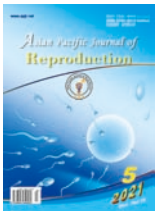

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Yq AZF microdeletions in male infertility: An update on the phenotypic spectrum, epidemiology and diagnostics

Awanish Jaiswal[1], Anurag Pandey[1✉], Mamta Tiwari[2], Akhtar Ali[3], Rohit Sharma[4]

[1]*Department of Vikriti Vigyan, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, U.P., India*

[2]*Department of Swasthavritta and Yoga, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, U.P., India*

[3]*Centre for Genetic Disorders, Institute of Science, Banaras Hindu University, Varanasi U.P., India*

[4]*Department of Rasa Shastra and Bhaishajya Kalpana, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, U.P., India*

ABSTRACT

According to the latest data, globally 15% of couples have infertility and male infertility contributes to 10% of all cases. Infertility can be caused by certain biological changes in the gonads and the reproductive system like azoospermia, oligospermia, asthenospermia, teratozoospermia and hypospermatogenesis. Genetic causes of azoospermia include chromosomal abnormalities, Y chromosome microdeletions and deletion or other mutations of Y-linked genes. The maximum number of the genes are located in the azoospermia factor region of the long arm (Yq) of the Y chromosome. Y chromosome microdeletion is known as the second major genetic cause of spermatogenetic failure. This article aims to review the latest updates on the involvement of Yq microdeletions in male infertility. The diagnostics, prevalence and phenotypic spectrum related to Yq gene microdeletions are discussed.

KEYWORDS: Azoospermia factor; AZF; Male infertility;Y chromosome microdeletion; Yq

1. Introduction

Infertility is one of the major problems around the world. Males are found to be responsible for 20%-30% of infertility cases[1]. The World Health Organization (WHO) estimates that 48-180 million couples worldwide currently are suffering from infertility[2,3]. The overall prevalence of primary infertility in India is between 3.9% and 16.8%[4]. In India, the prevalence of infertility varies in different states such as it is 3.7% in Uttar Pradesh, Himachal Pradesh and Maharashtra, 5% in Andhra Pradesh and 15% in Kashmir. The prevalence of infertility varies across the tribes and caste within the same region of country[5]. According to the International Committee for Monitoring Assisted Reproductive Technology (ICMART)-WHO, infertility is defined as a disease of the reproductive system represented by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected intercourse[6]. Idiopathic male infertility is one of the serious problems that draws the attention of geneticists to explore the problem and further find a permanent solution. To date, 122 genes and 110 pseudogenes have been identified in the Y chromosome[7]. However, the role of each gene in spermatogenesis is not defined as the number of genes responsible for microdeletion may be more, and they may lead to spermatogenesis failure[8]. The prevalence of azoospermia factor (AZF) microdeletion in male infertility ranges from 5.0% to 20.0% around the globe[9]. The long arm of the Y chromosome (Yq) microdeletion is found in 13% of azoospermic men and 1%-7% of severely oligospermic men[10]. Most of the infertility cases with Y chromosome microdeletion are sporadic[11]. The complex Y chromosome rearrangements can lead to disruption of genes in the pseudoautosomal region and may contribute to additional phenotypes such as short stature and haematological malignancies leading to syndromic forms of male infertility[12].

