

# Chromosomal and Hormonal Abnormalities in Azoospermic Patients in a Military Jordanian Cohort

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## ABSTRACT

**Objective:** To report the prevalence of chromosomal abnormalities and hormonal profile in azoospermic males referred to endocrine clinic from IVF center and urology clinic at King Hussein Medical Center.

**Methods:** Out of 1100 infertile males; thirty patients with azoospermia were subjected for chromosomal studies.

**Results:** Chromosomal abnormalities were found in 7/30 patients that constitute 23% of azoospermic group and 0.6% of the total group and almost 86% of them were Kline felter's syndrome. Hormonal profile showed a significantly higher FSH, LH and lower serum testosterone in those with chromosomal alterations versus those with azoospermia without chromosomal abnormalities.

**Conclusion:** The prevalence of chromosomal abnormalities is high in azoospermic patients and warrant routine chromosomal investigations.

**Key words:** Azoospermia, Chromosomal, Infertility.

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## Introduction

Azoospermia is the absence of sperms in the ejaculate with prevalence of approximately 1% of all men and in 10 to 15% of infertile males.<sup>(1)</sup>

The process of spermatogenesis requires an integrated interaction between gonadotrophic hormones Follicular Stimulating Hormone (FSH) and Luteinizing Hormone (LH). Plasma testosterone hormone and germ cells in addition to various somatic cells. Defects in spermatogenesis can result from impaired secretion or action of LH, FSH and testosterone, or from intrinsic defects in spermatogenesis inside the testes.<sup>(2)</sup>

Infertility due to male factors represents around 30-40% of all cases of infertility.<sup>(3)</sup>

Failed spermatogenesis makes up approximately 30% of male infertility cases.

<sup>(4,5)</sup>Genetic alterations, chromosome abnormalities, and Y chromosome microdeletions constitute the most common causes of male infertility.<sup>(4,5)</sup> Chromosomal defects are present in 5-7% in men with sperm counts  $< 10 \times 10^6/\text{ml}$ , increasing to 10 – 15% in those with azoospermia.<sup>(6)</sup>

Genetics contribute to infertility by affecting various physiological processes that include hormonal homeostasis, spermatogenesis, and sperm quality.<sup>(7)</sup>

The most common chromosomal abnormality is Klinefelter's syndrome<sup>(8)</sup> which is a genetic disorder involving germs cells from an early life<sup>(9,10)</sup> and Sertoli's and Leydig's cells from mid-puberty,<sup>(11)</sup> with an estimated prevalence of 1 in 600 males.<sup>(12)</sup>

The extrapolated statistics for Klinefelter's syndrome in Jordan is 11,222 for a population of 5.6 million in 2004. We extrapolate the

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world prevalence of 1:600 to our Jordanian male population to give an estimated number of patients affected by the disease. If we know that the male population is around half of the total population in Jordan, the estimated number would be 4667. Such estimate does not reflect the actual prevalence of Klinefelter's syndrome in any region.<sup>(13)</sup>

Twenty five percent (25%) of Klinefelter's syndrome cases are diagnosed post pubertal with rare cases identified before onset of puberty.<sup>(14)</sup> The major clinical stigmata of Klinefelter syndrome, 47, XXY (KS) and its mosaic pattern include progressive testicular failure inducing small firm testes, androgen deficiency, gynecomastia and azospermia.<sup>(15)</sup> Chromosomal abnormalities could be numerical like Klinefelter's syndrome with 47 XXY or 47 XYY and structural like translocations or microdeletion in the Azoospermia factor (AZF) region of the Y chromosome.<sup>(16)</sup>

The aim of this study is to determine the frequency of chromosomal abnormalities and hormonal profile in a subgroup of infertile military men with azospermia attending the infertility clinic at King Hussein Medical Center.

## Methods

Our study was conducted at male infertility clinic-King Hussein Medical Center in the period between January 2009 till June 2010. One thousand and one hundred (1100) patients have attended the clinic who had been married for at least one year of unprotected sexual intercourse. Thorough history was taken and physical examination was performed. Only subjects with non obstructive azospermia were enrolled in this study (n=30) and their identifications were based on clinical examination, hormonal investigations and sonographic imaging of testicles. There was no history of any drug ingestion for >6 months, negative history of alcohol consumption and no surgical procedures involving the reproductive system. At least two semen fluid analyses were done to confirm the presence of azospermia. Laboratory data including FSH, LH, total Testosterone, Prolactin levels, scrotal ultrasound and semen analysis were analyzed.

The protocol of analysis of seminal fluid is based on World Health Organization (WHO) 2010 guidelines. It includes at least two samples of semen obtained at least two weeks apart.<sup>(17,18)</sup> Centrifugation of the semen sample for 15 minutes at room temperature with speed of at least 3,000 g is required to confirm the diagnosis of azospermia.<sup>(17)</sup>

Chromosomal analyses of peripheral blood lymphocytes (PBL) were executed in all patients with azospermia by G-banding. Normally, 30 metaphase spreads were assessed, but when a marker chromosome was observed, an additional repeat chromosomal analysis was performed. Fluorescence in-situ hybridization (FISH) was then performed on metaphase chromosomes to affirm the origin of the marker chromosome and excess chromosomes. FISH was carried out according to manufacturer's recommendations using commercially available kits. At least 100 cells were examined to evaluate the number of sex chromosomes. X and Y

Chromosomes were identified by green and orange fluorescence respectively. Chromosomal study included both structural as well as numerical abnormalities.

