International Journal of Medical, Pharmacy and Drug Research (IJMPD)
Article DOI: https://dx.doi.org/10.22161/ijmpd.7.1.4
Peer-Reviewed Journal Int. J. Med. Phar. Drug Re., 7(1), 2023
ISSN: 2456-8015

Detection molecular for microdeletions of the Y chromosome in IVF technique

Mustafa Faeq Kazem Hussein*[1], Israa Sami Othman[2], Mohammed Abdulfatah Al-Ayashn[3], Abbas Abdul Wahhab Jumaah Al-Salihi[4] , Khaleel Ibrahim Ismael[5]

[1]Iraqi Ministry of Interior, Iraqi Police College, Department of Educational Affairs
[2]M.Sc. / Master's Degree Microbiology / Bacteria , Iraqi Ministry of Education, General Directorate of Education, Basra, AL-Yamama Prep School for Girls, Basra, Iraq.
[3]Iraqi Ministry of Health, Baghdad Health Directorate Al-Karkh, AL-Furat General Hospital, Baghdad
[4]Department of Applied Embryology, High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Nahrain University, Kadhimiya, Baghdad, Iraq
[5]Department of Clinical Reproductive Physiology, High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Nahrain University, Kadhimiya, Baghdad

Received: 15 Jan 2023; Received in revised form: 12 Feb 2023; Accepted: 22 Feb 2023; Available online: 28 Feb 2023

Abstract

Y chromosome microdeletion tests are the basis of success IVF technique to disclose the real problem for patients males (oligozoospermia) to reproductive treatment. The present study to showed the relationship between microdeletion and IVF technique, then early detection of Y chromosome microdeletion is auxiliary to venereal treatment, particularly in those young patients with AZFc deletion. The study was used 65 patients, males (oligozoospermia) and ten normal males, as control. DNA was recovered and treated, which was used for Y (CMD) through PCR SYBR Green real-time PCR for the amplification of three loci AZFa, AZFb, and AZFc regions. Our results showed only six samples (9 %) of single micro-deletions in the Y chromosome. Finally, the early diagnosis is very important for oligozoospermia patients whose will undergo the IVF technique. Kew word: Y chromosome, IVF technique, SYBER Green, Oligozoospermia.

Keywords*— *IVF technique, Y chromosome, DNA, Microdeletion, AZFa, AZFb, and AZFc.

I. INTRODUCTION

Y chromosome is considered the genetic cause that is the most spread for male infertility. According to published research, the prevalence of Y microdeletion in infertile males is roughly 7 %, with a range of 1-35 %. (1,2). From proximal to distal Yq, the long arm of the Y chromosome was found to include three AZF regions, namely AZFa, AZFb, and AZFc (3).

The Azoospermia Factor was considered as the second genetic reason for the spermatogenic failure of infertile males, where treatment of that infertile individuals' which causes in regions that represent AZFa, AZFb, and AZFc (4).In situations with substantially impaired spermatogenesis, a Y chromosomal microdeletion test is recommended. Improvements in molecular biology methods have made regular diagnosis more accessible. However, not all IVF clinics detect Y chromosome microdeletions (5).The "Azoospermia Factor" (AZFa, AZFb, and AZFc) regions are all linked to

spermatogenesis and are typically altered in 13 % of people. (6,7,8)

II. MATERIAL AND METHOD

5 ml Whole blood samples were collected from 65 patients with severe oligozoospermia and ten normal males as controls at Baghdad Center for Infertility in Baghdad city and kept at _20° C until use.

Extraction of DNA. Whole blood was extracted from 200 l of samples using a blood DNA extraction spin kit (Wizbiosolutions, Korea) according to the manufacturer's instructions, and in the last stage (100 l) of elution buffer was added and stored frozen at _20° C until use.SYBR of Green real-time which was implemented to find out microdeletions in these sections, which are AZFa, AZFb, and AZFc for AZF region in the chromosome, which these operations find out by DBY gene and RBMY gene, as well as DAZ

Primer designs

In this research, primers were created utilizing bioinformatics tools from worldwide databases (NCBI and IDT) and a variety of resources available on the internet (online tools and software) (table 1).

