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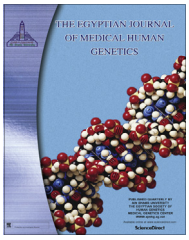

ORIGINAL ARTICLE

Pilot study for early prognosis of Azoospermia in relation to Y-STR Profiling

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KEYWORDS

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Abstract *Background:* Azoospermia constitutes 20% of male infertility situations and affects 1% of the total male population (Jarvi et al., 2010). This condition is classified into three major types; pre-testicular, testicular and post-testicular Azoospermia (Sermondade et al., 2012). Genetic defects causing Azoospermia are due to chromosomal or non-chromosomal alterations on the Y-Chromosome (Lee et al., 2011). Initial diagnosis of Azoospermia is established when no spermatozoa are detected on microscopic examination of semen (World Health Organisation, 1999).

Objective: To evaluate the correlation of Y-STR Profiling results and the prevalence of Azoospermia, to help for early prognosis of Azoospermia before puberty.

Methods: Buccal swab samples were taken from two groups of individuals (50 fertile and 50 Azoospermic patients), then DNA was isolated using QIAamp DNA Micro kit. DNA quantification was done using a Real-time PCR utilizing Quantifiler Kit. PCR was done using PowerPlex® Y PCR Amplification Kit, then amplified products were typed using a 3130 Genetic Analyzer.

Results: Five haplotypes in four different Y-STR loci were found to possess significantly higher occurrence percentages in Azoospermic than in fertile Saudi individuals, which can serve as a group of pre-diagnostic markers for early prognosis of Azoospermia in Saudi population.

Conclusion: There was a significant correlation of Y-STR Profiling results and the prevalence of Azoospermia condition, which supports the idea of using Y-STR Profiling in early prognosis of Azoospermia.

1. Introduction

Infertility is defined as a failure to conceive in a couple trying to reproduce for a period of 2 years without conception. Approximately 15% of couples are infertile, and among these couples, male factor infertility accounts for approximately 50% of causes. Male infertility is a multifactorial syndrome encompassing a wide variety of disorders. Male infertility is most likely the result of deletions and/or mutations of one or more of the myriad of genes required for spermatogenesis [1]. Azoospermia is the most severe form of male infertility, it is the medical condition of a man not having any measurable level of sperm in his semen. In humans, Azoospermia affects

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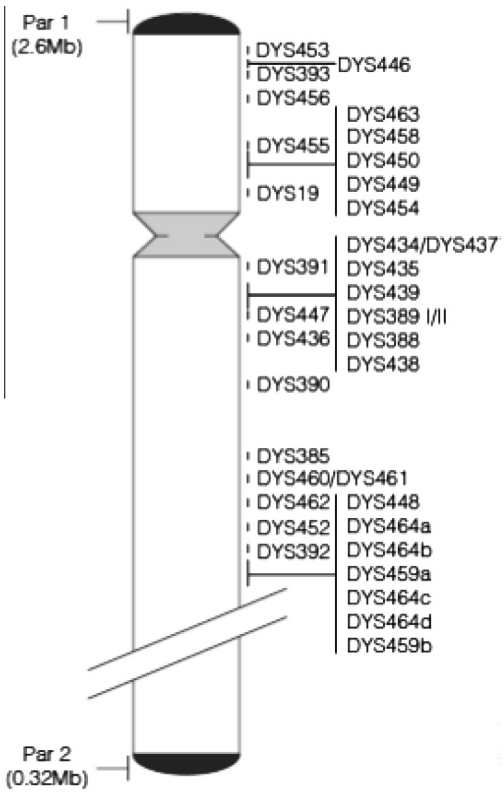

Figure 1 Relative positions of Y-STRs on human Y-Chromosome [20].

about 1% of the male population and may be seen in up to 20% of male infertility situations [2].

