatic cells (Wolgemuth 2003). Direct correlation of the rate of gonosomal mosaicism in somatic cells and spermatozoa in all men, fertile or infertile, suggests that a common mechanism controls the genesis of both cell types (Wolgemuth 2003; Critchlow et al. 2004). However, a correlation of aneuploidies rates in lymphocytes and spermatozoa can not be shown in the present study because all patients (n-19) were azoospermic. There is no clear correlation between sperm parameters, hormone parameters and aneuploidy frequency in any group. So, we emphasize that in the diagnosis of such syndromes like Klinefelter syndrome which is made by conventional cytogenetics, a large number of metaphases should be screened for low-level of gonosomal mosaicism by new techniques like FISH. This is a rapid and sensitive technique and can detect cryptic and low-level mosaicism. The correlation between easily available tissues like buccal cells and peripheral lymphocytes should be the subject of future investigations. FISH can help in achieving this goal and to determine the probability of successive testicular sperm retrieval in these patients. In conclusion, Klinefelter patients may have a low-level gonosomal mosaicism with 46,XY cell-line. This preliminary study also highlights the need for counting more than 100 metaphase spreads in KF cases which lack or have few features of hypogonadism.

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