Original Article

The Spectrum of Chromosomal Abnormalities and Endocrine Profile of Male Infertility with Nonobstructive Semen Abnormality: A Case–Control Study

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ABSTRACT

Background: Primary infertility is a common occurrence which affects approximately 15% of couples who desire to begin their family. Chromosomal abnormalities are well-established causes of pregnancy loss but may also have a role in explaining the cause of male infertility, especially with nonobstructive semen abnormalities. Hence, awareness regarding safety of artificial reproductive technology in these individuals due to underlying sperm aneuploidy is required. **Aims:** The aims of the study are to determine the prevalence of chromosomal abnormalities in primary infertile males with nonobstructive semen abnormalities and correlate with their endocrine profile. **Study Design:** A case–control study, in which 100 males with primary infertility and non-obstructive semen abnormalities were evaluated for chromosomal abnormality and hormonal profile; and were compared with 50 healthy males with normal semen analysis and at least one biological child. **Materials and Methods:** Blood T-lymphocytes were cultured using RPMI-1640 medium for obtaining metaphases and chromosomal analysis. **Statistical Analysis:** SPSS software and Student's *t*-test were used. A $p < 0.05$ was considered statistically significant. **Results:** Azoospermia (81%) was the most common nonobstructive semen abnormality. Overall prevalence of major chromosomal abnormalities and polymorphic variants was 16% and 7%, respectively. Klinefelter syndrome was the most common sex chromosomal numerical abnormality seen in 6.17% of cases with azoospermia. All healthy control males had 46, XY karyotype. Higher levels of follicle-stimulating hormone and luteinizing hormone and lower levels of testosterone along with testicular volumes were observed in infertile males with abnormal karyotype ($p < 0.05$). **Conclusion:** Primary infertile males with nonobstructive semen abnormality have high frequency of chromosomal aberrations, which justify the requirement of cytogenetic testing in these patients.

KEYWORDS: *Chromosomal abnormalities, endocrine profile, infertility, nonobstructive semen abnormality*

INTRODUCTION

The World Health Organization defines primary infertility as a disease of reproductive system wherein there is failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse.[1] Certainly, it is a common disability with worldwide variation as it affects 3%–15% of couples.[2] Fertility problems can affect both males and females;

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the male factors contribute up to 50% in couples seeking medical attention. Different etiologies have been documented for male and female infertility, of which fraction of cases have genetic predisposition, and minority of this fraction are carriers of demonstrable chromosomal aberrations (CAs), which can further lead to profound effect on gametogenesis and eventually lead to infertility.[3]

The frequency of CAs in the general population is approximately 0.6%,[4] whereas these abnormalities have higher prevalence in primary infertile males, seen in 2%–14% of cases.[5] The CAs can be either numerical or structural in nature involving either autosomes or sex chromosomes. Thus, cytogenetic evaluation by karyotyping is also recommended by the American Urological Association and European Academy of Andrology in all men specially with azoospermia or total motile sperm count below 5 million/cumm in a workup of male infertility.[6] Besides psychological benefits and answer to the query for underlying cause of infertility, chromosomal analysis also assists in decision-making of sperm retrieval for intracytoplasmic sperm injection (ICSI), androgenic substitution therapy, and follow-up for various endocrine or systemic dysfunctions, especially if one is diagnosed with Klinefelter syndrome (KS) during infertility workup.

Despite this, chromosomal study for primary infertility is still not routinely performed in our country due to lack of awareness, cost, and limited infrastructure; and thus, its prevalence and impact on primary male infertility in the Indian population are not well studied, due to which limited medical literature is there on the subject. In view of this, the primary aim of this case–control study was to assess the prevalence and types of CAs in infertile males with nonobstructive azoospermia (NOA) or oligozoospermia referred to the infertility clinic of a tertiary care center in Western Maharashtra, between January 2018 and February 2020, and to compare the findings with fertile and healthy control males in the reproductive age group. We also decided to explore the correlation between somatic chromosomal abnormalities and hormonal profile of these patients and control individuals. Although similar studies are there in the literature from India,[7-10] this is the first study of its kind that has compared the cytogenetic and endocrine profile of infertile males with healthy fertile male controls.