✉To whom correspondance may be addressed. E-mail: dr.anubhu@gmail.com

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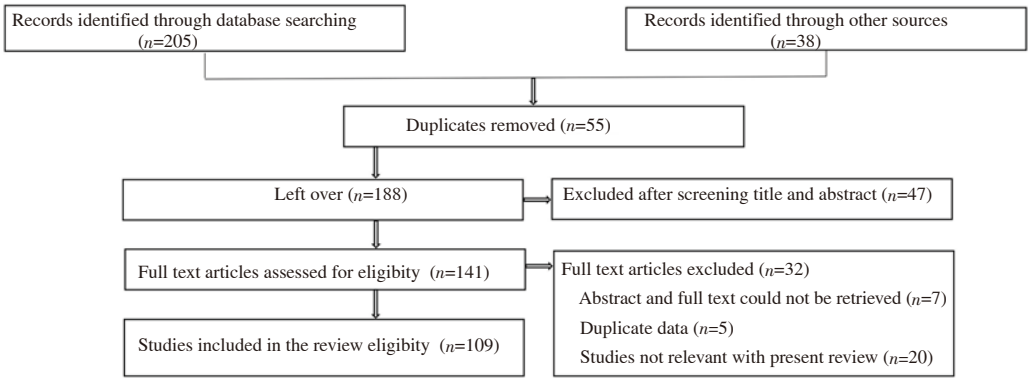

Figure 1. Flowchart of literature screening.

Terminal deletions in the Y chromosome are often associated with impaired spermatogenesis[13]. Three common microdeletions that occur in at least three regions of the Y chromosome are termed as AZF microdeletion *viz*. AZFa, AZFb, AZFc[14]. There are various sites of microdeletions on the Y chromosome, in which mainly ten hot spot sites are known defects, which lead to impaired sperm formation either in sperm count or the motility of sperm[15].

The region in gene sequence has exon, untranslated region and intron. During the primer designing, the area of the maximum chance of mutation is evaluated and the forward as well as the reverse sequence is designed. The primers were designed in such a way as to achieve the region-wise screening of the sequence on the Y chromosome. These are called the walkover on the Y chromosome to detect the boundary of microdeletion. These are later discussed in detail in the article. Polymorphic sequence variation was the first tool to detect the Y chromosome microdeletion. It can also be identified by a method like Southern Blotting, Microdeletion analysis by Polymerase Chain Reaction (PCR) method at Sequence Tagged Site.

2. Methodology

The literature available in the technical reports and online scientific records such as SciFinder, Google Scholar, MEDLINE, EMBASE, Scopus directory were explored for latest updates on the involvement of Yq microdeletions in male infertility by applying the following keywords: “azoospermia factor”, “AZF”, “male infertility”, “Y chromosome microdeletion”, “Yq microdeletion”, “diagnostics”, “prevalence”, “genotype”, “phenotype”, “epidemiology”, “oligospermia”, “gene”, “spermatogenesis”, “clinical examination”, “laboratory investigations”, “genetics” with their corresponding medical covered literature published up to May 2021. The search was restricted to the English language. The research methodology adopted for the selection of articles for this review is stipulated as flowchart in Figure 1.

3. Diagnostic tools of male infertility

The diagnostic steps related to male infertility range from basic pathological investigations up to genetic evaluations. However, before diagnosing Y chromosome microdeletion, it is essential to understand and evaluate the initial pathology of the disease. Collection of detailed history along with routine and systemic laboratory investigation is an essential basic workout for diagnosing male infertility before using molecular diagnostic tools of analysis. In the male, general health status appears to be associated with male infertility[16]. Here, an attempt has been made to summarise the overall diagnosis of male infertility in general and the algorithmic presentation of the same is depicted in Table 1[17–47].

With the advancement of molecular science, along with aforementioned basic diagnostic methods, the screening of Y chromosome microdeletion is also quite significant, as it has been nowadays considered a routine test for diagnosing male infertility. The role of Y chromosome in the diagnostics of infertility has been discussed comprehensively in this report.

4. Genetics in male infertility

Male infertility is a complex condition with a strong genetic and epigenetic background. The interaction between numbers of genes, physiology, endocrine and other controls of the gene expression along with factors like environmental and lifestyle factors which directly or indirectly affect the gene expressions may alter the infertility phenotype[48]. The testis histological phenotype is extremely heterogeneous and nearly about 2000 genes are involved in spermatogenesis; this proves the complexity of male infertility and the physiology of spermatogenesis[49]. The interaction between the thousands of genes, genetic expression, present-day lifestyle

Table 1. Summary of clinical examination, medical history and laboratory investigations in male infertility.

