Experimental
&
Clinical Article

Chromosome Abnormalities in Turkish Men with Primary Infertility

Tayfun GÜNGÖR[1], Mine KANAT PEKTAŞ[1], Müfit M. GÜNEL[2], Leyla MOLLAMAHMUTOĞLU[1]

Ankara, Turkey

OBJECTIVE: The present study aims to identify the prevalence and types of chromosome anomalies among Turkish men with primary infertility.

STUDY DESIGN: A case-control study was undertaken in 474 Turkish men with primary infertility and 450 phenotypically normal fertile men selected for the control group.

RESULTS: Azoospermia is defined to be the most frequent spermiogram abnormality within infertile men, followed by oligoasthenoteratozoospermia. Chromosomal abnormalities were demonstrated to occur significantly more in azoospermic subjects (28.3%) compared to other infertile subjects (11.5%) and fertile men (0.8%). Klinefelter syndrome was detected to be the most frequent chromosomal abnormality with an overall rate of 10.5%. Azoospermia Factor (AZF) microdeletions occur statistically similar among azoospermic men (4.2%) and other infertile men (2.3%), with a total frequency of 3.2%.

CONCLUSION: The high rate of chromosomal anomalies among infertile Turkish men strongly suggests the need for routine cytogenetic analysis prior to the application of assisted reproduction techniques.

Key Words: Azoospermia, Chromosomal abnormality, Klinefelter syndrome, Male infertility, Spermiogram

Gynecol Obstet Reprod Med;16:1 (32-36)

Introduction

Today, infertility the inability to achieve conception or sustain a pregnancy through to live birth is a very common and major health problem affecting about 10-20% of couples and 7% of all males.[1] A male factor is assumed to be responsible in about 50% of the infertile couples.[2] In at least 10% of these males, infertility is due to genetic causes.[3]

Chromosomal anomalies have been postulated as one of the principal genetic factors in male infertility.[4] It has been shown that chromosomal abnormalities occur five to ten times more frequently in male infertile population than in the general population.[5] The prevalence of chromosomal anomalies is highest in azoospermic men (10-15%), lower in oligospermic men (nearly 5%) and less than 1% in men with normal sperm quality.[6]

As the majority of chromosome abnormalities appear to affect male fertility through interference with sperm production and transport; severe disorders in sperm number, morphology and motility can point out the underlying chromosomal aberrations.[7]

Anomalies in sex chromosomes are the most frequently seen genetic abnormalities among azoospermic men. These anomalies most frequently include Klinefelter syndrome[8] and Y chromosome microdeletions, in especially Azoospermia Factor (AZF) regions.[9]

Klinefelter syndrome which is the most common chromosome anomaly among infertile men has a prevalence of 0.1-0.2% in the general population, 3% among infertile patients and 11% in patients with azoospermia.[10] This syndrome is characterized by one or more extra X chromosomes (47,XXY), deficient androgens, increased gonadotropins, small testes, sparse facial hair, gynecomastia, disproportionately long legs, reduced sexual activity and infertility.[11] This research aims to identify the prevalence and types of chromosome anomalies among Turkish men with primary infertility.

Material and Methods

The present study was approved by the Institutional Review Board and Ethics Committee of Dr Zekai Tahir Burak Women's Health Hospital where a case-control study was undertaken in a total of 474 Turkish men with primary infertility. The subjects were recruited from the andrology department of the study center from January 2007 to January 2008. All subjects were informed about the study and their written consents were obtained. Primary infertility was described as inability to achieve a pregnancy after at least one-year-long unprotected

Dr. Zekai Tahir Burak Women Health Research and Education Hospital
[1]Department of Infertility, [2]Department of Andrology, Ankara

Address of Correspondence: *Mine Kanat Pektas*
Ertugrul Gazi Mah. Kutlugun Sok. No: 37/14, 06590 Iccebeci, Ankara,
minekanat@hotmail.com

Submitted for Publication: *05.08.2009*
Accepted for Publication: *08.12.2009*

sexual intercourse. Infertile subjects who had obstructive azoospermia were excluded.

A total of 450 phenotypically normal fertile men were selected for the control group. The control and patient groups were matched with age and ethnic origin. Both the patient and control groups were subjected to detailed clinical examination. Data were obtained for each subject including age, ethnic origin, parental and couple consanguinity, medical and surgical histories, weight, height, examination of the external genitalia, hair distribution, marital status habits, libido, life style habits as smoking, alcohol or drugs, exposure to gonadotrophin and sexual and family histories.

