Open Access

Original Article

Evaluation of Hormonal Profile and Y Chromosome Microdeletion in Azoospermic and Severely Oligozoospermic Males Presenting with Primary Infertility

Khizar, Zujaja Hina Haroon, Muhammad Zeeshan Rana, Muhammad Anwar, Muhammad Usman Munir, Muhammad Younas

Department of Chemical Pathology, Armed Forces Institute of Pathology, Rawalpindi/National University of Medical Sciences (NUMS) Pakistan

ABSTRACT

Objective: To assess the frequency of Y chromosome microdeletions in males presenting with azoospermia and oligozoospermia using multiplex polymerase chain reaction and to identify the specific regions of the Y chromosome involved in these microdeletions to determine their association with reproductive hormone levels.

Study Design: Cross-sectional study.

Place and Duration of Study: Departments of Molecular Diagnostics and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from Jun 2021 to Jun 2022.

Methodology: Samples from a total of 112 patients having primary male infertility (76 patients diagnosed with Azoospermia and 36 patients with severe oligozoospermia) were included in this study. All patients underwent testing of serum testosterone, Follicle-stimulating hormone (FSH), Luteinizing hormone (LH) and prolactin. Multiplex PCR was done for detection of Y-chromosome microdeletions. A total of eight Sequence-Tagged Site (STS) markers, including ZFX/ZFY and sex-determining region (SRY) on Yp arm, were used as the internal positive control, as per the recommendations of the European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EQMN).

Results: We detected 3 (2.67%) cases of Y chromosome microdeletions from individuals having azoospermia, all involved AZFc region with no significant correlation found between Y-chromosome microdeletions and levels of reproductive hormones.

Conclusion: AZFc microdeletions were the most common type with better prognosis as compared to AZFa and AZFb deletions. This highlights the role of Y-chromosome microdeletions (YCMD) screening in non-obstructive azoospermic and severely oligozoospermic men. The level of testosterone was also found to be lower in individuals having microdeletions.

Keywords: Azoospermia, Oligozoospermia, Reproductive hormones, Y chromosome microdeletion.

How to Cite This Article: *Khizar, Haroon ZH, Rana MZ, Anwar M, Munir MU, Younas M. Evaluation of Hormonal Profile and Y Chromosome Microdeletion in Azoospermic and Severely Oligozoospermic Males Presenting with Primary Infertility. Pak Armed Forces Med J 2025; 75(4): 703-707. DOI: https://doi.org/10.51253/pafmj.v75i4.11241*

INTRODUCTION

The inability to conceive after 12 months of regular and unprotected intercourse is defined as infertility,[1] amounting to almost 15% of couples globally. Around 20-50% of these infertility cases are attributable to men.[2] Problems in ejecting semen, low sperm levels, abnormal sperm shape (morphology) and movement (motility), along with common lifestyle factors, such as cigarettes smoking, alcohol consumption, psychological stress, increased Body Mass Index (BMI) and advanced paternal age, are associated with male infertility, however, highly heterogeneous histological phenotypes in the testis and semen, with 2,000 genes involved in spermatogenesis alone, the most common genetic reason of male infertility (25%) is azoospermia.[3] Chromosomal aberrations, numerical or structural, are also a frequent cause.[4] Due to the presence of many ampliconic and palindromic sequences on the long arm of the Y chromosome (Yq), it is susceptible to self-recombination and to intra-chromosomal deletion, due to which, Y chromosome genes show copy number variation.[5] Y chromosome microdeletions,[6] occur as Y chromosome is the smallest chromosome, consisting of a long (Yq) and short (Yp) arm, harbouring Sex Determining Region (SRY), which controls spermatogenesis via genes located on Azospermia Factor (AZF) regions,[7] located on long arm of Y chromosome (Yq11), which is essential for normal spermatogenesis as AZF microdeletions cause approximately 14% of oligozoospermia and azoospermia cases. AZF region consists of three non-overlapping loci: AZF a, AZF b and AZF c from proximal to distal end.[8] Microdeletion in AZFb region causes spermatogenic arrest while deletion in AZFc region presents with histological and clinical features ranging from hypo-spermatogenesis to Sertoli-cell only syndrome (SCOS).[7,9] Our aim was to evaluate the prevalence of various Y chromosome microdeletions

Correspondence: Dr Khizar, Department of Chemical Pathology, Armed Forces Institute of Pathology, Rawalpindi Pakistan

Received: 29 Jan 2024; revision received: 02 May 2024; accepted: 12 Jun 2024

using multiplex Polymerase Chain Reaction (PCR) among men who are experiencing primary infertility due to non-obstructive azoospermia and severe oligozoospermia and to explore correlation between Y chromosome microdeletions and reproductive hormone levels.