This group of azospermic patients was further subdivided into two groups of those with chromosomal abnormalities (AZ+) versus those who didn't harbor chromosomal abnormalities (AZ-) and were compared among them regarding age and hormonal profiles.

### **Statistics:**

The mean  $\pm$ SD were calculated using descriptive statistics, standard deviation and intergroup comparison using T-student test. P-value < 0.05 is considered as significant.

## Results

The mean age ( $\pm$ SD) is  $34.1 \pm (3.8)$  years. The mean age of azospermic patients with abnormal karyotyping(AZ+) is 32.4 year  $\pm$ 4.2 versus 34.6 year  $\pm$ 3.6 for those with azospermia but normal karyotype (AZ-) (P-value =0.09). Chromosomal abnormalities were found in 7 out of 30 azospermic patients; six subjects with classical chromosomal abnormalities of 47XXY confirming Klinefelter's syndrome giving a

prevalence of 20% and one case of structural chromosomal abnormality in the form of 46XY,inv(9)P giving a prevalence of 3.3% ; this constitutes a total prevalence of 23.3% of azoospermic group. Normal chromosomal studies were detected in 77% of azoospermic subjects (see Table I).

Hormonal profile showed a significantly higher serum LH and FSH in the (AZ +) group versus (AZ-) group. Serum testosterone level was also significantly lower in the (AZ+) subgroup. Serum Prolactin level was discovered to be higher in (AZ+) subgroup versus (AZ-) subgroup. However, its level was not statistically significant (P-value =0.057) (see Table II).

**Table I:** Chromosomal abnormalities detected in azoospermic patients.

Chromosomal Abnormality	Azoospermia n=30(%)
47XXY	6 (20%)
46XY,inv(9) p	1 (3%)
46XY	23(77%)

## Discussion

This study is a unique one from Jordan as it detects chromosomal abnormalities in a group of infertile males. The occurrence of infertility is universal among different countries, regardless of their economical level.<sup>(19)</sup> The incidence of chromosomal aberrations is estimated to be 2.1-28.4% in infertile men versus 0.7-1% only in the general male population.<sup>(20)</sup>

Numerical and structural chromosome abnormalities represent a principal role in male infertility.<sup>(21)</sup> It is long-familiar that the sperm count is inversely related to the presence of chromosomal abnormalities.<sup>(22)</sup> Actually, the presence of abnormal karyotyping does not only compromise spermatogenesis but also results in the production of cytogenetically abnormal spermatozoa.<sup>(23)</sup> Even among infertile males with a normal blood karyotype, the incidence of chromosomal abnormalities restricted to the germ line is 5-10% in oligozoospermic subjects and 15-20% in an azoospermic males.<sup>(24)</sup>

The prevalence of chromosomal abnormalities changes in different studies from as low as 4.6% in azoospermic males in India<sup>(25)</sup> up to

35% in Ukraine.<sup>(26)</sup> Our study showed an intermediate rate of 23.3% (Table III).

Compared with other Arab countries, Yassine Naasse, *et al* from Morocco studied a cohort of 573 infertile males and found the frequency of chromosomal abnormalities in those with non obstructive azoospermia (n=444) was 10.2% which is much less than our study. The prevalence of chromosomal abnormalities in those with severe oligospermia was 0.35%<sup>(21)</sup> (Table III). While in a nearby country, Tunisia; Ghorbel M, *et al* showed a prevalence of chromosomal abnormalities of 22.2% that coincides with our results<sup>(27)</sup> (Table III).

Reviewing the literature in Arab world; Syria has recorded the highest prevalence of chromosomal abnormalities in azoospermic subjects at 28.4%,<sup>(28)</sup> while the lowest prevalence was reported in Gaza strip-Palestine at 9.4% in a thesis submitted for the degree of Master of science in biological sciences by Ashraf Jaber Shaqalaih<sup>(29)</sup> (Table III).

Again a study in a different ethnic group from China on 489 azoospermic patients revealed that the prevalence of chromosomal abnormalities reaching 20.86% of whom 14.9% are having klinefelter's syndrome.<sup>(30)</sup> In an another Jordanian study , the frequency of AZF microdeletions among azoospermic infertile males is 8.3%<sup>(31)</sup> that is almost similar to figures from Netherland(8.1%)<sup>(32)</sup> and China (8.6%).<sup>(33)</sup>

Hormonal profile of subjects with non obstructive azoospermia showed elevated serum levels of follicular-stimulating hormone (FSH). In a study by Schoor RA, *et al* showed that 89% of men with nonobstructive azoospermia had FSH levels greater than 7.6 mIU/ml that comes in accordance with our findings<sup>(34)</sup> (Table II). This study also showed a significant increase in serum levels of LH levels and reductions in serum T-Testosterone levels denoting significant testicular pathology( Table II).