Azoospermia can be classified into three major types; pre-testicular Azoospermia which is characterized by inadequate stimulation of otherwise normal testicles and genital tract. Typically, follicle-stimulating hormone (FSH) levels are low (hypogonadotropic) commensurate with inadequate stimulation of the testes to produce sperm. This condition is seen in about 2% of Azoospermia patients [3]. Testicular Azoospermia in which the testes are abnormal, atrophic, or absent, and sperm production is severely disturbed to absent. FSH levels tend to be elevated (hypergonadotropic) as the feedback loop is interrupted (lack of feedback inhibition on FSH). This condition is seen in 49–93% of men with Azoospermia [2]. Post-testicular Azoospermia in which sperms are produced but not ejaculated. This condition affects 7–51% of Azoospermic men. Idiopathic Azoospermia is the condition in which there is no known cause of Azoospermia. It may be a result of multiple risk factors, such as age and weight [4].

Y Chromosome is one of the smallest human chromosomes and consists of a short (Yp) and a long (Yq) arm. The Y Chromosome is male-specific, 60 megabases (Mb) in size, consisting of 60 million nucleotides, but has the least number of genes compared to any other chromosome. Of the 27 Y Chromosome genes identified, nine are located on the Yp and the remaining 18 on the Yq [5]. In 1976, Tiepolo and Zuffardi were the first to hypothesize a correlation between Y Chromosome deletions and male infertility [6].

The initial diagnosis of Azoospermia is established when no spermatozoa can be detected on high-powered microscopic examination of a pellet after centrifugation of the seminal fluid on at least two separate occasions. The World Health Organization (WHO) recommends that seminal fluid be centrifuged for 15 min at 3000×*g* or greater [7].

Genetic defects causing Azoospermia were categorized into two large categories: chromosomal and nonchromosomal. Chromosomal defects were further categorized into (1) structural abnormalities, such as Y Chromosome micro/macrodeletions, chromosomal inversions, and translocations; and (2) numerical abnormalities, also known as aneuploidy. Nonchromosomal defects included sperm mitochondrial genome defects and epigenetic alterations of genome [8].

Table 1 Fisher's exact test for comparing the percent of Y-STR haplotypes detected in Azoospermic and fertile individuals in the Y-STR locus (DYS19).

Y-STR locus	Haplotype	Group	No. of observations	Haplotype frequency	Estimate for difference	*Z*	*P*-value
DYS19	10	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	11	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	12	Azoospermic	1	0.02	0	0	0.500
		Fertile	1	0.02			
	13	Azoospermic	5	0.10	6	1.18	0.118
		Fertile	2	0.04			
	14	Azoospermic	37	0.74	−0.02	−0.23	0.591
		Fertile	38	0.76			
	15	Azoospermic	2	0.04	−0.12	−2.04	0.979
		Fertile	8	0.16			
	16	Azoospermic	4	0.08	0.06	1.39	0.082
		Fertile	1	0.02			
	17	Azoospermic	1	0.02	0.02	1.01	0.156
		Fertile	–	–			
	18	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	19	Azoospermic	–	–	–	–	–
		Fertile					

Table 2 Fisher's exact test for comparing the percent of Y-STR haplotypes detected in Azoospermic and fertile individuals in the Y-STR locus (DYS385a).

Y-STR locus	Haplotype	Group	No. of observations	Haplotype frequency	Estimate for difference	*Z*	*P*-value
DYS385a	7	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	8	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	9	Azoospermic	–	–	–	–	–
		Fertile	–	–	–	–	–
	10	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	11	Azoospermic	2	0.04	0.02	0.59	0.279
		Fertile	1	0.02			
	12	Azoospermic	1	0.02	−0.04	−1.03	0.848
		Fertile	3	0.06			
	13	Azoospermic	33	0.66	0.02	0.21	0.417
		Fertile	32	0.64			
	14	Azoospermic	3	0.06	0.02	0.46	0.323
		Fertile	2	0.04			
	15	Azoospermic	3	0.06	0	0	0.500
		Fertile	3	0.06			
	16	Azoospermic	4	0.08	−0.02	−0.35	0.637
		Fertile	5	0.10			
	17	Azoospermic	2	0.04	0.02	0.59	0.279
		Fertile	1	0.02			
	18	Azoospermic	2	0.04	−0.02	−0.46	0.677
		Fertile	3	0.06			
	19	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	20	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	21	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	22	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	23	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	24	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	25	Azoospermic	–	–	–	–	–
		Fertile	–	–			

Chromosomal abnormalities can be identified by karyotype of peripheral leukocytes in approximately 7% of infertile men. The prevalence of such abnormalities relates inversely to the sperm concentration; the prevalence is 10–15% in Azoospermic men. Sex chromosomal aneuploidy (Klinefelter syndrome) accounts for approximately two-thirds of chromosomal abnormalities observed in infertile men [9].