Methods of diagnosis	Factors screened	Scientific rationale/Importance	References
Medical history	Obesity	Peripheral conversion of testosterone to estrogen.	[17]
	Sickle cell	Testicular ischemia and ultimately damage.	[18]
	Diabetes	Autonomic neuropathy, neurogenic impotency and retrograde ejaculation.	[19]
	Liver disease	Diminished male secondary sexual character, testicular atrophy and gynecomastia.	[20]
	Chronic kidney disease	Femnanisation and hypogonadism.	[21]
Social history	Alcohol	Decrease testosterone level.	[22]
	Cigarette smoking	Decrease the seminal parameters-density, motility and morphology.	[23]
	Emotional stress	HPA axis activity, HPG axis activity.	[24]
	Anabolic steroids	Bind to the androgen receptor in the target tissue.	[25]
	Tight underwear, jeans	Decrease the temperature of the scrotum that hampers spermatogenesis.	[26]
Drug history	Antidepressants (SSRI)	Spermatogenesis, sperm motility and sperm density.	[27]
	Calcium channel blocker (nifedipine)	CCB inhibits sperm capacitation and prevents fertilization.	[28]
	Alpha adrenergic blocker (temsulosin)	Antegrade ejaculation/Anejaculation.	[29]
	Anti-epileptic (sodium valproate)	Affect the HPG axis.	[30]
	Antiretroviral (HAART, Saquinavir)	Increase abnormal semen morphology and decrease sperm motility.	[31]
Environmental and/or occupational exposure	Gasoline (Petroleum) /Heavy metals (lead/cadmium)	Disrupt the process of spermatogenesis, spermiogenesis and steroidogenesis in the testis by acting as a chemical, affecting the HPG axis/directly and damaging testicular tissue.	[32–34]
Semen analysis	Volume (2.0 mL-6.0 mL)	Aspermia- No sperm in ejaculate Hypospermia- <0.5mL (Improper collection, hypogonadism, retrograde ejaculation) Hyperspermia- > 6 mL of semen ejaculated (prolonged abstinence/excessive secretion from accessory sex gland)	[35]
	pH (7.2-7.8)	Any change in the range will occur due to inflammation of the prostate.	
	Viscosity <3 (scale 0-4)	High viscosity will resist sperm motility, concentration and antibody coating of spermatozoa.	
	Sperm concentration (20 million/mL)	Decrease in rate of pregnancy by intercourse/Intrauterine insemination with the decrease in sperm density.	
	Total sperm count (>40 million per ejaculate)	Absence of sperm in seminal plasma - Azoospermia, concentration less than 20 million/mL -oligospermia.	
	Motility (<50%progressive forward motility)	Disorders related to reduced motility can be due to the accessory reproductive gland.	
	Morphology (>14% normal form 4%)	Head defects: Large, small, tapered, pyriform, round, amorphous, vacuolated heads with the small acrosomal area, doubleheads, any combination of these. Neck and midpiece defects: Bent neck; asymmetrical insertion of midpiece into the head, thick, irregular midpiece; abnormally thin midpiece; any combination of these. Tail defect: Short, multiple, hairpin, broken, bent, kinked, coiled tails or any combination of these. Cytoplasmic defect: Greater than one-third of the area of a normal sperm head.	
	Viability (75% or more living 58%)	If motility is less than 5%-10%, it is known as ciliary dyskinesia defect in sperm flagella that causes low motility.	
	Leukocytes (less than 1 million/mL)	Indicate genital tract infection.	
Radiological investigations	Scrotal ultrasound	To rule out varicocele, epididymal abnormalities, testicular tumours, hemodynamic repercussion,undescended testis and other obstructive pathologies.	[36–38]
	TRUS	Testicular spermatogenesis and obstruction dilated vesicle and ejaculatory duct obstruction, prostatitis along with calcification and thick wall of the vesicles, absence of vas deference.	[39,40]
Urine examination	Post-ejaculation urinalysis	To rule out retrograde ejaculation.	[41]
Histological investigation	Testicular biopsy	To differentiate obstructive and non-obstructive azoospermia, diagnose testicular malignancies.	[42,43]
Biochemistry investigation	Serum testosterone	Low level directly affects male fertility.	[44]
	Lipid profile	Increased level alters sperm metabolism.	[45]
	Thyroid profile	Influences steroidogenesis as well as spermatogenesis.	[46,47]