A complete semen analysis was performed in all subjects according to the guidelines of the World Health Organization (1999).[12] Semen was collected by masturbation at the laboratory after 3-5 days of sexual abstinence, and examined as soon as liquefied. At least two abnormal semen analyses obtained four weeks apart had to be presented before a diagnosis of oligozoospermia or azoospermia could be made. Azoospermia was verified in at least two ejaculates, by pellet analysis after semen centrifugation (1000 g for 20 min). Sperm counts were performed using a Neubauer counting chamber, whereas the sperm morphology analysis was assessed by light microscope in semen smears stained by Leishman staining.[13]

Peripheral venous blood samples of 5 ml were taken from all subjects and these samples were collected in tubes containing disodium EDTA as anticoagulant and stored at 4ºC until DNA extraction. Chromosomal study using peripheral blood and trypsin-G, Q and C banding techniques following the International System for Human Cytogenetic Nomenclature was performed.[14] At least 30 metaphases were analysed for each patient and increased up to 100 metaphases in case of mosaicism. Genomic DNA was extracted from whole blood using standard method. A set of 36 Y-specific STSs (sequence tagged sites accessed from GenBank, USA) was tested in each patient using seven multiplex PCR reactions. These STSs included the following: the SRY (sY14) gene andsY81, sY82, sY86, sY84, sY88, sY182, sY151, sY94, sY95,sY97, sY102, sY105, sY109 sY117, sY121, SYPR3, sY124, sY127, sY128, sY130, sY133, sY134, sY143, sY152, sY153,sY145, sY147, sY149, sY242, sY239, sY208 sY254, sY255,sY157, sY159. Among them, sY242, sY239, sY208 sY254,sY255 were within DAZ gene. The ZFX/ZFY and CMCX primer sets were included as internal control. The PCR products were analysed on a 3% agarose gel (FMC Bioproducts, Rockland, ME, USA) containing ethidium bromide (0.5 µg/ml) and visualised by exposure to ultraviolet light. An STS or gene was considered absent, only after at least three amplification failures.

Fine needle aspiration testicular biopsy was performed under general anesthesia in all azoospermic patients. Sertoli-cell only syndrome was diagnosed, if all seminiferous tubules contained only Sertoli cells. Maturation arrest was diagnosed when tubules contained no spermatozoa but showed spermatogonia, spermatocytes and/or spermatids.

Collected data were analyzed by computerized Statistical Package for Social Sciences (SPSS for Windows, version 11.0, SPSS Inc. USA). The characteristics of distribution were tested with the Kolmogorov-Smirnov test. Pearson's chi-square test was applied to investigate the correlation between chromosomal abnormalities and impaired sperm parameters. P values less than 0.05 were considered to be statistically significant.

Results

The study population includes 474 infertile men and 450 phenotypically normal fertile men. Azoospermia is defined to be the most frequent spermiogram abnormality within infertile men, followed by oligoasthenoteratozoospermia. Table 1 shows the spermiogram disorders determined in a total of 474 men with primary infertility.

Table 1: Spermiogram Disorders in Men with Primary Infertility

Spermiogram Disorders	Number (Percent)
Azoospermia	212 (44.7%)
Oligoasthenoteratozoospermia	166 (35.0%)
Asthenoteratozoospermia	27 (5.7%)
Oligospermia	23 (4.9%)
Oligoasthenospermia	17 (3.6%)
Oligoteratozoospermia	12 (2.5%)
Teratozoospermia	9 (1.9%)
Asthenospermia	8 (1.7%)
Total	**474 (100.0%)**

Table 2 summarizes the prevalance and types of chromosomal anomalies diagnosed in the study population. Chromosomal abnormalities were detected in 28.3% (60 out of 212) of azoospermic subjects, 11.5% (30 out of 262) of other infertile men and 0.8% (4 out of 450) of fertile subjects. Klinefelter syndrome was found to be the most frequent chromosomal abnormality in the study population. Chromosomal abnormalities were demonstrated to occur significantly more in azoospermic subjects compared to other infertile and fertile subjects.

AZF microdeletions were diagnosed in %4.2 (9 out of 212) of azoospermic men and %2.3 (6 out of 262) of other infertile subjects, with a total frequency of 3.2%. The frequencies of AZF microdeletions were statistically similar among azoospermic men and other infertile men. Microdeletions were defined at AZFa, AZFb and AZFc regions in respectively three, six and four subjects. Two subjects were shown to have simultaneous microdeletions in AZFb and AZFc regions. Table 3 displays the results of fine needle aspiration testicular biopsy performed in azoospermic subjects.