Klinefelter's Syndrome (KS) is 45 times more common in infertile men than in the general male population and is the commonest numerical chromosome anomaly observed in male infertility<sup>(35)</sup> and this has been affirmed by our study where 86% of the cohort were

having Klinefelter's syndrome. The classical chromosomal pattern in subjects with Klinefelter's Syndrome is 47XXY, but 15% are classified as mosaic with 46XY/47XXY. The main components of this syndrome are azoospermia, seminiferous tubular dysgenesis and elevated serum levels of gonadotrophins.<sup>(36)</sup>

Individuals with Klinefelter's Syndrome frequently experienced azoospermia in seminal fluid analysis. However, there are rare reports stating the possibility of production of normal sperm count in such subjects.<sup>(37)</sup> Oligospermia has rarely been identified in cases affected by Klinefelter's syndrome, as the spermatogenesis was connected to the presence of normal 46, XY karyotype.<sup>(38,39)</sup>

The pathophysiologic mechanism leading to abnormal spermatogenesis in cases of KFS may result from an extra X chromosome,

which affects testicular development, Leydig cell insufficiency, and regulation of apoptosis of Sertoli and Leydig cells.<sup>(40)</sup> Spermatozoa retrieved from azoospermic subjects for ICSI may give embryos with unbalanced genetic makeup and therefore an increased risk of chromosomal abnormality for the offspring<sup>(41)</sup>. The presence of abnormal chromatin interferes with meiotic division is another pathophysiologic mechanism leading to abnormal sperm production.<sup>(42)</sup>

This study which is the first of its kind in Jordan looking into numerical and structural chromosomal abnormalities in non obstructive azoospermic males is limited by the small number of cases. Despite that no studies or data are available in Jordan; its results should be taken into consideration in evaluating infertile males and selection whom could be suitable for assisted fertilization in future.

**Table II:** Hormonal profile of those with chromosomal abnormalities and azoospermia and those with normal karyotype and azoospermia.

	Mean age(Year)	FSH	LH	Prolactin	T.Testosterone
Azoospermia with Chromosomal abnormalities N=7(AZ+)	32.4±4.2	25.3±12.8	19.8±6.8	19.5±10.4	286.4±89.9
Azoospermia with Normal Chromosomal study N=23(AZ-)	34.6±3.6	9.1±2.3	9.1±2.1	13.9±7.1	443.8±166.1
Total group	34.1±3.8	12.9±9.3	11.6±5.8	15.2±8.2	407.1±164.9
P-value	0.09	0.009	0.001	0.057	0.012

**Table III:** Review of the literature of prevalence of chromosomal abnormalities in non obstructive azoospermia

Authors (Reference)	Country, Year	Chromosomal abnormalities (%)
Akeel A. Yasseen, <i>et al</i> (43)	Iraq, 2001	11%
Punam Nagvenkar, <i>et al</i> (44)	Mumbai, India, 2005	14.3%
Elghezal, <i>et al</i> (45)	Tunisian, 2006	13.5%
Teraporn Vutyavanich, <i>et al</i> (46)	Thailand, 2007	4.6%
Ashraf Jaber Shaqalaih (29)	Gaza Strip- Palestine, 2007	9.4% (thesis)
H. Samli, <i>et al</i> (47)	Turkey, 2009	12%
Pınar Aslan Koşar, <i>et al</i> (48)	Isparta (South of Turkey), 2009	5.4 % ( 5/92 patients).
Alexander N. Yatsenko, <i>et al</i> (49)	Texas, USA, 2010	13.3% (35/264)
Moussa Alkhalaf, <i>et al</i> (50)	Kuwait, 2010	18.3%
	Syria, 2012	17.52% (17/97)
Walid Al-Achkar, <i>et al</i> (51)		
Myriam Ghorbel, <i>et al</i> (27)	Tunisian, 2012	22.2%(12/54)
Marwan Alhalabi, <i>et al</i> (28)	Syria, 2013	28.41%
Larysa Y. Pylyp, <i>et al</i> (26)	Ukrainian, 2013	35%
G. Sreenivasa, <i>et al</i> (52)	Southern India, 2013	14.5%
Dana Mierla, <i>et al</i> (53)	Romania, 2014	5.5%(6/108)
Ushang V. Kate, <i>et al</i> (54)	India, 2014	15.4%
Yassine Naasse, <i>et al</i> (21)	Morocco, 2015	10.5%
Jaganathan Suganya, <i>et al</i> (26)	India, 2015	*4.6 % ( 7/152 azoospermic males).
		**4/7: klinefelter's syndrome

## Conclusion

The prevalence of chromosomal abnormalities in azoospermic subjects in our cohort is 23%. Serum levels of LH and FSH were significantly higher and serum level of testosterone was significantly lower in azoospermic subjects with chromosomal abnormalities versus azoospermic subjects with normal karyotype. Our results strongly suggest that azoospermic patients should be karyotyped and receive counseling before they are referred for assisted reproduction techniques.

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