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Cytogenetic analysis and endocrine profile in patients with nonobstructive azoospermia or severe oligozoospermia

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Abstract
Objective: To study the prevalence of chromosomal anomalies in infertile males with severe oligozoospermia or non obstructive azospermia and its correlation with clinical and endocrine profile.
Patients and methods: Consecutive 30 male subjects (mean age 35.5 ± 7.1 years) with primary infertility attending at the infertility clinic, Urology department, Suez Canal University Hospital, Egypt were enrolled in the study. These patients had severe oligozoospermia (n = 9) or non obstructive azospermia (n = 21). Clinically testicular volume, scrotal Doppler ultrasound examination and endocrine evaluation (serum FSH, testosterone and prolactin) were determined. Cytogenetic analysis was performed by using the GTG (G-banded using trypsin and Giemsa) banding technique.
Results: Nine patients (30%) had chromosomal abnormality. Patients with Klinefelter Syndrome and de la Chapelle male syndrome represented 26.7% (n = 8) and 3.3% (n = 1) respectively. All patients diagnosed as Klinefelter group were azoospermic, while 57.1% of normal karyotyping were azoospermic and 42.9% were severe oligozoospermic ($p = 0.029$). Klinefelter group had significantly lower mean testosterone level than normal karyotyping group ($p = 0.016$). Also, Klinefelter group had significantly higher mean FSH and LH levels than normal karyotyping group ($p < 0.01$). The anomaly detected was 47, XXY chromosomal constitution, found in 8 (38%) out of 21 patients with non-obstructive azoospermia.
Conclusion: There is a high prevalence of chromosomal abnormalities in infertile males with non obstructive azoospermia. All patients with azoospermia and severe oligozoospermia (sperm count <5 million/ml) should undergo genetic screening. Our study indicates that even those presenting to infertility clinics can be heterogeneous in terms of karyotype and phenotype.

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Introduction

Infertility represents a considerable health issue affecting up to 17% of couples. Male factors contribute to 30–50% of the problem [1]. Chromosomal abnormalities are more frequent in infertile males compared to general male population [2]. These abnormalities have been reported in a higher frequency in males with severe oligozoospermia and non obstructive azoospermia than moderate or mild oligozoospermia [3].

Variable numerical and/or structural abnormalities of sex chromosomes were encountered in infertile males [4].

Klinefelter syndrome (47, XXY, mosaic) represents the most common karyotypic abnormality in men with oligozoospermia or azoospermia and can be identified clinically by characteristic body proportions, gynecomastia, firm and small testis and associated endocrine and nonendocrine disease [5].

The chromosomal anomalies, and its prevalence and impact on infertility in the Egyptian population is not yet well studied. The present work was therefore an endeavor to verify the prevalence and patterns of chromosomal anomalies in these patients in our province at Suez Canal region.

We performed cytogenetic analysis in case of male infertility having severe oligozoospermia or nonobstructive azoospermia. The karyotype findings were correlated with physical and endocrine profile.

Patients and methods

This study was conducted as a descriptive cross-sectional study including 30 consecutive males with primary infertility during 12 month period (March 2012–March 2013). The mean age of patients was 35.5 ± 7.1 years with a range of 19–58 years.

Males with primary infertility (n = 30) and having non-obstructive azoospermia (n = 21) or severe oligozoospermia (sperm counts ≤ 5 millions/ml).; n = 9) were evaluated at Urology Department, Suez Canal University Hospital. An informed written consent was obtained from every patient participating in the study. The study design was approved by the Ethics Committee of the Faculty of Medicine, Suez Canal University, Ismailia, Egypt.

Patients were subjected to detailed structured history taking, physical examination, testicular volume measurement and digital rectal examination.

Every patient provided two semen specimens, each after 4 days of sexual abstinence. On the basis of their mean sperm concentrations, the patients were categorized as having azoospermia or severe oligozoospermia (≤5 million sperms/ml).

For all patients, blood samples were obtained in early morning for the measurement of serum testosterone, FSH and LH by radioimmunoassay.

Peripheral blood samples were collected from all patients into heparinised test tubes. Cytogenetic analysis was performed using the GTG (G-banded using trypsin and Giemsa) banding technique [6].

