Seminal Fluid parameters abnormality in a sample of Iraqi Infertile Males with observation of the chromosomal abnormalities in an Azoospermia case by using Karyotyping

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Available from: http://dx.doi.org/10.21931/RB/CSS/2023.08.02.21

ABSTRACT

Male infertility is any health issue in a man that lowers the chances of his female partner getting pregnant. The present study aimed to observe the seminal fluid parameters abnormalities among primary and secondary infertility. The total number of participants is 93, consisting of 83 infertile males and 10 normal males as a control group. The patient’s group was classified into Azoospermia, Asthenospermia and Oligoasthenospermia according to their semen abnormalities. The total number of patients is divided into two groups, the primary (65), who have no children and those with secondary infertility (18), who are unable to conceive or carry a baby to full-term after having a previous successful pregnancy. According to the seminal analysis, there were 18 infertile males with Azoospermia, 26 infertile males with oligoasthenospermia, 39 infertile males with asthenospermia and 10 as a control group as represented. A one-way ANOVA is used to determine whether or not there is a statistically significant difference between the means of the analyzed independent groups. The mean of all parameters represents significant differences between the control group and other tested groups with few exceptions, e.g., the seminal volume in the control group showed no higher difference than Asthenospermia. Banding technique: G-band showing 46XY, der 2, der 3, der 4, der 7. And in der 5 but not in all cells. Abnormal karyotype for males with a derivative of one copy of chromosome 2, one copy of chromosome 3 was a derivative, a copy of chromosome 4 was a derivative and a derivative of chromosome 7.

Keywords: infertility 1; karyotype 2; Azoospermia 3; Asthenospermia 4; Oligoasthenospermia 5
INTRODUCTION

Male infertility is directly or indirectly responsible for 60% of cases involving reproductive-age couples with fertility-related issues \(^1\). Infertility is a disease of the reproductive system defined by a failure to achieve clinical pregnancy after 12 months or more of regular unprotected sexual intercourse \(^2,3\). The major causes of male infertility include varicocele, genital tract obstruction, testicular failure, cryptorchidism, idiopathic, gonadotoxic exposure, genetic conditions, infections, hormonal dysfunction, immunological conditions, ejaculatory/sexual dysfunction, cancer and systemic diseases \(^4,1\). The World Health Organization recommends defining fecundity as the ability of a couple to conceive after two years of attempting to become pregnant. Infertility can be divided into primary infertility and secondary infertility. Primary infertility is when the man has never impregnated a woman, sometimes known as primary sterility. Secondary infertility is the inability to have a second child after a first birth. Secondary infertility has been shown to have a high geographical correlation with primary infertility \(^3\). A normal semen sample should have a volume of 1.5 to 5ml with greater than 20 million sperms/ml. The number of abnormal sperm should be less than 40%, with greater than 30% of sperm cells in a sample demonstrating proper motility. An estimated 40 to 90% of male infertility is due to deficient sperm production of indefinable origin \(^5\). Many infertile men fail to impregnate their female counterparts because they lack sperm (Azoospermia) or too little sperm (oligozoospermia). Infertility may also be due to abnormal sperm morphology (tetratozoospermia) and insufficient sperm motility (asthenozoospermia) \(^6\). The present study aimed to measure the association between primary and secondary infertility with seminal fluid parameters abnormalities.

MATERIALS AND METHODS

Study design, sample size and selection criteria

The samples were collected over five months, from November 2019 to March 2020, from patients (83) with conceiving problems and low fertility who attended Kamal Al-Samaraye Hospital in Baghdad for a routine seminal fluid analysis, as revealed in supplementary data (1). Questionnaire information was included in the supplementary data (2). The control group consists (of 10) healthy fertile men from the same ethnicity with normal seminal fluid analysis and without systemic diseases. The ethics committee, Department of Biotechnology, College of Science, University of Baghdad and Kamal Al-Samarrai Hospital, Ministry of Health in Baghdad-Iraq approved this study.

Semen collection

The semen sample is collected by the man by self-masturbation near the laboratory or at home. It is recommended that semen is collected after a minimum of 3 days and a maximum of 7 days of abstinence \(^7\). Clear instructions regarding the collection of the semen sample must be provided before collection to ensure all fractions of the ejaculate are collected and complete \(^8\). The macroscopic examination included volume, color, viscosity and PH. The microscopic examination included the following parameters: Sperm Concentration, Sperm Motility and Sperm Morphology.

