AZF gene microdeletions: Case series and literature review

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Abstract

Objective: Approximately 10% of patients with non-obstructive azoospermia and 5% with non-obstructive severe oligozoospermia carry AZF region microdeletions (AZoospermic Factor) in the Y chromosome. The aim of this study is to analyze the clinical and pathological findings in this group of patients and compare them with the previous evidence.

Materials and methods: Retrospective study of 11 patients with diagnosis of azoospermia or oligozoospermia was performed and found the presence of AZFa, AZFb, and AZFc microdeletions or any combination of them.

Results: Microdeletions of AZFc region were found in 45% of cases, AZFa in 33% and a 10% showed a deletion of the three regions (a, b and c). 91% of them demonstrated azoospermia with low testicular volume in 62.5% cases.

Conclusion: Microdeletions of AZF regions are associated with azoospermia and a low expectation of sperm retrieval in testicular biopsy. On the other hand, they seem not to be related with significant modifications on the hormone profile.

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Microdelecciones del gen AZF: serie de casos y revisión de la literatura

Resumen

Objetivo: Aproximadamente un 10% de los pacientes con azoospermia no obstructiva y un 5% de pacientes con oligozoospermia severa presentan microdeleciones en las regiones azoospermic factor (AZF) del cromosoma Y. El objetivo principal de este estudio es analizar las características clínicas y patológicas de estos pacientes y compararlos con la literatura previa.

Material y métodos: Estudio retrospectivo de 11 pacientes con diagnóstico de azoospermia u oligozoospermia y presencia de microdeleciones AZFa, AZFb, AZFc o sus combinaciones.

Resultados: La microdeleción en la región AZFc apareció en un 45% de pacientes, AZFa en el 33% y un 10% presentaron mutación en las 3 regiones analizadas (AZFa, b y c). El 91% de los pacientes

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Introduction and objective

Microdeletion of the AZF region on the long arm of chromosome Y (Yq11.23) is the second leading cause of male infertility of genetic origin, preceded by Klinefelter syndrome. Although its prevalence in the general population is unknown, approximately 10% of patients with non-obstructive azoospermia and 5% of patients with severe oligozoospermia suffer from it. In some publications, the figure may reach 18% in azoospermic patients.

In 1976 the deletion of certain regions on the Y chromosome in infertile males was first described. It was not until 1995 when the final characterization of three distinct regions (designated a, b, and c) took place. In 1997, transcription units that would have a critical role in spermatogenesis, linking AZF regions to the spermatogenic process, were described.

Clinically, the common denominator of the deletion of any of these three regions or their combinations is primary infertility with azoospermia or severe oligozoospermia (<5 million/mL) in the absence of virilization disorders. The most reported alterations in the literature include decreased testicular size and rise in FSH levels. Therefore, the main objective that we propose in this review is to analyze the clinical and pathological features of patients with infertility and microdeletions of AZF in our experience.

Materials and methods

A retrospective study was performed from January 2009 to December 2013 from the database for clinical data in patients with non-obstructive azoospermia or severe oligozoospermia (<5 million/mL). Of the sample of such patients, we selected those who had genetic diagnosis of microdeletion of the AZF region (region a, b, and/or c).

The variables analyzed were age, pH, and semen volume (measured in mL), sperm concentration (million/mL), total motility, FSH, LH, and testosterone. The seminal values are obtained from the samples of fresh semen collected in the andrology laboratory, where microscopic analysis of the most successful sample of at least 2 semen analyses and training by means of swim-up technique were conducted. Hormone parameters were obtained by means of peripheral blood analysis using as reference values those of the laboratory of our center (FSH 2–12.4 mU/mL, LH 1.5–9.3 mU/mL, testosterone 3–10 ng/mL). The collected testicular volume was assessed by means of physical examination of a single andrologist. After obtaining the diagnosis of non-obstructive azoospermia, the karyotype and the microdeletions of chromosome Y are studied from peripheral blood sample using the PCR technique.