MATERIALS AND METHODS

This prospective case–control study included 150 males, which were further divided into two categories. Males in Category-A were infertile with NOA or oligozoospermia as per the following inclusion and exclusion criteria.

Category-B was comprised of 50 volunteered healthy males matched for age and ethnicity and with at least one biological child and normal semen analysis.

Written informed consent from all the participants and approval from local ethical committee and institutional review board to conduct the study (IEC#17-230) were obtained.

Inclusion and exclusion criteria

All the participants (n = 150) were interviewed for relevant medical history and detailed physical examination with investigations including semen analysis, hormonal profile, and scrotal sonography. Patients with primary infertility were diagnosed as azoospermia when there was absence of sperm in the ejaculate from centrifuged and fresh samples on more than two occasions at an interval of 1–3 weeks; and in men with severe oligozoospermia, karyotyping was performed when the sperm count was $<5 \times 10^6$/ml with similar interval and protocols as above.[11]

Infertile males with obstructive/surgical causes of azoospermia were excluded from the study namely varicocele-induced damage, undescended testis, trauma, mumps orchitis, previous scrotal or inguinal surgeries, and any history of gonadotoxic drugs intake.

Fifty healthy control males with normal semen analysis and proven fertility of at least one biological child were also studied.

Cytogenetic and hormonal analysis

5 ml of peripheral blood samples was withdrawn from all the participants, in each sodium heparin and sterile tubes, for cytogenetic study and hormonal assay, respectively. Chromosomal analysis was performed on peripheral blood T-lymphocytes cultured for 69–72 h, using RPMI-1640 medium with 15% fetal bovine serum and stimulated by 2% phytohemagglutinin. After 72 h, cells were harvested using hypotonic potassium chloride followed by GTG banding with trypsin. At least 20 metaphases were analyzed from each patient. When mosaicism was suspected, 30 additional metaphases were analyzed in each case. Chromosome study was done using image processor and software (Cytovision) version 7.2 build 147, and abnormalities were reported according to An International System for Human Cytogenomic Nomenclature (ISCN, version: 2016) at band level 500–550.[12]

Special assays such as C-banding and fluorescence *in situ* hybridization (FISH) were performed as and when required. FISH analysis was done for confirmation of low-level mosaicism on 500 viable interphase cells using commercially available in vitro diagnostic (IVD)

approved probes. Postcentromere probe validation in our laboratory, more than 4% of abnormal signals were considered as mosaic.

Reproductive hormones, including follicule-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone, were measured from early morning serum samples by electrochemiluminescence using Vidas® 2016 Serum Chemistry Analyzer (Biomérieux SA, Marcy L'etoile, France) immunoassay, according to the manufacturer's instructions. Normal reference ranges for men are: FSH, 1.03–9.7 mIU/ml; LH, 1.04–8.02 mIU/ml; and testosterone, 2.8–10.3 ng/ml. The testicular volume was calculated in all patients and controls as, length × width × depth × 0.71, using 7S Phased Array Transducer, GE Logiq P5 (North Carolina, USA) by an experienced radiologist.[13]

The major CAs (MCAs) were further divided into two major anomalies which include structural abnormalities and numerical abnormalities. Structural aberrations included balanced translocation, insertion, and Robertsonian translocation. Numerical abnormalities included mosaic (mos) form, non-mos form, and marker chromosome (+mar). Another category of CAs having heteromorphic forms was classified under polymorphic variants (PV), which included pericentric inversion, variations in size of stalk of acrocentric chromosomes, and length of heterochromatin regions.

Statistical analysis

For the sample size calculation, the alpha error was set at 0.05 and the Type II error at 0.20; hence, the calculation indicated that 100 patients would be required per group to detect a 20% difference in the study group compared with the control group. Data were collected, coded, entered, and analyzed using Microsoft Excel software. Data were imported into SPSS (Statistical Package for the Social Sciences) is name of software for data analysis. for analysis. Descriptive statistics was expressed in terms of means and proportions. To test the significance between two groups, Student's *t*-test was used. A $p < 0.05$ was considered statistically significant.

