Detection of the Microdeletions on Yq Chromosome in Egyptian Population with Idiopathic Male Infertility

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Abstract: Infertility is a common problem affecting 1:6 couples however; recent studies suggest values of 14-26% may be more realistic. Male infertility field has witnessed some remarkable advances including the publication of the sequence-tagged site (STS) map of the Y-chromosome, recognition that Y-specific genes may play an important role in the pathophysiology of male infertility, cloning of the Yq-specific candidate genes for male infertility, and evolution of intracytoplasmic sperm injection (ICSI) as a treatment modality for male infertility. A group of gene specific PCR primers was designed for nine genes in the Y-chromosome from the published STS map. These primers were used to screen 74 adult male Egyptian patients with idiopathic infertility with age ranged from 24-36 years and the duration of infertility in all of them ranged from 6 months up to 10 years. Our results showed that, by applying simple and multiplexing PCR for nine genes on Y-chromosome, fifty six patients were found to carry Y-chromosome microdeletions (75.67%) and the highest frequency was that of the DAZ gene which account for twenty seven patients (36.49%). Our results indicated that the major microdeletion on the Y chromosome can be detected in Egyptian population with idiopathic male infertility using simple and optimized multiplexing PCR as a sensitive, reliable and rapid technique

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1. Introduction

Recognizable causes of men infertility account for only 30-50% of the cases, and there is emerging evidence to suggest that a genetic basis for male infertility may exist in many men currently classified as idiopathic [Simoni *et al.,* 1999; Schmidt, 2005; Dong *et al.,* 2012]. Such a high prevalence of infertility is indeed surprising because the occurrence of infertility prevents further propagation of the defect, thus leading to the removal of the trait from the genetic pool. It is not clear how such a high prevalence of infertility is maintained in spite of ongoing removal of the affected individuals from the genetic pool [Rowe *et al.,* 2000].

Tiepolo and Zuffardi reported the occurrence of grossly cytogenetically detectable de novo deletions in six azoospermic individuals, describing for the first time the role of the Y chromosome in spermatogenesis. These observations led the authors to postulate the existence of a locus, called AZoospermia Factor (AZF), on Yq11 required for a complete spermatogenesis since the seminal fluid of these patients did not contain mature spermatozoa [Tiepolo and Zuffardi 1976; Ma *et al.,* 1992; Poongothai *et al.,* 2009]. This suggested that the deletions were the cause of the azoospermia and they postulated that a genetic factor located in Yq11 was important for male germ cell development. These gene or genes cluster was defined as "azoospermia factor" (AZF). However, the genetic complexity of the AZF locus could be revealed only with the development of STS and YAC-based mapping. These analyses permitted the detection of interstitial submicroscopic deletions not visible at the cytogenetic level and detectable only by STS-PCR or Southern hybridization. Such deletions are called microdeletions. Molecular mapping analyses on patients with microdeletions have complicated the original hypothesis of a single locus for spermatogenesis on Yq, suggesting that three non-overlapping regions in deletion intervals 5 and 6 may be deleted in infertile men. The location of AZF in Yq11 was further confirmed by numerous studies at cytogenetic and molecular level. Once the molecular map by Vergnaud *et al.* (1986) became available, AZF was localized to the deletion interval 6, a region in band q11.23 [Vergnaud *et al.,* 1986]. The publication of about 200 Y-specific STS allowed a much simpler Y chromosome screening for microdeletions to be performed [Vollrath *et al.,* 1992]. Thus, the original AZF region was further subdivided into three different non-overlapping sub-regions in Yq11 associated with male infertility, namely AZFa, AZFb, and AZFc [Vogt *et al.,* 1996]. Each one of these regions contains

several genes proposed as candidate genes involved in male infertility [SaoPedro *et al.,* 2003, Ballantyne *et al.,* 2010]. The aim of the present work is to study the frequency of the microdeletions on the Y chromosome and to type such deletions of candidate genes in Egyptian patients with idiopathic male infertility.

