

Original Article

Detection of azoospermia factor (AZF) microdeletion on Y chromosome in infertile men with azoospermia or severe oligozoospermia

Rabea Abol Magd Abobakr, Rashad Mahmoud Mostafa, Somaya Hosny Mahmoud, Hoda Yousry Abdallah, and Gehan Hussein Ibrahim.

Dermatology and Andrology Department Faculty of Medicine. Suez Canal University. Ismailia, Egypt.

Corresponding Author

Rabea Abol Magd Abobakr

Mailing Address: 419th 23July St. (Doshy tower), floor 7; Suez, Egypt.

Tel: 062/3351616-062/ 3334601-062/ 3322087

Mobile: 0020101682598

Email: rabeaabobakr@yahoo.com

Article Info: Date received : 14 / 9 / 2009

Date accepted : 12 / 10 / 2009

Abstract

Background: The origin of severe oligozoospermia and azoospermia is unknown in about 60-70% of cases. Azoospermia can be divided into obstructive and non-obstructive categories, patients with non-obstructive azoospermia are mostly due to genetic causes. Y chromosome deletions are possibly related to this problem. There are a very few Egyptian studies on similar subject, so this study aimed to detect AZF microdeletions and their location on Y chromosome in infertile patients.

Aim of work: was to detect Azoospermia Factor (AZF) microdeletions and their location on Y chromosome in infertile men with azoospermia or severe oligozoospermia and try to correlate these microdeletions with the patient's semen analysis findings, hormonal profile, and testicular histopathology.

Subjects and Methods: 40 patients enrolled in the study were examined for the presence of six STSs in AZF region located on the long arm of Y chromosome by (multiplex PCR) while amplification of SRY gene (sY14) was performed as an internal control. The six STSs used in this study were chosen according to the recommendations of the European Academy of Andrology (EAA), and the European Molecular Genetics Quality Network (EMQN), which proved to be the most frequent hot spots for microdeletions in infertile men.

Results: The AZF microdeletions did not reveal any microdeletions in the six sequence tagged sites (STSs) studied (sY84, sY86, sY127, sY134, sY254 and sY255), with prevalence of 0% AZF microdeletions. Discussion: The absence of microdeletions in our study compared to other studies might be attributed to the few

Key words: Azoospermia ,oligozoospermia, Y chromosome deletions , AZF microdeletions, infertile patients , STSs.

number of STSs selected in our study (6 STSs), the heterogeneity of STSs used among different studies and also due to the ethnic variations among different populations.

Introduction

Abnormalities in sperm production as severe oligozoospermia (presence of less than 10 million spermatozoa/ml in semen analysis⁽¹⁾ and azoospermia represent 10% of infertile males⁽²⁾. The origin of severe oligozoospermia and azoospermia is unknown in about 60-70% of cases⁽³⁾. Azoospermia can be divided into obstructive and non-obstructive categories, patients with non-obstructive azoospermia are mostly due to genetic causes⁽⁴⁾.

Human Y chromosome, one of the smallest chromosomes in the genome, is endowed with about 60 million DNA base sequences. The main function of Y chromosome in the propagation of species is through sex determination and control of spermatogenesis⁽⁵⁾.

Y chromosome deletions that are possibly related to male infertility were first reported in 1976⁽⁶⁾ using chromosome banding technique which revealed deletions on the distal part of the long chromosome arm (Yq) located on Yq11. Furthermore, these findings indicated that the genetic factor responsible for spermatogenesis is also located in this locus (Yq11) of the Y chromosome. This region was defined as the azoospermic factor (AZF)⁽⁷⁾.

Vollrath et al⁽⁸⁾. reported the first Polymerase Chain Reaction (PCR) based sequence-tagged sites (STSs), interval map of the Y chromosome. STSs means short, tagged tracts of Deoxyribonucleic acid (DNA) sequence that are used as landmarks in genome mapping. In most instances, 200 to 500 base pairs of sequence define STS that is operationally unique in the human genome (i.e., can be specifically detected

by the PCR in the presence of all other genomic sequences)⁽⁹⁾.

Three non-overlapping regions, referred to as AZFa, b, c from proximal to distal Yq have been defined as spermatogenesis loci. The loss of one of these loci, caused by spontaneous mutation in the paternal germ line, leads to severely disturbed fertility. The deleted regions are usually of submicroscopic dimensions and are known as AZF microdeletions. Deletions of the AZFc region occur significantly more often than AZFa or AZFb⁽¹⁰⁾.

