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RESEARCH ARTICLE

Molecular and Hormonal Profile of AZF Microdeletions in Senegalese Infertile Men Presenting Non-Obstructive Azoospermia

Boubacar FAYE[1], Mama SY[2], Chantal MAHOU[1], Racha Kamenda IBONDOU[2], Abdou DIOP[1], Aboubacar BA[3], Makhtar NIANG[3], Mame Libasse Laye NIANG[1], Babacar NDIAYE[1], Simon Pierre SAGNA[1], Ndeye Marieme TOP[4], Thierno Abdoulaye DIALLO[1], Cheikh LOUCOUBAR[4], Inès VIGAN-WOMAS[3], Gora DIOP[5], Abdoulaye SECK[1,6]

[1]Medical Biology Laboratory of Pasteur Institute of Dakar, Senegal.
[2]Laboratory of Cytology, Cytogenetics and Reproductive Biology, Faculty of Medecine, Cheikh Anta University of Dakar, Senegal.
[3]Department of Immunophysiopathology and Infectious Diseases, Pasteur Institute of Dakar, Senegal.
[4]Department of Epidemiology, clinical Research and Data Science, Pasteur Institute of Dakar, Senegal.
[5]Department of Animal Biology, Faculty of Science and Technology, Senegal.
[6]Faculty of Medicine, Pharmacy and Dentistry, Cheikh Anta University of Dakar, Senegal.

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Corresponding Author: Boubacar FAYE, Medical Biology Laboratory of the Pasteur Institute of Dakar, Senegal.

Abstract

Introduction: Azoospermia characterized by the total absence of sperm in the ejaculate affects 5 to 15% of infertile men. Genetic abnormalities such as Y chromosome microdeletion are reported with 15 to 20% prevalence within men with non-obstructive azoospermia (NOA). Three regions located on the long arm of the Y chromosome named AZFa, AZFb and AZFc have been the most incriminated.

Objective: The objective of this study was to determine the prevalence of AZF microdeletions within patients with NOA and analyze their hormonal profile;

Material and Methods: Multiplex polymerase chain reaction for AZF microdeletion was performed with hormonal analysis (LH, FSH and Testosterone) using ELISA. 6 STSs markers of chromosome Y microdeletion were used. Comparisons between outcome groups were performed using Student's t-test. Our results revealed a prevalence of 41.2% of cases of microdeletions on the Y chromosome (14/34) with: 7/14 AZFa deletion, 6/14 AZFc deletion and 1/14 AZF ac deletion. The FSH level was significantly higher in patients with Y microdeletion (21.03±5.59IU/L) compared with patients without microdeletion (18.27±12.04UI/L) (p=0.0073). The FSH level was higher in patients with AZFc microdeletions (21.89±7.36) vs patients with AZFa microdeletions (20.68±4.52) or AZFac. Patients with microdeletion had significant higher level of LH concentration (9.12±4.64UI/l vs17.36±0.63UI/L). Testosterone concentration was significantly lower in patient with AZF microdeletion (18.28±14.80nmol/L vs of 14.42±4.58nmol/L).

Conclusion: Regarding the high prevalence of AZF microdeletion assessed in our preliminary study, (41,2%), research of AZF microdeletion appears as a relevant test for the management of NOA in our context and should be proposed in routine.

Keywords: AZF- Male Infertility, Azoospermia -Y Chromosome Microdeletion, - Assisted Reproductive Technology (ART).