2. Material and Methods

Our study was approved by The Meri Hospital, Faculty of Medicine, University of Alexandria and informed consent was obtained from each patient. The study includes 74 adult male patients with idiopathic infertility. The age of these patients ranged from 24 to 36 years. The mean age of the overall group was 34 years and the duration of infertility in all patients ranged from 6 months up to 10 years and the female partner was completely free. All patients were studied with a comprehensive history and general investigation for exclude possible causes of testicular damage, such as cryptorchidism, varicocele, seminal tract infections, drug use, endocrinopathies, post-mumps orchitis, testicular trauma or torsion. All cases with azoospermia and oligospermia from obstructive causes and cases with constitutional cytogenetic abnormality were excluded from this study. Semen samples were obtained following a 3 days of sexual abstinence and complete semen analysis was performed according to guidelines of the World Health Organization [Rowe *et al.,* 2000]. FSH, LH and testosterone plasma concentrations were measured by RIA [Babson, 1991]. The normal ranges of hormonal data in adult male are of the following values: FSH: 1.6-12.5 mIU/ml, LH: 0.8-9.1 mIU/ml, Testosterone: 2.6-20 ng/ml.

Testicular biopsy was obtained from patient using Bupivicain (0.25%; Astra USA Ins.) as a local spermatic cord block and was supplemented with a small amount by a small amount of i.v. sedation. A transverse incision was made in the left hemiscrotum and carried down to the tunica albuginea of the testis where a slightly smaller opening into the parenchyma was created. The testis was compressed and the exposed seminiferous epithelium was sharply excised. The harvested tissue sample was put in a fixative then processed into paraffin by the standard histopathology laboratory method. Histopathological sections were prepared from the paraffin blocks, cut at 5 microns in thickness and stained by Haematoxylin and Eosin (H&E), then examined under the light microscope [Holstein *et al.,* 1994].

Genomic DNA was isolated and purified from whole blood using a QIAamp Blood Mini Kit (Qiagen, Hilden, Germany). The purified DNA was analyzed on agarose gel (1%) electrophoresis stained with 0.5 µg/ml ethidium bromide and visualized by UV. 200 ng of the purified DNA was used for simple and multiplexed PCR. A total of 9 STSs distributed on the AZF region of the Y chromosome namely; sY254, sY255, sY117, sY14 (as internal control), DBY, USP9Y, RBM9Y, EIF1AY, DAZ were selected for the detection in this study and had been confirmed to be related to infertile men. Primer sequences were designed from the UniSTS database on the National Library of Medicine website. These primers were synthesized by Pharmacia-Amersham Chemical Co.

The 9 pairs of primers were as follow; EIF1AY: forward; 5'-GCAAACTGATTTATTTTCATTGTTT-3', reverse; 5'-CAGCAAATATTATGGTCTTTTATCC-3'; sY117: forward; 5'-GTTGGTTCCATGCTCCATAC-3', reverse; 5'-CAGGGAGAGAGCCTTTTACC-3'; DBY: forward; 5'-TATTGGCAATCGTGAAAGAC, reverse; 5'-AATATGTGGAGGACAGTTAC-3'; sY14: forward; 5'-GAATATTCCCGCTCTCCGGA-3', reverse; 5'-GCTGGTGCTCCATTCTTGAG-3'; USP9Y: forward; 5'-GGTAGCTCTATTTAGCAGTT-3', reverse; 5'-TTGGTTTGGAGCAGTTTATC-3'; RBM1A1: forward; 5'-ATGCACTTCAGAGATACGG-3', reverse; 5'-CCTCTCTCCACAAAACCAACA-3'; DAZ: forward; 5'-GGAAGCTGCTTTGGTAGATAC-3', reverse; 5'-TAGGTTTCAGTGTTTGGATTCCG-3'; Sy254: forward; 5'-GGGTGTTACCAGAAGGCAAA-3', reverse; 5'-GAACCGTATCTACCACCGAGC-3'; Sy255: forward; 5'-GTTACAGGATTCGGCGTGAT-3', reverse; 5'-CTCGTCATGTGCAGCCAC-3'.