METHODOLOGY

This was cross-sectional study done at Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from June 2021 to June 2022. Data collection was done after gaining approval from Institutional Review Board vide Reference Number FC-CHP-24/READ-IRB/21/666. Non-probability convenient sampling technique was used and estimated sample size of 112 was calculated through the World Health Organization (WHO) sample size calculator, by keeping confidence interval at 95% and power at 80% with prevalence of Y chromosome microdeletions at 6.1%.[10]

Inclusion Criteria: Adult males aged 18 to 50 years with primary infertility and a sperm count less than 5 million × 106/mL were eligible for inclusion in the study if they provided informed, written consent.

Exclusion Criteria: Individuals with azoospermia caused by obstruction or oligozoospermia resulting from infective or congenital factors were excluded.

Semen analysis was conducted by automated analyser at least twice for each participant. Semen was checked for standard sperm quality parameters (semen count, sperm volume, sperm motility and morphology) as per WHO guidelines. Based on at least two semen analysis reports, participants were divided into two groups: those having no sperm in ejaculate were regarded as azoospermic and ones with sperm count of 5 million ×106/mL or less per ml were labelled severely oligozoospermic. Reproductive hormone profile (total serum testosterone, FSH, LH and prolactin) was measured by chemiluminescence principle using ADVIA Centaur autoanalyzer. For Y-chromosome microdeletion analyses, 2.5 ml peripheral venous blood sample was acquired in EDTA tube. Extraction of genomic DNA was done using commercially available GeneJET DNA Purification Kit (Thermo-fischer) whose yield was tested by NANO DROP spectrophotometer. To diagnose complete AZF deletions accurately, two multiplex polymerase chain reactions were used. The set of PCR primers used consisted of 8 STS (Sequence Tagged Sites) markers which included ZFX/ZFY and sex-determining region (SRY) on Yp arm,[11] as shown in Table-I. On detection of a deletion, the multi-plex PCR was repeated along with the single-plex amplification of that specific marker to confirm the deletion.

Statistical Package for the Social Sciences (SPSS) version 24 was used for statistical analysis. Quantitative variables were examined using descriptive statistics like median and Interquartile Range (IQR), whereas qualitative variables were analysed using percentages and frequency for which comparison between outcome groups was done. Mann-Whitney test was used to compare biochemical parameters (Testosterone, FSH, LH and Prolactin) with Y chromosome microdeletions and independent sample t-test was applied for comparison of reproductive hormone levels with microdeletion where a *p*-value of ≤0.05 showed statistical significance.

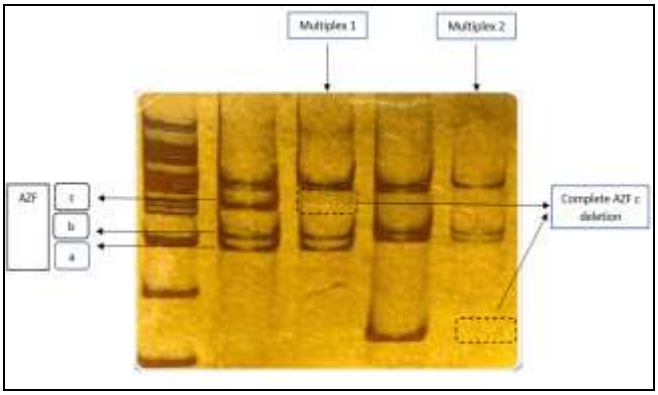

Figure-1: 1st column is DNA ladder,2nd and 4th columns are external controls and Multiplex 1 and Multiplex 2 are patients' samples. In Multiplex 1, sY 254 is absent while Multiplex 2 shows, sY 255 is absent

RESULTS

A total of 112 males with primary infertility were included in the study. The frequency of Y chromosome microdeletions was found to be 2.67%. The median (IQR) age of the infertile males was 32.29(5.49) years. Based on sperm count in semen analysis, 76(75.24%) participants had azoospermia while 36(24.2%) had severe oligozoospermia. Participants were divided into three categories based on age, with 33(33.3%) individuals being <30 years, 62(62.6%) being 30-45 years in age and 4(4%) being >45 years old. The median levels for serum testosterone, FSH, LH and prolactin were 12.3 nmol/L (IQR 8.30), 6.50 mIU/ml (13.80), 5.50 mIU/ml (5.40) and 198 uIU/ml (112) respectively. Y-chromosome microdeletions were noted in 3 participants, with a