RESULTS

In this case–control study, 100 primary infertile males with semen abnormalities and 50 healthy control males with proven fertility were recruited for chromosomal analysis and their endocrine profile. Distribution and characteristics of somatic chromosomal anomalies in different categories of infertility are summarized in Tables 1 and 2. The mean age of our patients was 32.72 ± 3.77 years (range from 25 to 45 years). There was no significant age difference between patients with normal karyotype and abnormal karyotype [Table 3].

Somatic chromosomal study

The overall prevalence of MCA in the present study was 16% and PV was observed in 7% of cases. NOA was the most common presentation (81%) of semen abnormality, in which 63 (77.77%) cases had normal karyotype, 7 (8.64%) cases had numerical chromosomal abnormalities, 5 (6.17%) cases had structural abnormalities, and 6 (7.40%) cases with PV. Structural abnormalities were more common in autosomes ($n = 4$), which has resulted either from reciprocal balanced translocations [Figure 1] involving two nonacrocentric chromosomes (2%); Robertsonian translocation [Figure 2] between two acrocentric chromosomes 21 and 22 (1%) and also an insertion (1%) [Figure 3]. Oligozoospermia was reported in 19% of cases, out of which 14 (73.69%) cases presented with a normal karyotype, and 2 (10.5%) cases each with numerical and structural chromosomal abnormalities and single case with PV.

KS was the most common sex chromosomal numerical abnormality (5%) presenting with NOA. Two cases were mos KS which was confirmed on FISH [Figure 4] and one case had additional abnormality of the presence of a supernumerary marker chromosome. 47,XYY syndrome was seen in three men including one mos form. Mos 47,XYY/46, XY [Figure 5] was less common (1%) and had oligozoospermia, whereas non-mos forms (2%) had presented with azoospermia. Two individuals had structural abnormalities of the Y chromosome: one had a balanced translocation with a metacentric chromosome [Figure 6] and another had a terminal deletion of the long "q" arm. An isolated case of 46,XX male syndrome was there [Figure 7a]. Metaphase FISH revealed translocation of SRY gene on a large metacentric chromosome [Figure 7b]. Pericentric inversion of chromosome 9 was the most common PV.
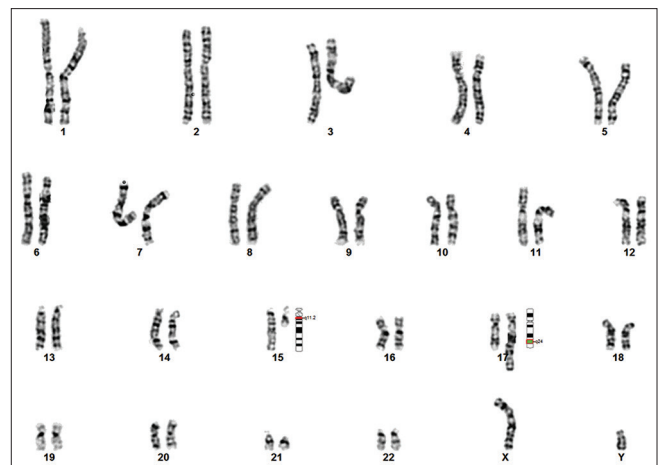

Figure 1: 46,XY, t(15;17)(q11.2;q24)

Table 1: Category of patients with nonobstructive semen abnormalities and control group					
Patients category	*n*	Normal KT, *n* (%)	Numerical abnormalities, *n* (%)	Structural abnormalities, *n* (%)	PV, *n* (%)
NOA	81	63 (77.77)	7 (8.64)	5 (6.17)	6 (7.40)
Oligozoospermia	19	14 (73.69)	2 (10.52)	2 (10.52)	1 (5.27)
Total	100	77	9	7	7
Control group	50	50	Nil	Nil	Nil

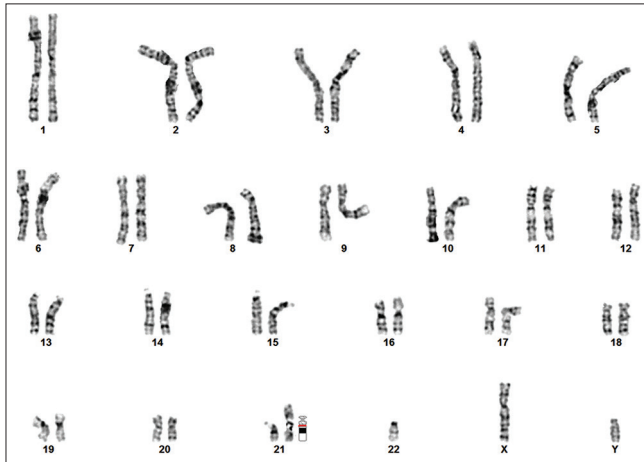NOA=Nonobstructive azoospermia, PV=Polymorphic variants