Microdeletions of the Y-Chromosome may be found in 10–15% of men with Azoospermia. Such microdeletions are too small to be detected by karyotyping but can be identified using PCR techniques to analyze sequence-tagged sites that have been mapped along the entire length of the Y-Chromosome [10]. Genetic counseling may be offered whenever a genetic abnormality is suspected, in either the male or the female partner, and should be provided whenever a genetic abnormality is detected [11].

Y-STRs are Short Tandem Repeats found on the male-specific Y Chromosome (Fig. 1). They are often used in forensics, paternity, and genealogical DNA testing as they have the ability to link male individuals from the same paternal lineage. Y-STR Profiling involves simple and reliable PCR techniques and is tolerant of very degraded samples, besides it can be employed as an investigative tool presenting solutions for solving several mysterious forensic cases [12]. The forensic utility of Y-STRs results from their high levels of polymorphism in human populations, their small size in base pairs (~100–400 bp), and the ability to type multiple Y-STRs in a single PCR reaction [13].

The aim of the present study was to evaluate the correlation of Y-STR Profiling results and the prevalence of Azoospermia condition.

2. Subjects and methods

2.1. Samples

One hundred buccal swab samples from one hundred individuals all subjected to semen analysis were included in this study and are categorized into two groups:

Group I (control group): consists of 50 buccal swab samples taken from men aged from 25 to 40 years from the Saudi population after their informed consents, and it was

Table 3 Fisher's exact test for comparing the percent of Y-STR haplotypes detected in Azoospermic and fertile individuals in the Y-STR locus (DYS385b).

Y-STR locus	Haplotype	Group	No. of observations	Haplotype frequency	Estimate for difference	*Z*	*P*-value
DYS385b	7	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	8	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	9	Azoospermic	–	–	–	–	–
		Fertile	–	–	–	–	–
	10	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	11	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	12	Azoospermic	–	–	−0.02	−1.01	0.844
		Fertile	1	0.02			
	13	Azoospermic	–	–	−0.02	−1.01	0.844
		Fertile	1	0.02			
	14	Azoospermic	2	0.04	0.02	0.59	0.279
		Fertile	1	0.02			
	15	Azoospermic	–	–	−0.02	−1.01	0.844
		Fertile	1	0.02			
	16	Azoospermic	3	0.06	0	0	0.500
		Fertile	3	0.06			
	17	Azoospermic	10	0.20	−0.06	−0.71	0.763
		Fertile	13	0.26			
	18	Azoospermic	14	0.28	−0.02	−0.22	0.587
		Fertile	15	0.30			
	19	Azoospermic	18	0.36	0.14	1.56	0.059
		Fertile	11	0.22			
	20	Azoospermic	1	0.02	−0.04	−1.03	0.848
		Fertile	3	0.06			
	21	Azoospermic	2	0.04	0.02	0.59	0.279
		Fertile	1	0.02			
	22	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	23	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	24	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	25	Azoospermic	–	–	–	–	–
		Fertile	–	–			

proved from the results indicated in the report of their semen analysis that they are all having normal sperm count and characteristics and they are all fertile persons.

Group II (patients group): consists of 50 buccal swab samples taken from men aged from 25 to 40 years from the Saudi population after their informed consents, and it was proved from the results indicated in the report of their semen analysis that they all have Azoospermia (sperm count = 0).

A photocopy for the clinical report of semen analysis done for each individual (from both groups) was obtained from a private clinical lab in Riyadh prior to taking buccal swab samples in the duration from September to March 2014. All buccal swab samples taken were collected and stored at −20 °C till DNA extraction was performed.