CCB: calcium channel blocker; HAART: highly active antiretroviral therapy; HPA: hypothalamic-pituitary-adrenal axis; HPG: hypothalamic pituitary-gonadal axis; SSRI: selective serotonin reuptake inhibitor; TRUS: transrectal ultrasonography.

and environmental factors may affect the phenotypic expression of a gene which may play a contributory role in male infertility[50]. To diagnose genetic abnormality in azoospermia or severe oligospermia, techniques such as karyotyping 'fluorescence *in–situ* hybridization (FISH) methods' chromosome microdeletion screening with PCR technique and cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation test are possibly performed[51].

5. Y chromosome analysis

The polymorphism of the Y chromosome is high in terms of copy number (length) and its length in terms of Mb is 60 Mb[52]. To date, 156 transcription unit coding genes and 27 different proteins encoded by Y chromosomes have been identified[53]. There are 7 deletion intervals in Y chromosomes in which region 5 and 6 are critical for spermatogenesis[54]. Euchromatic DNA is about 23 Mb long, the

long arm is 14.5 Mb in length and the short arm is 8 Mb in length[55]. Euchromatic sequences have been divided into three classes, namely, 1) Transported from X chromosomes in the processes of evolution of Y chromosomes; 2) Partial Sequence on Y chromosome which is similar to part of the X chromosome; 3) Frequent unit across the proximal short arm (Yp) of the Y chromosome and most of part of distal long arm Yq regions[56].

6. Methods for Y chromosome analysis

6.1. Testing for Y chromosome microdeletions

The male factors responsible for infertility has various components like infection, immunological factors, anatomical malformations, or chemical insult[57]. Besides these components, genetic anomalies may also be one of the important causes of male infertility. There is a correlation between failures of spermatogenesis with the microdeletion of the long arm of the chromosome[58,59]. There is the region-wise distribution of each part of the Y chromosome known as the AZF which is further divided into a, b, c regions. Y chromosome microdeletion most frequently involves the AZFc region (60%), less frequently involves the AZFb region (16%) and only rarely involves the AZFa interval (5%). Larger Y chromosome microdeletions involving two or three AZF regions are diagnosed in 14% of cases. The comparison of several reports on different genes related to the AZF region of the Y chromosome is illustrated in Table 2.

Table 2. Comparison of different work done on different genes related to the azoospermia factor (AZF) region of the Y chromosome.

