Cytogenetic Evaluation of Infertile Men: A Retrospective Study

İnfertil Erkeklerde Sitogenetik Değerlendirme: Retrospektif Bir Çalışma

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ABSTRACT Objective: Infertility was described as failure to conceive after at least 1 year of unprotected intercourse. The incidence of chromosomal abnormalities is 10 to 20 fold higher (2.2-14.3%) in males with infertility compared to the general male population (0.7-1%). In this study, we aimed to investigate the contribution of chromosomal aberrations to male infertility. **Material and Methods:** We analyzed male patients diagnosed with infertility referred to the Fırat (Euphrates) University, Faculty of Medicine, Department of Medical Biology and Genetics within a 3-year period between 2004 and 2006 by conventional cytogenetic methods and reviewed the cytogenetic results of these patients. **Results:** We reviewed 94 male patients with infertility. Chromosomal abnormalities were detected in 13 (13.8%) cases and polymorphisms were present in 7 (7.4%) cases . Chromosomal abnormalities were Klinefelter syndrome (47, XXY) and 47,XYY syndrome. Polymorphisms were pericentric inversion of the chromosome 9 and Yqh polymorphisms. **Conclusion:** These data show that cytogenetic evaluation is necessary for an accurate approach to elucidating the causes of infertility and physicians should also be aware of the diversity of chromosomal abnormalities that play important roles in the etiology of infertility. It is clear that there are many genetic factors causing infertility such as Y chromosome microdeletions, some mutations of the CFTR gene, mutations in SOX9, KALIG1 etc., but cytogenetic examinations should be made prior to molecular studies.

Key Words: Cytogenetics; chromosome aberrations; infertility, male

ÖZET Amaç: İnfertilite, en az 1 yıllık korunmasız düzenli cinsel ilişkiye rağmen çocuk sahibi olamama durumudur. Kromozomal anomali insidansı, normal erkek popülasyona (%0.7-1) göre infertil erkeklerde 10-20 kat (%2.2-14.3) daha yüksektir. Bu çalışmada, kromozomal anomalilerin erkek infertilitesine katkısını değerlendirmek amaçlanmıştır. **Gereç ve Yöntemler:** 2004-2006 yılları arasındaki 3 yıllık dönemde, Fırat Üniversitesi Tıp Fakültesi Tıbbi Biyoloji ve Genetik Anabilim Dalına infertilite tanısıyla başvuran erkek hastalar konvansiyonel sitogenetik metotlarla analiz edilerek sonuçları değerlendirildi. **Bulgular:** İnfertil 94 hasta incelendi. 13 (%13.8) olguda kromozomal anomali, 7 olguda ise polimorfizm (%7.4) tespit edildi. Kromozomal anomaliler Klinefelter sendromu (47,XXY) ve 47,XYY iken, polimorfizm olarak Yqh polimorfizmi ve 9 no.lu kromozomun inversiyonu saptandı. **Sonuç:** Bu sonuçlar, infertilite nedenlerini araştırmada sitogenetik değerlendirmenin doğru bir yaklaşım için gerekli olduğunu göstermektedir ve klinisyenler infertilite etiyolojisinde önemli bir yeri olan kromozomal anomaliler açısından da dikkatli olmalıdırlar. Y kromozom mikrodelesyonu, bazı CFTR gen mutasyonları, SOX9, KALIG1 gen mutasyonları gibi infertiliteye yol açan pek çok genetik faktör olduğu açıktır, fakat moleküler çalışmalardan önce mutlaka sitogenetik inceleme yapılmalıdır.

Anahtar Kelimeler: Sitogenetik; kromozomal düzensizlikler; infertilite, erkek

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Infertility was described as failure to conceive after at least 1 year of unprotected intercourse.[1] Male infertility affects 10% of couples of reproductive age worldwide and is treatable in many cases.[2] The etiology of

male infertility is complex and may include anatomic problems such as congenital bilateral absence of the vas deferens, endocrinologic problems, immunologic problems such as antispermatozoal antibodies and genetic causes.[3-5] In spite of the fact that male infertility has various causes, genetic factors are considered significant.[6,7]

Reports indicate that genetic causes such as chromosomal and gene disorders make a significant contribution to infertility.[8] The numerical and structural chromosomal abnormalities are frequent in oligospermia and azoospermia cases with unknown etiology.[7] The incidence of chromosomal abnormalities is 10 to 20 fold higher (2.2-14.3%) in males with infertility compared to the general male population (0.7-1%).[9] In this study, we aimed to investigate the contribution of chromosomal aberrations to male infertility.

MATERIAL AND METHODS

We reviewed the cytogenetic results of male patients referred to our department with the diagnosis of infertility according to the results of urological examinations and semen sampling, within a 3-year period from 2004-2006, retrospectively. For karyotyping of the patients, 5 mL peripheral blood samples were drawn into heparinized injectors. Cytogenetic analysis of 72-hour PHA-stimulated cultures of peripheral blood lymphocytes and GTG-banding was performed using standard protocols. The preparations were examined using light microscopes. 20 metaphase plaques were evaluated for each case. The chromosomal abnormalities were reported according to the International System for Human Cytogenetic Nomenclature (ISCN) 2005. In case of mosaicism, 50 cells were examined. Fluorescent In Situ Hybridization (FISH) methods were used to determine mosaicism in the 47,XYY case. Y chromosome probe (Vysis, Downers Grove, IL, USA) was used for FISH and 500 interphase cells were examined to determine the rate of mosaicism. The chromosomal abnormalities were described according to the ISCN.[10] Percentage values were used to determine the rate of polymorphisms and chromosomal abnormalities. This research was conducted in accordance with the Helsinki Declaration principles. The study protocol was approved by the Institutional Ethical Review Board and informed consent from each patient was obtained.

