Molecular Detection of Y- Chromosome Micro Deletion Among Infertile Male Patients Attending Some Health Facilities in Selected States of Northwest, Nigeria

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Abstract- Background: ***The aim of the study was to molecularly detect Y chromosome micro-deletions among male infertile patients and established the prevalence of the Y chromosome micro-deletion and among the semen parameters. Methods: A total of 383 infertile males were studied for Y chromosome micro-deletions using multiplex PCR assay. Molecular analysis was performed on the seminal fluid. Results: Out of 383 infertile male patients, 46 (14.00%) had Y micro-deletions. One hundred seven of 765 (13.99%) non-obstructive azoospermic patients and 27 of 133 (20.30%) severe oligozoospermic patients had Y micro-deletions. Among the 134 infertile men with Y micro-deletions, the most frequent micro-deletions were detected in the AZFc region, followed by AZFbc, AZFb, AZFa, AZFabc(Yq), Yp(SRY)+Yq, and partial AZFc regions. Karyotype analysis was available for 130 of the 134 patients with Y micro-deletions. Of them, 36 (27.69%) patients had sex chromosomal abnormalities. Levels of FSH and LH in patients with AZFc micro-deletion were significantly lower, while those in patients with Yp(SRY)+Yq were significantly higher than in patients without Y micro-deletions. Level of testosterone in patients with AZFabc (Yq) or Yp (SRY)+Yq was significantly lower than that in patients without Y micro-deletions. However, there was no significant difference in the levels of reproductive hormones between all patients with and without Y micro-deletions. Conclusion: These results highlight the need for Y chromosome micro-deletion screening for correct diagnosis of male infertility. Obtaining reliable genetic information for assisted reproductive techniques can prevent unnecessary treatment and vertical transmission of genetic defects to offspring. Recommendation: Screening for Y-chromosome micro deletions is highly suggested in fertility clinics where assisted reproduction therapy (ART) viz; IVF etc are conducted to reduced rate of failure as a result of compromised sperm DNA integrity through Y-chromosome micro-deletions.***

Indexed Terms- Male infertility, oligoasthenoteratozoospermia, oligoasthenozoospermia, Severe Asthenozoospermia, Y chromosome micro-deletion, Northwest, Nigeria.

I. INTRODUCTION

Male infertility is the failure of a male individual to cause conception to take place after one year of unprotected sexual intercourse with a healthy fertile female (Albert *et al*., 2014; WHO, 2023). This public health problem affects approximately 10%-15% of couples worldwide, and male related factors are responsible for most of this case (WHO, 2023). Several factors have been implicated in male infertility such as erectile dysfunction, infections, anti-sperm antibodies, exposure to chemical agents and radiations, testicular cancer, varicocele, genetic factors, hormonal abnormalities etc (WHO, 2023).

Therefore, male infertility is a compound problem consisting of several factors. Thus, about 30%-50% of the male infertility cases have unknown causes. Micro-deletion of the azoospermia factor (AZF)