Table-I: Gene specification and Primer set for Y Chromosome Microdeletion Analysis, (n=112)

Multiplex PCR Set	STs	Locus	Region	Sequence	Size(bp)
Multiplex 1	sY86, sY127, sY254	DYS273	AZFa	GTGACACACAGACTATGCTTC ACACACAGAGGGACAACCC	318
		DYS218	AZFb	CTAGGCTCACAAACGAAAAG CTGCAGGCAGTAATAAGGG	277
		DAZ	AZFc	GGGTGTTACCAGAAGGCAAAATC GAACCGTATCTACCAAAGCAGC	380
		ZFX	X	CCATTCACACGAAAGACTATCC AGACCTGACTGTAAAATCTCCC	585
		SRY	Yp	GAATATTCCCGCTCTCCGG GCTGGTGCTCCATTCTTGAG	470
Multiplex 2	sY84, sY134, sY255	DYS148	AZFa	AGAAGGGTCTGAAAGCAGG GCCTACTACCTGGAGGCTTC	326
		DYS224	AZFb	GCTTAAAATGTTTGAGAAGCC CATCATGCTATGCACTTCAG	249
		DAZ	AZFc	GTTACAGGATTCGGCGTG CTCGTCATGTGCAGCCAC	123
		ZFX	X	CCATTCACACGAAAGACTATCC AGACCTGACTGTAAAATCTCCC	585
		SRY	Yp	GAATATTCCCGCTCTCCGG GCTGGTGCTCCATTCTTGAG	470

Table-II: Descriptive Statistics (Median, IQR) of Hormonal Profile, (n=112)

Hormonal Profile	Total	Azoospermia	Oligozoospermia	*p*-value
Testosterone (nmol/L)	12.3(8.30)	12.15(8.68)	12.90(7.10)	0.387
FSH (mIU/ml)	6.50(13.8)	6.80(15.05)	5.90(8.60)	0.872
LH (mIU/ml)	5.50(5.40)	5.55(5.80)	4.90(3.20)	0.280
Prolactin (uIU/ml)	198(112)	207(111)	180(110)	0.533
Microdeletions	3	3	0	0.21

**FSH: Follicle-stimulating hormone, LH: Luteinizing hormone*

Table-III: Comparison of Reproductive Hormone Levels with Microdeletion, (n=112)

Hormonal Profile	Micro-deletion		*p*-value
	Yes	No	
Testosterone(nmol/L)	13.67±7.67	13.85±6.44	0.065
FSH (mIU/ml)	19.20±4.40	12.82±18.78	0.068
LH (mIU/ml)	9.87±1.18	7.42±7.21	0.447
Prolactin (uIU/ml)	168.67±22.59	210.72±93.02	0.932

**FSH: Follicle-stimulating hormone, LH: Luteinizing hormone*

frequency of 2.67% overall. All the cases of microdeletions were detected among infertile males with azoospermia. Among the two groups, 3.94% (3/76) of azoospermic patients were having Y-chromosome microdeletion while no microdeletion was detected in males with severe oligozoospermia. Among the participants found to have Y-chromosome microdeletion, AZFc region was involved in all the cases, making it the most common microdeletion being detected. On chromosomal studies, all the patients having microdeletions were found to have normal male karyotype (46XY). In participants with microdeletions, the level of testosterone was 12.6 nmol/L, while the levels of FSH were 6.5 mIU/mL and LH were 5.4 mIU/mL. It was observed that testosterone levels were relatively lower in participants with microdeletions compared to those without deletions, while FSH levels were elevated in

participants with microdeletions compared to those without deletions.