Karyotyping preparation

One DNA sample from an azoospermia case was selected for Karyotyping. Chromosomes were prepared from 72-hour peripheral blood cells stimulated culture with phytohemagglutinin PHA (prepared in the Iraqi Center for Cancer and Medical Genetics. Iraq). Stander procedures for cultures, harvests and slid preparation, were modified and performed in our laboratory according \(^9,10\).
Briefly, 5 ml of heparinized peripheral blood was cultured in RPMI1460 (Sigma-Aldrich, MO) supplemented with 20% fetal bovine serum (Gibco, NY), and antibiotics (penicillin and streptomycin). Then, the culture was exposed to 20µg/ml Colcemid (Kreatech, Netherland) for 30 minutes, followed by hypotonic treatment (KCL 0.075N) for 30 minutes. A fixation procedure with methanol: Glacial acetic acid (3:1) v:v was performed freshly. Chromosomal were analyzed with GTG-banding, and karyotyping was described according to ISCN, 2013 10,11

RESULT
Study samples data

In the current study, the total participants are 93 males, consisting of 93 males within the patients group and 10 within the control group. The patients incorporated in this study were classified into three groups: Azoospermia, Asthenospermia and Oligoasthenospermia according to their semen abnormalities as stated in the WHO reference guide 12. The control group included males (10) who had normal seminal fluid analysis tests. The age means ± standard deviation of the patient groups was (31.39±7.499) years, and for the control group was (31.28 ±6.72) years. The total number of patients was divided into two groups, the primary 65 (78.3) who had no children, which consisted of 18 (21.7) Azoospermia, 28 (33.7) Asthenospermia and 19 (22.9) Oligoasthenospermia. In contrast, the secondary infertility group who are unable to conceive or carry a baby to full-term after having a previous successful pregnancy was 18 (21.7), consisting of 11 (13.3) Asthenospermia and 7 (8.4) Oligoasthenospermia. According to the seminal analysis, there are 18 (21.7) infertile males with Azoospermia, 39 (47) males with Asthenospermia and 26 (31.3) males with Oligoasthenospermia. The control group (10) had normal seminal fluid analysis, as represented in Table (1).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>Azoospermia</th>
<th>Asthenospermia</th>
<th>Oligospermia + Asthenospermia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>18 (21.7)</td>
<td>28 (33.7)</td>
<td>19 (22.9)</td>
<td>65 (78.3)</td>
</tr>
<tr>
<td>Primary</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>18 (21.7)</td>
</tr>
<tr>
<td>Secondary</td>
<td>-</td>
<td>-</td>
<td>11 (13.3)</td>
<td>7 (8.4)</td>
<td>18 (21.7)</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>18 (21.7)</td>
<td>39 (47)</td>
<td>26</td>
<td>93 (100)</td>
</tr>
</tbody>
</table>

Table 1. Distribution of the studied patients.
Table 2. Distribution of the studied population among age, family history, varicocele, duration of infertility and childhood history.

Table (3) shows differences in seminal characteristics among the studied groups (P < 0.05). High abnormality in parameters of sperms was represented in Azoospermia and oligospermia patients than in the control group. The sperm count and sperm motility status were significantly reduced in azoospermia and oligospermia patients compared with the control group. Sluggish sperm was significantly increased in oligospermia compared to Azoospermia. Dead sperm were significantly increased in oligospermia in comparison with the control group. It also had a negative impact on sperm count, sperm concentration, and motility.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Volume</th>
<th>T. count</th>
<th>Motility</th>
<th>Abnormal</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=10)</td>
<td>2.50±0.22a</td>
<td>51.70±5.11a</td>
<td>65.80±3.40a</td>
<td>19.50±3.02b</td>
<td>80.50±3.02a</td>
</tr>
<tr>
<td>Azoospermia (n=18)</td>
<td>1.47±0.16b</td>
<td>0.00±0.00c</td>
<td>0.00±0.00d</td>
<td>0.00±0.00c</td>
<td>0.00±0.00c</td>
</tr>
<tr>
<td>Oligoasthenospermia N=26</td>
<td>1.78±0.12b</td>
<td>5.02±0.63c</td>
<td>14.92±1.40c</td>
<td>79.27±2.04a</td>
<td>20.73±2.04b</td>
</tr>
<tr>
<td>Asthenospermia N=39</td>
<td>2.38±0.16a</td>
<td>37.95±3.67b</td>
<td>22.23±1.19b</td>
<td>73.43±2.42a</td>
<td>26.57±2.50b</td>
</tr>
<tr>
<td>LSD</td>
<td>0.54</td>
<td>10.48</td>
<td>4.65</td>
<td>7.73</td>
<td>7.90</td>
</tr>
</tbody>
</table>

Table 3. Seminal fluid differences among studied groups. Means with a different letter in the same column are significantly different (P<0.05).