RESULTS

We reviewed 94 male patients with infertility referred to our department between 2004 and 2006. Out of 94 patients, 30 (31.9%), 30 (31.9%) and 34 (36.2%) were normozoospermics, oligozoospermics and azoospermics, respectively. Chromosomal abnormalities were detected in 13 (13.8%) cases. The most frequent chromosomal anomaly was Klinefelter syndrome (46,XXY) (12.8%); 1 and 11 cases with this anomaly were in the oligozoospermic and azoospermic groups, respectively. There was a statistically significant relationship between the oligozoospermia and azoospermia groups ($p< 0.05$). In one patient, mosaic 47,XYY was detected in cytogenetic analysis. The rate of mosaicism was 47,XYY(%76)/46,XY (%24) in the FISH study. The most frequent polymorphism was the increase in the heterochromatin region of chromosome Y, detected in 5 (5.3%) patients. Abnormal karyotypes and polymorphisms detected in this study were shown in Table 1 and Table 2, respectively. There was a statistically significant difference between the normozoospermia, oligozoospermia groups and the azoospermia group ($p< 0.05$).

TABLE 1: Abnormal karyotypes found in infertile men.

Oligozoospermia	Azoospermia
47,XXY (1 case)	47,XXY (11 cases)
	47,XYY (87%)/ 46,XY(13%) (1 case)

TABLE 2: Polymorphisms found in infertile men.

Normozoospermia	Oligozoospermia	Azoospermia
*46,XYq+ (2 cases)	46,XYq+ (2 cases)	46,XYq+ (1 case)
46,XY,inv(9)(p11q13)	46,XY,inv(9)(p11q13),	
(1 case)	inv(9)(p11q13) (1 case)	

* Increase in the heterochromatin region of chromosome Y.

DISCUSSION

The prevalence of chromosomal abnormalities is 13.8% in the general population.[10] In different studies, the rates varied between 5.2% and 13.4%.[1,11-13] There is azoospermia and severe oligozoospermia in 40-50% of infertile males.[14] The rate of chromosomal abnormalities was similar (13.8%) in all infertile males. The rate of chromosomal abnormalities was reported to be 18.9% in azoospermic patients.[15] In the present study, we also found a significantly higher incidence of chromosomal abnormalities (35.3%) in the azoospermic group. In cases of non-obstructive azoospermia, there is a 15% risk of chromosomal abnormality, including both aneuploidies and structural rearrangements, including rare complex chromosomal rearrangements (CCRs).[14,16]

The vast majority of the abnormal karyotypes, especially 47,XXY, and polymorphisms including sex chromosomes (including variants of chromosome Y) were similar to the results of other studies.[6,17] One oligozoospermic patient (%3.3) was karyotyped as 47,XXY. The relationship between many normal variants of chromosomes, such as pericentric inversions, 9qh+, Yqh+, and impaired spermatogenesis is not clear.[1] The most frequently occurring heterochromatic variant is pericentric inversion, inv(9)(p11q13), with a prevalence of 1–1.65% in the general population and 1.17-5% in infertile males. The heterochromatic variant 9qh was observed in 0.3-14.3% of infertile males compared to the 1.4% in the normal population. Lissitsina et al. also reported that they could be among the several unknown factors, which impair spermatogenesis.[1] The incidence of pericentric inversion of chromosome 9 was 3.3% in normospermic patients in our study. Homozygosity for pericentric inversion of chromosome 9 was present in one (3.3%) oligozoospermic patient. The parents of this patient were carriers for pericentric inversion of chromosome 9. This is the first case in the literature with homozygosity for pericentric inversion of chromosome 9 and primary infertility.

The variation in the length of the Y chromosome is usually due to variation in the distal part of the long arm that is known to contain heterochromatin. The increase in the length of the heterochromatin of the Y chromosome is the most frequent variant in sex chromosomes and satellites are present in autosomal chromosomes.[18] The incidence of Yq polymorphisms was also high, 6.7%, 6.7% and 2.9% in normozoospermic, oligozoospermic and azoospermic patients, respectively.

In late 2004 and 2005, we had some contacts with many urology clinics in our region and we suggested infertile men to be referred to a genetic department to be evaluated cytogenetically. This attempt increased the number of infertile patients presenting to our department (data shown in Table 1) and the number of abnormal karyotypes. This study also shows that cytogenetic examination of infertile men is essential. It is clear that there are many genetic factors leading to infertility such as microdeletions of chromosome Y, some mutations of the CFTR gene, mutations in SOX9, KALIG1 etc., but cytogenetic examinations should be made prior to molecular studies.[19]

Cytogenetic analysis and genetic counseling would be helpful in infertile males with azoospermia and oligospermia by determining the genetic factors causing infertility and by assessing the genetic risks of the offspring provided by assisted reproductive techniques.

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