2.2. DNA extraction

Total genomic DNA was extracted from buccal swab samples from both groups of individuals included in the study using a QIAamp DNA Micro kit (Qiagen) with the "DNA Purification from buccal swabs (Spin Protocol)" [14]. The extracted DNA was stored at 4 °C for less than 2 weeks until further processing.

2.3. DNA quantitation

Two microliters of each extracted sample was quantified with a Quantifiler Kit (Life Technologies) according to manufacturer's protocol [15] using a Real-time PCR 7500 system (Applied Biosystems).

2.4. DNA amplification

The PowerPlex® Y PCR Amplification Kit was used to amplify the following twelve Y-STR loci: DYS19, DYS385a, DYS385b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438 and DYS439 located on the Y-Chromosome [16]. DNA was amplified according to manufacturer's recommendations using a GeneAmp® PCR System Veriti thermal cycler (Applied Biosystems). The

Table 4 Fisher's exact test for comparing the percent of Y-STR haplotypes detected in Azoospermic and fertile individuals in the Y-STR locus (DYS389I).

Y-STR locus	Haplotype	Group	No. of observations	Haplotype frequency	Estimate for difference	*Z*	*P*-value
DYS389I	10	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	11	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	12	Azoospermic	1	0.02	−0.04	−1.03	0.848
		Fertile	3	0.06			
	13	Azoospermic	43	0.86	0.08	1.05	0.148
		Fertile	39	0.78			
	14	Azoospermic	6	0.12	−0.04	−0.58	0.718
		Fertile	8	0.16			
	15	Azoospermic	–	–	–	–	–
		Fertile	–	–			

Table 5 Fisher's exact test for comparing the percent of Y-STR haplotypes detected in Azoospermic and fertile individuals in the Y-STR locus (DYS389II).

Y-STR locus	Haplotype	Group	No. of observations	Haplotype frequency	Estimate for difference	*Z*	*P*-value
DYS389II	24	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	25	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	26	Azoospermic	–	–	–	–	–
		Fertile	–	–	–	–	–
	27	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	28	Azoospermic	–	–	−0.02	−1.01	0.844
		Fertile	1	0.02			
	29	Azoospermic	6	0.12	−0.24	−2.93	0.998
		Fertile	18	0.36			
	30	Azoospermic	35	0.70	0.32	3.39	0.000
		Fertile	19	0.38			
	31	Azoospermic	6	0.12	−0.04	−0.58	0.718
		Fertile	8	0.16			
	32	Azoospermic	3	0.06	−0.02	−0.39	0.653
		Fertile	4	0.08			
	33	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	34	Azoospermic	–	–	–	–	–
		Fertile	–	–	–	–	–

amount of template DNA added to the reaction mixture was dependent upon the Real-Time PCR results, with an optimal concentration of 0.5 ng/µl and a volume of 19.45 µl DNA sample. A negative control was made with 19.45 µl of deionized water and a positive control was made by vortexing the tube of 2800 M control DNA, then diluting an aliquot to 0.5 ng in 19.45 µl deionized water into a reaction tube, then adding PCR amplification mix for PowerPlex Y kit [17].

The amplification reaction for PowerPlex® Y multiplex system was run in the following conditions: 95 °C for 11 min as an initial hold; 96 °C for 1 min; (ramp 100% to 94 °C for 30 s, ramp 29% to 60 °C for 30 s, ramp 23% to 70 °C for 45 s) for 10 cycles; (ramp 100% to 90 °C for 30 s, ramp 29% to 58 °C for 30 s, ramp 23% to 70 °C for 45 s) for 22 cycles; 60 °C for 30 min; and 4 °C forever as a final hold [17]. Amplified samples were stored at 4 °C for less than 2 weeks until further processing.

