Detection Y Chromosome Microdeletions Among Iraq Population in Infertile Patients with Azoospermia and Severe Oligospermia

Samah A Hammood1, Saleh M Al-Khafaji2, Alaauldeen S M AL-Sallami1

1Department of Biology-College of Science /University of Kufa/Iraq
2Department of Anatomy & Histology - College of Medicine/ University of Kufa/Iraq.

ABSTRACT
Objective: To detection of microdeletions of Y chromosome and study the frequency of microdeletions in infertile men with non-obstructive azoospermia or severe oligozoospermia (Middle Euphrates center) in Iraq population. Material and methods: 153 males were included in the study, the cases were divided into groups according to the infertility etiology and semen analysis according to World Health organization, the frequencies and the characteristics of Y chromosome microdeletions were investigated in groups. Multiplex PCR was applied to detect the microdeletions. Results: Y chromosome microdeletion was detected in 42 (40.7%) of 153 cases. Microdeletions in azoospermia showed more frequently detected 28 (52.8%), followed by severe oligospermia 14 (28%). Microdeletions in the AZFc region were the most common 12 (22.64%), followed by AZFb 11 (20.75%) and AZFa 5 (9.43%) in azoospermia compared to severe oligospermia AZFc 6 (12%) AZFb 4 (8%) and AZFa 4 (8%). Conclusion: Y chromosome microdeletions were detected quite frequently in certain infertility subgroups. Therefore, detailed evaluation of an infertile man by physical examination, semen analysis, hormonal evaluations and when required, karyotype analysis may predict the patients for whom Y chromosome microdeletion analysis is necessary and also prevent cost increases. Recommendation: This study emphasizes that analysis of microdeletions should be carried out for all patients with idiopathic azoospermia and severe oligospermia who are candidates for intracytoplasmic sperm injection.

Keywords: Y chromosome Microdeletions, Infertility, Azoospermia, Severe oligozoospermia.

INTRODUCTION
Infertility is a distracting major health problem that affects the human beings especially when the cause of infertility is the male partner5. It is defined as inability of a sexually active couple not using contraception, to achieve pregnancy within one year of regular intercourse attributed to deficiencies in the semen quality5. It accounts for about 30-40% of overall cases of infertility and it affects approximately 7% of all males worldwide5. Azoospermia is defined as the total absence of spermatozoa in the ejaculate, classification of can be based on obstructive and nonobstructive forms5. In many instances non-obstructive azoospermia was related to the history of clinical unilateral or bilateral varicocele5. The role of Y chromosome in male infertility was first elucidated in 1976 when6 have proposed the existence of a gene factor which controls spermatogenesis that is localized within the euchromatic region of the Y chromosome long arm (Yq11), which was called the Azoospermia Factor. (AZF), because the first six men observed with microscopic terminal deletions in Yq, by routine karyotyping, were azoospermic. Many genes controlling spermatogenesis were mapped within these AZF regions5. Interstitial and terminal deletions in AZFa, or AZFb, or AZFcalone or in any combination of the Yq are all associated with dramatic non-obstructive spermatogenic failure, therefore, there is a clear cause-effect relationship between AZF loci deletion/s and male infertility5. The two main genes located in the AZFa region are USP9Y and DBY (also called DDX3Y)5. Deletions in the AZFa region that remove both of these genes cause Sertoli cell–only syndrome, a condition characterized by the presence of complete Sertoli cells in the testes but a lack of spermatozoa in the ejaculate10. Deletions of the AZFb region cause arrest of spermatogenesis at the primary spermatocyte stage11, indicating that the region is essential for fertility (Nuti and Krausz,2008). The main gene in the AZFb region is RBMY, and there are six copies of the gene located on the Y chromosome12. While Deletions in the AZFc region produce a wide range of phenotypes, many of which are associated with low sperm concentration due to reduced spermatogenesis13. AZFc deletions cause approximately 12% of non obstructive azoospermia and 6% of severe oligozoospermia14. The AZFc region contains DAZ genes involved in spermatogenesis, this gene has four copies on the Y chromosome which are thought to serve a variety of roles throughout the spermatogenic process because they are expressed in all stages of germ cell development15.

MATERIAL AND METHOD

*Author for Correspondence: salih.alkhafaji@uokufa.edu.iq;
Table 1: List of primers used to detection AZF microdeletions.