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1. Introduction

Infertility is defined as the inability of a couple to conceive a child after 12 months of regular unprotected sexual intercourse [1]. The World Health Organization estimates that around 15% of couples of childbearing age are concerned and male infertility represents 50% of the etiologies **[2,3]**. Male infertility is a multifactorial syndrome encompassing a wide variety of factors. For more than half of infertile men, the etiology is unexplained (idiopathic) and only 4 to 10% are diagnosed with a genetic abnormality; [4, 5]. Molecular studies carried out in severe azoospermic and oligozoospermic men suffering from idiopathic infertility have suggested a correlation between Y chromosome deletions and spermatogenesis abnormalities [1,6,7,8]. The region involved, responsible for Azoospermia or severe Oligozoospermia was called "azoospermia factor locus" or azoospermia factor region; (AZF). Three major loci have been identified in the AZF: AZFa, AZFb and AZFc, containing 16 coding genes involved in the process of spermatogenesis [1,6,7,8]. However, despite the presence of AZF microdeletions, sperm may be available depending on the deleted region of AZF.Indeed a complete deletion of the AZFb region is a negative prognostic factor. On the other hand, in case of AZFc deletion, the chances of finding spermatozoa in the testicular tissue are around 50%. [9]. These patients could benefit from an assisted reproductive technology (ART). In the literature, reported prevalence of microdeletions vary from 3 to 18% with some ethnic variations. [10,11, 12].

The objective of this study was to determine the frequency of AZF microdeletions in the occurrence of non-obstructive azoospermia in infertile Senegalese men received at the medical biology laboratory of the Pasteur Institute in Dakar.

2.Materials and Methods

2.1 Ethical Approval

This study was approved by the Research Ethics Committee (CER) of Cheikh Anta Diop University of Dakar, Senegal under the reference: Protocol 0365/2018/CER/UCAD. Written consent was obtained from each participant.

2.2 Patients

The study was carried out at the medical biology laboratory of the Pasteur Institute in Dakar. Thirty-nine (39) patients were selected for molecular screening of AZF including 34 patients and 5 controls. Participants were selected based on the following criteria: primary infertility, non-obstructive azoospermia and normal karyotyping. Five more patients, with proven fertility diagnosed with normozoospermia were selected for control.

2.3 Semen Analysis

Sperm was obtained by masturbation after a period of abstinence (3 -5 days). Analysis was performed according to the 6th edition of WHO guidelines for semen analysis (version 2021) [2].10 µL of each sperm sample were then examined using the SCA (Sperm Class Analyzer) machine. Azoospermia was confirmed after centrifugation at 12,000 rpm for two minutes and examination of the pellet was done after spreading on a glass slide.

2.4 Molecular Investigation

2.4.1 DNA Extraction

Genomic DNA was extracted from blood samples collected in EDTA tubes using the Zymo Research DNA purification kit (Quick-DNA Miniprep plus Kit), according to the manufacturer's recommendations. The extracted DNA was assessed by nanodrop to determine quantity and quality before being stored at -20ºC.

Reverse) and 8.5 µl of H2O. PCR amplifications were carried out using the Gradient Thermal cycler.

The revelation of the PCR products was carried out on 2% agarose gel dissolved in 1X TBE. The electrophoretic migration conditions were 120 Volts for 35 min.

2.5 Hormonal Analysis

We analyzed the levels of the following hormones: luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone.

The dosages of the hormones FSH and LH were carried out by the ARCHITECT automatic machine using the chemiluminescence technique, while the testosterone dosage was carried out by the VIDAS automatic machine using the ELFA (Enzyme Linked Fluorescent Assay) technique.

2.6 Statistics

Student t test was used to compare continuous variables presented as mean with standard deviation. P value less than 0.05 ($p < 0.05$) was considered as statistically significant. Analysis was performed using R software version 3.2.2 for windows.

Table 1. *STS sites and primer sequences used for the search for microdeletions of the Y chromosome (Simoni et al., 1999)*

Regions	STS	Sequence 5' -3'	Size (bp)
AZFa	sY86-F	GTG ACA CAC AGA CTA TGC TTC	318
	sY86-R	ACA CAC AGA GGG ACA ACC CT	
AZFa	sY84-F	AGA AGG GTC TGA AAG CAG GT	326
	sY84-R	GCC TAC TAC CTG GAG GCT TC	
AZFb	sY127-F	GGC TCA CAA ACG AAA AGA AA	274
	sY127-R	CTG CAG GCA GTA ATA AGG GA	
AZFb	sY134-F	GTC TGC CTC ACC ATA AAA CG	301
	sY134-R	ACC ACT GCC AAA ACT TTC AA	
AZFc	sY254-F	GGG TGT TAC CAG AAG GCA AA	380
	sY254-R	GAA CCG TAT CTA CCA AAG CAG	
AZFc	sY255-F	GTT ACA GGA TTC GGC GTG AT	123
	sY255-R	CTC GTC ATG TGC AGC CAC	

F: Forward, R: Reverse

3. Results

3.1 AZF Microdeletion Profile

The mean age of patients was 38± 4.6 years old.

