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Male Infertility and Cytogenetic Disorders: A Cross-Sectional Study

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Infertility is a very common health problem. In this study, the relationship between cytogenetic disorders, semen parameters and reproductive hormone levels of infertile men from Western region of Algeria, Tlemcen, was evaluated. A total of 27 infertile males were studied for the cytogenetic evaluation. Also, 12 fertile males as a control group were studied. Karyotyping was performed on peripheral blood lymphocytes according to the standard methods. Semen analysis and endocrine evaluation were carried out. The prevalence of abnormal chromosomal karyotype in the patients with abnormal sperm parameters was 11.11%. The most frequent cause was Klinefelter's syndrome (03/27). The levels of testosterone (T) in patient group were significantly lower than those of fertile group ($p<0.0001$). In addition, the levels of Follicle-Stimulating Hormone (FSH) ($p<0.0001$), and Luteinizing Hormone (LH) ($p<0.0001$) were significantly higher in patients than those in the fertile group. The same results of hormone levels were found when compared the patients with normal and abnormal karyotype. Cytogenetic disorders have severe adverse of sperm concentration and reproductive hormone levels. These findings suggest the need for routine genetic testing and counseling prior to the employment of assisted reproduction techniques.

Key words: Male infertility, chromosomal abnormalities, sperm concentration, reproductive hormone levels, karyotyping

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INTRODUCTION

Infertility is a very common health problem, affecting approximately 15-20% of couples trying to conceive (Dohle *et al*., 2012). In the past years, there was a belief that infertility is a woman's problem but in fact, male factors contribute to about half of infertility cases (De Kretser and Baker, 1999; Akgul *et al*., 2009; Elfateh *et al*., 2014). Idiopathic male infertility accounts for more than 30% of all male infertility cases (Dohle *et al*., 2012). A large majority of these cases have associated genetic disorders that range from chromosomal aneuploidy or structural rearrangements to mutations or microdeletions (Amol *et al*., 2013). Several studies have shown increased chromosomal aberrations in 5-7% of patients with oligospermia and 10-15% in patients with azoospermia (Akgul *et al*., 2009; Ravel *et al*., 2006). Chromosomal aberrations are mainly represented by sex chromosomal defects, which are twice as high in infertile men compared with controls (Katagiri *et al*., 2002). At least 5% of azoospermic males have been found to have Klinefelter's syndrome with 47, XXY aneuploidy (Amol *et al*., 2013).

These cytogenetic disorders affect the semen quality and cause various degree of male infertility. The atrophy of the testis and the decreasing of the sperm count observed in Klinefelter's syndrome patients may attributed to atresia of the germ cells containing two X chromosome, which theoretically results from fatal gene dosage caused by the extra X chromosome (Burgoyne, 1978; Elfateh *et al*., 2014).

The aim of this study was to evaluate the relationship between cytogenetic disorders, semen quality and reproductive hormone levels of infertile men from Western region of Algeria, Tlemcen.

MATERIALS AND METHODS

This is a cross-sectional study performed during the period from September, 2013-December, 2014.

Patients: In this study 27 infertile men whose infertility was related to testicular problems were involved. All subjects were evaluated clinically at the Department of Urology of University hospital, Tlemcen, Algeria. The mean age was 34.6±5.68 and cases were between 27 and 48 years old.

All of patients underwent an andrological workup, which included medical history, physical examination, hormonal estimation and semen analysis according to World Health Organization recommendations and standards (WHO., 1999). The purpose of the study was explained to the patients and informed consent forms were signed by them. The institutional ethics committee approval was obtained before beginning the study.

Control subjects: Twelve normal fertile men included as control subjects. Every man in the control group had fathered at least one child.

Hormone analysis: The levels of the reproductive hormone Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH) and Testosterone (T) were measured by electrochemiluminescence immunoassays (ECLIA) using the Elecsys 2010 chemistry analyzer (Roche, Germany), based on the manufacturer's instructions. The normal reference ranges for LH, FSH and testosterone hormone were 1.7-8.6 (mU mL^{-1}), 1.5-12.4 (mU mL^{-1}) and 2.80-11.5 (ng mL^{-1}), respectively.

