Cytogenetic assessment of Iranian infertile men with undescended testis: A retrospective study

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ABSTRACT
Objective: Undescended testis (UDT) is a urogenital disease that affects fertility. This study looked into the cytogenetic abnormalities of Iranian infertile patients with UDT.

Methods: Our study included 522 infertile patients with UDT (case group) and two control groups, one with 300 infertile men without UDT and another with 268 fertile men.

Results: Chromosomal abnormalities were found in 45 patients with UDT (8.62%). Seven of the alterations were considered as normal features. Klinefelter syndrome and mosaicism were the most common anomalies. Chromosomal abnormalities were found in 31 infertile men in the control group (10.33%), 13 of which deemed normal and 18 (6%) anomalous. Nine chromosomal abnormalities were found in the second control group with fertile men (3.35%), six deemed normal and three (1.11%) anomalous.

Conclusion: Despite the high rate of abnormalities in infertile controls (6%) and the higher rate seen in infertile individuals with UDT indicate a significant prevalence of chromosomal abnormalities in the Iranian population, particularly when the literature suggests that the normal rate of abnormal karyotypes should be within the 0.7-1% range in the general population. The incidence of abnormal karyotypes increased when infertile patients had additional conditions such as UDT.

Keywords: cytogenetics, undescended testis, cryptorchidism, male infertility, Iranian population

INTRODUCTION
Undescended testis (UDT) or cryptorchidism is one of the most prevalent urogenital defects in boys that lead to sexual development deficiency. Testicles usually descend during the last weeks of gestation or a few weeks after birth (Ghirri et al., 2002). The process unfolds in two phases, in which the testicles descend from the intra-abdominal location into the extra-abdominal scrotal sac of the boy (Kaleva & Toppari, 2005). The first phase is trans-abdominal and androgen-independent, while the second is androgen-dependent. The first phase occurs from the 8th to the 15th week and the second from the 25th to the 35th week of gestation. The reasons for UDT are still unknown. UDT has been linked to a number of hormone disorders and related factors (Cox et al., 2008), including testosterone, gonadotropin-releasing hormone (GnRH), follicle stimulating hormone (FSH), luteinizing hormone (LH), anti-Müllerian hormone (AMH), insulin-like 3 protein (INSL3), and HOXA 10 (Niedzielski et al., 2016).

The prevalence of UDT situates between 2-4% in full-term boys (Komarowska et al., 2015). Two possible consequences of UDT are testicular cancer and infertility (Giwercman et al., 1987; Deng et al., 2019). UDT can be unilateral or bilateral. The incidence of infertility is significantly higher in patients with bilateral UDT than in subjects with unilateral UDT (Hollowell, 2014). According to previous studies, subjects with unilateral UDT are usually more successful at having children than their counterparts with bilateral UDT (Cendron et al., 1989). Genetic disorders account for 15-30% of all cases of male infertility (Neto et al., 2016). Genetic screening may benefit patients with azoospermia or oligospermia (Cavkaytar et al., 2012). Karyotyping as a first step in genetic investigation helps doctors to determine whether their patients present with chromosomal aberrations. Chromosomal abnormalities can be generally categorized as numerical or structural.

Numerical abnormalities include cases in which the patient is missing one or more chromosomes or has one or a few extra chromosomes. Structural aberrations include duplications, deletions, inversions, translocations, insertions, rings, and isochromosomes (Genetic Alliance & District of Columbia Department of Health, 2010). Since chromosomal disorders are commonly seen with infertility, we assumed that the incidence of chromosomal abnormalities might be high in infertile patients with UDT. This study was the first to examine the cytogenetic alterations of infertile patients with UDT to understand the association between UDT and karyotype abnormalities in the Iranian population.

MATERIALS AND METHODS
Patients and clinical data
This retrospective study included 522 infertile men with undescended testis (UDT) in the case group and two control groups, the first with 268 fertile men who underwent sex selection for family balancing at the Royan Institute and had at least one child, and the second with 300 infertile men without UDT or urogenital disease who sought fertility treatment at the Royan Institute. Our study included individuals seen at the Royan Institute from 2010 to 2015. All participants gave written consent before joining
the study. The Ethics Committee of the Rovan Institute ap-
poved the study. The individuals included in this study had
previously undergone physical examination, hormone test-
ing, semen analysis, and karyotyping. The subjects in the
fertile control group had normal spermograms, FSH, LH, and
testosterone levels. A specialist performed the physi-
cal examination of patients with UD and reported the type
of UD and additional information about the appearance of
sex organs with the aid of ultrasound examination. The
patients with UD did not have other urogenital diseases
such as hypospadias or ambiguous genitalia. Subjects with
UD were further divided into bilateral and unilateral UD.
The hormone tests of infertile patients were performed by
electrochemiluminescence (ECL) and included FSH, LH, and
testosterone. Semen analysis was performed after 2-5
days of sexual abstinence at the andrology laboratory ac-
cording to the World Health Organization (WHO) criteria
(2010). Sperm concentration, semen volume, pH, motility, and morphology were thoroughly checked. Infertile
patients were categorized as having oligospermia/se-
vere oligospermia (sperm counts of less than 5 million per
ml) or azoospermia (zero sperm count). Statistical analysis
was performed on SPSS version 22. Differences between
groups with a p-value ≤0.05 in the chi-square test were
deemed significant.