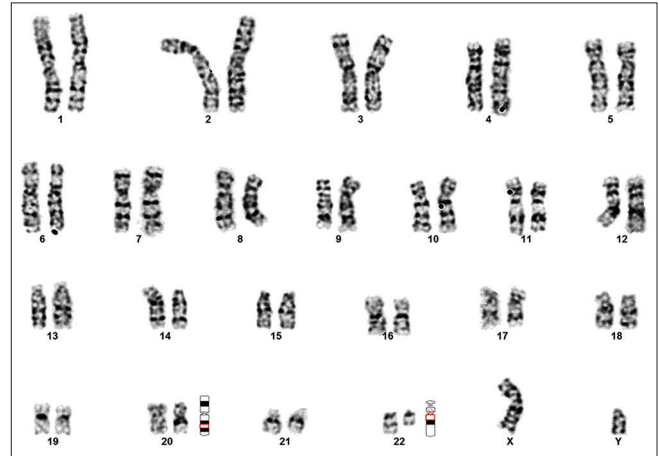
Figure 2: 45,XY, rob,(21;22)(q10;q10)

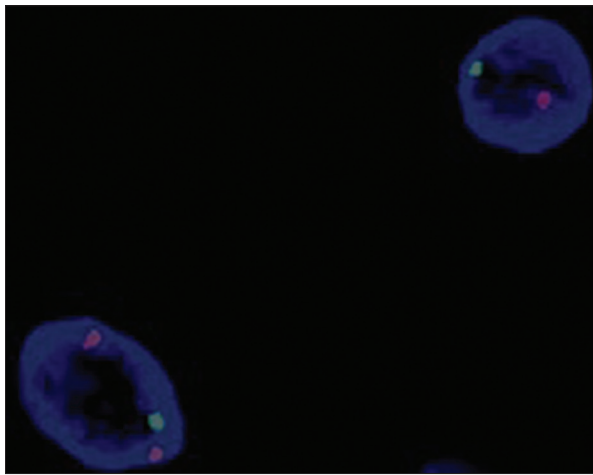**Figure 3:** 46,XY, der(20) ins(22;20)(q11.2;q13)

Figure 4: Mosaic Klinefelter syndrome: Lower interphase cell shows two X and one Y chromosome represented by red signals and a green signal respectively. Upper cell shows normal XY constitution
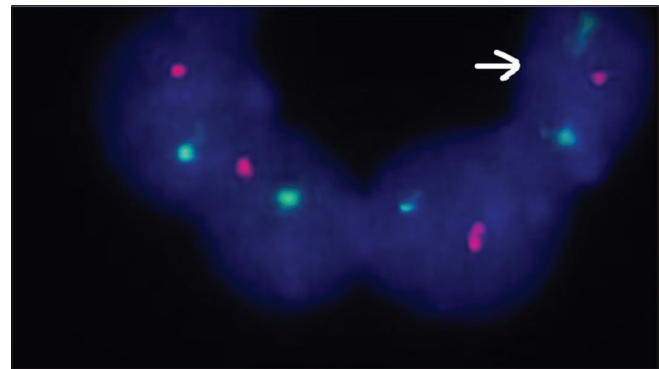

Figure 5: Mosaic XYY syndrome: Interphase cell showing one X chromosome and two Y chromosomes represented by a red signal and two green signals, respectively (white arrow)

All the healthy control men had a normal male karyotype of 46,XY.

Hormonal profile

Mean sperm count of the infertile men with normal and abnormal karyotype was 0.39 ± 1.16 and 0.49 ± 1.2 million/ml, respectively. Mean FSH, LH, and testosterone levels and bilateral testicular volumes in healthy control males and men with normal karyotype and abnormal karyotype are tabulated [Tables 2 and 3]. Higher levels of FSH and LH and lower level of testosterone and mean testicular volumes were observed in infertile males with abnormal karyotype constituted by MCA, which was statistically significant ($p < 0.05$).