Semen analysis: Semen samples were obtained from the male cases undergoing standardized investigation for the diagnosis of the cause of infertility. The samples were collected by masturbation following an abstinence period of 3-5 days. The semen were then allowed to liquefy at 37°C and processed immediately thereafter using the WHO recommended guideline (WHO., 1999). Semen volume was estimated by weighing the collection tube with the semen sample and subtracting the weight of the empty preweighed tube, assuming that 1 mL semen = 1 g.

For sperm motility assessment, 10 µL of well-mixed semen was placed on a clean glass slide kept at 37°C and covered with a 22×22 mm coverslip. The preparation was placed on the heated stage of a microscope at 37°C and immediately examined at 400× magnification. The sperm were classified as progression motile (WHO class AB motility), locally motile (WHO class C motility) or immotile (WHO class D motility).

For the assessment of the sperm concentration, the samples were diluted in distilled water. The sperm concentration was subsequently assessed using a Bürker-Türk haemocytometer (Paul Marienfeld GmbH and Go.KG, Lauda-Königshofen, Germany). Based on sperm count the cases were classified into different groups. Azoospermia is defined as the total absence of sperm cells and oligozoospermia is the condition which occurs when the sperm cell count is less than 20×10^6 cells mL^{-1}.

From each semen sample, a smear for morphology evaluation was made according to David classification of normal and abnormal human spermatozoa (David *et al*., 1975).

Karyotyping analysis: Chromosome investigations were performed on cultures of peripheral blood lymphocytes using standard techniques (Ferguson-Smith, 1974). Lymphocytes were cultured for 72h in RPMI-1640 medium with phytohemagglutinin (PHA) at 37°C and colcemid was added before harvest. The cultured lymphocytes were treated with hypotonic solution (0.075 M potassium chloride) and then fixed in Carnoy's fixative (methanol:acetic acid = 3:1 v/v). Chromosomal analysis was performed with RTG banding. At least 25 metaphases were analyzed for each patient and up 100 metaphases were analyzed in case of mosaicism. The karyotypes were interpreted using the recommendation of International System for Human Cytogenetic Nomenclature (Shaffer and Tommerup, 2005).

Statistical analysis: Statistical analysis was performed with SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). The categorical variables were presented by numbers and frequencies while the continuous variables were presented as Mean±standard deviation (SD). Student's T test was used to test the significance of differences between mean values of two quantitative variables. The p-value <0.05 was considered to be statistically significant.

RESULTS

The comparison of age, semen parameters and hormonal levels between patient and control groups was shown in Table 1. The results showed no significant difference of age between the two groups (p = 0.67). Mean sperm count was 12.27±8.46 (million mL^{-1}) in patient group, with immotile form (Mean 54.47±35.87%) and normal form (Mean 26.03±26.98%). The difference was significant when compared this group with the control group (p<0.0001). Also, there was a significant difference in serum levels of FSH, LH and T when compared with the patients and the control group (p<0.0001).

Chromosomal frequency finding among fertile and infertile men was summarized in Table 2. Among the 27 infertile men studied, 03 showed chromosomal abnormality corresponding to a frequency of 11.11%. All of chromosomal abnormalities in patient group were found to be gonosomal. Two patients were diagnosed as a non mosaic form Klinefelter's syndrome (47.XXY) and one patient was diagnosed as a mosaic form Klinefelter's syndrome (47.XXY/46.XY).

Comparing the serum levels of FSH, LH and T in patients with normal and abnormal karyotype, the results showed that, the hormonal levels of FSH and LH in patients with abnormal karyotype were significantly higher than those in the patients with normal karyotype (for FSH levels, p = 0.003 and for LH levels, p = 0.04) (Fig. 1 and 2). On the other hand, the levels of T were significantly lower than those in the patients with normal karyotype (p<0.0001) (Fig. 3).

DISCUSSION

Infertility is a couple's problem. Male factors have been estimated to contribute to infertility in 25-50% of infertile couples (Begum and Ehsan, 2013). Male infertility is not one disorder but is a syndrome that results from many congenital and acquired illnesses.