### Cytogenetic analysis

Karyotyping was performed on trypsin-banded meta-
phase chromosomes with a standard protocol of 550 band
resolutions from peripheral lymphocyte cultures. Then, 50
random metaphase spreads were analyzed for each per-
son. More than 50 metaphase spreads were checked in
patients suspected for mosaicism. Karyotypes were de-
fined using the International System for Human Cyto-
genic Nomenclature (ISCN 2016). Chromosome variations
such as centromeric heterochromatin variants were con-
sidered as normal variations based on the ISCN 2016 (Mc-
Gowan-Jordan et al., 2016) and previous studies (Zhou et
al., 2006). Although previous studies have correlated inv
(9) (p12q13) with male infertility due to spermatogenesis
disorders, this finding was considered a normal feature in
our study in accordance with the ISCN (Sasiadek et al.,
1997; Mozdarani et al., 2007).

### Statistical analysis

The Chi-square test was used in statistical analysis. A
p-value ≤0.05 was considered significant.

### RESULTS

Our study included Iranian men divided as follows: 522
infertile patients with UD; 300 infertile men without UD;
and 268 fertile men. Participant ages ranged from 25 to
61 years at the time of diagnosis; participants were aged
40±5.6 years on average. The included patients belonged
to different ethnic groups. Physical examination, ultra-
sound, or patient medical records indicated that 292 indi-
viduals had unilateral UD (55.94%) and 230 subjects had
bilateral UD (44.06%). Semen analysis of patients with
UD showed that 348 (66.66%) were azoospermic, 110
(21.07%) were oligospermic, and 64 (12.26%) had severe
oligospermia. The group of infertile individuals had 204
(68%) subjects with azoospermia, 70 (23.33%) with oli-
gospermia, and 26 (8.66%) with severe oligospermia. The
hormone profile of patients with UD revealed increased
gonadotropin levels and lower to nearly normal plasma
testosterone levels.

Mean FSH and LH levels were 26.89±22.91 and
11.89±9.13 mIU/mL, respectively, while the mean testos-
sterone level was 3.47±2.44 ng/mL. The hormone profiles
of infertile individuals showed mean FSH and LH levels of
13.15±12.84 and 10.21±9.88 mIU/mL, respectively, and
an average testosterone level of 3.61±2.13 ng/mL. The
ranges for normal hormone levels based on WHO criteria
were as follows: FSH (1.5-12) mIU/mL; LH (1-10) mIU/
ML; testosterone (2-8) ng/mL. Cytogenetic analysis re-
vealed chromosomal alterations in all three groups, with
45 individuals (8.62%) in the case group, nine (3.35%) in
the fertile control group, and 31 (10.33%) in the infertile
group without UD. Normal chromosomal alterations were
seen in seven individuals (1.34%) in the case group, six
cases (2.23%) in the fertile group, and 13 (4.33%) sub-
jects in the infertile group.

Normal variations were excluded from statistical analy-
sis. Thirty-eight subjects with UD (7.3%), three (1.11%)
fertile individuals, and 18 (6%) infertile patients had dif-
ferent kinds of pathogenic chromosomal abnormalities.
Table 1 describes in detail the chromosomal alterations
seen in the case and control groups. Numerical chro-
omosomal abnormalities in the case group featured Klinefelter
syndrome and chromosomal mosaicism in 18 (3.44%) and
10 cases (1.91%), respectively. Interestingly, Klinefelter
syndrome was the most common chromosomal anomaly.
One patient with UD had 47, XXY syndrome. Nine of the
patients with mosaic karyotypes had sex chromosome mo-
saicism; one had mosaicism of unknown origin (probably
linked to the Y chromosome); and one patient had struc-
tural and numerical chromosomal abnormalities (Table 1).