It remains unclear which of the genes of the AZF region are indeed pathologically relevant as several ones exist as multiple copies which are homologous to a high degree. The so called DAZ (Deleted in Azoospermia) gene cluster in AZFc seems to be the most important⁽¹¹⁾. The proportion of infertile men with deletions in one or more sub regions in the different countries is ranging from 0.7-34.5%⁽¹²⁾.

As AZF microdeletions have an impact on fertility and carry a risk of transmitting these microdeletions to future male offspring when assisted reproductive techniques (ART) are used. Moreover as there are a very few Egyptian studies on similar subject, so this study aimed to detect AZF microdeletions and their location on Y chromosome in infertile Egyptian patients with azoospermia and severe oligozoospermia.

Aim of work

The aim of the study was to detect Azoospermia Factor (AZF) microdeletions and their location on Y chromosome in Egyptian infertile men with azoospermia or severe oligozoospermia and try to correlate these microdeletions with the patient's semen analysis findings, hormonal profile, and testicular histopathology.

Subjects and Methods

This cross sectional analytic study included

40 infertile males who attended the Andrology clinic of Suez Canal University Hospital. Age ranged from 20 up to 45 years having severe oligozoospermia (less than 10 million sperms/ml) or azoospermia according to their semen analysis. Giving no history of exposure to any source of radiation e.g. personnel working in X-ray or computed tomography (C.T.) centers. And their defective spermatogenesis is not secondary to Infection, obstruction of the seminal tract, pituitary failure or any cause of testicular damage. Each patient undergo history taking, physical examination, laboratory investigations, testicular biopsy and genetic analysis.

Four milliliters venous blood was collected from each subject. 2ml in a tube containing EDTA as anticoagulant which kept at -20°C till DNA extraction was performed. The other 2ml were centrifuged and serum was separated and kept at -20°C for hormonal analysis including testosterone, FSH, and LH using a competitive Electrochemiluminescence immunoassay "ECLIA" principle⁽¹³⁾.

Diagnosis of azoospermia was based on the absence of sperm in at least two separate semen analysis and after centrifugation of semen samples at 1000 rpm for 5 minutes. Severe oligozoospermia was diagnosed by the presence of less than 10 million spermatozoa/ml in semen analysis⁽¹⁾. Qualitative and quantitative analysis of germinal epithelium in 25 tubules. Patterns were assessed by Levin classification⁽¹⁴⁾.

All patients enrolled in the study were examined for the presence of six STSs in AZF region located on the long arm of Y chromosome while amplification of SRY gene (sY14) was performed as an internal control. The procedure (multiplex PCR) was done according to Sargin et al⁽¹⁵⁾, in four steps as Genomic DNA Extraction⁽¹⁶⁾, Determination of DNA concentration and purity, Detection of the AZF microdeletions and finally Analysis and Visualization. The six STSs used in this study were chosen according to the recommendations of the European Academy of

Andrology (EAA), and the European Molecular Genetics Quality Network (EMQN), which proved to be the most frequent hot spots for microdeletions in infertile men with low sperm counts⁽¹⁷⁾. We examined six STSs in AZF region located on the long arm of Y chromosome and an internal control in the SRY gene (sY14) in 40 patients; positive (fertile males) and negative control (female subject) were also included.

Ethical Considerations:

- Written consent was taken from every case sharing in the study.
- The participants were informed about the purpose of the study.
- Blood samples were taken on a single occasion to reduce the number of injections.
- All the steps of the test were explained to the patient with all its possible complications.
- All patients' data and test results are confidential.

Results

The present study included 40 infertile male patients, 25 (62.5%) azoospermic and 15 (37.5%) severe oligozoospermic infertile males. The ages ranged from 20-45 years old with mean age 30.9 and standard deviation ± 5.32 . The highest population of patients was between the age of 20-<30 years old, 52.5% of the study population. 50% were smokers or drug addicts but none were alcohol consumers. All patients were married with primary infertility and normal sexual history. had well developed secondary sexual characteristics and 10% had gynecomastia. 7.5% of the patients having small firm testis.

LH and Testosterone levels were normal in most of patients (97.5%) and low in only one patient (2.5%). FSH levels were normal in 57.5% and elevated in 42.5% of patients. No difference among oligozoospermic and azoospermic patients regarding testosterone levels, with a variation in mean and SD values among oligozoospermic and azoospermic patients for both LH and FSH levels (Table 1).

On examining testicular pathology the most frequent finding was maturation arrest, 35% of the patients. Hypo spermatogenesis and germ cell aplasia were equally detected, 22.5% of the patients. The remaining 20% of the patients had normal testicular biopsy (Fig. 1).