Cytogenetic abnormalities have to known to cause male infertility but the exact mechanism by which chromosomal anomalies induces infertility is still not clear. Many studies have documented the chromosomal abnormalities which range from 2.2-15.7% for infertile men (Azimi *et al*., 2012). In our

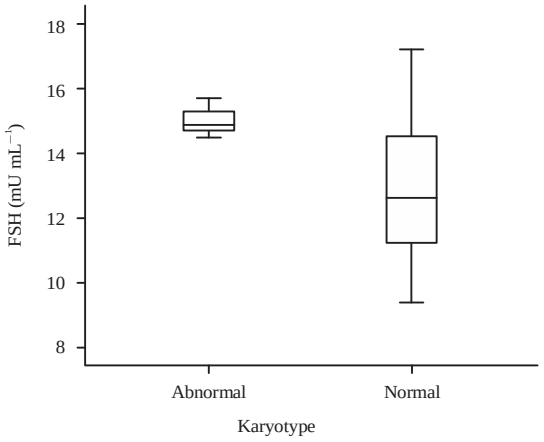

Fig. 1: Serum levels of FSH in patients with normal and abnormal karyotype (p = 0.003). Box plot indicates median and interquartile range, FSH: Follicle stimulating hormone

Table 1: Comparison of age, semen parameters and hormone levels between patient and control group

			Motility* (%)			Morphology (%)				
	Age (year)	Sperm count (million mL^{-1})	AB	C	D	Normal	Abnormal	FSH (mU mL^{-1})	LH (mU mL^{-1})	T (ng mL^{-1})
Patient group										
Mean±SD	34.66±5.68	12.27±8.46	26.27±29.81	8.13±4.90	54.47±35.87	26.03±26.98	62.85±33.95	13.14±2.19	9.10±1.88	3.17±1.73
Control group										
Mean±SD	33.83±5.40	95.83±20.28	71.25±8.29	15.83±5.96	12.08±6.20	78.75±7.91	21.25±7.91	5.27±1.22	4.69±0.79	6.14±1.30
p-value	0.67	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

*Sperm were classified as progression motile (WHO class AB motility), locally motile (WHO class C motility) or immotile (WHO class D motility), FSH: Follicle stimulating hormone, LH: Luteinizing hormone, T: Testosterone

Table 2: Chromosomal frequency finding among patient and control group

	No.	Frequency (%)
Patient group (N = 27)		
46.XY	24	88.88
47.XXY	02	07.40
47.XXY/46.XY	01	03.70
Control group (N = 12)		
46.XY	12	100.00

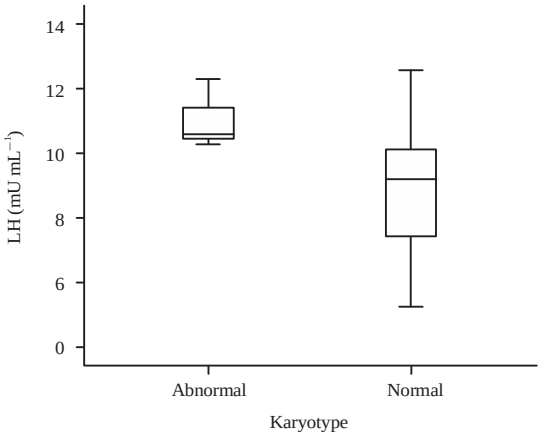

Fig. 2: Serum levels of LH in patients with normal and abnormal karyotype (p = 0.04). Box plot indicates median and interquartile range, LH: Luteinizing hormone

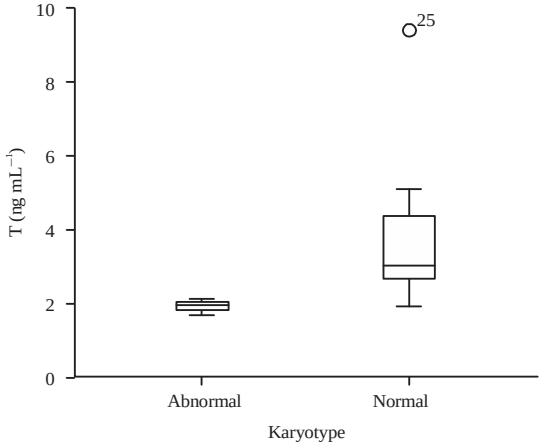

Fig. 3: Serum levels of T in patients with normal and abnormal karyotype (p = 0.0001). Box plot indicates median and interquartile range. Case number 25 represent outlier, T: Testosterone

study we observed that 11.11% of the infertile males are associated with chromosomal abnormality.