S.No.	Title	Target gene	Method	Conclusion	References
1	*DDX3Y* gene rescue of a Y chromosome AZFa deletion restores germ cell formation and transcriptional program	*AZFa* (*DDX3Y* and *USP9Y*)	Whole-genome RNA sequencing of purified GCLCs revealed an enrichment of genes	*DDX3Y* gene rescue of Y chromosome *AZFa* shows restoration of the germ cell formation and transcriptional program.	[60]
2	The sperm quality and clinical outcome were not affected by sY152 deletion in Y chromosome for oligozoospermia men after ICSI treatment	*sY152*	Multiplex PCR analysis	sY152 is the specific marker in the AZFc region in which deletion can lead to oligozoospermia and azoospermia.	[61]
3	Development of a multiplex quantitative fluorescent PCR assay for identification of rearrangement in the AZFb and AZFc regions.	*AZFb* and *AZFc* regions	Quantitative fluorescent PCR (QF-PCR) assay	AZF band AZFc region has a number of palindromic sites; deletion and duplication affect spermatogenesis.	[62]
4	The role of DAZ family in spermatogenesis	*DAZ* (*AZFc*)	Vivo UV-crosslinking followed by IP and RT-PCR,	*DAZ* family gene has a vast variety of functions which may be determined by the concern.	[63]
5	Microdeletion of a Y specific marker, Yfm1 and implication for a role in spermatogenesis	*Yfm1*	PCR, DNA sequencing	There are many loci of Yfm1 which corresponds to the *DAZ* gene which may play a crucial role in azoospermia or oligospermia.	[64]
6	Human Y chromosome variation and male dysfunction	*SRY* gene	DNA sequencing	*AZF b/c*-deleted male infertility is not caused due to absence of Y chromosome but instead microdeletion of the AZF b/c.	[65]
7	Comparative analysis of human masculinity	Male-specific Y (*MSY*) genes	SNP with PCR method	*DAZ* genes are considered as testis-specific with multiple copies.	[66]
8	Human AZFb deletions cause distinct testicular pathologies depending on their extensions in Yq11 and Y haplgroup: New cases and review of literature	Y genes, *EIFA1Y*, *HSFY*, *PRY*, *RBMY1*, *RPS4Y*, *SMCY*	STS with PCR method	STS markers Y153 deletions in g1 besides presence in g2 and g3 are therefore impossible to identify in routine PCR.	[67]
9	A fibre FISH contig spanning the non-recombining region of the human Y chromosome	Gene families, *CDY*, *DAZ*, *RBMY*, *TSPY* and *XKRY* along the NRY	FISH method	In Y chromosomes it is easy to detect aberration such as terminal and interstitial deletion ring formation para and pericentric inversion.	[68]
10	AZF microdeletions and partial deletions of AZFc region on the Y chromosome in Moroccan men	*AZF (b+c)*	PCR method	Deletion found with the patient with oligospermia/azoospermia.	[69]

DDX3Y: DEAD-box helicase 3 Y-linked; *USP9Y*: ubiquitin specific peptidase 9 Y- linked; GCLCs: germ cell-like cells; *DAZ*: deleted in azoospermia; *HSFY*: heat shock transcription factor; *PRY*: polycystine related Y; *RBMY1*: RNA binding motif protein; *RPS4Y*: ribosomal protein S 4 linked Y; STS: sequence tag sites; SNP: single nucleotide polymorphism; FISH: florence *in situ* hybridization; *CDY*: chromodomain protein Y linked; *TSPY*: testis specific protein Y linked; XKRY: XK related Y linked; NRY: nonrecombinant region of the Y chromosome.

Table 3. Primer sequences for Y chromosome microdeletion.

Name	Sequence	Product size (Mb)	Position on Y chromosome
SY83L	CTTGAATCAAAGAAGGCCCT	275	AZFa
SY83R	CAATTTGGTTTGGCTGACAT	275	AZFa
SY69L	GGAACAGCATCTTGCTCTGT	234	AZFa
SY69R	ACTATGGGAGACCAAGGCTC	234	AZFa
SY84L	AGAAGGGTCTGAAAGCAGGT	326	AZFa
SY84R	GCCTACTACCTGGAGGCTTC	326	AZFa
SY117L	GTTGGTTCCATGCTCCATAC	262	AZFb
SY117R	CAGGGAGAGAGCCTTTTACC	262	AZFb
SY127L	GGCTCACAAACGAAAAGAAA	274	AZFb
SY127R	CTGGCAGGCAGTAATAAGGGA	274	AZFb
SY131L	ACATATCCCTTGCCACTTCA	143	AZFb
SY131R	ACATATCCCTTGCCACTTCA	143	AZFb
SY152L	AAGACAGTCTGCCATGTTTCA	125	AZFc
SY152R	ACAGGAGGGTACTTAGCAGT	125	AZFc
SY254L	GGGTGTTACCAGAAGGCAAA	326	AZFc
SY254R	GAACCGTATCTACCAAAGCAGC	326	AZFc
SY255L	GTTACAGGATTCGGCGTGAT	126	AZFc
SY255R	CTCGTCATGTGCAGCCAC	126	AZFc
SY157L	CTTAGGAAAAAGTGAAGCCG	285	AZFc
SY157R	CCTGCTGTCAGCAAGATACA	285	AZFc
SY158L	CTCAGAAGTCCTCCTAATAGTTCC	231	AZFc
SY158R	ACAGTGGTTTGTAGCGGGTA	231	AZFc