Maturation arrest was the most common finding of testicular biopsy among patients with azoospermia as it accounted for 40% (25% of all studied patients) of patients with azoospermia, the second most common finding of testicular biopsy was germ cell absence or aplasia which accounted for 36% (22.5% of all studied patients) of patients with azoospermia. Normal testicular biopsy and hypospermatogenesis had equal percentage 12% each (7.5% of all studied patients) among azoospermic patients.

As for oligozoospermic patients,

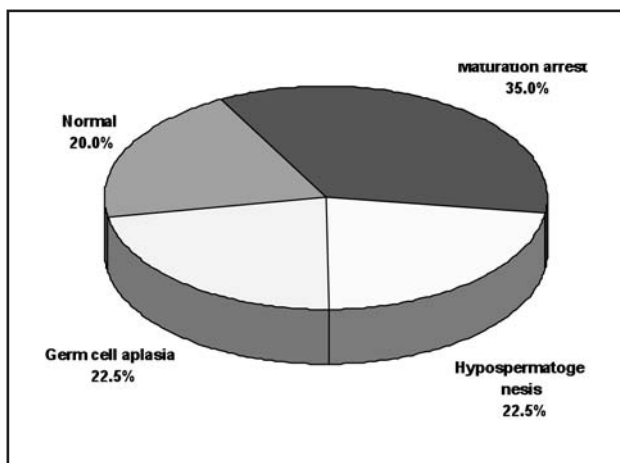


FIG .1. Results of testicular biopsy among the studied patients.

hypospermatogenesis was the most common testicular biopsy finding (40%) (15% of all studied patients) followed by maturation arrest (26.5%) (10% of all studied patients) and finally the normal picture accounted for 33.5% of all oligozoospermic patients (12.5% of all studied patients) with no patients had germ cell aplasia among oligozoospermic patients Table (2).

Half of the patients with normal testicular biopsy had high FSH levels, while, 64.3% of patients with maturation arrest had normal FSH levels and the remaining 35.7% had high FSH levels. In hypospermatogenesis group, 66.7% had normal FSH levels and the rest 33.3% had high FSH levels, finally almost half of patients (55.6%) with germ cell absence or aplasia had high FSH levels and the remaining 44.4% had normal FSH levels Table (3).

The AZF microdeletions in this study did not reveal any microdeletions in the six sequence tagged sites (STSs) studied (sY84, sY86, sY127, sY134, sY254 and sY255), with prevalence of 0% AZF microdeletions.

Discussion

Our results revealed that 62.5% of the infertile male patients were azoospermic and 37.5% were severe oligozoospermic which were close to the other results^(15,18) who found that (78%, 75%) respectively of their infertile patients were azoospermic and 23% 25% respectively were severe oligozoospermic.

FSH levels in our result were elevated in 57.5% which are nearly comparable to previous studies^(18,19). This increase most probably indicates disturbance of seminiferous tubules function with testicular failure and poor prognosis.

In contrast to our results, normal mean FSH

levels were found among infertile male patients with severe oligozoospermia or azoospermia in some studies^(20,21) who reported a mean value of 14.1 mIU/ml and 15.54 mIU/ml respectively. Their explanation was that it might be due to idiopathic non endocrine causes of the infertility among these patients.

AZF microdeletions in this study did not reveal any microdeletions in the six sequence tagged sites (STSs) studied (sY84, sY86, sY127, sY134, sY254 and sY255), which were chosen depending on the recommendations of the European Academy of Andrology (EAA), and the European Molecular Genetics Quality Network (EMQN) as these sites are the most hot spots found to be frequently deleted in infertile men with low sperm counts.

A meta-analysis study⁽²²⁾ has mentioned that the prevalence of Y chromosome microdeletions is ranging from 0.7–34.5% with an average of 8.2%. The after coming studies⁽²³⁾⁽¹²⁾⁽¹⁷⁾⁽²⁴⁻²⁵⁾ concerning AZF microdeletions which have been done in different countries confirmed this meta-analysis.

Surprisingly, a recent study done on Iranian infertile men⁽²⁶⁾ showed a prevalence of 52% of AZF microdeletions in their sample population which were 50 infertile males.

The absence of microdeletions in our study compared to other studies might be attributed to the few number of STSs selected in our study (6 STSs), the heterogeneity of STSs used among different studies and also due to the ethnic variations among different populations. Additionally, a reason for this absence could be that sometimes the abnormality of AZF region is a point mutation rather than a deletion which can not be detected by STSs multiplex PCR method.