<table>
<thead>
<tr>
<th>Region</th>
<th>Primer</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRY(Yp)</td>
<td>SRY-F</td>
<td>5'-GAA TAT TCC CGC TCT CCG GA -3'</td>
</tr>
<tr>
<td></td>
<td>SRY-R</td>
<td>5'-GCT GGT GCT CCA TTC TTG AG -3'</td>
</tr>
<tr>
<td>AZFa</td>
<td>sY86-F</td>
<td>5'- GTG ACA CAC AGA CTA TGC TTC -3'</td>
</tr>
<tr>
<td></td>
<td>sY86-R</td>
<td>5'- ACA CAC AGA GGG ACA ACC CT -3'</td>
</tr>
<tr>
<td>AZFb</td>
<td>sY127-F</td>
<td>5'- GCC TCA CAA ACG AAA AA -3'</td>
</tr>
<tr>
<td></td>
<td>sY127-R</td>
<td>5'- CTG CAG GCA GTA ATA AGG GA -3'</td>
</tr>
<tr>
<td>AZFb</td>
<td>sY134-F</td>
<td>5'- GTC TGC CTC ACC ATA AAA CG -3'</td>
</tr>
<tr>
<td></td>
<td>sY134-R</td>
<td>5'- ACC ACT GCC AAA ACT TTC AA -3'</td>
</tr>
<tr>
<td>AZFc</td>
<td>sY255-F</td>
<td>5'- GAA CCG TAT CTA CCA A GC AGC -3'</td>
</tr>
<tr>
<td></td>
<td>sY255-R</td>
<td>5'- GTG TAC ATG TGC AGC CAC -3'</td>
</tr>
<tr>
<td>AZFc</td>
<td>sY254-F</td>
<td>5'- GAA TAT TCC CGC TCT CCG GA -3'</td>
</tr>
<tr>
<td></td>
<td>sY254-R</td>
<td>5'- GGG TGT TAC CAG AAG GCA AA -3'</td>
</tr>
<tr>
<td>AZFc</td>
<td>sY150-F</td>
<td>5'- TTG ATC TAT CTG CCT GAG TGC -3'</td>
</tr>
<tr>
<td></td>
<td>sY150-R</td>
<td>5'- TTG AAT TAT CTG CCT GCT GAG TGC -3'</td>
</tr>
</tbody>
</table>

F- Forward primer, R- Reverse primer, SRY- Sex-determiningthe region of Ychromosome, STS- sequence-tagged site, bp- base pair.

Table 2: Y chromosome microdeletion rates according to the results of semen analysis.

<table>
<thead>
<tr>
<th>Sperm count groups</th>
<th>Microdeletion n, (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoospermia</td>
<td>Absent (25)</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Present (36)</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Total (61)</td>
<td>61</td>
</tr>
<tr>
<td>0-5 million/mL</td>
<td>Absent (0)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Present (14)</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Total (28)</td>
<td>28</td>
</tr>
<tr>
<td>&gt;20 million/mL</td>
<td>Absent (0)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Present (0)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total (0)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>Absent (61)</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Present (42)</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Total (103)</td>
<td>103</td>
</tr>
</tbody>
</table>

This study was conducted in the laboratories of Faculty of Medicine, and in the laboratory of the Fertility Center's in AL-Sader Medical city in the Province of Najaf, AL-Najaf Health Directorate / Ministry of Health Iraq during the period from 1 April/2018 to 30 August/2018. The mean and stander deviation of age of infertile patients was (33± 1.24) years, the semen samples collected are 153 samples which divided after analysis according to the procedure described by the WorldHealth Organization (16) to 53 azoospermia, 50 samples from severe oligospermia infertile patients and control group (fertile) were 50 samples (Normozoospermia). The blood samples were obtained from persons through drag 3ml of blood by using of medical sterile syringes from brachial vein, and placed in ethylene diamine tetra acetic acid (EDTA) tube for DNA extraction kit(Promega-U.S.A) according to the manufacturer's instructions. PCR was carried out using master mix from Promega PCR kit (Promega, U.S.A) and STS primers (Bioneer ,Korea) for the regions used were: for AZFa sY86 , for AZFb sY127 and sY134 for AZFc sY254, sY255 and sY150(table-1). This primer set was suggested by and is prescribed by the European academy of andrology (EAA) and European molecular genetics quality network (EMQN). The internal control was STS primer sY14 for sex determining region of the Y (SRY). Thermo cycling for PCR was carried out at 95 °C for 3 min; followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, extension at 72 °C for 1.5 min and a final extension at 72 °C for 10 min. PCR products were separated on agarose gel electrophoresis, stained with ethidium bromide, and visualized using UV light.

RESULTS

Y chromosome microdeletion was detected in 42 (40.7%) of 153 cases. Microdeletions in azoospermia showed more frequently detected 28 (52.8%), followed by severe oligospermia 14 (28 %), No Y chromosome microdeletions were detected in cases with control group. The results are shown in Table (2).

Microdeletions in the AZFc region were the most common 12 (22.64%), followed by AZFb 11(20.75%) and AZFa 5(9.43%) in azoospermia showed figure (1) and figure (2) compared to severe oligospermiaAZFc 6 (12%) AZFb 4 (8%)and AZFa 4 (8%) showed figure (3) and figure (4). The results are shown in Table (3).

The results showed rates of STS had AZFb sY134 and AZFc sY255 deleted more frequently in azoospermia patients followed byAZFa sY86 , AZFbs Y127, AZFc sY150 and AZFc sY254 respectively shows table (4) . The results showed rates of STS had AZFcsY254, sY255 and AZFc sY150, deleted more frequently in severe oligospermia patients followed by, AZFb sY134 ,AZFb sY127, and AZFAs Y86 showed table (5).

