Chromosomal abnormalities associated with male infertility in Baghdad Iraq

Saad Mohamed Nada  Hazim I AL-Ahmed Hussien Jassim Obade*

Biotechnology research center/ University of Nahrain
*College of Sciences/ University of Babylon

E-mail: Dr.hazim_al_ahmed@yahoo.com

Abstract
In the current study, 142 patients (109 azoospermia and 33 severoligospermia) were studied in order to explore the cytogenetic cause’s background and the hormonal study of male infertility in Baghdad Strip of Iraq. Of the 142 infertile males, 14 patients showed abnormal chromosomal karyotypes corresponding to a frequency of 9.85% (14/142), the occurrence of chromosomal abnormalities was only confined to the azoospermia patient group. Patients with abnormal karyotypes represented 12.84% (14/109) of the azoospermia patients. Nine had a 47, XXY karyotype of Klinefelter’s syndrome [47XY;64.28% (9/14)], [47XY+mar;14.28% (2/14)], [46XY,del13(p12) and (del21(p12);7.14% (1/14)], [46XY,tra(5;12, -6); 7.14% (1/14)] and one with chromosomal instability that showed multiple mosaic karyotypes 7.14% (1/14). The hormonal levels of nine patients with Klinefelter’s syndrome (KFS) were diagnosed in the present study, and the results showed elevated FSH and LH concentrations compared with control. While serum testosterone values were below the control.

Key words: male infertility, cytogenetic, abnormal chromosom

Introduction
Male causes for infertility are found in about 50% of infertile couples [1,2]. Reduced male fertility can be a result of congenital and/or acquired abnormalities. They include infections of the genital tract, varicocele, developmental and anatomical abnormalities, endocrinopathies, immunological factors, environmental exposures, and genetic abnormalities. Frequently, however, male infertility is difficult to diagnose, and about 60-75% of cases remain idiopathic. These idiopathic cases present with no previous history associated with fertility problems and have normal findings on physical examination [3].

Congenital abnormalities include a history of testicular maldescent, karyotype abnormalities, and azoospermia (sperm concentration is 0 × 10⁶/ml) due to congenital agenesis of the vasa deferentia [4]. Karyotype abnormalities like in Klinefelter's syndrome that characterized by the presence of one or a number of extra X chromosomes, and in Down syndrome that associated with moderate to severe reduction in sperm production, also a number of rare complex genetic syndromes can affect fertility in men [5]. In case of Y-chromosome gene deletion, micro deletion are more prevalent in infertile individuals, and deletions can cause severe spermatogenic defects ranging from non-obstructive azoospermia to oligozoospermia [6].

Aneuploidies can be easily diagnosed after performing G banding using trypsin and Glemsa (GTG) karyotyping. Therefore, GTG karyotyping is certainly a mandatory test in the diagnostic workup of any infertile man. Klinefelter’s syndrome is the most frequent sex chromosome abnormality [7,8]. Adult men with Klinefelter’s syndrome have small firm testicles devoid of germ cells. The phenotype can vary from a normally virilised man...
to one with androgen deficiency, including female hair distribution, scanty body hair and long arms and legs
because of late epiphyseal closure. Leydig cell function is commonly impaired in men with Klinefelter's
syndrome [9].

The hypothalamus-pituitary endocrine system regulate the hormonal events that required to the normal testicular
function. Hypothalamus stimulated the pituitary gonadotropins which are: Luteinizing Hormone (LH) stimulate
the production of testosterone , and Follicle-Stimulating Hormone (FSH) which stimulate the production of
seminiferous fluid [5]. Normal levels of LH and FSH are necessary for maintenance of spermatogenesis,
disorders of the pituitary or hypothalamus will cause inadequate gonadotropin stimulation of the testis and that
will lead to problems with fertility [5].

Normal FSH concentration may indicate obstruction of sperm transport. Elevated FSH concentration may
suggest severe defects in spermatogenesis, but in men with reduced testicular volume and signs of
hypoandrogenism with the presence of high FSH level may indicate primary testicular failure, but if FSH is not
elevated in these men that may due to failure of the hypothalamo-pituitary function or to pituitary tumor [10].

Materials and Methods
In the present study we used 142 patients (109 azoospermia and 33 severe oligospermia) and 15 fertile male as
control group were studied in order to explore the cytogenetic cause’s background and hormonal levels of male
infertility in Baghdad Strip of Iraq.

Blood Sampling
Five ml of blood was collected by vein puncture obtained from some Baghdad Hospitals and clinics doctors
from February 2013 till May 2014. Each collected blood sample was dispensed into tubes Heparinzed tubes for
cytogenetic studies and obtained plasma for hormonal studies.