6.2. FISH method

FISH is the cytogenetic technique for visualizing specific locations on Y chromosomes[70]. The first step is the fixation of the cell with a formalin-based fixative that crosses protein-protein or protein-nucleic acid cross ring after that designed probe is being added. The probe has to be complementary to the chromosome's region of interest. This can be either done with a short double standard RNA probe or a short double standard DNA probe with an enzyme known as DNAs or random DNA nick can be created. A special nucleotide is being provided with florescent protein after that ligase is being added to seal the floor with fluorescent nucleotide. PCR can amplify the probe. Denaturation of the cell is done by heating it to 950 ℃. After that, the temperature is cooled down, allowing the labelled probe to specifically bind to the target DNA sequence. Once the hybridization is completed, the unhybridized probes are washed away. The result can be analyzed under a fluorescence microscope[71]. The probe is designed so that it can only bind if it is complementary to the DNA. If any sort of mutation is present in the gene, no hybridization is possible for the probes that lie within these regions, even a deletion in the given region is enough for failure to bind at the site of interest[72]. No fluorescence signal should have occurred in this case. In the latest research and specific in genetic disorders, FISH is commonly used to create a karyotype for detecting chromosomal aneuploidy; therefore, a long probe is designed to cover the whole chromosome and stain them in different colours. Trisomy of chromosome number 21 Down syndrome can be detected with this method. In the same method, it is also used to detect the microdeletion in Y chromosomes[73].

6.3. PCR and gel electrophoresis method

For the mutational analysis of Y chromosome microdeletion, the following methods are adopted, namely, 1) Extracting of DNA from the blood sample, 2) Primer designing, 3) Perform PCR, 4) Gel electrophoresis to evaluate the microdeletion. The genomic DNA was extracted with the whole blood and processed by using the digestion of cellular protein and subsequently salting out the protein with sodium chloride and ethanol precipitation of DNA[74]. Now, the primer will be designed to check the microdeletion of the region of *AZF* which is divided into *AZFa*, *AZFb*, *AZFc* (sY69, sY83, sY84 corresponds to AZFa; sY117, sY127, sY131 corresponds to *AZFb*; sY152, sY157, sY158, sY254, sY255 corresponds to *AZFc*). As per the recent report, the primer has been designed to find out the mutation on the Y chromosome (Table 3)[75].

6.4. Clinical implications of Y chromosome microdeletion

In recent research, cytological detectable deletion was found in the men of Yq azoospermia[76]. After long discussion and researches, it was termed as *AZF* of three different regions *AZFa*, *b*, *c*, and different breakpoints have been accessed[77]. It is an assumption that *via* intra-chromosomal homologous rearrangement of genetic material by crossing over, most of the *AZF* microdeletion is generated which occurs between a series of repeated sequences blocks having almost identical structures generated[78]. Each of the deletions usually removes genes in a region or by the destruction of a major gene whose expression alone is responsible for spermatogenesis that is why it is still very difficult to attribute spermatogenic function to a definite gene[79]. A clear classification of the person with the specific type of deletion can be precluded by known patterns of deletion which are variable in detail[80]. The association between the length of deletion and quality of semen/testicular histology is null[81]. So far in the advancement of science, there has been no evidence of any phenotypic abnormities of microdeletion in all respect besides the defective spermatogenesis[82,83]. A person with Yq microdeletion

needs assisted reproductive technology/intracytoplasmic sperm injection (ART/ICSI), though the success rate of that is very low to date[84]. It has been acknowledged that microdeletion is more marked in son than father[85].