DISCUSSION

Y chromosome deletions are one of the most common genetic causes of male infertility, the prevalence of AZF microdeletions differet worldwide among both infertileazoospermic and severe oligospermic males; it generally ranges from 1-13% worldwide but may reach up to 55%. In this study, the frequency of AZF microdeletion was 40.7% in 153 cases, this is fluctuate than that reported in some previous studies such the study by. This difference between the results may be due to some factors such as ethnic differences, patient selection criteria, methodological aspects, and even the type and number of markers used in the studies. Moreover, the frequency of microdeletions detected in the present study was leftover.
The range reported by previous studies from Turkey (1.3–9.1%) was quite different from the results obtained in this study. The patients were selected based on the diagnostic criteria used in the study. Therefore, different selection criteria and composition of the study could lead to variations in the frequency of AZF deletions. Moreover, the accurate or wrong diagnosis of the disease can also result in frequency variations in different reports. There is also heterogeneity in selecting PCR markers both in type and number in different methods.

In the present study, microdeletions in the AZFc region were the most common (22.64%), AZFb (20.75%) and AZFa (9.43%) regions. The frequent appearance of AZFmicrod deletions was consistent with previous studies and the distribution rate of other microdeletions was similar. It is well known that the deletions of the AZF regions cause spermatogenic impairment and that the complete deletion of any of them is usually associated with the total depletion of spermatogenic cells. The AZFa deletion was found to associate with complete absence of germ cells and presence of Sertoli cells in the seminiferous tubules while AZFb and AZFc deletions are associated with developmental arrest of germ cells at pachytene stage or at spermatid stage respectively. Also AZFc was found to associate with hypo spermatogenesis, maturation arrest and a variety of testicular phenotypes. When the cases were analyzed according to semen analysis, microdeletion was most frequently (52%) found in the azoospermic group as expected. Furthermore, no microdeletions were detected in cases with sperm counts above 20 million/mL. Microdeletions occur in about one in 4000 men in the general population but their frequency is significantly increased among infertile men. Study also agreement with a similar study in which the cases were assessed according to semen analysis, AZF microdeletions were detected in the moderate oligozoospermic group, even if its frequency was quite low.

The importance of genetic studies of male infertility in Iraq has been demonstrated by conducting studies to investigate the AZF and SRY regions microdeletions and hormonal disturbance in 43 azoospermic and 20 healthy and fertile men. All cases showed deletions with undetected chromosomal abnormalities. Five of these deletions have been detected in 18.2% of azoospermic men have shown deletions with undetected chromosomal abnormalities. Several of these deletions have been detected in men with a history of post puberty mumps and were also detected in patients with undistinguishable correlation parallel to the detected microdeletions.

The high percentage of the AZFc distributed among azoospermia in our study suggests that it is possible that AZFcsY255 AZFb sY134 is predominant in Iraqi azoospermic population. The azoospermia affects by diseases infection, hormone disturbance, protein deficiency reflects or suggesting that sY255 of the AZFc and sY134 of the AZFb in azoospermia patients fragile sites toward imbalance factors of male infertility.
causes of azoospermia such as failure of spermatogenesis and obstruction of the ductal system particularly the vas deferens have been investigated\textsuperscript{36}. It was reported that

Figure 1: Multiplex PCR Product for azoospermia SRY (472 bp) as internal control, AZFa SY 86 (326bp), AZFb SY127,134 (274bp, 301bp) and AZFc SY 255,254,150 (123bp, 380bp,158bp) ,lane (L symbolizes the 100 bp DNA ladder).

Figure 2: Multiplex PCR for azoospermia SRY (472 bp) as internal control ,AZFa SY 86 (326bp), AZFb SY127,134 (274bp, 301bp) and AZFc SY 255,254,150 (123bp, 380bp,158bp),lane (L symbolizes the 100 bp DNA ladder).

Figure 3: Multiplex PCR for Severoligospermia SRY (472 bp) as internal control ,AZFa SY 86 (326bp), AZFb SY127,134 (274bp, 301bp) and AZFc SY 255,254,150 (123bp, 380bp,158bp),lane (L symbolizes the 100 bp DNA ladder).
obstruction of the vas deferens was not a major cause of azoospermia\(^3\). Infection of the seminal fluid has been implicated as the major cause of azoospermia in infertile males\(^3\).

**CONCLUSION AND RECOMMENDATION**

Y chromosome microdeletions detection is very necessary for infertile male suffering from azoospermia and severe oligospermia before any decision for In Vitro Fertilization. Other solutions can be applied for infertile males with those deletions AZFa or AZFb sperm retrieval is inevitably impossible in cases with. Moreover, there is always risk of transmission of the microdeletions mutation to any male child and genetic counseling is necessary prior to the treatment.

**REFERENCES**

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**Figure 4**: Multiplex PCR for Severoligospermia, SRY (472 bp) as internal control, AZFa SY 86 (326bp), AZFb SY127,134 (274bp, 301bp) and AZFc SY 255,254,150 (123bp, 380bp,158bp), lane (L symbolizes the 100 bp DNA ladder).