The most common type of karyotype abnormality in infertile case is represented by Klinefelter's syndrome and Y chromosome long arm micro-deletions represent the most frequent chromosomal structural abnormalities (Ravel *et al*., 2011; Ghorbel *et al*., 2012; Maiburg *et al*., 2012). In this study, all patients with abnormal karyotype presented true Klinefelter syndrome. This abnormality is a form of primary testicular failure with testicular hypotrophy and elevated gonadotropin plasma levels and it presents the most common form of male hypogonadism (Lee *et al*., 2007).

The occurrence of karyotypic abnormalities among infertile men depends on a number of factors. The most important is the criterion for selection of patients based on the sperm count. It is well-known that the sperm count is inversely related to the existence of chromosomal anomaly (Kosar *et al*., 2010). Our results retrospective analysis of cytogenetic results of 27 infertile patients diagnosed with semen parameters defects revealed Klinefelter syndrome in three patients. As mentioned in the literature, chromosomal abnormality incidence was higher in the azoospermic group (22.2%) than in the oligozoospermic group (13.6%), this confirms that the incidence of chromosomal abnormality increases as sperm count decreases (Ceylan *et al*., 2009). Sreenivasa *et al*. (2013) reported that the prevalence of Klinefelter syndrome among infertile men is very high, up to 5.2% in sever oligozoospermia and 14.5% in azoospermia (Sreenivasa *et al*., 2013). It has always been assumed that more than 90% of mon-mosaic 47.XXY males are azoospermic (Kosar *et al*., 2010). Indeed, in Ferlin *et al.* (2005) report, 74.4% of mosaic 47.XXY/46.XY patients were azoospermic, whereas the remaining had severe oligospermia (Ferlin *et al*., 2005).

Moreover the semen status of infertile men in our study was characterized by reduce of sperm concentration and presence of immotile and abnormal form of spermatozoa. These findings are consistent with those of previous reports (Saidi *et al*., 2008; Aleisa, 2013). Influence of chromosomal disorders on sperm motility and morphology has not been clearly clarified in most of the previous studies (Matsuda *et al*., 1991; Yoshida *et al*., 1997). In 2011 a study by Brahem *et al*. (2011) reported that, for all teratozoospermic patients a normal karyotype and an absence of Y chromosome microdeletion was overt (Brahem *et al*., 2011).

The relationship between age and semen parameters has been reported by many previous studies (Sartorius and Nieschlag, 2010; Nie *et al*., 2012). They concluded that male age had a negative correlation with sperm motility, that increasing in male age strongly correlates with decreasing of both normal sperm motility and sperm morphology. In our results the patients were older than the control group but no significant difference has been noted. Also the semen parameters were significantly lower than those of fertile group ($p<0.0001$). This would support the results and the final conclusion of the result would be efficient.

Furthermore, our study noted that infertile men had the higher FSH and LH levels and lower testosterone levels. These results are in agreement with those of previous studies (Kosar *et al*., 2010; Amol *et al*., 2013). Serum FSH and LH levels may have prognostic implications on testicular function but it is not known whether these parameters have any prognostic implications on cytogenetic abnormality of infertile patient. However, our results clarified this relationship in all studied genetic abnormalities carriers, where genetic abnormalities is reflected in, the levels of testosterone that were significantly lower than they that should be. Influence of genetic abnormalities on FSH and LH varies according to the type of abnormalities (Elfateh *et al*., 2014). The levels were significantly elevated than those of fertile group.

The present study had several limitations, including a relatively small sample size and a small number of hormonal parameters. However, the major strengths of the study include its population-based design (as opposed to most other case-control studies) and the standardized protocol with quality control measures.

CONCLUSION

Chromosomal analysis is an important investigation in male subjects with infertility. The data showed that the chromosomes disorders have severe adverse influence on sperm concentration and reproductive hormone levels. These findings strongly suggest that such patients should at least be karyotyped and receive counseling before they are referred for assisted reproduction techniques. Such investigation is a pre-requisite to minimize the risk of transmitting genetic abnormalities to future generations, such as intellectual disability, genital ambiguity and/or birth defects.

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