PCR was carried out in 25 µl total volume that contain 12.5 µl 2XPCR master mix (Fermentas Chemical Co.), 200 ng purified genomic DNA, 30 pmole of each pair of gene specific primers and 3.5 µl of nuclease free water to give 25 µl final volume. PCR conditions for the following primers EIF1AY, sY117, DBY, sY14, sY254 and sY155 was as follow; 95 ºC for 5 minutes followed by 30 cycles at 94 ºC for 30 seconds, 50 ºC for 30 seconds and 72 ºC for 30 seconds. This was followed by one cycle at 72 ºC for 10 minutes. For the USP9Y, RBM1A1 and DAZ genes the PCR conditions was as follow; 95 ºC for 5 minutes followed by 30 cycles at 94 ºC for 1 min., 50 ºC for 1 min. and 72 ºC for 1.5 min. This was followed by one cycle at 72 ºC for 10 minutes. Multiplexed PCR was carried out in a total volume of 50 µl contain 25 µl High Fidelity 2XPCR master mix (nnnn), 200 ng purified genomic DNA, 30 pmole of each pair of gene specific primers in combination of two and three pairs at a time in the same reaction tube

and the volumes were adjusted to 50 µl with nuclease free water. PCR conditions for the following primers combinations EIF1AY, USP9Y sY117, DBY, sY14, sY254, and sY255 were as follow; 95 ºC for 10 min. followed by 50 ºC for 10 min. then 30 cycles of 94 ºC for 45 seconds, 50 ºC for 45 seconds and 72 ºC for 45 seconds. Finally one cycle at 72ºC for 10 minutes. Amplification conditions for DAZ and RBM1A1 primers pairs were carried out as follow; 95 ºC for 10 min. followed by 50 ºC for 10 min. then 30 cycles of 94 ºC for 1 min., 50 ºC for 1 min. and 72 ºC for 1.5 min. Finally one cycle at 72 ºC for 10 minutes. All PCR products were analyzed on 2% agarose gel stained with ethidium bromide and visualized by ultraviolet.

3. Results

Results of serum hormones and semen analysis are shown in Table 1. It was found that all of the studied patients had a normal LH and testosterone levels on the otherhand, the serum level of FSH was higher among two patients (12.5 and 11.11 mIU/ml respectively).

Table 1. Hormonal and semen profile of some selected patients:

Patient Code	Age	Seminal Analysis	FSH	LH	Teste-sterone
M26	24	Azoospermia	12.5	5.7	N
M25	36	Azoospermia	17.6 ↑	19.3 ↑	N
M32	32	Azoospermia	5	11	3.3
M29	33	Azoospermia	N	N	N
M65	30	Azoospermia	3.16	7.67	26.0
M64	36	Azoospermia	10.3	4.8	N
AM	36	Oligo-azoospermic recently	0.89 ↓	N	N
M34	34	Azoospermia	5.1	2.9	N
M62	unknown	Azoospermia	12.5	9.88	3.98
M23	36	Azoospermia	10.25	6.42	N
M31	32	Azoospermia	36.9 ↑	16.2 ↑	4.9
M41	35	Azoospermia	7.2	N	N
M24	32	Azoospermia	10.79	9.26	N
M22	31	Azoospermia	10.9	N	4.5
M30	33	Azoospermia	4.24	N	5.7
M31	36	Azoospermia	N	N	N
M47	34	Azoospermia	N	N	N
M10	unknown	Oligo-azoospermic after 2 years	5.6	N	N
M61	unknown	Azoospermia	N	N	N
M4		Azoospermia	N	N	N

Moreover, it was found that both of these patients have a mixed pattern histological finding, SCOS and early maturation arrest. Semen analysis of all patients under investigation showed that, 72 had azoospermia and 2 had severe oligospermia (sperm count less 5 x 106 per ml ejaculate) as shown in Table 1.

Results of histopathological examination of testicular tissues for patients under investigation showed that, three patients had mixed pattern phenotype (SCOS/early maturation arrest), one patient had mixed pattern (SCOS/late maturation arrest), one patient had hypospermatogenesis/ maturation arrest at variable stage, two patients had SCOS, 10 patients had maturation arrest; four patients at spermatocyte stage and six at spermatid stage as shown in Fig.1.