Table 1: Primers were used for qPCR detection and SYBR GREEN.

Detection site	Name	Primers	T. m
SY254	AZFc (DAZ1)	F: GCGGAATCCAAACACTGAAAC R: ACAGAGGGAAGGATGACTAGAA	62
SY255	AZFc (DZA1)	F: CGTGTTCGTCATTTGAGCTAAG R: GAGGTGAAAGAGGCAGAGATAG	62
SY127	AZFb (RBMY)	F: AGGGCCTCGGATGTCTTAT R: CTCTCCCAACTTCTGCCATATC	62
SY134	AZFb (RBMY)	F: GATATGGCAGAAGTTGGGAGAG R: AGAGAAGGCGGATTCCTTTG	62
SY 84	AZFa (DBY)	F: GTTGGCACCGTTCTTTCTAAAC R: AGGATTACACCCAAACAGGTG	62
SY 86	AZFa (DBY)	F: CACCTGTTTGGGTGTAATCCT **R:** GGCGTCCATACCTTCCATTT	62
SRY	Control	F: GGAGAAGCTCTTCCTTCCTTTG R: CTATCCTGGACGTTGCCTTTAC	62

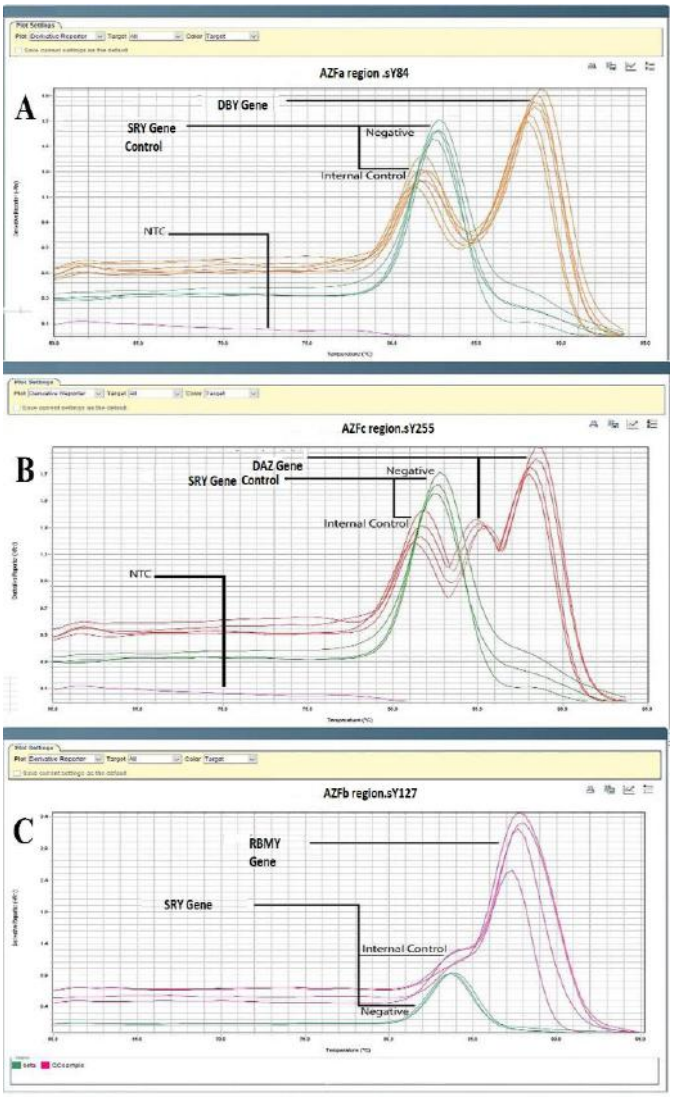
In the investigations and implementation of the Y chromosome by using qualitative SYBR of Green PCR.