2.5. STR genotyping

One microliter of PCR product from each sample was mixed with 9.5 µl of Hi-Di formamide and 0.5 µl ILS 600 size standard. The mixture was denatured at 95 °C for 3 min and cooled to 4 °C for 3 min. Electrophoresis was performed on an ABI 3130 Genetic Analyzer using POP4 polymer. Samples were injected for 5 s at 3 kV, and then run at 15 kV for 2000 s at a constant temperature of 60 °C. The raw data were collected using 3130 Data Collection software, version 3.0 and analyzed using GeneMapper ID-X software, version 1.0. Genotypes were determined by comparing the size of the

Table 6 Fisher's exact test for comparing the percent of Y-STR haplotypes detected in Azoospermic and fertile individuals in the Y-STR locus (DYS390).

Y-STR locus	Haplotype	Group	No. of observations	Haplotype frequency	Estimate for difference	*Z*	*P*-value
DYS390	18	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	19	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	20	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	21	Azoospermic	1	0.02	−0.06	−1.39	0.918
		Fertile	4	0.08			
	22	Azoospermic	4	0.08	−0.02	−0.35	0.637
		Fertile	5	0.10			
	23	Azoospermic	42	0.84	0.18	2.12	0.017
		Fertile	33	0.66			
	24	Azoospermic	3	0.06	−0.06	−1.05	0.854
		Fertile	6	0.12			
	25	Azoospermic	–	–	−0.04	−1.44	0.926
		Fertile	2	0.04			
	26	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	27	Azoospermic	–	–	–	–	–
		Fertile	–	–			

Table 7 Fisher's exact test for comparing the percent of Y-STR haplotypes detected in Azoospermic and fertile individuals in the Y-STR locus (DYS391).

Y-STR locus	Haplotype	Group	No. of observations	Haplotype frequency	Estimate for difference	*Z*	*P*-value
DYS391	7	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	8	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	9	Azoospermic	–	–	−0.02	−1.01	0.844
		Fertile		0.02			
	10	Azoospermic	18	0.36	−0.1	−1.02	0.847
		Fertile	23	0.46			
	11	Azoospermic	24	0.48	0.02	0.20	0.421
		Fertile	23	0.46			
	12	Azoospermic	8	0.16	0.1	1.62	0.053
		Fertile	3	0.06			
	13	Azoospermic	–	–	–	–	–
		Fertile	–	–			

unknown fragments to the allelic ladders provided by the manufacturer [17]. All the work in this study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in human.

2.6. Statistical analysis

To compare the haplotype frequencies for each haplotype in each Y-STR locus included in the PowerPlex® Y PCR amplification kit, between the group of Azoospermic and the group of fertile individuals, a Fisher's exact test was performed to assess the level of significance (0.05) using the following two hypothesis [21] and the results are shown in Tables 1–12:

Null hypothesis H_0. The percent of occurrence of the haplotype in Azoospermic individuals ($P_{Azoospermic}$) $\leqslant$ the percent of occurrence of the haplotype in fertile individuals ($P_{Fertile}$).

Alternative hypothesis H_1. The percent of occurrence of the haplotype in Azoospermic individuals ($P_{Azoospermic}$) $>$ the percent of occurrence of the haplotype in fertile individuals ($P_{Fertile}$).

3. Results and discussion

From the statistical results represented in Tables 1–12, we observe that five haplotypes in four Y-STR loci have been detected with various occurrence percentages in Azoospermic individuals, more greater than their occurrence percentages

Table 8 Fisher's exact test for comparing the percent of Y-STR haplotypes detected in Azoospermic and fertile individuals in the Y-STR locus (DYS392).

Y-STR locus	Haplotype	Group	No. of observations	Haplotype frequency	Estimate for difference	*Z*	*P*-value
DYS392	7	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	8	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	9	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	10	Azoospermic	4	0.08	−0.06	−0.96	0.832
		Fertile	7	0.14			
	11	Azoospermic	40	0.80	0	0	0.500
		Fertile	40	0.80			
	12	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	13	Azoospermic	5	0.10	0.08	1.71	0.044
		Fertile	1	0.02			
	14	Azoospermic	–	–	−0.02	−1.01	0.844
		Fertile	1	0.02			
	15	Azoospermic	1	0.02	0	0	0.500
		Fertile	1	0.02			
	16	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	17	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	18	Azoospermic	–	–	–	–	–
		Fertile	–	–			

Table 9 Fisher's exact test for comparing the percent of Y-STR haplotypes detected in Azoospermic and fertile individuals in the Y-STR locus (DYS393).