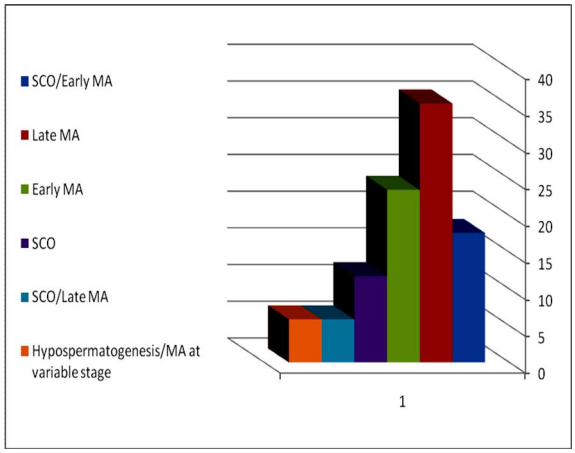

Figure 1: 3D histogram showing the frequencies of different phenotype **for patients under study.**

Detection of candidate genes by simple and multiplexing PCR; Selected STS or gene-specific designed PCR primers were first optimized separately on DNA samples isolated and purified from control fertile individual with proven paternity to test the success of the detection process before utilizing these primers for the diagnosis of the microdeletions in patient with idiopathic infertility and before applying the multiplex PCR assay. Fig. 2 and 3 show the results of simple PCR using DBY, sY14, s11

Figure 3 shows the result of a simple PCR for patient with EIF1AY, sY117 and DBY primers. It was found that these infertile patients showed microdeletions for these three exons as shown in Figure 3 lane 9 to 14.

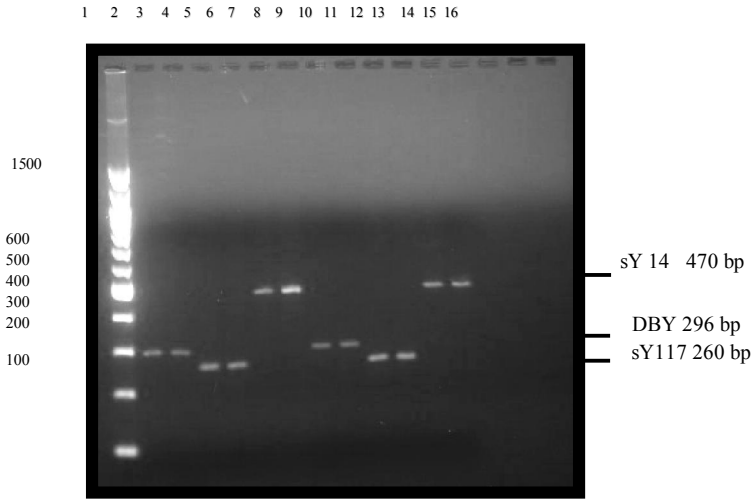

Figure 2: Agarose gel (3%) electrophoresis of PCR products Lanes 2-5 for two control DNA samples of a proven paternity individual with primers DBY (Lanes 2,3 and 8,9), SY117 (Lanes 4,5 and 10,11) and sY14 (Lanes 6,7 and 12,13). Lane 1 represents 100 bp ladder DNA molecular weight markers and Lanes 14-16 represent negative PCR controls without primers.