The five structural abnormalities observed were Rob-
ertsonian and reciprocal translocations, deletion of the (Y)
chromosome, and an inversion. Another small group of
abnormal karyotypes comprised four cases of sex revers-
sal (0.76%) with the 46, XX karyotype instead of the 46,
XY normal karyotype, which features were described in
a published study developed at the Rovan Institute (Moham-
dadpour Lashkari et al., 2017). The analysis of abnormal
karyotypes in the infertile control group revealed that 13
(4.33%) individuals had Klinefelter syndrome, a number as
high as the one observed in the case group. The group also
featured two individuals with mosaicism, two with trans-
locations, and one with duplication. Thirty patients in the
case group had unilateral UD and eight had bilateral UD.
The incidence of abnormal karyotypes was higher among
individuals with unilateral UD. Hormone profiles showed
that patients with UD and abnormal karyotypes had high-
er mean FSH and LH levels (27.98±19.11 and 17.52±13.7
mIU/mL, respectively) and lower to nearly normal testos-
sterone levels (2.64±1.69 ng/mL). Cytogenetic analysis re-
vealed 27 cases of azoospermia and 11 of oligospermia.
The incidence of azoospermia was higher than the inci-
dence of oligospermia in patients with UD (Figure 1). The
statistical analysis of chromosomal alterations revealed a
significant difference (p-value ≤0.05) between case and
control groups.

### DISCUSSION

Although UD is a multifactorial condition, the genetic
factors linked to the condition are still a topic of discussion.
This retrospective study looked into cytogenetic alter-
ations in Iranian infertile patients with UD and compared
them against fertile and infertile controls. The novelty in
this study lies in the fact that it is the largest cytogenetic
study ever performed in an Iranian population. Our study
found chromosomal abnormalities in 7.3% of the infertile
individuals with undescended testicles. The rates of chro-
omosomal aberrations in infertile controls without UD and
fertile controls were 6% and 1.11%, respectively. Sta-
tistical analysis revealed a significant difference between
the case and control groups (p-value ≤0.05). An earlier
study analyzed 110 patients with UD and/or hypospadias.
### Table 1. Chromosomal alterations observed in study participants

| Chromosomal Alterations* | Normal Variations | Chromosomal Abnormalities | Combined numerical and structural |
|--------------------------|-------------------|--------------------------|---------------------------------|
| Type                     | No of Cases       | Type                     | No of Cases                     | Type                      | No of Cases |
| **Infertile Patients with UDT N=522** |                   |                           |                                 |                           |             |
| 46,XY, inv (9)(p12q13)   | 4                 | Klinefelter Syndrome     | 18                              | 46,XY; t(1;16)(p10;q10)    | 1            |
| 46,XY, inv(3)(p11q11.2)  | 2                 | Jacobs Syndrome          | 1                               | 46,XY; t(13;16)(q12.1;q22) | 1            |
| 46,X, inv(Y)(p11.2q11.2) | 1                 | Different types of Klinefelter Syndrome mosaicism | 4                             | 45,X; der(13;13)(q10;q10)  | 1            |
|                          |                   | 47,XX,+mar[4]/46,XX[12] | 1                               | 46,XY; inv(4)(p15.32p14)   | 1            |
|                          |                   | 45,X[ ]/46,XY[ ]        | 1                               | 46,XY; del(Y)(q11.222)     | 1            |
| **Fertile Men (1st Control Group) N=268** |                   |                           |                                 |                           |             |
| 46,XY, inv(9)(p12q13)   | 2                 | 47,XXY[1]/45,X[1]/46,X Y[13] | 1                             | 46,XY; inv(10)(p12.3q21.3) | 1            |
| 46,XYq+                 | 1                 | 48,XXYY[1]/45,X[1]/46, X Y[13] | 1                             |                           | -            |
| 46,XY, 1qh+             | 1                 | 46,XY, 9qh+              | 1                               |                           | -            |
| 46,XY, 21psk+           | 1                 | 46,XY, 9psk+             | 1                               |                           | -            |
| 46,XY, 15ps+            | 1                 | 46,XY, 15psk+            | 1                               |                           | -            |
| **Infertile Patients without UDT (2nd control group) N=300** |                   |                           |                                 |                           |             |
| 46,XY, inv(9)(p12q13)   | 7                 | Klinefelter Syndrome     | 13                              | 46,XY; dup(7)(q11,21q11.22)| 1            |
| 46,XY, 1qh+             | 1                 | Jacobs Syndrome          | 1                               | 46,XY; t(6;12)(q25.1;q21.31)| 1            |
| 46,XY, 9qh+             | 1                 | Different types of Klinefelter Syndrome mosaicism | 1                             | 46,XY; t(6;17)(p21.33;p10) | 1            |
| 46,XY, 16q+             | 1                 | 47,XXY[2]/46,XY[13]      | 1                               |                           | -            |
| 45,X[21]/46,XY[18]      | 1                 | 45,X[21]/46,XY[18]       | 1                               |                           | -            |
| 46,XY, 14psk, 14psk+    | 1                 |                           |                                 |                           | -            |
| 46,XY, 15psk, 15psk+    | 1                 |                           |                                 |                           | -            |
| 46,XY, 15psk+           | 1                 |                           |                                 |                           | -            |