Y-STR locus	Haplotype	Group	No. of observations	Haplotype frequency	Estimate for difference	*Z*	*P*-value
DYS393	8	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	9	Azoospermic	1	0.02	0.02	1.01	0.156
		Fertile	–	–			
	10	Azoospermic	10	0.20	0.20	3.54	0.000
		Fertile	–	–			
	11	Azoospermic	3	0.06	0.06	1.79	0.037
		Fertile	–	–			
	12	Azoospermic	28	0.56	−0.14	−1.47	0.929
		Fertile	35	0.70			
	13	Azoospermic	5	0.10	−0.16	−2.13	0.983
		Fertile	13	0.26			
	14	Azoospermic	–	–	−0.02	−1.01	0.844
		Fertile	1	0.02			
	15	Azoospermic	2	0.04	0.02	0.59	0.279
		Fertile	1	0.02			
	16	Azoospermic	1	0.02	0.02	1.01	0.156
		Fertile	–	–			

in fertile individuals. The statistical results for those five haplotypes are assembled in Table 13.

From the statistical results shown in Table 13, we found that the calculated *P*-value was less than the statistical level of significance ($\infty = 0.05$) in the five haplotypes; 30, 23, 13, 10 and 11 present in the Y-STR loci; DYS389II, DYS390, DYS392 and DYS393(2) respectively. This led to the acceptance of the alternative hypothesis (H_1) of Fisher's exact test, which states that the percent of occurrence of each of these five haplotypes in Azoospermic individuals is significantly higher than the percent of the occurrence of the same haplotype in fertile individuals. Accordingly, those haplotypes can be selected and grouped as pre-diagnostic markers which can predict for the incidence of Azoospermia condition among Saudi individuals.

The prevalence of any of these five haplotypes in the Y-STR profile of a Saudi male individual, may increase the probability of occurrence of Azoospermia condition in this

Table 10 Fisher's exact test for comparing the percent of Y-STR haplotypes detected in Azoospermic and fertile individuals in the Y-STR locus (DYS437).

Y-STR locus	Haplotype	Group	No. of observations	Haplotype frequency	Estimate for difference	*Z*	*P*-value
DYS437	13	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	14	Azoospermic	48	0.96	0.04	0.85	0.199
		Fertile	46	0.92			
	15	Azoospermic	2	0.04	−0.02	−0.46	0.677
		Fertile	3	0.06			
	16	Azoospermic	–	–	−0.02	−1.01	0.844
		Fertile	1	0.02			
	17	Azoospermic	–	–	–	–	–
		Fertile	–	–			

Table 11 Fisher's exact test for comparing the percent of Y-STR haplotypes detected in Azoospermic and fertile individuals in the Y-STR locus (DYS438).

Y-STR locus	Haplotype	Group	No. of observations	Haplotype frequency	Estimate for difference	*Z*	*P*-value
DYS438	8	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	9	Azoospermic	4	0.08	−0.02	−0.35	0.637
		Fertile	5	0.10			
	10	Azoospermic	40	0.80	0.02	0.25	0.403
		Fertile	39	0.78			
	11	Azoospermic	5	0.10	−0.02	−0.32	0.625
		Fertile	6	0.12			
	12	Azoospermic	1	0.02	0.02	1.01	0.156
		Fertile	–	–			
	13	Azoospermic	–	–	–	–	–
		Fertile	–	–			

Table 12 Fisher's exact test for comparing the percent of Y-STR haplotypes detected in Azoospermic and fertile individuals in the Y-STR locus (DYS439).