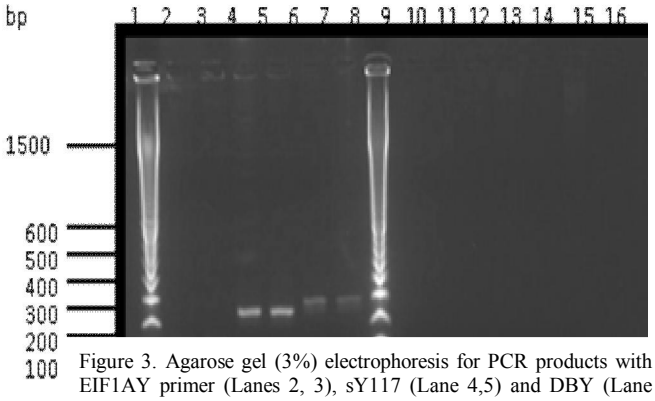

Figure 3. Agarose gel (3%) electrophoresis for PCR products with EIF1AY primer (Lanes 2, 3), sY117 (Lane 4,5) and DBY (Lane 6,7). Lanes 9-14, represents PCR products of infertile patient with microdeletions in these three exons. Lanes 1 and 8 represent 100 bp ladder molecular weight DNA markers and Lanes 15 and 16 represent negative PCR control without primers.

In an attempt to amplify multiple exons at a time, multiplex PCR was conducted. Figure 5 shows the result of multiplex PCR using DAZ, 1300 bp and RBM1A1, 800 bp on Y chromosome for a control fertile individual of proven paternity. Intense specific multiplex PCR products was obtained using DAZ and RBM1A1 primers at the corresponding exact sizes, 1300 and 800 bp respectively (Fig. 4 Lanes 2 and 3). Similar results were obtained with primers USP9Y, sY117 and EIF-1AY in one trial and primers USP9Y, sY117 and DAZ 255 in another trial as shown in Fig. 4 Lanes 2-5. In all cases specific multiplex products were obtained corresponding to the exact sizes of the amplified exons under investigation.

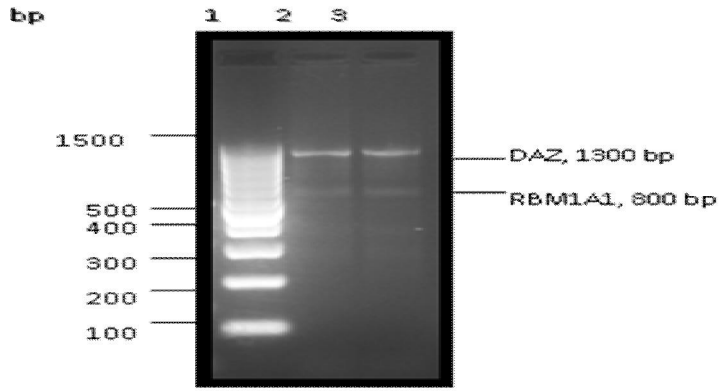

Figure 4. Agarose gel (2%) electrophoresis of multiplex PCR products for a control DNA sample with primers DAZ, 1300 bp and RBM1A1, 800 bp Lanes 2 and 3. Lane 1 represents 100 base pair ladder DNA molecular weight markers.

Multiplex PCR was performed in a DNA samples isolated and purified from idiopathic infertile men and the result is shown in Fig. 5. It was noticed that the DNA samples isolated from infertile men showed microdeletions in these two exons (sY14 and sY117) as indicated by negative PCR products (Fig. 5, Lanes 2-4) compared to DNA samples isolated from control fertile men which showed a definite, clear PCR products corresponding to sizes of 470 and 260 base pair under identical reaction conditions (Fig. 5, Lanes 5 and 6). This result was in agreement with the previous results that were obtained with the simple PCR reactions for those three patients.

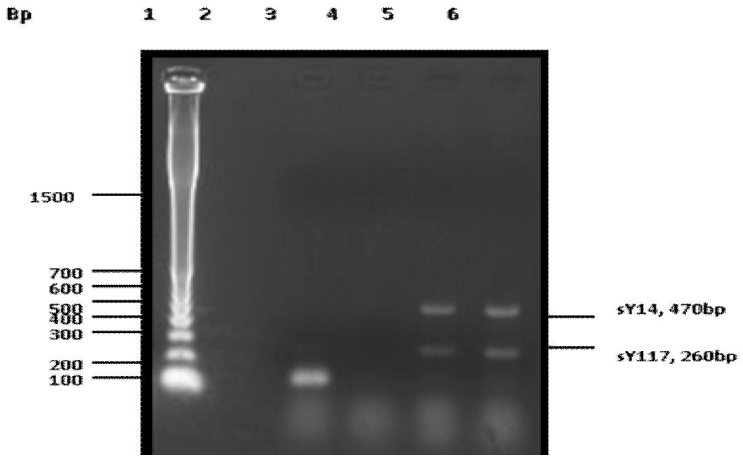

Figure 5. Agarose gel (2%) electrophoresis of multiplex PCR products with primers sY14 and sY117 for idiopathic infertile men (Lanes 2-4) and control fertile men (Lanes 5 and 6). Lane 1 represents 100 bp ladder DNA molecular weight markers.