The karyotypes were described according to the ISCN (International System for Human Cytogenetic Nomenclature). Scrotal Doppler ultrasound was performed to evaluate the testicular size, vascularity and the evidence of varicocele. Testicular volume was calculated using Hansen formula for a prolate spheroid: length (L) × width (W) × height (H) × 0.52. Normal testicular volume is 12–30 ml [7].

Statistical analysis

Collected data were coded, entered and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 16.0) software for analysis. Baseline characteristics of the study population were presented as frequencies and percentages (%) in qualitative data or mean values and standard deviations (SD) in quantitative data. Differences between frequencies and means were compared by Chi-square and paired samples *t*-tests, respectively. A p value of <0.05 was considered significant.

Results

There was no significant difference between both groups of normal and abnormal karyotyping regarding age distribution (p > 0.05). The mean age of Klinefelter group was slightly higher than normal group, but without significant difference (p > 0.05) (Table 1).

Azoospermia was reported in 21 patients (70%), while severe oligozoospermia was reported in 9 patients (30%). The mean sperm count of the patients with severe oligozoospermia was 1.98 ± 1.85 millions/ml with a range of 0.3–5 million/ml.

Nine out of thirty studied patients (30%) had chromosomal abnormality. Patients with Klinefelter Syndrome (n = 8; 26.7%) and one patient (3.3%) with de la Chapelle male Syndrome.

Table 1 Age distribution in normal karyotyping (n = 21) and Klinefelter (n = 8) groups.

Age (years)	Normal karyotyping (n = 21)		Klinefelter (n = 8)		Used test	p-value
	No.	%	No.	%		
19–<30	2	9.6	0	0.0	$X^2 = 1.7$	0.44
30–<40	15	71.4	5	62.5		
40–58	4	19.0	3	37.5		
Total	21	100.0	8	100.0		
Mean ± SD	34.3 ± 5.8		39.38 ± 9.1		t = 1.8	0.08
Range	19–44		31–58			

Table 2 Hormonal and clinical examination in infertile men with normal or abnormal karyotyping.

Category	Patients with normal karyotyping n = 21 *p*-value	patients with abnormal karyotyping n = 9	p-value
FSH (mIU/ml)	14.2 ± 4.0 (2.7–64.4)	36.9 ± 16.5 (4.3–60.4)	0.044*
LH (mIU/ml)	9.04 ± 6.7 (7.2–19.5)	19.2 ± 7.6 (3.0–28.7)	0.048*
Testosterone (ng/dL)	4.2 ± 1.6 (0.5–6.6)	2.7 ± 0.6 (2.1–3.9)	0.016*
Testicular volume in ml	15.8 ± 4.1 (7.2–19.5)	5.96 ± 2.11 (2.8–9.3)	0.0003*
Varicocele n (%)	10 (47.6)	3 (33.3)	0.33

* Stastically significant difference if $p < 0.05$

All patients diagnosed as Klinefelter group were azoospermic having numerical chromosomal abnormalities; 47, XXY karyotype.

All patients of Klinefelter group (100%) had small testicular volume, while only 23.8% of normal karyotyping had small testicular volume. The mean testicular volume of Klinefelter group was significantly smaller than normal group (5.96 ml versus 15.8 ml, respectively) ($p = 0.0003$). There was no significant difference between both groups regarding varicocele frequency ($p > 0.05$) (Table 2).

Noteworthy, 57.1% of normal karyotyping were azoospermic and 42.9% were severe oligozoospermic.

Hormonal assay of the studied population (n = 30). The mean testosterone, FSH, and LH was (3.8 ± 1.5 IU), (20.8 ± 17.5 IU), and (12.1 ± 8.3 IU), respectively.

Infertile men with Klinefelter syndrome had significantly lower mean testosterone level than normal karyotyping group ($p = 0.016$). Also, Klinefelter group had significantly higher mean FSH and LH levels than normal karyotyping group ($p < 0.05$) (Table 2).