region located on the long arm of the Y chromosome (Yq11) is considered the most common genetic cause of male infertility (Kim *et al*., 2017). The AZF region is divided into three non-overlapping sub regions called AZFa, AZFb, and AZFc, all of which are required for normal spermatogenesis (Kim *et al*., 2017; Suede *et al*., 2023). Micro-deletions in these three regions are associated with various spermatogenetic alterations including Sertoli cell-only syndrome (SCOS), maturation arrest, and hypo spermatogenesis. Specifically, micro-deletion of AZFa is synonymous to SCOS and azoospermia. The absence of AZFb is associated with maturation halt during meiosis, while micro-deletion of AZFc give rise to so many clinical and histologic characteristics, oligozoospermia, oligoasthenoteratozoospermia, asthenozoospermia, oligoasthenozoospermia, asthenoteratozoospermia and SCOS (Vincenzo *et al*., 2022). Extensive studies have been carried on Y microdeletions on patients with abnormal semen parameters such as oligozoospermia, oligoasthenoteratozoospermia, asthenozoospermia, oligoasthenozoospermia, asthenoteratozoospermia and SCOS (Vincenzo *et al*., 2022)., with a reported prevalence of 3% to 28% (Rabinowitz *et al*., 2021; Damdinsuren *et al*., 2022). Therefore, disruption of AZF can be viewed as the most common molecularly diagnosable cause of spermatogenic failure in the setting of individuals with abnormal semen parameyers such as azoospermia, severe oligozoospermia, oligoasthenoteratozoospermia, asthenozoospermia, oligoasthenozoospermia, asthenoteratozoospermia and SCOS (Damdinsuren *et al*., 2022; Hammami *et al*., 2024). However, Y micro-deletions can be transmitted from infertile fathers to their male offspring, who could also experience infertility, through the procedure of ICSI. Thus, it is important to evaluate Y chromosome micro-deletions in male infertility before carrying out assisted reproduction in order to reduce failure rate, emotional stress and time wastage.
All patients filled structured questionnaire, consent form, semen analysis, and molecular Y chromosome micro-deletions analyses. Semen samples were obtained by masturbation and coitus interupt into a sterile container after 3-5 days of coitus abstinence. Specimens were transported at room temperature to the laboratory and analyzed for sperm count, sperm volume, pH, motility, morphology, and detected for the present or absent of fructose according to the guidelines of the WorldHealth Organization (WHO). After semen analysis, patients were categorized into oligoasthrnoteratozoospermia, oligoasthenozoospermia, oligozoospermia, normozoospermia. A structured questionnaire was used to collect information about each subject's medical history and demographic characteristics. Appropriate ethical approval was obtained from the Ethics Committees of the various health facilities. Written informed consent was obtained from each participant before samples were collected. Molecular analysis: Semen samples were collected into sterile clean plain containers and genomic DNA was extracted from the seminal fluids using Zymo DNA extraction Kit (Qiagen GmbH, Germany). Y chromosome micro deletions were detected using multiplex. PCR amplification with specific sequence-tagged sites (STS) using 16 sets of primers. This allowed evaluation of the following sites: sY14 (sex determining region Y, SRY gene) and ZFY (X-linked gene encoding a zinc-finger protein) for reproduction in order to provide appropriate information to patients. The aim of this study was to detect Y chromosome micro deletions by using multiplex polymerase chain reaction (PCR) in 383 infertile males and evaluating the prevalence among semen parameters.

II. METHODS

Subjects and semen analysis: Three hundred and eighty three (383) infertile males samples who were attending any of the selected Health facilities were examined for molecular detection of Y chromosome micro deletions using Gama globulin as control regions; sY254 (DAZ gene; AZFc region; sY186 (AZFa), and sY127 (AZFa) while the second reaction targets sY84 (AZFa), sY134 (AZFb), and sY127 (AZFc). Primers targeting HBB (β-globin; Forward: ACTGGG CATGTGGAGACAGAGA, Reverse: TGTTTCCCATTC TAAACTGTAC) was also used as an amplification control for each reaction. After the PCR, amplicons was separated and detected by gel electrophoresis.

Primers and probes for PCR

Primer	Primer sequence (5′ - 3′)	Band size (bp)
sY86 (Mix 1)	F: GTG ACA CAC AGA CTA TGC TTC R: ACA CAC AGA GGG ACA ACC CT	320
sY127 (Mix 1)	F: GGC TCA CAA ACG AAA AGA AA R: CTG CAG GCA GTA ATA AGG GA	274
sY254 (Mix 1)	F: GGG TGT TAC CAG AAG GCA AA R: GAA CCG TAT CTA CCA AAG CAG C	400
sY84 (Mix 2)	F: AGA AGG GTC TGA AAG CAG GT R: GCC TAC TAC CTG GAG GCT TC	326
sY134 (Mix 2)	F: GTC TGC CTC ACC ATA AAA CG R: ACC ACT GCC AAA ACT TTC AA	301
sY255 (Mix 2)	F: GTT ACA GGA TTC GGC GTG AT R: CTC GTC ATG TGC AGC CAC	126
β-globin gene	F: ACT GGG CAT GTG GAG ACA GAG A R: TGT TTC CCA TTC TAA ACT GTA C	460