DISCUSSION

In this study, the Multiplex PCR for Y chromosome microdeletions was performed to assess its prevalence among primary infertile males having azoospermia and severe oligozoospermia. In infertile subjects, Klinefelter syndrome is the most common karyotype abnormality, while Y chromosome long arm microdeletions are the most prevalent non-numerical chromosomal abnormality, where occurrence of Y chromosome microdeletions was found to be 2.67%(3/112). AZF region microdeletions of long arm of Y chromosome are among the significant causes of non-obstructive azoospermia and oligozoospermia, presenting as male factor infertility, where Y chromosome microdeletions frequency is approximately 2% in general population, increasing up to almost 16% in cases of azoospermia and severe oligozoospermia,[12] while the occurrence of these Y chromosome microdeletions is reportedly much lower, around 1 in 4000 (0.025%).[13] This correlation between Y chromosome deletions and male infertility, first hypothesized by Zuffardi and Tiepolo in 1976 has now been well established owing to advanced molecular techniques.[14] especially as the frequency of AZF microdeletions (2.67%) detected in our study is similar to ranges reported in other studies, such as, 2.5 % in Qatar,[15] and 2.27% in Saudi Arabia.[16] However, our result is slightly lower than the reported frequency of 16.1% in India,[17] and 20.6% in Iran.[18] A study conducted in Lahore, Pakistan, for frequency of microdeletions reported that no case of Y-chromosome microdeletion could be found while one of the two samples of EAA Quality Control Schemes exhibited deletion.[19] The discrepancy in Y chromosome microdeletion frequency can be attributed to several factors like smaller sample size, selection bias, number and type of markers (STS) used, patient selection criterion, ethnic background of the subjects and environmental or occupational exposure.[20] The possible mechanism that may account for this variation of Y chromosome microdeletions is interplay of genetic and environmental factors where the existence of repetitive DNA sequences may be the most likely explanation for varying degrees of frequency as no recombination happens in AZF region of Y chromosome.[21] All the cases of microdeletions in our study were found in AZFc region making it the most common type of deletion and being associated with both azoospermia and oligozoospermia phenotype, as reported earlier, AZFc microdeletion is invariably the most common type reported in studies.[2,22] It is common for non-obstructive azoospermia patients to have elevated serum FSH and LH, although patients having normal reproductive hormone levels can also carry microdeletions at the AZF loci.[23] In our study, levels of FSH were comparatively higher while levels of testosterone were lower normal in patients with microdeletions. The recent development of techniques like Intracytoplasmic sperm injection (ICSI) and testicular sperm extraction (TESE) can help infertile male partner with azoospermia or oligozoospermia to achieve successful fertilizations and pregnancy.[24]

LIMITATIONS OF STUDY

This study is limited from being generalised to the local population due to its sample size and being a single centre study.

CONCLUSION

This study demonstrated that Y chromosome microdeletion had frequency of 2.67% among primary infertile men in our population while the most common type of microdeletion found was AZFc microdeletion hence highlighting role of YCMD screening in males having non-obstructive azoospermia and severe oligozoospermia.

ACKNOWLEDGEMENT

We would like to acknowledge all those who participated directly or indirectly in the study.

Conflict of Interest: None.

Funding Source: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

K & ZHH: Data acquisition, data analysis, critical review, approval of the final version to be published.

MZR & MA: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

MUM & MY: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. World Health Organization. WHO fact sheet on infertility. Glob Reprod Health 2021; 6(1) : e52. https://doi.org/10.1097/GRH.0000000000000052