Cytogenetic Analysis
Lymphocyte cultures were set up in the laboratory by adding 0.5 ml of heparinized blood to 4.5 ml of modified
RPMI-1640 medium Quantum PBL (Problem-based leaning) supplemented with L-Glutamine, Fetal calf serum
and penicillin and streptomycin (10000 I.U), and phytohemaglutinin (PAA, Austria). Cells were incubated for
70 h in a 5% CO2 incubator. Colcemide (PAA, Austria) at concentration 1 μg/ml was added to the cultures and
incubated at 37°C inside water path for 35-40 min. The cultures were then centrifuged at 10000 rpm for 10 min.
The pellet was resuspended in hypotonic solution (KCl, 0.075M, PAA, Austria) and immediately centrifuged
at 10000 rpm for 10 min, and resuspended in freshly prepared, ice-cold fixative containing methanol: acetic acid
(3:1) (Merck, Darmstadt, Germany), left for 20 min at room temperature. The solution was then centrifuged at
10000 rpm for 10 min, and the pellet was resuspended in freshly prepared ice-cold fixative containing
methanol:acetic acid (3:1). If the solution was not clear after additional centrifugation, the last step was repeated
until a clear solution was obtained. After decantation to reduce the volume to about 1 ml, the pellet was mixed
with the remaining fixative and dropped from about 30-80 cm with a Pasteur pipette onto an ethanol washed
slide; the fixative was removed by slight blowing, decantation and air-drying. Subsequently, the slides were
stained in 5% Giemsa solution for 10 min. [11]

Karyotyping
GTG (Gimsa-Trypsin) banding technique was performed. When the banding of the chromosomes was not
successful, the protocol was repeated. After staining, at least 20 metaphase plaques were analysed for each
sample by using cytovision.

Hormonal Assay
Testosterone, FSH, LH, and prolactin were determined by using miniVIDAS Bio merieux/ Italia.

Statistical Analysis
The statistical SPSS version (13) program was used to analyze the level of hormones Significant differences
were obtained according to ANOVA.

Results and discussion
Cytogenetic analysis by GTG banding
Among the 142 infertile patients included in this study, 14 patients showed abnormal chromosomal karyotypes
which represent 9.85% (Table.1.and Figure.1) The occurrence of chromosomal abnormalities was only
confined to the azoospermia patients group. Patients with abnormal karyotypes represented 12.84% (14/109)
of the azoospermia patients. Nine had a 47, XXY karyotype (Klinefelter's syndrome) and represented 6.33%,


64.28% (9/14) of the chromosomal abnormalities patient group, and 8.25% (9/109) of the azoospermia patient group. Figure (2). Two patient (case 53,126) had 47,XY, +mar karyotype, and represented 1.40% (2/142) of the patient study population, 14.28% (2/14) of the chromosomal abnormalities patient group, and 1.83% (2/109) of the azoospermia patient group. Figure (3). One patient (case 93) had [46,XY,del13(p12) and del21(p12)] karyotype, Figure (4) and One patient (case 87) had 46,XY, tra(5;12, -6) karyotype, Figure (5) and One patient had a chromosomal instability with a heterogeneous scale of mosaic aneuploides, Figure (6), represented 0.7% (1/142) of the patient study groups, 7.14% (1/14) of the chromosomal abnormalities patient group, and 0.91% (1/109) of the azoospermia patient group.

Table (1): Abnormal cytogenetic results among azoospermia patient

<table>
<thead>
<tr>
<th>Case</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>47,XXY</td>
</tr>
<tr>
<td>31</td>
<td>47,XXY</td>
</tr>
<tr>
<td>54</td>
<td>47,XXY</td>
</tr>
<tr>
<td>63</td>
<td>47,XXY</td>
</tr>
<tr>
<td>69</td>
<td>47,XXY</td>
</tr>
<tr>
<td>77</td>
<td>47,XXY</td>
</tr>
<tr>
<td>96</td>
<td>47,XXY</td>
</tr>
<tr>
<td>121</td>
<td>47,XXY</td>
</tr>
<tr>
<td>128</td>
<td>47,XXY</td>
</tr>
<tr>
<td>53</td>
<td>47,XY,+mar</td>
</tr>
<tr>
<td>126</td>
<td>47,XY,+mar</td>
</tr>
<tr>
<td>72</td>
<td>Chromosomal instability (multiple karyotypes)</td>
</tr>
<tr>
<td>93</td>
<td>46,XY,del13(p12) and del21(p12)</td>
</tr>
<tr>
<td>87</td>
<td>46,XY,tra(5;12)</td>
</tr>
</tbody>
</table>

Fig. (1): Showing one X and one Y chromosome in addition to 22 pairs of somatic chromosomes (case 3)
Fig. (2): Karyotype of one Klinefelter's syndrome patient showing 47,XXY (case 31)

Fig. (3): 47,XY,+mar karyotype showing extra marker chromosome (case 126)

Fig. (4): 46,XY, del(13)(p12) and del(21)(p12) karyotype (case 93)
Klinefelter’s Syndrome Patients (KFS)

Nine patients with Klinefelter’s syndrome (KFS) were diagnosed in the present study all showed elevated FSH and LH concentrations and means (32.14±3.84 mIU/ml), (20.32±2.38 mIU/ml) compared with control (5.73±1.85 mIU/ml), (4.28±1.03 mIU/ml), respectively Figure (7). Serum testosterone values mean was (3.21±1.15 ng/ml) all were below the control (13.25±3.16 ng/ml) Figure (8).