(Simoni *et al*., 1999, *Chen et al*., 2022; Zhai *et al*., 2022)

STS multiplex PCR sets and their amplified fragments. PCR was carried out in 10 µl reaction volumes containing 50 ng of genomic DNA, 1×PCR buffer, 1.5 mM MgCl2, 1mM of each dNTP, 10 pmol of each specific primer, and 1 unit of AmpliTaq Gold DNA polymerase (Thermo Fisher, USA). The PCR conditions consisted of an initial denaturation at 95°C for 10min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 62°C for 1 min 30 s, extension at 65°C for 1min 30 s, and a final extension at 65°C for 10 min on an ABI PRISM 2700 thermal cycler (Thermo Fisher). Amplification products were separated by gel electrophoresis on 3% NuSieve gels containing ethidium bromide (0.1 mg/ml) and were visualized under ultraviolet light. To ensure quality of the test, each PCR reaction, and normal female and fertile male DNA samples were used as negative and positive controls, respectively. Water was equally used as blank control to rule out any DNA contamination.

Statistical analysis: Data were expressed as percentages mean (%). Comparisons between outcome groups were performed using Student's t-test for continuous variables and Chi-square test. Statistical significance was considered when $p \leq 0.05$. Statistical analysis was carried out using the Statistical Package for Social Sciences version 22.0 (SPSS Inc., Chicago, IL, USA).

III. RESULTS

Results from the multiplex PCR of Mix 1 and Mix 2 showed an overall Y-chromosome micro-deletion prevalence of 8.0% (n=4). Three (6%) were AZFc Y-chromosome micro-deletion while 1(2%) was AZFb Y-chromosome micro-deletion. The deletion was detected by simultaneous absence of band for the AZF in both Mix 1 and Mix 2. There was an overall prevalence of 14.6% Y-chromosome micro deletions. Normospermic cells reveal no micro-deletion while the oligoasthenoteratospermic cells had the highest number of micro-deletion 29 followed by asthenospermic cells 17 with asthenospermic cell has the least number 9 but have the highest percentage 9(16.67) table 1.

The distribution of the Y-chromosome micro-deletions among primary and secondary infertility indicates that those subject with primary infertility had the highest number of micro-deletion 43 out of 56 Y-chromosome micro-deletions with AZFc as the most dominant while secondary infertility had a lowest total of 13out of 56 with AZFb as dominant and generally the distribution was statistically not significant with P-values of 0.58, 0.70 and 0.23 table 2.

Table 1: Frequency of Y-chromosome micro-deletions among the study population

Parameter	Frequency	Percentage (%)
AZFa Y-chromosome micro-deletion	9	2.35
	19	4.96
AZFc Y-chromosome micro-deletion		
AZFb Y-chromosome micro-deletion	28	7.31
	52	14.6
Total	383	

Table 2: Frequency of Y- chromosome micro-deletion in relation to semen parameters

Parameters	Number (n)	Deletion	Percentage (%)
Normospermia	184	00	0.0
OAT	81	29	7.6
Asthenozoospermia	60	17	4.4
OA	4	01	0.3
AT	54	09	2.3

Key: OAT – Oligoasthenoteratozoospermia, OA – Oligoasthenozoospermia, AT - Asthenoteratozoospermia

4.12: Frequency of Y-chromosome micro-deletion in relation to primary and secondary infertility

PARAMETERS	No. examine	INFERTILITY 1 fertility (%)	2 fertility (%)	$\chi 2$	df	p-value
AZF a deletion	09	7(70.0)	2(20.0)	0.302	1	0.58
No deletion	373	269(72.1)	104(27.9)			
AZF b deletion	19	13(68.4)	6(31.6)	1.152	1	0.70
No deletion	364	264(72.5)	100(27.5)			
AZF c deletion	28	23(82.1)	5(17.9)	1.455	1	0.23
No deletion	355	254(71.5)	101(28.5)			