The present study showed a high variation in genotype pattern of idiopathic infertile Egyptian patients. Results obtained in this study showed that, only one patient namely M32 had an AZFa genotype (1/74, 1.35%), sixteen patients had AZFb genotype (16/74, 21.63%), eleven patients had AZFc (11/74, 16.21%) and twenty eight patients (28/74, 37.83%) showed mixed pattern genotype; two namely M55 and M61 had AZFa + AZFb (2/74, 2.7%), one patient namely had AZFa + AZFc (1/74, 1.35%), twenty-ones had AZFb + AZFc (28.37%) and four patients namely M66, M63, M60 and M43 showed AZFa + AZFb + AZFc genotype (4/74, 5.4%) . Moreover, DBY is deleted in eight patients (10.81%), DAZ is deleted in twenty seven patients (36.49%), RBM is deleted in twenty one patients (28.37%) and EIF1AY is deleted in seventeen patients (22.98%). Nine patients had deletion of RBM gene only (9/21, 42.85%) and four

patients carried deletion of EIF1AY only (4/21, 19.04%) Results are summarized in figure 6.

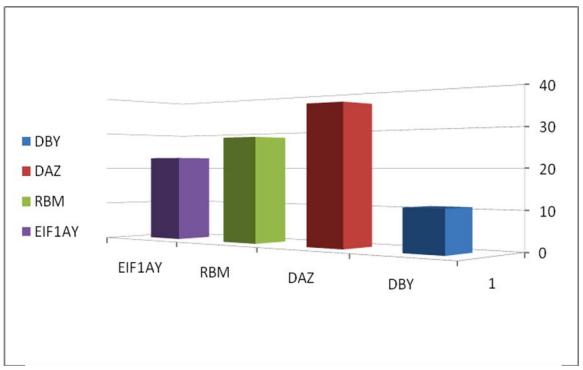

Figure 6: 3D histogram shows the frequencies of gene deletion for idiopathic infertile men under investigation.

4. Discussion

In our study serum level of FSH hormone was elevated (> 12.5 mIU/ml) among 2 patients (M25 and M31) (11.11%) and both have a mixed pattern histological finding; SCO and early maturation arrest with small testicles. Normal FSH levels are required for normal spermatogenesis and the selective elevation of FSH level in patients with idiopathic azoospermia can be explained by the possible role of inhibin and other testicular factors in regulating FSH secretion that is impaired in germinal cell loss and other testicular damage. But even severe histopathological damage of the seminiferous tubules is accompanied by normal FSH concentration (McLachlan *et al.,* 1996**)**. Therefore, patients with idiopathic or severe oligozoospermia and have normal serum level of FSH hormone should be excluded from screening of microdeletions of the three AZF intervals.

The present study included seventy four males with idiopathic infertility. By PCR analysis fifty six patients were found to carry Y microdeletions (75.67%), this percentage is higher than that reported by Vogt *et al.* (66%) [Voget *et al.,* 1996] and higher than the frequency reported by Kent-First *et al*. [Kent-First *et al.,* 1999]. We noticed that the highest frequency was that of DAZ gene that accounts for twenty seven patients (36.49%). Such frequency was matching that reported by Najmabadi *et al* .(1996) who screened 60 patients with 26 STSs, eleven patients were found to carry Y microdeletions four of them (36.36%) with deletions encompassing the DAZ gene. Higher frequency of deletions was detected by Ferlin *et al* ., 1999 as 42.5% and even higher by Foresta *et al.,* 1997 that rises up to 72.7%. Much lower frequencies were recorded in studies conducted by Reijo *et al*., 1995 that accounts for 13.4%. In the present study three patients namely M4, M15 and M27 (3/74, 4.05%) carry deletions encompassing the whole DAZ locus indicating the complete absence of AZFc intervals. All of them are azoospermic. M4 and M5 carry AZFb+c combined genotype. On the other hand, RBM deletions were found to account up to 28.37%, a value that is relatively lower to that reported by Foresta *et al.,* 2000 who found that four patients out of eleven (36.37%) to carry deletions involving RBM.