Table 2: Distribution of Chrmosomal Abnormalities in Infertile and Fertile Subjects

	Azoospermic Subjects (n=212)	Other Infertile Subjects (n=262)	Fertile Subjects (n=450)	p
46, XY (normal)	152 (71.7%)	232 (88.5%)	446 (99.2%)	0.06
47, XXY (Klinefelter syndrome)	**35 (16.5%)**	**15 (5.7%)**	**0**	**0.03***
Mosaic 46,XY/47, XXY	9 (4.2%)	7 (2.7%)	1 (0.2%)	0.02*
47, XYY	7 (3.3%)	5 (1.9%)	0	0.05
46, XX (male)	**4 (1.9%)**	**2 (0.8%)**	**1 (0.2%)**	**0.03***
45, X/46, X, iso (Yp)	**3 (1.4%)**	**1 (0.4%)**	**1 (0.2%)**	**0.03***
46, XY, t(9;22)	1 (0.5%)	0	1 (0.2%)	0.08
45, XY, t(13; 15) (p11; p11)	1 (0.5%)	0	0	0.06

Table 3: Results of Testis Biopsy in Azoospermic Subjects

Testis Biopsy Results	Number (Percent)
Normal	29 (13.7%)
Sertoli cell only syndrome	131 (61.8%)
Maturation arrest	52 (24.5%)
Total	212 (100.0%)

A total of 157 live pregnancies were conceived by ART in the infertile group within one year. However two cases of Down syndrome (trisomy 21), one case of Edwards syndrome (trisomy 18), one case of Turner syndrome (45, XO) and one case of Klinefelter syndrome (47, XXY) were diagnosed prenatally among the aforementioned assisted pregnancies. On the other hand, two cases of Down syndrome and one case of Turner syndrome were prenatally among 450 naturally conceived pregnancies of the fertile group.

Thus chromosomal abnormalities were detected to be significantly more frequent in the assisted pregnancies of the infertile group when compared to those achieved in the fertile group (3.2% vs 0.7%, p=0.005).

Discussion

Constitutional chromosome abnormalities are found in %14.1 of the azoospermic and 5.1% of the oligozoospermic patients in a study done by Retref et al.[15] Chromosomal abnormalities were detected in 11% of azoospermic and 6.15% of oligoasthenoteratozoospermic South Indian infertile men with a sum of 7.9 % by Rao et al. They observed a strong and statistically significant association of chromosomal anomalies with azoospermia.[16]

Nagvenkar et al. have found constitutional chromosomal abnormalities in 14.3% of azoospermic and 6.5% of oligozoospermic Indian men, with an overall rate of 10.2%.[17]

In a more recent study held by Elghezal et al., 13.5% of Tunisian male infertile patients with non-obstructive spermatogenesis disorders are found to have chromosomal abnormalities.[18] Lissitsina et al. revealed chromosome alterations in 47.8% of Estonian infertile men. Major chromosomal abnormalities were 10 times more frequent (13.4%) in infertile males (15.6% in azoospermics and 12% in oligozoospermics).[19] A study conducted in Mexican men revealed that 18.9% of azoospermic cases were accompanied by an abnormal karyotype. The most frequent chromosomal anomaly was detected as Klinefelter syndrome which was identified in 15.4% of cases.[20]

Two studies held to assess infertile Turkish men reveals different results. In a research done by Duzcan et al, the median incidence of sex chromosome aneuploidy in the oligozoospermic patients is computed to be 4.5% (range, 0.8-7.3%), compared to 0.7% in normal males.[21] Samlı et al have detected chromosomal abnormalities in 12% of azoospermic patients and 4% of oligozoospermic patients. 9.6% of the azoospermic and 2.4% of the oligozoospermic patients are found to have gonosomal abnormalities in the same study[22]

The increased incidence of chromosomal aberration is well established by the present study. That is, chromosomal abnormalities were detected in 28.3% of azoospermic subjects, 11.5% of other infertile men and 0.8% of fertile subjects. Klinefelter syndrome was the most frequent infertility-related chromosomal abnormality diagnosed in 16.5% of azoospermic subjects and 5.7% of other infertile men, with an overall rate of 10.5%. AZF microdeletions occurred in 15 out of 474 subjects with a frequency of 3.2%. Microdeletions were defined at AZFa, AZFb and AZFc regions in respectively three, six and four subjects. Two subjects were shown to have simultaneous microdeletions in AZFb and AZFc regions.