After reassessing the set of data obtained, patients with azoospermia are given the possibility of sperm retrieval by means of testicular biopsy. This is done on an outpatient basis under local anesthesia and making three incisions located in the upper, middle, and lower pole of the largest testis. Of each of the incisions, two samples, which are analyzed in situ by biologists from the center with the aim of identifying suitable sperm for intracytoplasmic sperm injection (ICSI) and cryopreserving them, are taken. One more sample is taken that is sent to pathology for structural analysis.

The samples are processed in microtubes to be used later in ICSI. Through face-to-face or telephone interview, the patient is informed of the intention of the study and, after verbal consent on their part, they are questioned on the assisted reproduction treatment offered and it is performed to the couple. All patients were informed and gave their consent to participate in this study.

Results

The data are collected in Table 1.

The mean age of the patients studied was 36.8 years. 90.9% of patients had azoospermia in the spermogram. One patient had a concentration of 0.25 million sperms/mL. Progressive motility in the spermogram was only informed in the non-azoospermic patient and it was 0%. The mean seminal volume and pH were normal (4.3 mL and 8.02, respectively).

Regarding the hormone study, the mean FSH was 16 mU/mL (SD: 8.25), and testosterone 7.1 ng/mL (SD: 2.3). The mean LH was 7 mU/mL (SD: 3.6). 45.5% of the patients had a decreased testicular volume, the same as 45.5% had normal testicular volume. Only one patient had atrophic testicular volume (Fig. 1).

At the genetic level, one patient had an altered karyotype (XY-q) and another one a 46XY/45X mosaicism. The most frequent alteration of the Y chromosome was the microdeletion of the AZFc region with 45.5% (5 patients); 3 patients (27.3%) presented alteration in the AZFa region. There was not a single patient with microdeletion in the AZFb region. Two patients showed complete alteration of the three regions with a normal hormonal study and normal testicular volume; in one patient there was AZFbc combined alteration.

In the 3 patients in whom testicular biopsy was performed, the most frequent pathological finding was the syndrome of Sertoli cells only or Del Castillo syndrome (2 patients with altered AZFc and AZFa, respectively). One patient was diagnosed with maturation arrest. In no case sperm suitable for assisted reproductive therapy could be recovered in practiced testicular biopsy.
Table 1  Clinical and analytical characteristics of the study sample.

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FSH and LH expressed in mU/mL. Testosterone expressed in ng/mL.
Adop: adoption; Atr: atrophic testicular volume; Dec: decreased testicular volume; MA: maturation arrest in testicular biopsy; N: normal testicular volume; SCO: Sertoli-cell-only in testicular biopsy; DS: donor semen; ART: assisted reproduction therapy.

Figure 1  Testicular volume expressed as a percentage.

The assisted reproduction option most commonly used by couples was artificial insemination with donor semen (72.7%).

Discussion

Only 3% of cases of infertility in men are due to the impaired function or sperm number, usually expressed as azoospermia or severe oligoazoospermia.

The AZF gene is divided into three regions described as AZFa, AZFb, and AZFc, all located on the long arm of chromosome Y (Yq11.23). Some studies postulate the existence of a fourth region, AZFd, located between AZFa and AZFb regions. However, in other studies, this possibility is not contemplated. We have identified several critical genes that have been called sequence-tagged sites (STS), whose deletion causes the clinical effect. The USP9Y, DBY, and DDX3Y genes belong to the AZFa region. RBMY1 and PRY belong to the AZFb region. The deleted azoospermia (DAZ) gene, located in the AZFc region is expressed in all stages of sperm maturation.

It does not seem that the polymorphisms of the Y chromosome haplogroups are related to AZF alterations. A study on a Danish population of oligo-azoospermic men without AZF deletion revealed a significantly higher proportion of haplogroup 26+ compared to the general population, suggesting a mechanism of infertility different from Y microdeletions.