Discussion

Chromosomal anomalies are higher in infertile men. Studies from the literature showed 6.54% chromosomal anomaly rate. These cytogenetic studies have reported variable frequency (5–27%) of major chromosomal anomalies in male infertility, depending on the criteria of patients [8]. In our study, 30% of all cases revealed chromosomal alteration. Low rates of chromosomal abnormality ranged from 1.9% to 4% among European countries were reported [9]. Relatively higher rates of chromosomal anomalies were reported in other countries. In India, Nagvenkar et al., in their prospective study investigated 88 infertile men (42 men with azoospermia and 46 men with sperm count <5 million/ml). Constitutional chromosome abnormalities were identified in 14.3% of azoospermic and 6.5% of oligozoospermic men [10]. In a study on 179 men with infertility in Turkey(representing European and Asian geography), 21 (11.49%) cases had chromosomal abnormalities, including 13 (7.2%) cases with Klinefelter's syndrome [11]. In another study on 102 infertile men (41 males with azoospermia and 61 males with oligozoospermia) Gunduz et al. from Turkey, reported constitutional chromosomal abnormality in 14 (34.1%) azoospermic patients and in 2 (3.3%) oligozoospermic patients. The 47, XXY karyotype was the commonest in the azoospermic group [12]. Our results are in accordance with these findings, demonstrating high figures of chromosomal abnormalities, compared to other studies from different countries.

Lissitsina et al. reported that chromosomal abnormalities were higher in patients with azoospermia than those with oligozoospermia [13]. In our patients, chromosomal abnormalities were detected in 42.5% of 21 patients with azoospermia and 0% of 9 oligozoospermic cases.

Klinefelter's syndrome,represented the majority of karyotype abnormality in infertile cases, characterized by primary testicular failure with reduced testicular volume and elevated gonadotropin plasma levels. In our study, 8 out of thirty cases had nonmosaic 47, XXY karyotype. Klinefelter's syndrome (either pure or mosaic form) was found in just 12 patients (0.6%) out of 1792 infertile Dutch men [14]. This low rate may be due to fair and easy access of patients to clinical genetics laboratories. Ethnic and racial factors may play role in that significant difference in occurrence of chromosomal abnormalities among different countries.

It has been assumed that more than 90% of nonmosaics 47, XXY males are azoospermic [15]. However, our study reported 100% of the nonmosaic 47, XXY karyotype to be azoospermic.

The frequency of patients with de la Chapelle male Syndrome (46 xx) in our study was (n = 1; 3.3%). He had azoospermia, low testosterone and high LH and FSH.

This disorder shows a prevalence of 1:9000 to 1:20,000. Applying molecular methods, it has been demonstrated that about, 3 of 46 XX-males have Y chromosomal material translocated onto the tip of one X chromosome [16].

In general, 46, XX males are significantly shorter than Klinefelter patients or healthy men, resembling female controls in height and weight. XX-males seem to have normal intelligence, ejaculate analysis reveals azoospermia [17]. The 46, XX males, with reduced testosterone production, have to receive appropriate testosterone replacement therapy.

The mean value of testicular volume in patients with chromosomal abnormality in our study was 5.96 ± 2.11 ml. Testosterone level 2.7 ± 0.6, FSH level 36.9 ± 16.5 and LH = 19.2 ± 7.6 IU/ml. All of them were in accordance with published literature [18–20].

Our results reflect a regional pattern of our patients in Ismailia province. It is the first study performed to evaluate chromosomal, hormonal patterns of patients with severe oligozoospermia or azospermia in Suez Canal region. Further multicenter studies among Egyptian males focusing on that issue may be suggested to clarify this obvious higher incidence of chromosomal abnormality among infertile men with non obstructive azoospermia.

Conclusion

There is a high prevalence of chromosomal abnormalities in infertile males with non obstructive azoospermia. All patients with azoospermia and severe oligozoospermia (sperm count <5 million/ml) should undergo genetic screening. Our study indicates that even those presenting to infertility clinics can be heterogeneous in terms of karyotype and phenotype. Early detection of these abnormalities is essential as these patients require in addition to the genetic counselling and treatment of infertility, androgen replacement therapy and identification of associated health problems.

Conflict of interest

None declared.

Authors contributions

Mohamed Hassan Ali (Ali MH), MD, PhD first and corresponding author, creating idea of the research, revise the study design and interpret collected data, preparing the manuscript, reviewing and supervising the research article.

Mohamed Soliman (Soliman M), MD, MSc; evaluation of patients, Data collection, preparation of manuscript.

Adel Hussein Metwally (Metwally AH), MD, PhD;Senior Author,reviewing and supervising the research article.

Ammar Ghobeish (Ghobeish A), create the idea of the research, formulate the design of the study, Senior Supervisor of the research team.

Ethical committee approval

An informed written consent obtained from every patient participating in the study. The study design was approved by the Ethics Committee of the Faculty of Medicine, Suez Canal University, Ismalia, Egypt.

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