Diversity in the testicular biopsy results which were found in the present study were not associated with any type of AZF microdeletions

a conclusion proved previously^(20-22,27) who stated that in general there is no clear correlation between the presence or localization of the deletions and the testicular biopsy pattern.

On the contrary of our results, Dada et al⁽²⁸⁾ correlated the position of the AZF microdeletion with the phase in which spermatogenesis was arrested and suggested that each AZF locus acts at a different phase of spermatogenesis and deletion of each locus causes spermatogenic arrest at a particular stage.

The absence of selected microdeletions in our study with limited number of STSs, and the presence of reasonable incidence in other Egyptian studies like that done in⁽²⁹⁾ on 80 patients involving 20 STSs, Y chromosome microdeletions were detected in only four patients.. and the other⁽³⁰⁾ recently done in 2009 on 100 infertile men, 68 were oligospermic and the remaining 32 were azospermic. Four had microdeletions. The frequency of microdeletions in oligospermic patients was 2/68 (2.9%) (AZFb: sY134 – sY127). The frequency of microdeletions in azospermic patients was 2/32 (6.2%) (AZFa sY86 & AZFb 134); the search for AZF microdeletions with expanding range of STSs, is a mandatory preliminary step to define accurately the etiology of spermatogenic failure and to determine the frequency and site of gene microdeletions and at that point negative results for AZF microdeletions is important and relieving, and can encourage the infertile male patients to proceed with assisted reproduction technology without taking the hazard of transmitting microdeletions to their male offsprings.

Finally, we can suggest that the multiplex PCR described in the present study may be a suitable, cost-effective and less time consuming method for screening the AZF microdeletions which cannot be predicted on the basis of clinical findings or even from the results of semen analysis and has important ethical consequences if the patient is a candidate for assisted reproductive techniques.

Table (1): FSH levels among patients with oligozoospermia and azoospermia.

	FSH		Total
	Normal	High	
	No. (%)	No. (%)	No. (%)
Oligozoospermia	9 (22.5%)	6 (15.0%)	15 (37.5%)
Azoospermia	14 (35.0%)	11 (27.5%)	25 (62.5%)
Total	23 (57.5%)	17 (42.5%)	40 (100.0%)

Table (2): Results of testicular biopsy among oligozoospermic and azoospermic patients.

	Oligozoospermia	Azoospermia	Total
Normal	5 12.5%	3 7.5%	9 20.0%
Maturation arrest	4 10.0%	10 25.0%	14 35.0%
Hypospermatogenesis	6 15.0%	3 7.5%	10 22.5%
Germ cell absence or aplasia	0 0%	9 22.5%	9 22.5%
Total	15 37.5%	25 62.5%	40 100.0%

Table (3): Testicular biopsy results and hormonal profile values among the studied patients.

Testicular biopsy	Testosterone		LH		FSH		Total
	low	Normal	Normal	High	Normal	High	
Normal	0 0%	8 20.0%	8 20.0%	0 0%	4 10.0%	4 10.0%	8 20.0%
Maturation arrest	0 0%	14 35.0%	14 35.0%	0 0%	9 22.5%	5 12.5%	14 35.0%
Hypo spermatogenesis	0 0%	9 22.5%	9 22.5%	0 0%	6 15.0%	3 7.5%	9 22.5%
Germ cell absence or aplasia	1 2.5%	8 20.0%	8 20.0%	1 2.5%	4 10.0%	5 12.5%	9 22.5%
Total	1 2.5%	39 97.5%	39 97.5%	1 2.5%	23 57.5%	17 42.5%	40 100.0%

References

1. Robin HF, Anne ZS, Freya EM, et al. Etiology of azoospermia in a large nonreferral inner-city population. *Fertility and Sterility*, 2006; 86(1): 197.
2. Dohle GR. Azoospermia. In: *Andrology for the clinician*. 1st ed. Springer Berlin Heidelberg, 2006: 81.
3. Mittal RD, Singh G, Srivastava A, et al. Y chromosome micro-deletions in idiopathic infertility from Northern India. *Annales de Génétique*, 2004; 47:331.
4. Roy A, Lin Y, Martin MM. Genetics of Idiopathic male Infertility. In: *The Genetics of male infertility*. 1st ed. Humana press, 2007: 99.
5. Zheng Li, Haines CJ, Han Y. Micro-deletions of the human Y chromosome and their relationship with male infertility. *Journal of Genetics and Genomics*, 2008; 35(4): 193.
6. Tiepolo L, Zuffardi O. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Human Genetics*, 1976; 34: 119.
7. Vergnaud G, Page DC, Simmler MC, et al. A deletion map of the human Y chromosome based on DNA hybridization. *American Journal of Human Genetics*, 1986; 38:109.
8. Vollrath D, Foote S, Hilton A, et al. The human Y chromosome: a 43-interval map based on naturally occurring deletions. *Science*, 1992; 258: 52.
9. Bor P, Hindkjaer J, Ingerslev HJ, et al. Multiplex PCR for Screening of Microdeletions on the Y chromosome. *Journal of Assisted Reproduction and Genetics*, 2001; 18(5): 291.
10. Ferlin A, Arredi B, Foresta C. Genetic causes of male infertility. *Reproductive Toxicology*, 2006; 22: 133.
11. Noordam MJ, Repping S. The human Y chromosome: a masculine chromosome. *Current Opinion in Genetics & Development*, 2006; 6 (3): 225.