All patients were screened for AZF microdeletions of Y chromosome. No microdeletions were found in control subjects. Electrophoretic migration profile of multiplex PCRs A and B are represented in figure 1. The PCR results revealed that the STS (Sequence Tagged Site) SY134 and SY127 concerning AZFb region were not amplified.

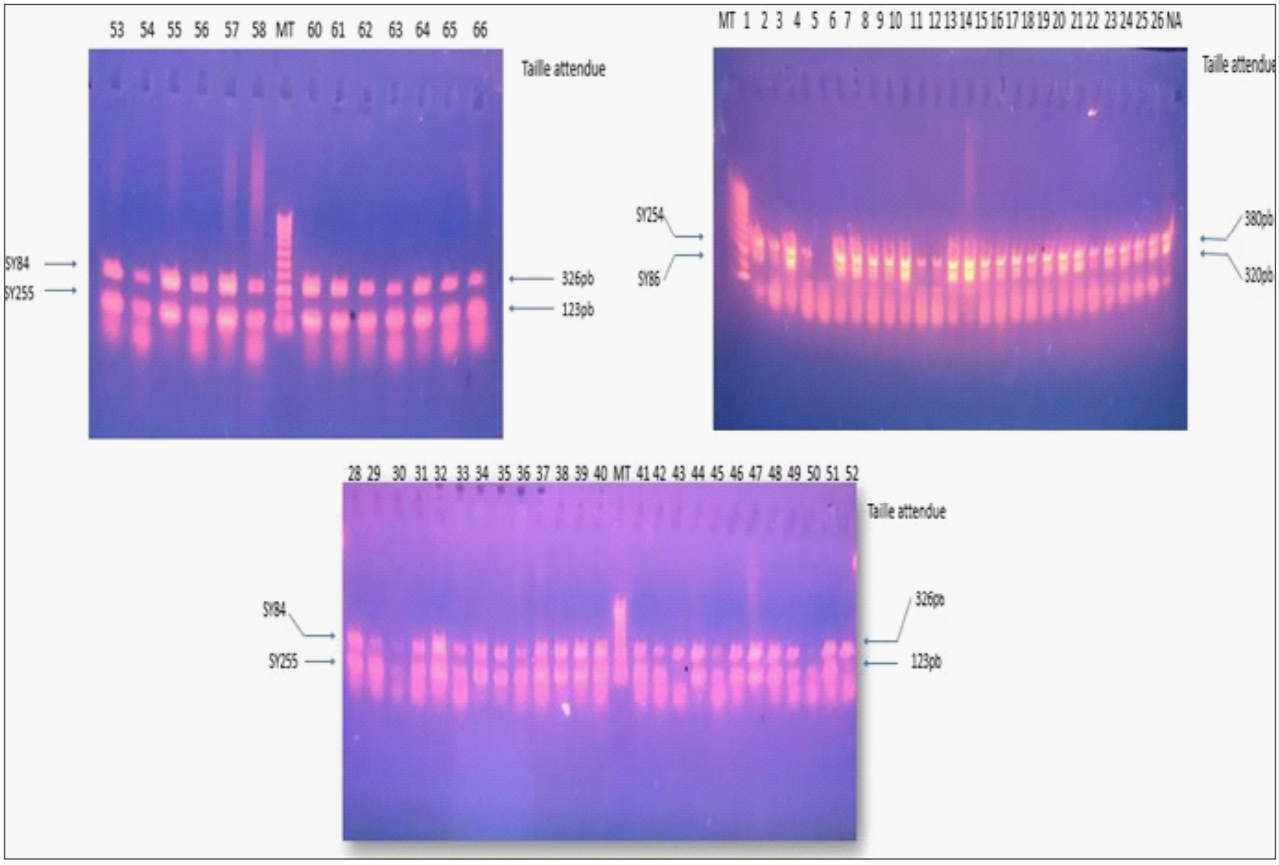

Figure 1. *Electrophoretic migration profile of multiplex PCRs A and B. MT (Size Marker): 100bp*