In Table (3), the seminal fluid analysis shows the distribution of seminal parameters (volume, total count, motility and abnormal morphology) among Azoospermia, Oligo-asthenospermia and Asthenospermia. The highest percentage of the volume of semen, total count and motility in the control group were 2.5, 51.7 and 65.8 respectively and less abnormal morphology in comparison to the Oligoasthenospermia was (79.27), and Asthenospermia was (73.43), while Azoospermia showed no values for all parameters except the mean of volume was (1.47).
Figure (1): seminal fluid analysis showing the distribution of seminal parameters. (A) distribution of the seminal volume, (B) total count, (C) motility and (D) abnormal morphology among Azoospermia, Oligo-asthenospermia and Asthenospermia.

Statistical analysis
Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). One-way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means.

Karyotyping
Karyotyping (Figure 2) by using the banding technique: G-band showing 46XY, der 2, der 3, der 4, der 7 and in der 5 but not in all cells. Abnormal karyotype for males with a derivative of one copy of chromosome 2, one copy of chromosome 3 was a derivative, a copy of chromosome 4 was a derivative and a derivative of chromosome 7.
DISCUSSION

Table (1) shows that the patients have sterility either due to Asthenospermia, which indicates reduced sperm motility as <40% sperm motility or less than 32% with progressive motility, or due to Azoospermia which affects about 1% of the male population and characterized by a man whose semen contains no sperm.

The distribution of the studied population among age, family history, varicocele, duration of infertility and childhood history is represented in Table (2). There are no differences between ages among all groups. It may be because most of the patients admitted to the hospital for treatment were at the age they should be fertile. This result disagrees with a study that represented that the mean age was 35.88 ± 0.57 years. Also, there are no differences between the duration of infertility among groups. The duration of infertility was 5.278 ± 4.46, 6.208 ±4.916 and 6.1± 4.55 in Azoospermia, Asthenospermia and Oligo-asthenospermia, respectively, which is in agreement with a study by who showed that the duration of infertility was 5.44 ±0.29 years. Azoospermia family history showed that only 2 (11.11 % ) cases out of 18 have a history with infertility, while Asthenospermia and Oligo-asthenospermia showed 7 (17.94 % ) and 8 (30.76 %) out of 39 and 26 cases respectively. This results in agreement with a study by who reported (11.8%) permanent involuntary childlessness in at least one first or second-degree relative (brother, sister, half-sib, uncle, aunt).

Childhood histories like hypospadias, congenital anomalies, and the onset of puberty; current systemic illnesses like diabetes mellitus, cystic fibrosis, cancer, and infections; surgical history like scrotal, pelvic, retroperitoneal, and inguinal surgeries; sexual history like a history of sexually transmitted diseases; medications and allergies, and exposure to gonadotoxins like both chemical and environmental toxins, such as heat. Varicocele was observed to be associated with infertility in all groups with no significant differences, which explains its role in infertility. Study shows that varicocelectomy increases semen quality and pregnancy outcomes in couples with documented infertility only if
the male partner has a clinically palpable varicocele and affected semen parameters\textsuperscript{18}.

As shown in Table (3), a one-way ANOVA is used to determine whether or not there is a statistically significant difference between the means of the analyzed independent groups. In general, means with a different letter in the same column are significantly different (P<0.05), and the mean of all parameters represents significant differences between the control group and other tested groups with few exceptions, e.g., the seminal volume in the control group showed no high difference than Asthenospermia. Male infertility is characterized by an abnormality in seminal volume, sperm motility, morphology, PH, color, velocity, sperm concentration and sperm count using visual examination, microscope, and counting chambers as reported by the WHO reference values for human semen\textsuperscript{12}. Accordingly, the parameters included the following: Appearance (grey to opalescent); volume (2.0 ml or more); PH (7.2–7.8); sperm concentration (>15 × 10^6 spermatozoa/ml); total sperm count (39 × 10^6 or more/ejaculate); motility (50% or more with forward progression); morphology (4% or more with normal form); and white cell count or pus cell (<1 × 10^6/ml)

\textbf{Karyotyping}

No numerical abnormalities were observed (46XY). Structural abnormalities was observed and associated with der 2, der 3, der 4, der 7 and der 5, but not in all cell. Abnormal karyotype for males with a derivative of one copy of chromosome 2, one copy of chromosome 3 was a derivative, a copy of chromosome 4 was a derivative and a derivative of chromosome 7. Pylyp et al. (2013) detected numerical abnormalities (two mosaic cases with karyotype 47,XXY/46, XY and 47,XYY/46, XY). A study by\textsuperscript{19} showed that KF was the most common chromosomal abnormality, followed by YCMD in azoospermi patients. YCMD was the most common chromosomal abnormality for the severe oligozoospermia group, followed by Robertsonian translocations.

\textbf{CONCLUSION}

The presence of chromosomal abnormalities in infertile males suggests that it is important to use conventional chromosomal analysis in addition to routine seminal analysis tests, especially before any assisted reproductive techniques.

\textbf{References}


Received: May 15, 2023/ Accepted: June 10, 2023 / Published: June 15, 2023