Correlation of serum FSH and LH levels in infertile men with normal karyotype in comparison with PV in chromosomes was significant ($p < 0.05$). However, in our study, we observed that testicular volumes and serum testosterone levels between males with normal karyotype and PV had no statistically significant correlation ($p = 0.832$) [Table 4]. However, all the infertile men diagnosed with KS had small testicular volume and is statistically significant [Table 5].

DISCUSSION

In the present study, we found that the prevalence of MCA and PV was 16% and 7%, respectively,
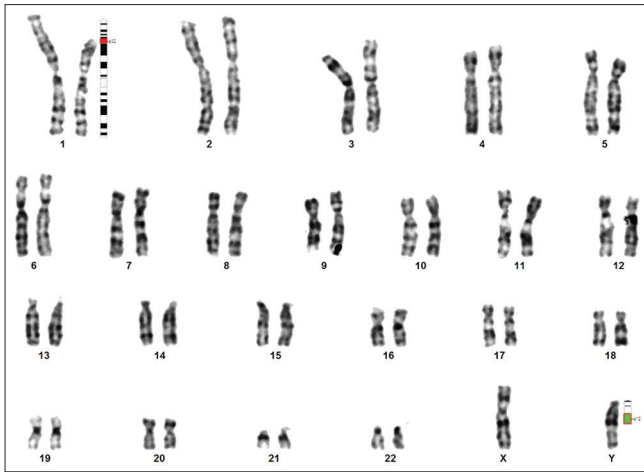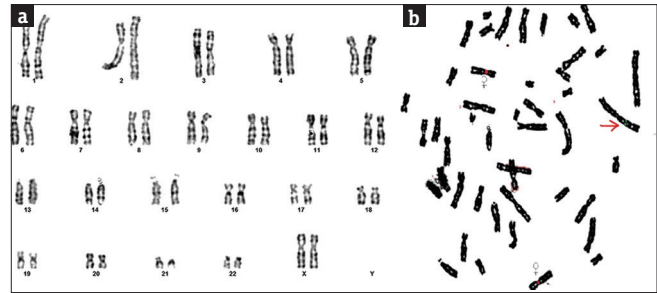
Figure 6: 46,X,t(Y;1)(q12;p32)

Figure 7: (a) De La Chapelle male syndrome (46, XX). (b) Inverted DAPI image showing two X chromosomes (♀) represented by centromeric probe (red signals). The green signal depicts SRY gene translocated to a large metacentric chromosome (red arrow)

among infertile males. It is also observed that infertile males with abnormal karyotype had elevated levels of gonadotropins and lower levels of testosterone and testicular volumes.

The technique of ICSI has revolutionized the management of male infertility and adopted by *in vitro* fertilization (IVF) clinics worldwide. However, since the beginning, there are concerns regarding its safety while utilizing sperms from infertile men, due to unbalanced chromosomal rearrangements. Hence, it has been recommended by various workers in the field of human reproduction that karyotyping should be performed in all infertile men, specifically with NOA, before they undergo testicular sperm retrieval.[14]

The prevalence of genetic abnormalities in male infertility varies from 4% to 13%, especially in men with defective sperm production,[15] among which the most common cytogenetic aberrations involve the gonosomes.[10,16] This can be seen in the form of numerical or structural abnormality, involving X or Y chromosomes. Another category of chromosomal defects in such individuals may be due to structural (balanced reciprocal translocations, inversions, insertions, or heteromorphisms) or uncommonly numerical abnormalities of autosomes (presence of a supernumerary marker chromosome).