* Four cases of unfertile patients with UDT diagnosed with sex reversal 46, XX syndrome are not mentioned in this table.
The authors reported seven abnormal karyotypes among patients (6.4%) (Yamaguchi et al., 1991). Another study found seven abnormal karyotypes (4.4%) in a group of 160 patients with UDT (Sasagawa et al., 1996). Karyotyping of an 11-month-old boy with bilateral undescended testes revealed he had 47, XYY syndrome (Suzuki et al., 1999), as seen in one of our patients. A total of 984 individuals with hypospadias and/or UDT were studied in 2002; 884 of them had UDT and a reported rate of chromosomal anomalies of 1.84%.

In agreement with previous studies, the authors recommended that patients with UDT should undergo chromosomal analysis, but mentioned testing was more beneficial for patients with combined congenital abnormalities since they were at greater risk of having higher rates of abnormalities (Moreno-Garcia & Miranda, 2002). We also recommended karyotyping for individuals with UDT. Interestingly, although our study enrolled fewer individuals, the prevalence of abnormal karyotypes was significantly higher in our population (7.3%). The differences between the two populations - our study enrolled 522 infertile individuals with UDT only - may explain this apparent discrepancy. Another study included 48 individuals with UDT and hypospadias. Eight (16.7%) had abnormal karyotypes. Surprisingly, despite the high frequency of chromosomal abnormalities in their patients, the authors reported that karyotyping was not needed for all individuals with hypospadias or UDT (McAleer & Kaplan, 2001). Cox et al. (2008) reported that karyotyping is not required in patients with only unilateral or bilateral UDT or in patients with UDT and distal hypospadias. The authors recommended karyotyping only for patients with proximal hypospadias and UDT, since they accounted for a greater proportion of chromosomal abnormalities (16%). The observations described by McAleer & Kaplan (2001) and Cox et al. (2008) did not match our findings, since our patients with UDT only had a high incidence of abnormal karyotypes (7.3%). This number appears to indicate a significant prevalence of chromosomal abnormalities in the Iranian population when compared with 6% and 1.11% found in infertile and fertile controls, respectively, and even more so when the literature suggests that the normal rate of abnormal karyotypes should be within the 0.7-1% range in the general population (Nussbaum et al., 2016).

In a group of 94 boys with Klinefelter syndrome, 83.7% had the 47, XXY karyotype and 7.1% had 47, XXY/46, XY mosaicism. UDT was the most diagnosed disease among prepubertal individuals with Klinefelter syndrome. The phenotypes diagnosed in pubertal patients included small testes, UDT, and gynecomastia (Pacenza et al., 2012). Our study also found an association between UDT and Klinefelter syndrome, a combination seen in 18 cases (3.44%) of infertile individuals in our population. Klinefelter syndrome was likewise observed in 13 infertile individuals without UDT (4.33%). Other types of sex chromosomal mosaicism were seen in 1.91% of the individuals in the case group, a higher proportion than in the general population. Our results showed that other chromosomal abnormalities such as sex reversal and structural aberrations may be related to UDT, although with lower incidence compared with Klinefelter syndrome. The rate of chromosomal abnormalities in our study was high among infertile controls without UDT, with an incidence of 6% vs. 7.3% in the case group. It seems, however, that patients with more significant involvement - such as UDT - are more likely to have chromosomal abnormalities. The comparison of hormone profiles of patients with UDT and individuals in the case group with UDT and abnormal karyotypes revealed that all subjects with UDT had increased FSH and LH levels and lower to nearly normal testosterone levels. These features may be a consequence of UDT, not a trait resulting from having abnormal karyotypes.

CONCLUSION

In agreement with previous studies, our study found a significant association between UDT and karyotype abnormalities, although the proportion of individuals with karyotype abnormalities was higher in the Iranian population included in our study (7.3%) than the proportion seen in the general population. Karyotyping should be offered to patients with UDT undergoing fertility treatment, since they may have chromosomal abnormalities and infertility caused by chromosomal anomalies. Preimplantation genetic diagnosis (PGD) is strongly recommended for azoospermic patients tested positive for chromosomal abnormalities in microsurgical testicular sperm extraction (Micro-TESE) and individuals with oligospermia.
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CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

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