2. Oz O. Evaluation of Y chromosome microdeletions and chromosomal anomalies in infertile men. Horm Mol Biol Clin Investig 2021; 42(3): 279–283. https://doi.org/10.1515/hmbci-2021-0003
3. Krausz C, Riera-Escamilla A. Genetics of male infertility. Nat Rev Urol 2018; 15(6): 369–384. https://doi.org/10.1038/s41585-018-0003-3
4. Marzouni ET, Harchegani AB, Layali I. Chromosomal aneuploidies and associated rare genetic syndromes involved in male infertility. J Mens Health 2021; 17(3): 7–17. https://doi.org/10.31083/jomh.2021.016
5. Colaco S, Modi D. Genetics of the human Y chromosome and its association with male infertility. Reprod Biol Endocrinol 2018; 16:14. https://doi.org/10.1186/s12958-018-0330-5
6. Huang IS, Fantus RJ, Chen WJ, Wren J, Kao WT, Huang EY, et al. Do partial AZFc deletions affect the sperm retrieval rate in non-mosaic Klinefelter patients undergoing microdissection testicular sperm extraction? BMC Urol 2020; 20: 183. https://doi.org/10.1186/s12894-020-00587-5
7. Elsaid HO, Gadkareim T, Abobakr T, Mubarak E, Abdelrhem MA, Abu D, et al. Detection of AZF microdeletions and reproductive hormonal profile analysis of infertile Sudanese men pursuing assisted reproductive approaches. BMC Urol 2021; 21(1): 69. https://doi.org/10.1186/s12894-021-00834-3
8. Deng CY, Zhang Z, Tang WH, Jiang H. Microdeletions and vertical transmission of the Y-chromosome azoospermia factor region. Asian J Androl 2023; 25(1): 5. https://doi.org/10.4103/aja2021130
9. Witherspoon L, Dergham A, Flannigan R. Y-microdeletions: a review of the genetic basis for this common cause of male infertility. Transl Androl Urol 2021; 10(3): 1383–1392. https://doi.org/10.21037/tau-19-599
10. Gholami D, Jafari-Ghahfarokhi H, Nemati-Dehkordi M, Teimori H. Y chromosome microdeletions frequency in idiopathic azoospermia, oligoasthenozoospermia, and oligospermia. Int J Reprod Biomed 2017; 15(11): 703–712.
11. Simoni M, Bakker E, Krausz C. EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions. State of the art 2004. Int J Androl 2004; 27(4): 240–249. https://doi.org/10.1111/j.1365-2605.2004.00495.x
12. Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: a committee opinion. Fertil Steril 2015; 103(3): e18–25. https://doi.org/10.1016/j.fertnstert.2014.12.103
13. Krausz C, Hoefsloot L, Simoni M, Tüttelmann F. EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013. Andrology 2014; 2(1): 5–19. https://doi.org/10.1111/j.2047-2927.2013.00173.x
14. Tiepolo L, Zuffardi O. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. Hum Genet 1976; 34(2): 119–124. https://doi.org/10.1007/BF00278879
15. Arafa MM, Majzoub A, AlSaid SS, El Ansari W, Al Ansari A, Elbardisi Y, et al. Chromosomal abnormalities in infertile men with azoospermia and severe oligozoospermia in Qatar and their association with sperm retrieval intracytoplasmic sperm injection outcomes. Arab J Urol 2018; 16(1): 132–139. https://doi.org/10.1016/j.aju.2017.11.019
16. Beg MA, Nieschlag E, Abdel-Meguid TA, Alam Q, Abdelsalam A, Haque A, et al. Genetic investigations on causes of male infertility in Western Saudi Arabia. Andrologia 2019; 51(6): e13272. https://doi.org/10.1111/and.13272
17. Dutta S, Paladhi P, Pal S, Bose G, Ghosh P, Chattopadhyay R, et al. Prevalence of Y chromosome microdeletion in azoospermia factor subregions among infertile men from West Bengal, India. Mol Genet Genomic Med 2021; 9(10): e1769. https://doi.org/10.1002/mgg3.1769
18. Bahmanimehr A, Zeighami S, Jahromi BN, Anvar Z, Parsanezhad ME, Davari M, et al. Detection of Y chromosome microdeletions and hormonal profile analysis of infertile men undergoing assisted reproductive technologies. Int J Fertil Steril 2018; 12(2): 173–178. https://doi.org/10.22074/ijfs.2018.5183
19. Siddique A, Qureshi HJ, Siddique S, Husnain A. Y-Chromosomal microdeletions in a local infertile male population of Pakistan. Pak J Physiol 2009; 5(1): 29–33. https://doi.org/10.69656/pjp.v5i1.694
20. Sachdeva K, Saxena R, Majumdar A, Chadda S, Verma IC. Use of ethnicity-specific sequence tag site markers for Y chromosome microdeletion studies. Genet Test Mol Biomarkers 2011; 15(6): 451–459. https://doi.org/10.1089/gtmb.2010.0213
21. Zaimy MA, Kalantar SM, Sheikhha MH, Jahaninejad T, Pashaiefar H, Ghasemzadeh J, et al. The frequency of Yq microdeletion in azoospermic and oligospermic Iranian infertile men. Iran J Reprod Med 2013; 11(6): 453–458.
22. Fayez A. Molecular characterization of some genetic factors controlling spermatogenesis in Egyptian patients with male infertility. Infertil 2012; 3(3): 69–77. https://doi.org/10.5005/jp-journals-10016-1045
23. Zhang F, Li L, Wang L, Yang L, Liang Z, Li J, et al. Clinical characteristics and treatment of azoospermia and severe oligospermia patients with Y-chromosome microdeletions. Mol Reprod Dev 2013; 80(11): 908–915. https://doi.org/10.1002/mrd.22226
24. Kleiman SE, Yogev L, Lehavi O, Hauser R, Botchan A, Paz G, et al. The likelihood of finding mature sperm cells in men with AZFb or AZFb-c deletions: six new cases and a review of the literature (1994–2010). Fertil Steril 2011; 95(6): 2005–12. e4. https://doi.org/10.1016/j.fertnstert.2011.01.123