All of the patients were azoospermic, and all had small testes
No signs of hypergonadotropism were detectable in the KFS boys during prepuberty or early puberty. After midpuberty, however, concomitantly with elevations in basal FSH and LH levels, their response to GnRH stimulation became abnormal [12]. These observations are in agreement with earlier findings [13,14] and suggest diminished testicular inhibition of gonadotropin secretion. In addition, the boys with KFS developed after midpuberty low T/LH ratios. This timing is in agreement with gradual appearance of Leydig cell hyperplasia during midpuberty [15]. Such changes in activity of the hypothalamic-pituitary-testicular axis thus probably represent a state of compensated hypergonadotropic hypogonadism attributed to diminished responsiveness of Leydig cells to LH with advancing puberty.

In the present study, both numerical and structural chromosomal aberrations were found in our patient study population. The occurrence of the chromosomal abnormalities was only confined to the azoospermia patient group; this could be the effect of the small size and slightly unbalanced nature of our patient study population (azoospermics 109 and severely oligozoospermia 33). The prevalence of chromosomal anomalies among the studied infertile men was found to be 9.85%. This lies within the previously published range (3.6%-22.6%), as shown in Table (2). Association between human male infertility and chromosomal anomalies has been known for a long time [16,17]. Chromosomal abnormalities are more frequently observed in the population of azo/o/oligozoospermic males than in the general population [18]. Thus, it would not be unusual to find chromosomal abnormalities in men attending infertility clinics.

<table>
<thead>
<tr>
<th>Source</th>
<th>Study subjects (n)</th>
<th>Chromosomal abnormalities (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kondoh et al., 1992</td>
<td>130</td>
<td>13.8</td>
</tr>
<tr>
<td>Pandiyan and Jequier, 1996</td>
<td>1210</td>
<td>3.6</td>
</tr>
<tr>
<td>Günüz et al., 1998</td>
<td>102</td>
<td>15.7</td>
</tr>
<tr>
<td>Kleiman et al., 1999</td>
<td>72</td>
<td>16.6</td>
</tr>
<tr>
<td>Akeel et al., 2001</td>
<td>64</td>
<td>12.5</td>
</tr>
<tr>
<td>Penna Videa. et al., 2001</td>
<td>84</td>
<td>22.6</td>
</tr>
<tr>
<td>Nagavenker et al., 2005</td>
<td>88</td>
<td>10.2</td>
</tr>
<tr>
<td>Pina-Neto et al., 2006</td>
<td>165</td>
<td>9.6</td>
</tr>
<tr>
<td>Hellani et al., 2006</td>
<td>257</td>
<td>3.9</td>
</tr>
<tr>
<td>Mohammed et al., 2007</td>
<td>289</td>
<td>8.0</td>
</tr>
<tr>
<td>Shaqalah, 2007</td>
<td>85</td>
<td>9.4</td>
</tr>
<tr>
<td>Samir et al., 2011</td>
<td>217 azoospermia</td>
<td>16.28</td>
</tr>
<tr>
<td></td>
<td>92 oligoospermia</td>
<td>5.56</td>
</tr>
<tr>
<td>This study, 2014</td>
<td>142</td>
<td>9.85</td>
</tr>
</tbody>
</table>

The distribution of chromosomal abnormalities detected in the present study showed that Klinefelter's syndrome (47,XXY) was the most prevalent abnormality, representing 64.28% (9/14) of our positive cases. This result was in agreement with several previously published studies [19,20]. Our result was not unexpected since Klinefelter's syndrome was described as the most frequent genetic cause of male infertility [21,22]. The exact mechanism by which chromosomal anomalies induce infertility is not clear. It is likely that the presence of abnormally distributed chromatin may interfere with meiotic division, therefore, reduces sperm production [23]. Testicular histology in such patients may reveal areas of atrophy and hyalinization of the seminiferous tubules as well as some areas with tubules of normal appearance that contain a reduced number of mature spermatozoa [24]. Spermatogenesis bearing abnormal chromosomes may cause abnormal embryonic development that can in turn cause early pregnancy loss [25]. Moreover, these chromosomal aberrations may have serious implications for infertile males who seek the help of intra-cytoplasmic sperm injection (ICSI) due to the possibility of transmission of these abnormalities to the offspring [26,27].

To conclude, chromosomal abnormalities found with relatively high prevalence in our infertile males are the major cause of their male infertility, and justify the requirement of cytogenetic analysis for every infertile male, particularly azoospermics, seeking children.

References