These variations among the incidences of chromosomal abnormalities belonging to a certain ethnic group might account for the size and heterogeneity of the study samples. The relevantly higher incidence of Klinefelter syndrome in our study may be attributed to the fact that our hospital is a tertiary centre that contains a department of genetics to which the

complicated cases of infertility throughout the country are usually referred. Although the frequency of chromosomal anomalies in our research sample is much higher than that of the samples in previous Turkish studies,[20,21] it is similar with the findings of studies conducted in different countries of the world.

Chromosomal anomalies occur significantly more frequent among azoospermic men compared to other infertile and fertile subjects. This finding is also consistent with those reported by studies done in different countries. AZF microdeletions occur among azoospermic Turkish men statistically similar to Chinese and Caucasian men.[23-25] That's why; it can be suggested that Klinefelter syndrome and other genetic anomalies occur as frequent in infertile Turkish men as in other infertile men.

The dramatic advance in assisted reproductive technology has enabled many infertile couples to have children. Such development has evoked the concerns about the genetic risk because the infertile male or female is a potential carrier of chromosomal abnormalities definitely. Nevertheless the detection of such an abnormality is of fundamental significance for the diagnosis of infertility, the required treatment, the appropriate management of the forecoming pregnancies and the evaluation of the risk for the next generation. Therefore, the cytogenetic screening of both partners is addressed to be essential prior to the application of assisted reproductive techniques (ART).

Since the partners of affected men have an elevated risk of miscarriage and birth of children with aneuploidy and congenital anomalies, karyotyping should be offered to men with azoospermia or severe oligospermia before their sperms are used for ART. Moreover the male offspring of men with Y chromosome microdeletions can be expected to inherit these defects and the related clinical consequences. Therefore screening for Y chromosome microdeletions may be applied in all men with azoospermia or severe oligospermia who would be candidates for any type of ART. However routine genetic screening does not seem to be cost-effective so that the cost of karyotyping is not routinely covered by the insurance policies of infertile couples undergoing ART. That's why; genetic screening should be recommended for infertile men diagnosed with azoospermia or severe oligospermia (<5 million sperms/ml) in the absence of organic lesions.

As the majority of chromosome abnormalities appear to affect male fertility by disturbing spermatogenesis, seriously impaired values in a spermiogram can indicate a chromosomal aberration. The high rate of chromosomal anomalies among infertile males, especially with azoospermia or severe oligozoospermia strongly suggests the need for routine cytogenetic analysis prior to the employment of assisted reproductive techniques because such an analysis allows genetic counselling and pre-implant or prenatal diagnosis.

Primer İnfertil Türk Erkeklerindeki Kromozom Bozuklukları

AMAÇ: Sunulan çalışmada, primer infertilite nedeniyle hekime başvuran Türk erkeklerindeki kromozom bozukluklarının belirlenmesi amaçlanmaktadır.

GEREÇ VE YÖNTEM: Sunulan çalışmada primer infertilite saptanan 474 Türk erkeği ile fenotipik olarak normal görünen ve fertil olan 450 Türk erkeği, kromozom bozuklukları bakımından karşılaştırılmıştır.

BULGULAR: Primer infertil Türk erkekkelrinde en sık görülen spermiogram bozukluğu olan azospermiyi oligoastenoteratozospermi izlemektedir. Kontrol grubuna ve diğer infertil erkeklere kıyasla, azospermik erkeklerde kromozom bozukluklarının daha sık olduğu bulunmuştur (%0.8 vs %11.5 vs 28.3%). Çalışmada incelenen tüm erkeklerde en çok görülen kromozom bozukluğu olan Klinefelter sendromunun sıklığı %10.5 olarak hesaplanırken azospermik erkeklerde bu değer %16.5 olmaktadır. Çalışmaya dahil edilen tüm erkeklerin %3.2'sinde belirlenen Azospermi Faktörü (AZF) mikrodelesyonları, azospermik erkeklerde ve diğer infertil erkeklerde benzer oranda görülmektedir (%4.2 vs %2.3).

SONUÇ: Primer infertil Türk erkeklerindeki kromozom bozukluğu sıklığının yüksek bulunması, yardımcı üreme teknikleri uygulanmadan önce rutin olarak gerçekleştirilen sitogenetik analizin gerekliliğini ve önemini vurgulamaktadır.