Males affected by this deletion do not come to the andrologist in adulthood because of infertility, a fact clearly linked to the alteration. It has not been shown that there are other developmental abnormalities of primary or secondary sexual characteristics, as well as changes in the hypothalamus–pituitary–gonadal axis. As indicated in the Introduction section, the hormonal profile is currently under debate. There are some items that find differences in some of the parameters studied, differences that could not be reproduced and widely validated. For example, Frydelund-Larsen et al. found an increased value of FSH in the carrier group of the microdeletion, a finding consistent with what was observed in our sample. The Y chromosome microdeletion is not part of any complex syndrome.

It is acceptable to begin the study of Y chromosome microdeletions when the sperm concentration in the semenogram is lower than 2 million/mL, since the possibility of being a carrier of a microdeletion above this number is practically non-existent. The suitable sperm retrieval rate is significantly higher in the microscopic technique (micro-TESE) compared with conventional TESE and it should be offered to carriers of AZFC, but not to carriers of AZFa, AZFb, and AZFbc. For AZFc, the recovery rate by micro-TESE is 54%, being non-existent if the missing region is AZFa or AZFb. The possibility of finding sperm suitable for ICSI is not related to hormone levels or other clinical features in these patients. In 2004, Choi et al. published a study in which they compared the rates of fertilization and pregnancy in patients with AZF compared to a male control group with secretory azoospermia and normal genetic study, showing...
that there were no statistically significant differences in the rates of fertilization and pregnancy for patients with AZFc microdeletion (33.3% vs 37.5%, \( p = 0.57 \)), once the sperm was obtained by TESE or ejaculate.\(^6\)

The diagnostic method of microdeletions adopted by means of convention is the double polymerase chain reaction (PCR) complex,\(^7\) of a sample of peripheral blood, although it is also possible to perform it from a semen sample.\(^8\) Note that in our sample less than one-third of the patients underwent testicular biopsy, since the rest were rejected due to the low possibility of obtaining suitable spermatozoa, so in this sense, our data are limited and little extrapolated. For the same reason, we were unable to draw conclusions about the outcome of the ICSI in these patients.

The complete deletion of the AZFa (5%) leads to the Sertoli-cell-only syndrome. However, the complete deletion of the AZFb (3–10%) results in a syndrome of maturation arrest in the spermatocyte–spermatid stage. Due to the high variability and complexity of the AZFc region, composed mostly of ampiclons, the genetic description of it has been a real challenge. The complete deletion of the c region (60% of Y deletions) produces azoospermia or severe oligozoospermia, with sperm concentration below 1 million/mL. This disorder has a prevalence of 1/4000. However, the partial deletion of the AZFc region can result in a multitude of clinical and histological changes, given its great complexity.\(^9\)

Zhang et al. analyzed a sample of 120 patients with Y microdeletions without finding a common pattern in the histological findings of the patients with AZFc deletion.\(^10\)

The proportion of mutations in our sample is significantly different compared to that reported in other series. AZFa occurred in 27.2% of cases compared to the 5% observed in other studies,\(^11\) to the detriment of AZFb and AZFc. The histological alterations found in patients who underwent TESE match what was described in the literature.

In our sample, the fertility options chosen by our patients (72% chose artificial insemination with donor sperm or adoption) account for the low recovery rate of sperm, making the ICSI only possible in a small percentage of patients. However, when this is possible (AZFc), the overall rate of pregnancies to term shows no difference from the rest of the azoospermic men who opted for ICSI.\(^12\) Moreover, some authors warn that the ICSI involves not only a vertical transmission invariably of alteration to male fetuses, but that it also increases the risk of expansion and new deletions,\(^13\) although the descendants do not have any congenital somatic alteration.\(^2\)

Conclusion

Y chromosome microdeletions are a relatively common cause of secretory azoospermia in infertile men, and they are clinically translated as exclusive germine alteration. The increase in the number of studies and the growing interest in this condition begin to provide sufficient evidence to provide the possibility of performing micro-TESE in these patients to increase the chances of success, for biopsy of both testes, or to expand the study with DNA fragmentation.

Conflict of interest

The authors declare that they have no conflict of interest.

References