In the present study, azoospermia (81%) was a more common presentation in men with primary infertility due to nonobstructive causes. The overall prevalence of somatic MCA was 16%, excluding PVs, which is in concurrence with another Indian study.[17] In azoospermic men, the prevalence of MCA was 14.8%, which was also similar to other European studies.[18,19] Usually, males with azoospermia (*n* = 81) have higher rate of MCA as compared to oligozoospermia, though in our

study frequency was higher in the latter group (21.6%) due to a smaller sample size (*n* = 19). Review of literature shows a variable frequency of MCA in infertile males from different cytogenetic studies, ranging from 5% to 27%, depending on various inclusion and exclusion criteria, semen characteristics, ethnic and racial factors, sample size, access for cytogenetic testing, and even consideration of PV under MCA.[19]

Gonosomal (sex chromosomes) abnormalities are frequent as a genetic cause in male infertility. It is noteworthy in our study that 11% of infertile men with either azoospermia or oligozoospermia with no relevant medical history had sex chromosomal abnormality, which was represented, either by numerical (*n* = 8) or structural changes (*n* = 3) in gonosomes [Table 2].

Out of all the MCA in male infertility, KS was the most common gonosomal numerical abnormality seen only in azoospermic males, accounting for 6.17% cases, which was also seen as a leading genetic cause of male infertility in other studies.[10] In the present study, 3.7% and 2.4% of azoospermic males were non-mos and mos KS. All males with KS were asymptomatic and were diagnosed only when they underwent infertility assessment; only one (189 cm) out of five males had tall stature. The mean FSH and LH were significantly higher in KS patients [Tables 2 and 5] as compared to infertile males with normal karyotype (*p* < 0.001) in contrast mean testosterone level was significantly reduced (*p* < 0.001), which was in concurrence with another larger study by Kim *et al*.[20] In KS males, there is theoretical risk of gonosomal aneuploidy in about 50% of sperms and thus surgical sperm retrieval is not successful in all individuals.[21]

Among other numerical sex chromosome abnormalities, in our cohort, we had 3% of cases of 47,XYY syndrome (less commonly known as Jacob's syndrome). All the patients were asymptomatic with normal behavior and phenotype. Although the prevalence of this syndrome causing azoospermia is

Table 2: Patterns of numerical and structural chromosomal abnormalities along with testicular volumes and follicular stimulating hormone level

Chromosomal abnormalities	Karyotype	Presentation	n (%)	FSH (mIU/ml)	Testes volume (ml)	Total
Numerical abnormalities	47,XXY	NOA	3	29.27±7.93	4.27±0.68 (right)	9
					5.53±1.10 (left)	
	mos 47,XXY/46,XX	NOA	1	19.3	5.6 (right)	
					6.5 (left)	
	mos 47,XXY/47,XY,+mar/46,XX	NOA	1	17.2	5.3 (right)	
					6.7 (left)	
	47,XYY	NOA	2	12.49±14.2	17.15±0.92 (right)	
					17.85±0.49 (left)	
	mos 47,XYY/46,XY	Oligoazoospermia	1	13.28	15.6 (right)	
					15.8 (left)	
	47,XY,+mar	Oligoazoospermia	1	9.89	16.5 (right)	
					16.8 (left)	
Structural abnormalities	46,XY,der(20)ins(22;20)(q11.2;q13)	Oligoazoospermia	1	9.3	16.8 (right)	7
					16.2 (left)	
	45,XY,rob(21;22)(q10;q10)	NOA	1	11.9	8.8 (right)	
					8.3 (left)	
	46,XY,t(15;17)(q11.2;q24)	NOA	1	22.15	15.8 (right)	
					16.5 (left)	
	46,X,t(Y:1)(q12;p32)	NOA	1	4.36	10.6 (right)	
					11.1 (left)	
	46,X,del(Y)(q11.2)	NOA	1	13.07	11.2 (right)	
					11.8 (left)	
	46,XY,t(1;9)(q21;q13)	Oligoazoospermia	1	10.9	17.5 (right)	
					16.8 (left)	
	46,XX	NOA	1	20.11	10.8 (right)	
					11.5 (left)	
PVs	46,XY,inv(9)(p11q13)	NOA	3	8.46±1.24	17.47±1.44 (right)	7
					17.77±1.3 (left)	
	46,XY,22pstk+	NOA	1	40.15	17.8 (right)	
					17.3 (left)	
	46,XY,16qh+	Oligoazoospermia and NOA	2	6.39±4.53	17.6±1.27 (right)	
					17.95±0.49 (left)	
	46,X,Yqh+	NOA	1	6.7	17.2 (right)	
					18.8 (left)	