Meanwhile no deletion has been detected for USP9Y, but such finding can not negate a prospective role of this gene. RBM frequency deletion was found to account for 28.38% (21/74 patients). Among sixteen patients showing AZFb genotype, five patients (31.25%) have only RBM gene deleted in AZFb interval and show early maturation arrest (at the spermatocyte stage) which is in agreement with the suggested role of RBM cited in the literature [Navarro-Costa *et al.,* 2010]. The other eleven patients showed deletion of sY117, EIF1AY and RBM. Seventeen patients had deletion of EIF1AY representing about 22.98% of the study population and only four patients out of the sixteen ones (25%) carrying AZFb genotype had this gene deleted. One patient namely M35 out of the sixteen ones (6.25%) exhibits AZFb genotype with EIF1AY deleted alone. These findings in addition to the speculated role of EIF1AY mentioned in the literature can be considered a reliable evidence for the contribution of this gene in development of AZFb genotype and subsequently associated phenotype namely early spermatocyte arrest [Affara, 2010].

sY117 is a very proximal STS (nearer to centromere) whose deletion has been detected in nine patients out of the sixteen showing AZFb genotype. The combined deletion of sY117, EIF1AY and RBM was detected in one patient namely M46, a finding that indicates the complete absence of AZFb interval but unfortunately the histopathological feature of this patient was unavailable.

Mixed pattern phenotypes were detected in this study showing SCO/early maturation arrest with a corresponding genotype AZFb/AZFc. Silber *et al* and Renee Reijo et al have reported similar findings where patients carrying AZFb+c showed mixed SCO/maturation arrest [Reijo *et al.,* 1996, Silber *et al.,* 1998]. Furthermore, patients showing AZFc genotypes in our study have a less severe histopathological feature showing late maturation arrest up to round spermatid stage with very few mature sperms detected. Both genotypes shared the deletion of DAZ genes. It is noticed that when the deletion is limited to 6D-6F intervals, a mild phenotype (late maturation arrest with few mature sperms) was shown whereas larger deletion extending

beyond 6D-6F appear to be associated with a total absence of testicular spermatozoa.

It is clinically known that men with DAZ regions deletions are mostly azoospermic and that spermatogenesis, as determined by formal histology in these men is variable ranging from a pattern of Sertoli cell-only syndrome (SCO) to the presence of condensed spermatids in some tubules, in addition it has been reported by Mulhall *et al.,* 1997 that testicular spermatozoa have been found in 50% of DAZ deleted patients. The presence of testicular spermatozoa in DAZ deleted patients of our study came in agreement with what was found in Mulhall *et al.,* study. Therefore we can deduce that spermatogenesis albeit at reduced output can still proceed in DAZ deleted patient. DAZ gene is expressed in early germ cell population (gonocytes and spermatogonia). This localization suggests that the DAZ gene cluster plays a role in the proliferation, maintenance or differentiation of those early germ cell populations, meanwhile our study demonstrates that an intact DAZ gene cluster is not absolutely required for completion of the later stages of spermatogenesis. DAZ gene cluster therefore plays a pivotal role in the quantitative rather than qualitative regulation of the spermatogenic epithelium. Different explanations have been postulated to interpret such variation of phenotype. First, the presence of a functional DAZ homologue (DAZL1) on human chromosome 3p specifically expressed in testis whose activity may compensate for deleted DAZ. Second, deletions may not remove all functional copies of DAZ genes; moreover DAZ transcript may be the product of an mRNA splicing process. Third the absence of other genes within the DAZ clusters has not been confirmed. Fourth, mosaicism seems to play an important role in such variation of phenotype. Since abnormal Y chromosomes are inherited from fertile fathers, microdeletions can arise in offsprings either from *de novo* random meiotic errors in paternal germ cell lineages or from paternal constitutional mosaics as a result of mitotic accidents. Fifth, a further hypothesis is the existence of abnormalities of the promoter, transcription or post-transcription phases but nothing is known about these aspects of regulation of DAZ expression [Aarabi, 2009].