Fourteen patients (n=14) presented microdeletions of the AZF region (41.18% of the studied samples). Among these 14 patients, AZFa microdeletion was the most frequent with 50% of prevalence (n=7), followed by AZFc microdeletions with 42.86% (n=6). AZFac combination was found with 7.14% of cases (n=1). Some patients presented a combination of microdeletion; thus 6 profile have been identified. (Table 2).

Table 2. *Characterization of Y chromosome microdeletions in patients with non-obstructive azoospermia*

Electrophoresis profiles	AZF (Sequence Tag Sites) microdeletions				AZF deletions observed
	AZFa (SY 84)	AZFa (SY 86)	AZF(SY 254)	AZFc (SY 255)	
Profile A (n= 20)	+	+	+	+	None
Profile B (n= 1)	-	+	+	+	
Profile C (n= 3)	+	-	+	+	Del AZFa
Profile D (n= 3)	-	-	+	+	
Profile E (n= 4)	+	+	+	-	Del AZFc
Profile F (n= 2)	+	+	-	-	
Profile G (n= 1)	-	-	-	-	Del AZFa + AZFc

(+): no deletion and (-): presence of deletion.

3.2 Hormonal Profile

Patients were divided into two groups. The group A concerned patients without microdeletions and the group B patients with microdeletion. FSH level was significantly higher in group B compared to group A (21.03±5.59UI/L vs 18.27±12.04UI/L) (p=0.0073) (Figure 2).

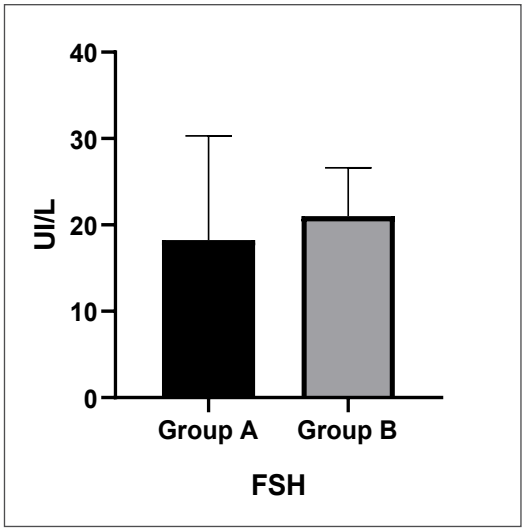

Figure 2. *Profile of FSH levels in group A and B*

The mean level of FSH was higher in patients with AZFc microdeletions (21.89±7.36) compared with patients with AZFa microdeletions (20.68±4.52) and those with AZFac combination (Figure 3). (p=0.8417).

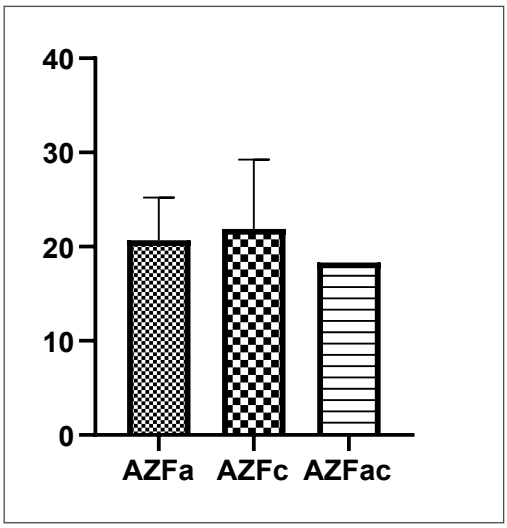

Figure 3. *FSH levels and profile of AZF deletion*

In group B, LH levels were also significantly higher (17.36±0.45UI/L vs 9.12±4.64UI/l), (p<0,05). Testosterone levels were significantly lower in group B (14.42±3.74nmol/L vs 18.28±14.80nmol/L), (p<0,05). Means levels of hormone expressed in groups A and B are presented in table 3.