FSH: Follicle-stimulating hormone, NOA: Nonobstructive azoospermia

Table 3: Age, hormonal profile, sperm count, and testicular volume in control group and infertile men with normal and abnormal karyotypes

Category	Control group (n=50)	Patients with normal KT (n=77)	Patients with abnormal KT (n=16)	t	df	p
Age (years)	33.3±2.23	32.73±3.85	32.69±3.56	0.035	91	0.972
FSH (mIU/ml)	4.9±1.3	7.06±3.44	15.18±10.27	3.73	91	<0.001
LH (mIU/ml)	4.5±1.1	5.15±2.37	9.33±4.7	4.1	91	<0.001
Testosterone (ng/ml)	5.4±2.1	4.94±1.51	3.44±1.66	3.87	91	<0.001
Sperm count (million/ml)	16.7±3.8	0.39±1.16	0.49±1.2	3.63	91	0.72
Testicular volume (ml), right	18.5±2.3	16.95±4.12	11.35±5.34	4.682	91	<0.001
Testicular volume (ml), left	17.6±1.9	16.98±3.81	11.94±5	4.559	91	<0.001

FSH=Follicle-stimulating hormone, LH=Luteinizing hormone

Table 4: Age, hormonal profile, sperm count, and testicular volume in infertile men with normal karyotype and polymorphic variants

Category	Patients with normal KT (*n*=77)	Patients with polymorphic variants (*n*=7)	*t*	df	*p*
Age (years)	32.73±3.85	34.85±2.2	1.43	82	0.155
FSH (mIU/ml)	7.06±3.44	12.14±12.55	2.7	82	0.008
LH (mIU/ml)	5.15±2.37	8.41±6.6	2.8	82	0.006
Testosterone (ng/ml)	4.94±1.5	4.82±1.53	0.212	82	0.832
Sperm count (million/ml)	0.39±1.16	0.32±0.9	0.139	82	0.890
Testicular volume (ml,) right	16.95±4.12	17.51±1	0.367	82	0.714
Testicular volume (ml), left	16.98±3.81	17.9±0.9	0.631	82	0.530

FSH=Follicle-stimulating hormone, LH=Luteinizing hormone

Table 5: Testicular volume in infertile men with a normal karyotype and Klinefelter syndrome

Category	Patients with normal KT (*n*=77)	Patients with Klinefelter syndrome (*n*=5)	*t*	df	*p*
Testicular volume (ml), right	16.95±4.12	5.4±2.06	6.191	80	<0.001
Testicular volume (ml), left	16.98±3.81	6.22±1.28	6.262	80	<0.001

variable in literature and males with XYY syndrome can be fertile, there are reports on this disorder with variable fertility issues either due to aneuploidy or hyperdiploidy during gametogenesis at different stages of maturation.[22]

There was a rare case (1%) of De La Chapelle male syndrome (46,XX) we found in our study. The individual was phenotypically male with short stature (164 cm), decreased testosterone (1.28 ng/ml), elevated gonadotropins levels, bilateral small testes, and azoospermia. Other authors have also reported frequency of 1.36% in males with XX chromosomal constitution having NOA.[23]

Structural abnormalities were more common in autosomes (*n* = 4) in our study. It has been well established that carriers of balanced translocations are phenotypically normal; however, due to various malsegregation patterns (adjacent 2:2 or 3:1 or rarely 4:0), there is formation of bivalents during gametogenesis which may alter the gene expression pattern and lead to spermatogenic arrest.[24] In the present study, balanced genetic abnormalities in autosomes were responsible for 2.46% and 10.52% of NOA and oligozoospermia, respectively [Table 1]. The prevalence rate of autosomal abnormality for NOA is in concurrence with another larger study[25] but higher than those published for males with oligozoospermia due to lesser sample size.[20]

We found that chromosome 1 was involved in two cases of balanced translocation; one with sex chromosome Y [Figure 6] which has resulted into azoospermia, second with chromosome 9 which had oligozoospermia. Rearrangements at multiple breakpoints involving chromosome 1 have also been linked in the literature to male infertility, especially with azoospermia.[26]