In the present study, AZFa was associated with hypospermatogenesis, AZFc showed maturation arrest at late spermatid stage with few mature spermatozoa. AZFb showed phenotypes ranging from early (at primary or secondary spermatocytes) to late maturation arrest (at spermatid stage), seven patients showed extended deletions AZFb+c, two of them (M_{25} and M_{31}) had mixed pattern phenotype Sertoli cell only syndrome and early maturation arrest, one patient showed Sertoli cells only syndrome, one patient showed hypospermatogenesis and maturation arrest at variable stages and one patient showed early maturation arrest and two patients their histopathology were unavailable. Patient M_{30} suffering 1ry infertility since 1.5 years, has a normal hormonal profile, its semen analysis revealed azoospermia and showed only deletion at sY117 (proximal STS). Meanwhile, the patient has a histopathological profile that reveals normal spermatogenesis. A similar finding has been reported by Jon L. Pryor *et al.* (1997).

The absence of DAZ appears therefore to be insufficient to determine the complete loss of spermatogenic line, but rather seems to produce a reduction in the number of these cells or an alteration of their maturation process. It is possible that the testicular damage caused by DAZ deletions is progressive and that oligozoospermic patients may become azoospermic later in life (Ferlin *et al.,* 2007). This finding was observed with patient M_{13} that within 2 years, its sperm count has fallen 4 millions/cc to 1.5 millions/cc. M_{13} carried AZFc/partial AZFb deletion but no histological profile was available. The patient had normal hormonal profile.

Deletions in AZFa and in AZFb cause azoospermia in two thirds of all cases, and more rarely severe oligozoospermia. Therefore the phenotype associated with such deletions seems to be more severe than that observed in AZFc deleted patients. The testicular histology of AZFa deletion patients with azoospermia always shows Sertoli cell only syndrome while in patients with severe oligozoospermia it resembles severe hypospermatogenesis (no maturation arrest is seen). AZFb patients could have more variable defects, and in about half of cases a spermatogenic arrest is observed. The variable spermatogenic alterations observed in AZFb patients may indicate multiple functions of RBMY during spermatogenesis or alternatively, that others genes namely EIF1AY and others located in this region may act in combination to RBMY and that their presence or absence modulates the phenotype.

Based on our findings we can draw a general outline to be followed in concern of Y chromosome deletion analysis and its implication. The result of deletion analysis will roughly direct towards a certain methodology. AZFa deleted patients are extremely rare and usually show Sertoli only cell syndrome. The probability of finding sperms in AZFb deleted patient is virtually null. Rather the round spermatid found by diagnostic biopsy or testicular sperm extraction may be used in Round Spermatid Injection (ROSI) or Round Spermatid Nucleus Injection (ROSNI). Therefore, it is recommended for AZFb deleted patients to undergo diagnostic biopsy rather than the more invasive testicular sperm extraction or sex

selection. The situation is different regarding azoospermic patients with AZFc or partial AZFb deletions where hidden islands of spermatogenesis are found. Meanwhile the progressive decline in sperm numbers over months associated with oligozoospermic patient showing AZFc can be overcome by cryo-preservation. Males that are conceived by intracytoplasmic sperm injection or in vitro fertilization techniques from father with oligozoospermia or azoospermia would also benefit from knowledge of their Y status. Since AZFc deletions may be associated with a decline in sperm production over time, cryo-preservation of semen in early adulthood should be considered. Moreover, the identification of the genetic defect will render future medical or surgical therapies unnecessary.

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