Table 3. *Hormonal profile and AZF microdeletion*

Patients (n=34)	FSH	LH	Testosterone
Group A: patients without AZF deletion	18.27±12.04UI/L	9.12±4.64UI/l	18.28±14.80nmol/L
Group B: patients with AZF deletion	21.03±5.59UI/L	17.36±0.45UI/L	14.42±3.74nmol/L

4. Discussion

4.1 Prevalence of AZF Microdeletion

The overall prevalence of Y chromosome microdeletion in our series was 41.2%. This prevalence was lower than those found in Sudan in 2021 and in Iraq with 58.8% and 47.8% respectively. However, it was higher than the prevalence found in India in 2014 (13.1%) and in Tunisia (11.1%).

Region	Prevalence	Authors
Senegal	41.2%	Our study
Sudan	58,8%	Elsaid et al., 2021
Iraq	47,8%	Al-Janabi et al., 2020
India	13,1%	Prafulla et al.,2014
Tunisia	11,1%	Chabchoub et al.,2019

4.2 Profile of AZF Microdeletion

The overall prevalence of Y chromosome microdeletion was 41.2% with a predominance of AZFa microdeletion with 50% of cases, followed by AZFc microdeletion with 42.86% and AZFac with 7.14%

We observed a predominance of AZFa microdeletions followed by AZFc in Senegal, as in Sudan. This profile was different in India and Tunisia with the predominance of AZFc microdeletion with respectively 9.83% and 7.41% [12, 21,14,15]. These results could be explained by the differences in ethnic origin of the populations studied and the STS markers used. Since other unidentified factors may also have been involved, additional studies are needed to clarify other causes of Y chromosome microdeletions [16,17,18,13,20]. The probability of finding mature germ cells by testicular sperm extraction (TESE) depends on the type of microdeletion of the Y chromosome. Indeed, a complete deletion of the AZFa or AZFb regions represents a negative prognostic factor, whereas in the case of AZFc deletion or partial deletion of AZFb, the chance of finding sperm in the testicular tissue is 50% [13].

The AZFa microdeletion, associated with SCO Sertoli cell syndrome is more deleterious in terms of spermatogenesis with zero probability of finding sperm on testicular biopsy. On the other hand, microdeletions of the AZFb and AZFc regions are associated with an arrest of maturation or even variable damage to spermatogenesis but still combined with the probability of finding spermatozoa during micro testicular biopsy in order to perform an ART (Assisted Reproductive Technology [19]

The frequency of cases of microdeletions varies from one region to another and could be explained by several factors such as low recruitment of patients included in the study, the recruitment criteria of the subjects, the STS markers used, the different geographical and environmental factors between these different countries may plays a role in the heterogeneity of the results. Differences which may exist concerning professional exposures, ethnicity of the populations studied, as confirmed by studies of Mohammed [16], Vogt [17] and Sachdeva [18].

Indeed, the probable causes of this difference between the different cases of microdeletions of the AZF regions of the Y chromosome could be both genetic and environmental [13, 20]. In our cohort we did not find AZFb microdeletion. This could be a pattern of low prevalence in our population and is to confirm by investigations in a larger cohort

5. Conclusion

The prevalence of microdeletions of AZF regions was 41.2% (14/34) with 7/14 for AZFa and 6/14 for AZFc region. Regarding to the high prevalence of AZF micro deletion in our series, we recommend screening test for Y chromosome microdeletions in the management non-obstructive azoospermia in our context. Therefore, genetic counseling and appropriate guidelines are necessary for the management of this patients specially in the case, a treatment by assisted reproductive technology is programmed.

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