The present study had two cases of male infertility associated with de novo +mar. First case had dual abnormalities, mos KS with +mar in more than 10% cells (mos 47,XXY/47,XY,+mar/46,XX) who presented with azoospermia; and another case with oligozoospermia was having 47 modal chromosome number (47,XY,+mar) in all the metaphases. Both markers appeared to be derived from acrocentric chromosomes on GTG banding. Further molecular cytogenetic characterization could not be done in these cases due to nonavailability of extensive multicolor FISH panels in our center and unwillingness from patients due to cost factor. In the present study, +mar was seen in 5.26% of cases of oligozoospermia, which is slightly lower than the detection rate of +mar in healthy infertile males with oligozoospermia which is 7% in literature.[27] The exact mechanism which leads to semen abnormalities is not well understood, but it can be speculated that carriers of constitutional chromosomal defects have genetic imbalances in gametes and subsequently potential unviable offspring.

PV were seen in 7% of NOA, which was lesser in frequency as compared to other studies.[28,29] The most common variant in the present study was pericentric inversion of chromosome 9. Other variants were prominent constitutive heterochromatin in chromosome 16 and satellite-stalk region prominence in chromosome 22. These variants were not included in MCA in the present study, because the chromosomes with these variations, especially inv (9), behave normally during meiosis with 1;1 segregation pattern,[30,31] and it has been found that PV eventually do not influence the outcome in IVF-embryo transfer treatment.[32]

In this study, patients with both NOA and oligozoospermia groups with an abnormal karyotype

had elevated FSH and LH levels, whereas testosterone level was reduced. However in our cohort, infertile males having variants in chromosomal morphology did not show any significant correlation ($p > 0.05$) with testosterone level when compared with men having a normal karyotype.

Elevated serum levels of gonadotropins are reliable indicators of germinal epithelial cell damage and are usually associated with NOA. In our study, we also found higher levels of mean FSH and LH with reduced testosterone levels as compared to fertile healthy controls. The deranged hormonal profile in primary infertile males due to idiopathic nonobstructive etiology in the present study has also shown statistically significant correlation with MCA in comparison with infertile males with a normal karyotype ($p < 0.001$), and these data are in accordance with other studies.[33,34] In our study, we also found that infertile males with abnormal karyotype had smaller testicular volumes as compared to the control group ($p < 0.001$) [Tables 2 and 3]. Among the individuals with MCA and small testicular volumes, KS was the predominant genotypic abnormality in the present study, followed by structural abnormalities in chromosome Y ($n = 2$), Robertsonian translocation ($n = 1$), and De La Chapelle male syndrome ($n = 1$) [Tables 2 and 5]. This phenotypic association with genetic abnormality was also found in different studies by Kim *et al.* and Koşar *et al.*[20,33] The PV also had significant correlation with gonadotropin levels ($p < 0.05$) in comparison with fertile controls; however, the differences between testosterone level and testicular volumes between the two groups were not significant ($p > 0.05$). In the present study, deranged hormonal profile together with small-sized testicles and MCA is characteristically seen in men with NOA.

Limitations of the study
The present study should be considered in light of certain limitations. First, being a single-center study, it comprised small number of patients that might be insufficient to represent the entire population of infertile males from different ethnicities. Second, Y chromosome microdeletion could not be performed in the entire cohort; hence, relevant data could not be reproduced. Nevertheless, the study highlights association between hormonal levels and varied spectrum of major chromosomal abnormalities in infertile males compared with their healthy counterparts.

CONCLUSION
The present case–control study revealed a significant association between CAs in infertile males with nonobstructive semen abnormalities. Serum

gonadotropin levels with chromosomal abnormalities were significantly higher than infertile males with normal karyotype and healthy fertile control males, whereas serum testosterone level and testicular volume were lower in patients with MCA in comparison with the fertile control group.

Therefore, we recommend that the treating physician should consider cytogenetic workup in nonobstructive semen abnormality with deranged hormonal profile. Screening of underlying genetic defects in this era of artificial reproduction will assist not only in the prevention of vertical transmission of genetic defects to offspring but also in prognostication of therapeutic outcomes and eventually counseling of couples for a successful outcome.

Ethical approval
Institutional ethical committee approval and informed consent was obtained from the patients for publication of this article.

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Availability of data and materials
The data generated during the current study are available from the corresponding author on reasonable request.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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