The magnitude that utilized the SYBR Green Pcr amplification was conducted out in all situation DNA tests utilizing 20 µL response mixtures comprising one µL of DNA Sample, 0.5 µL (for DBY, RBMY, DAZ, and SRY) of the various specific primers, ten µL 1 X Wizpure qPCR master (SYBR) with rox dye (which also included Taq DNA polymerase, dNTP mix, 2.5 mM MgCl2, and SYBR Green I dye) (Wizbiosolutions, Korea) and 0.4 µL ROX source dye finally introduced RNase -Free water up to 20 µL. The multiplication settings were set using software version 8.0.1.8 and began with a 300-second incubation at 95°C to activate the DNA Polymerase, followed by 40 cycles of 30 seconds of denatured proteins at 95°C, 30 seconds annealed at 60° C, and 10 seconds elongation at 72°C (Real-time PCR analyzer.

III. RESULTS

Table 2: The micro-deletion in DBY, RBMY, DAZ, and control genes with CT value and number of patients.

Detection site	Name gene	Result	CT value	Number patient
SY84,SY86	AZFa	Deleted	27.93	15
SY127,SY134	AZFb	Deleted	30.65	31
			25.90	27
SY254,SY255	AZFc	Deleted	27.94	18
			26.44	51
			33.92	60
Control	SRY	No deleted	29.86	66



The oligozoospermic individuals in our trial with AZFc microdeletion will most likely be able to produce sperm by self-ejaculation and hence have the next generation through IVF. However, the quality and quantity of sperm in AZFc microdeletion individuals decreased with age. This shows that early detection of Y chromosomal microdeletions might help guide reproductive therapy. The sooner we receive the inspection, the more time we will have to devote to clinical infertility therapy. As we all know, the genetic background may have a role in IVF results (figure 1). As a result, the existence of genetic illnesses, such as Y chromosome microdeletion, has a significant impact on infection in aided venereal medicine (9).

Figure (1): Real-time PCR using SYBER Green to four genes: A) The DBY gene in the AZFa region (detection site SY84), B) The DAZ gene in the AZFc region (detection site SY255), and C) The RBMY gene in AZFb (detection site SY127).

Optimization

A comprehensive test was conducted in which the ability of the primer to amplify each gene at the annealing temperature, which ranges between 58 - 65 ° C, by using DNA from a fertile man, as it is considered as a positive control during its use in the development stage.

IV. DISCUSSION

In our analysis, we noticed that six samples (9 %) exhibited single micro-deletions in the Y chromosome as well as certain hazardous locations inside the Y chromosomes long arm. As a result, this study was meant to investigate the relationship between these microdeletions and IVF, as well as how early diagnosis benefits Y chromosome patients

seeking reproductive therapy. The current study discovered just four (4 out of 40) single microdeletions in the Y chromosome. There were two of these microdeletions in the RBMY gene, one in the DBY gene and one in the DAZ gene. There were no Y chromosomal deletions in any of the 40 control samples tested from fertile men (0 %). This study in Egyptian populations with, comparing our results, shows a high rapprochement in the proportion of microdeletions in the Y chromosome, particularly in the DBY and RBMY genes, and a small difference in the ratio in the DAZ gene because the number of samples in our study is greater than the current study (10). There was also evidence of another ongoing probe. Three of the patients evaluated had partial microdeletions in the AZFa region, with an 8.8 % frequency, in the G34990 section (one patient) and the sY85 section (two patients) by using triplex qPCR with Eva Green DNA-binding dye to determine the melting temperature (Tm) of the STS previously with only one patient deletion in Sy 84 at the AZFa region (11). The prevalence of Y chromosomal microdeletion has been reported to be 5.42 %, 8 %, 7.7 %, and 10.8 % in Turkish, Iranian, Korean, and Chinese individuals, respectively, showing that the incidence of Y chromosomal microdeletion in infertile males varies according to cultural and geographical variances (12,13,7). With a frequency of 62.2 % in our investigation, we determined that AZFc is the most common AZF microdeletion type in all patients with Y chromosomal microdeletion in southwest China (14).