P > 0.05 – not statistically significant

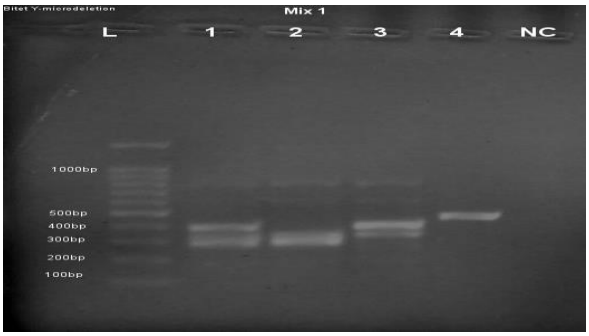

Plate 4.1a: A representative of Agarose gel electrophoresis patterns showing PCR amplification products from multiplex PCR for Y-microdeletion. L: A molecular weight size marker (100bp+). Lane 1: Normal male positive for AZFa, AZFb and AZFc as indicated by 326, 301 and 126 base pairs respectively. Lane 2: positive for AZFa and AZFb as indicated by 326 and 301 base pairs respectively while AZFc is deleted. Lane 3: positive for AZFa and AZFc as indicated by 326 and 126 base pairs respectively while AZFb is deleted. Lane 4: β -globulin Control band (450bp); NC: negative control (Female).

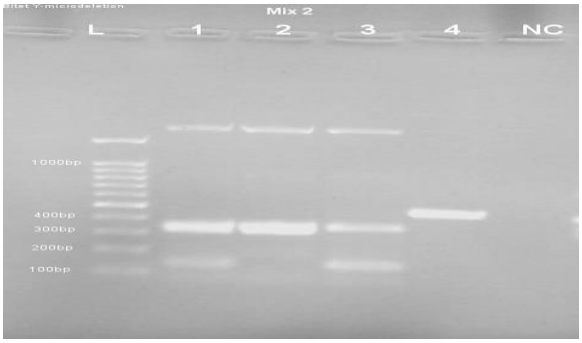

Plate 4.1b: Mix 2: A representative of Agarose gel electrophoresis patterns showing PCR amplification products from multiplex PCR for Y-microdeletion. L:

A molecular weight size marker (100bp+). Lane 1: Normal male positive for AZFa, AZFb and AZFc as indicated by 326, 301 and 126 base pairs respectively. Lane 2: positive for AZFa and AZFb as indicated by 326 and 301 base pairs respectively while AZFc is deleted. Lane 3: positive for AZFa and AZFc as indicated by 326 and 126 base pairs respectively while AZFb is deleted. Lane 4: β -globulin Control band (450bp); NC: negative control (Female).

IV. DISCUSSION

Y- Chromosome micro-deletion plays a crucial role in male infertility (Rabinowitz *et al*., 2021; Damdinsuren *et al*., 2022). Spermatogenesis depend on some genes contained in the region of the AZF, these includes; AZFa, AZFb and AZFc (Roabinowitz *et al*., 2021; Damdinsuren *et al*., 2022). Studies of Tiepolo and Zuffardi, (1976) reveal that Y-Chromosome micro-deletion affect testicular differentiation and maturation, it also play a vital role in predicting sperm extraction from the testes (Damdinsuren *et al*., 2022). Similar findings show that Y- Chromosomes micro-deletions are related to spermatozoa motility and morphology (Damdinsuren *et al*., 2022; Hammami *et al*., 2024).

This study got a prevalence of 14. 8% of Y-Chromosomes micro-deletion among the study population 36(383), AZFa deletion with 9(9/383: 2.35%); AZFb deletion with 19(19/383: 4.96%), and AZFc deletion is with 28(28/383: 7.31%) while normospermia and healthy fertile individual have zero percent deletion in the Northwestern Nigeria and is the most current place where this study was carried out in the geopolitical zone to assess Y- Chromosome micro-deletion among infertile men. AZFc deletion account for the most common deletion and this finding is in concordance with many research outcomes that AZFc is the most common and silent deletion (Hammami *et al*., 2024, Rabinowitz *et al*., 2021; Damdinsuren *et al*., 2022; Chen *et al*., 2023).