12. Hellani A, Al-Hassan S, Iqbal MA, et al. Y chromosome microdeletions in infertile men with idiopathic oligo- or azoospermia. *Journal of Experimental and Clinical Assisted Reproduction*, 2006;3: 1.
13. Wu A. *Tietz Clinical Guide to Laboratory Tests*. 4th ed. WB Saunders, Philadelphia, 2006: 410.
14. Levin HS. Testicular biopsy in the study of male infertility. Its current usefulness, histologic techniques, and prospects for the future. *Human Pathology*, 1979; 10: 569.
15. Sargin CF, Berker-Karatüzüm S, Manguolu E, et al. AZF microdeletions on the Y chromosome of infertile men from Turkey. *Annales de Génétique*, 2004; 47: 61.
16. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*, 1988;16: 1215.
17. Rejeb R, Maazoul F, Trabelsi M, et al. Y chromosome microdeletions in Tunisian infertile males. *Pathologie Biologie*, 2008; 56:111.
18. Geidam AD, Yawe KD, Adebayo AE, et al. Hormonal profile of men investigated for infertility at the University of Maiduguri in northern Nigeria. *Singapore Medical Journal*, 2008; 49: 538.
19. Emokpae MA, Uadia PO, Mohammed AZ, et al. Hormonal abnormalities in azoospermic men in Kano. Northern Nigeria. *Indian Journal of Medical Research*, 2006; 124: 299.
20. Oates RD, Silber S, Brown LG, et al. Clinical characterization of 42 oligospermic or azoospermic men with microdeletion of the AZFc region of the Y chromosome, and of 18 children conceived via ICSI. *Hum Reprod*, 2002; 17: 2813.
21. Peterlin B, Kunej T, Sinkovec J, et al. Screening for Y chromosome microdeletions in 226 Slovenian subfertile men. *Human Reproduction*, 2002; 17: 17.
22. Foresta C, Moro E, Ferlin A. Y chromosome microdeletions and alterations of spermatogenesis. *Endocrine Reviews*, 2001; 22: 226.
23. Mohammed F, Al-Yatama F, Al-Bader M, et al. Primary male infertility in Kuwait: a cytogenetic and molecular study of 289 infertile Kuwaiti patients. *Andrologia*, 2007; 39: 87.
24. Ateyah A, Amer M, Helmy N, et al. Incidence of chromosomal anomalies and Y microdeletions in 50 Egyptian males with non-obstructive azoospermia. *Egyptian Journal of Andrology & Reproduction*, 2002; 16(1): 13.
25. El Awady MK, El Shater SF, Ragaa E, et al. Molecular study on Y chromosome microdeletions in Egyptian males with idiopathic infertility. *Asian Journal of Andrology*, 2004; 6: 53.
26. Malekasgar AM, Mombaini H. Screening of Y chromosome microdeletions in Iranian infertile men. *Journal of Human reproductive Sciences*, 2008; 1: 2.
27. Friel, Houghton JA, Maher M, et al. Molecular detection of Y chromosome microdeletions: an Irish study. *International Journal of Andrology*, 2001; 24: 31.
28. Dada R, Gupta NP, Kucheria K. Yq Microdeletions - Azoospermia Factor Candidate Genes and Spermatogenic Arrest. *Journal of Biomolecular Techniques*, 2004; 15: 176.
29. Shahira Riad Nowier, MM El-sheikh, Hoiyda A Abdel Rasool, et al. Prevalence of Y Chromosome Microdeletion in Males with Azospermia And Severe Oligospermia in Egypt. *Research Journal of Medicine and Medical Sciences*, 2009 ; 4(2): 189.
30. Ali Hellani, Saad Al-Hassan, Adel Al-Duraihim, et al. Y chromosome microdeletions: are they implicated in teratozoospermia? *Human Reproduction Hum. Reprod*, 2005.