Y-STR locus	Haplotype	Group	No. of observations	Haplotype frequency	Estimate for difference	*Z*	*P*-value
DYS439	8	Azoospermic	1	0.02	0.02	1.01	0.156
		Fertile	–	–			
	9	Azoospermic	3	0.06	0.04	1.03	0.152
		Fertile	1	0.02			
	10	Azoospermic	1	0.02	0	0	0.500
		Fertile	1	0.02			
	11	Azoospermic	30	0.60	0	0	0.500
		Fertile	30	0.60			
	12	Azoospermic	12	0.24	−0.1	−1.11	0.866
		Fertile	17	0.34			
	13	Azoospermic	3	0.06	0.04	1.03	0.152
		Fertile	1	0.02			
	14	Azoospermic	–	–	–	–	–
		Fertile	–	–			
	15	Azoospermic	–	–	–	–	–
		Fertile	–	–			

individual in Saudi population. Since all STR loci obey the law of independent assortment stated by Mendel [19] and are therefore inherited independently, so the occurrence of more than one haplotype of those five haplotypes in Table 13 in the Y-STR profile of a Saudi male individual, will give rise to the prediction of Azoospermia condition in this individual with more increasing percentage.

When comparing our Y-STR haplotype frequency results of the group of the five haplotypes chosen as pre-diagnostic markers for Azoospermia in fertile individuals, with the

Table 13 Statistical results for pre-diagnostic Y-STR haplotype markers for Azoospermia.

Y-STR locus	Haplotype	Group	No. of observations	Haplotype frequency	Estimate for difference	*Z*	*P*-value
DYS389II	30	Azoospermic	35	0.70	0.32	3.39	0.000
		Fertile	19	0.38			
DYS390	23	Azoospermic	42	0.84	0.18	2.12	0.017
		Fertile	33	0.66			
DYS392	13	Azoospermic	5	0.10	0.08	1.71	0.044
		Fertile	1	0.02			
DYS393	10	Azoospermic	10	0.20	0.20	3.54	0.000
		Fertile	–	–			
	11	Azoospermic	3	0.06	0.06	1.79	0.037
		Fertile	–	–			

Table 14 Comparison of Y-STR haplotype frequencies in four different populations.

Y-STR locus	Haplotype	Turkish population	Chinese population	Libyan population	Saudi population
DYS389II	30	0.40	0.22	0.17	0.38
DYS390	23	0.38	0.30	0.47	0.66
DYS392	13	0.08	0.20	0.03	0.02
DYS393	10	–	–	–	–
	11	0.02	–	0.02	–

corresponding haplotype frequency results in the same Y-STR loci in three different populations (Table 14), we found that there is a close proximity in the Y-STR haplotype frequency values between the four populations [18,22,23].

When comparing the Y-STR haplotype frequency values in the three haplotypes; 30, 23 and 13 in the three Y-STR loci; DYS389II, DYS390, and DYS392 respectively, in the four populations, we found a great convergence between the corresponding haplotype frequency values in each Y-STR locus in the four different populations.

In the Y-STR locus (DYS393); there was a complete absence of the haplotype "10" in all populations, and regarding the haplotype "11" in the same locus, it was absent in both the Saudi and the Chinese populations, while it possesses a very low frequency reaches (0.02) in both Turkish and Libyan populations.

Moreover, all frequency values in the five Y-STR haplotypes in all populations were less than the corresponding frequency values of the Azoospermic Saudi individuals included in the study except for the haplotype "13" in the Y-STR locus (DYS392) in the chinese population which was found to have a frequency value reached (0.20) in contrast to the corresponding frequency values (0.08), (0.03), and (0.02) in the Turkish, Libyan, and Saudi populations respectively. This supports the idea that the five Y-STR haplotypes chosen in our study, can be utilized as pre-diagnostic markers for Azoospermia prediction in more populations after similar statistics done.

4. Conclusion

It was found that there are five haplotypes located within four different Y-STR loci that can serve as a group of pre-diagnostic markers for early prognosis of Azoospermia in Saudi population.

Consequently, we can conclude that performing Y-STR Profiling including the four Y-STR loci; DYS389II, DYS390, DYS392 and DYS393 for newborn Saudi males, will be beneficial in predicting Azoospermia condition, in case that there is one or more of this group of five haplotypes in those four Y-STR loci is detected in the Y-STR genotyping results.

Conflict of interest

The author declares no conflict of interest.

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