We detected AZF deletion frequencies in subjects with Oligoasthenoteratospermia 7.6%, Asthenozoospermia 4.4%, Oligoasthenospermia 0.3%, Asthenoteratospermia 2.3% and Normospermia 0.0%, this finding tallies with previous reports which found prevalence of 1% - 35%depending o9n the male subfertility (Kihaile *et al*., 2005; Hammami *et al*., 2024; Gholami *et al*., 2017; Barisic *et al*., 2021; Rabinowitz *et al.,* 2021; Damdinsuren *et al.,* 2022; Chen *et al*., 2023).

Comparing our current study with other similar published reports, we realized that ethnicity and population plays a role in the variation in the distribution of different Y chromosome micro deletion (Dutta *et al*., 2021). The average prevalence of Y Chromosome micro deletions in South European population (Italy) was about 15%, the prevalence of Y chromosome micro deletion in North European population (France, Netherland, Germany, Sweden and Austria was relatively low between 1% - 4% Dutta *et al*., 2021, Damdinsuren, *et al*., 2022). USA has a prevalence of 9.4% among the infertile men population while Spain has 3.3% prevalence (Dutta *et al*., 2021). Brazil with a prevalence of 10.0% while in the Asian countries such as China, Taiwan, Japan, India, Saudi Arabia and Kuwait had 19.4%, 10.0 – 11.7%, 15.8%, 9.6 – 12.0%, 3.2% and 3.3% respectively among their infertile men populations (Damdinsuren *et al*., 2022). The study among Chinese populations reported a prevalence of 16.9% in infertile male subjects which is relatively closer to our findings (Dutta *et al*., 2021, Damdinsuren *et al*., 2022).

The prevalence of Y chromosome in Sri Lanka was about 1.5%, Pakistan 3.54%, India is 15%, Tunisia and Tanzania in Africa 2.7%, 3.27% respectively(Hammami *et al*., 2014, Dutta *et al*., 2021, Damdinsuren *et al*., 2022).

In relation to types of infertility; this study had primary infertility with the highest number of Y – chromosome micro deletions 43 (11.2%) while secondary infertility had the least 13 (3.4%). This variation could be associated with variation in the number of subjects with primary infertility compared with number of secondary infertility among the study population and those with primary infertility may be genetically inherited while the Y- micro deletions among secondary infertility may be induced from the environment probably due increased usage of herbicide and pesticides within the region (Gabrielsen and Lamb, 2020; *Carsso et al*., 2023); though there are paucity of data on the association between primary and

secondary infertility in relation to Y-chromosome micro deletions.

To the best of our knowledge, there are paucity of previous reports of Y Chromosome micro deletion in the Northwestern Nigerian infertile male population being exposed to such kind of screening, therefore, this could be the first report from the male infertile population of the Northwestern Nigeria that documents an overall prevalence of 14.8% AZF micro deletion which agrees with many published reports globally. This finding correspond with many finding outcomes in the sense that micro deletions are restricted to individual infertile male with abnormal semen parameters (Oligozoosperia, Oligoteratospermia, Asthenospermia, asthenoteratospermia etc) while Normospermia and fertile individual have no microdeletions (Dutta *et al*., 2021, Rabinowitz *et al*., 2021, Damdinsuren *et al*., 2022).

Variations in Y chromosome micro deletions frequency as reported above could be due to sample size fluctuation, selection bias of subjects, differences in diagnostic protocol and methods, choice of sequence tagged sites (STS), differences in demographic and environmental factors (Dutta *et al*., 2021, Rabinowitz *et al*., 2021, Damdinsuren *et al*., 2022).

CONCLUSION

This study demonstrated that Y chromosome micro deletion is a major contributor to azoospermia. Detection of Y chromosome micro deletions is of great use for guiding clinical diagnosis, help in selecting treatment schemes, and reducing the incidence of genetic diseases. In this study, the importance of Y chromosome micro deletion screening and genetic counseling is strongly emphasized for infertile men prior to employment of assisted reproduction techniques.

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