Anahtar Kelimeler: Azospermi, Kromozom bozukluğu, Klinefelter sendromu, Erkek infertilitesi, Spermiogram

References

1. Siffroi JP. Genetic aspects of male sterility. J Gynecol Obstet Biol Reprod. 2005;34 (1 Pt 2): 1S15-9.
2. Mau-Holzmann UA. Somatic chromosomal abnormalities in infertile men and women. Cytogenet Genome Res. 2005;111(3-4):317-36.
3. Quilter C. Chromosomal abnormalities and male infertility. Indian J Med Res. 2005;122(1):11-2
4. Engel W, Adham IM, Navernia K, et al. Genetic causes of male infertility. Verh Dtsch Ges Pathol 2004; (88):130-5
5. Delgado Martin JA, Blazquez Izquierdo J, Silmi Moyaro A. The management of azoospermic patient. Arch Esp Urol 2004;57(9):1021-33.
6. Sharlip ID, Jarow JP, Bekler AM, et al. Best practice policies for male infertility, Fertil Steril 2002:77:873.
7. Maduro MR, Lamb DJ. Understanding new genetics of male infertility. J Urol. 2002;168(5):2197-205.
8. Huynh T, Mollard R, Trounson A. Selected genetic factors associated with male infertility. Hum Reprod Update.

2002; 8(2):183-98.

9. Fernandez-Salgado E, Alvarez-Nava F, Borjas-Fajardo F, et al. Molecular analysis of microdeletions of the Y chromosome in Venezuelan males with idiopathic infertility. Invest Clin. 2006; 47(4):395-403.
10. Gonsalves J, Turek PJ, Schlegel PN, et al. Recombination in men with Klinefelter's syndrome. Reproduction 2005; 130(2): 223-9.
11. Gooren LJ, de Ronde W. Some new aspects of the Klinefelter syndrome. Ned Tijdschr Geneeskd. 2006; 150(49):2693-6.
12. World Health Organization, Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction, 4th, Cambridge University Press, 1999.
13. Jarow JP, Espeland M. Evaluation of the azoospermic patient. J Urol 2002;142:62.
14. Mitelman F. An Internatioal System for Human Cytogenetic Nomenclature. ISCN 1995, Karger, Basel, Switzerland.
15. Retref AE, van Zyl JA, Menkveld R. Chromosome studies in 496 infertile males with a sperm count below 10 million/ml. Hum Genet 1984;66(2-3):162-4.
16. Rao RL, Babu KA, Kanakavalli MR. Prevalence of chromosome defects in azoospermic and oligoasthenoteratozoospermic South Indian infertile men attending an infertility clinic. Reprod Biomed Online 2005;10(4):467-72.
17. Nagvenkar P, Desai K, Hinduja I. Chromosomal studies in infertile men with oligozoospermia and non,obstructive azoospermia. Indian J Med Res 2005; 122(1): 34-42.
18. Elghezal H, Hidar S, Braham R, et al. Chromosome abnormalities in one thousand infertile males with nonobstructive sperm disorders. Fertil Steril 2006; 86(6):1792-5.
19. Lissitsina J, Mikelsaar R, Punab M. Cytogenetic analyses in infertile men. Arch Androl. 2006;52(2): 91-5.
20. Mera-Espinoza JP, Davalos-Rodriguez IP, Rivera-Ramirez H, et al. Chromosomal abnormalities in patients with azoospermia in Western Mexico. Arch Androl 2006; 52(2): 87. 21. Duzcan F, Aybek Z, Tepeli E, et al. Sex chromosome aneuploidy rates in the somatic cells of infertile men. J Reprod Med. 2006;51(6):489-92.
22. Samlı H, Samlı MM, Solak M, et al. Genetic anomalies detected in patients with non-obstructive azoospermia and oligozoospermia. Arch Androl. 2006;52(4):263-7.
23. Zhou-Cun A, Yang Y, Zhang SZ, et al. Chromosomal abnormality and Y chromosome microdeletion in Chinese patients with azoospermia or severe oligozoospermia. Yi Chuan Xue Bao 2006;33(2):111-6.
24. Ferlin A, Arredi B, Speltra E. Molecular and clinical characterization of Y chromosome microdeletions in infertile men: a ten year experience in Italy. J Clin Endocrinol Metab. 2007;92(3):762-70.
25. Mohammed F, Al-Yatama F, Al-Bader M. Primary male infertility in Kuwait: a molecular and cytogenetic study of 289 infertile Kuwaiti patients.Andrologia. 2007; 39 (3): 87-92.