V. CONCLUSION

The microdeletion of the Y chromosome test is significant for oligospermia patients undergoing IVF to determine the success of the operation will be successful. The early identification of Y chromosomal microdeletions in infertile men not only illuminates the pathogenesis of oligospermia but also assists in the patient involvement for both the infertile man and his future male children.

REFERENCES

[1] Foresta C, and Moro E, Ferlin A. Prognostic value of Y deletion analysis. The role of current methods. Hum Reprod. 2001;16 (8):1543–7.

[2] Foresta C, and Moro E, Ferlin A. Y chromosome micro deletions and alterations of spermatogenesis. Endocr Rev. 2001;22 (2):226–39.

[3] Vogt PH, Edelman A, Kirsch S, et al. Human Y chromosomeazoospermia factors (AZF) mapped to different sub regions inYq11. Hum Mol Genet. 1996;5 (7):933–43.

[4] Buch B, Galán JJ, Lara M, Ruiz R, Segura C, Real LM,Martínez-Moya M, Ruiz A. Scanning of Y-chromosomeazoospermia factor loci using real-time polymerase chainreaction and melting curve analysis. Fertil Steril. 2003;80:907-13.

[5] Laboratoire de Biologie de la Reproduction, HoÃpital Nord, 42055 Saint Etienne, 2Laboratoire de GeÂneÂtique Mole Âculaire and FaculteÂ de. Y chromosome micro deletion screening in infertile men in France: survey of French practice based on 88 IVF centres. Human Reproduction Vol.19, No.4 pp. 788±793, 2004.

[6] Yuen R, Merkoulovitch A, MacDonald JR, Vlasschaert M, LoK, Grober E, Marshall CR, Jarvi KA, Kolomietz E, and SchererSW. Development of a high-resolution Y-chromosome microarray for improved male infertility diagnosis. Fertil Steril.2014;101:1079-85.e3.

[7] Zaimy M, Kalantar SM, Sheikhha MH, Jahaninejad T,Pashaiefar Ghasemzadeh J, Zahraei M. The frequency of Yq micro deletion in azoospermic and oligospermic Iranian infertile men. Iran J Reprod Med. 2013;11:453-8.

[8] Kozina V, Cappallo-Obermann H, Gromoll J and Spiess AN. A One-Step Real-Time Multiplex PCR for Screening Y Chromosomal Micro deletions without Downstream Amplicon Size Analysis. Plos One. 2011; 6:e23174.

[9] Zhu XB, Gong YH, He J, et al. Multicenter study of Y chromosome micro deletions in 1,808 Chinese infertile males using multiplex and real-time polymerase chain reaction. Andrologia 2017;49:12726–32.

[10] Said M.M., Fahmi A.A., Hemeda H.M., Aly B., AbdelAal R.H., Nasr S.M., El Gamal M., Fawzy I.O., El Tayebi H.M. and Abdelaziz A.I. Detection of Ychromosome micro deletions in recurrent abortions among Egyptian females using SYBR Green Real Time PCR. Journal of American Science 2013;9 (9):150-156]. (ISSN: 1545-1003).

[11] Tatiana Puga Torres1, Xavier Blum Rojas1, Medardo Blum Narváez1, Edith López Montanero2, Alexandra NarváezSarasti2. Triplex Real-time Polymerase Chain Reaction Optimization for AZF Y-chromosome Micro deletion Analysis. JBRA Assisted Reproduction .2015;19 (2):37-43

[12] Fu L, Xiong DK, Ding XP, et al. Genetic screening for chromosomal abnormalities and Y chromosome

microdeletions in Chinese infertilemen. J Assist Repro'd Genet 2012;29:521–7.

[13] Dincer M, Karahasanoglu A, Uzun Cilingir I, et al. Frequency of Y chromosome microdeletions and chromosomal abnormalities I infertile Turkish men. Genet Couns 2013;24:431–4.

[14] Ting Liu, MD, PhDa,b,*, Yu-Xin Song, MDa, Yong-Mei Jiang, MD, PhDa . Early detection of Y chromosome micro deletions in infertile men is helpful to guide clinical reproductive treatments in southwest of China. Medicine. 2019; 98:5.