7. Y chromosome microdeletion and male infertility

The human Y chromosome harbours genes that are responsible for testis development and also for the initiation and maintenance of spermatogenesis in adulthood. The long arm of the Y chromosome (Yq) contains many ampliconic and palindromic sequences making it predisposed to self-recombination during spermatogenesis and hence susceptible to intra-chromosomal deletions. Such deletions lead to copy number variation in genes of the Y chromosome resulting in male infertility[86].

Male infertility can be caused by several factors, apart from that the genetic alterations have emerged as one of the leading causes of male infertility. In infertile males, the genetic defects commonly observed are karyotypic abnormalities, gene copy number variations, single-gene mutations and deletions of the Y chromosome (Yq microdeletions). These genetic defects impede the development of the male gonads or urogenital tract during development, and cause arrest of germ cell production and maturation, resulting in producing non-functional spermatozoa. Amongst the various factors, Yq microdeletions are the leading genetic causes of male infertility[87]. Based on data of several thousands of patients, Yq microdeletions are found in a high proportion of patients with azoospermia or severe oligozoospermia; therefore, Yq deletion screening has now become a routine test for infertile males in many countries to identify the cause of male infertility.

8. Azoospermia and oligospermia

The absence of sperm in ejaculation is defined as azoospermia. In the general population, it is estimated to affect 1% of the male population[88]. The treatment of infertility due to the other causes is revolutionized by ART and IVF along with ICSI[89]. By the use of viable spermatozoa recovered from epididymal and testicular biopsy, the male with the post-meiotic defect may retain fertility[90].

Oligospermia is defined as a total sperm count of less than 15 million/mL in male ejaculates[91]. It usually associates with abnormalities in motility and morphology. Categorization based on the density is of not much importance as the sperm count 1-5 million/mL is sufficient for natural conception[92]. However, Y chromosome microdeletion has been reported in severe oligospermia patients, but still further research is required in this field.

In the etiological cause of azoospermia and severe oligospermia, genetic factors predominate from multifactorial diseases. In spite of large information about gene expression essentially in the testis and a higher number in infertile mice, very little pieces of knowledge about the pathophysiology of deduced sperm production is available. The reduced sperm production along with its primary cause, genetic and epigenetic consequences is still in controversy. To understand spermatogenesis, to provide adequate genetic counselling and to adopt the best course of action for the patient, the identification of genetic abnormality is useful.

Since azoospermia is untreatable that why it is more important to focus on this condition following testicular and epididymal biopsy, ICSI can be anticipated when spermatogenesis is not completely abolished. After the detail, molecular analysis of the *AZF* locus has been further subdivided into three regions but somewhere extra four regions have been described: *AZFa*, *AZFb*, *AZFc* and the fourth suspected region is *AZFd*[93,94].

Spermatogenesis arrest may be due to microdeletion in the AZF region. However, more research is needed to explore the genotype-phenotype relation and *AZF* gene microdeletion. Sequence tag site-polymerase chain reaction (STS-PCR) is the gold standard for microdeletion[95]. Multi analytic suspension arrayis also considered a highly efficient method to diagnose the Y chromosome microdeletion[96]. ART, ICSI, testicular sperm extraction make reproduction possible for many couples, but the chance of vertical transmission of *AZF* microdeletion increases[97].

9. *AZF* gene

Spermatogenesis is controlled by many genes that are specific to the Y chromosome only. Most of the genes are present in the specific region known as the AZF region. Micro deletion is known to be the most frequent structural chromosomal abnormalities and is the major cause of male infertility. *AZF* microdeletion can be recognized by STS-PCR, suspension array technology and array genomics comparative hybridization[98]. Suspension array technology is used in single nucleotide polymorphism genotyping and genetic diseases. It uses microsphere beads to prepare an array. Suspension array technology allows side by side testing of multiple gene variants through the use of microsphere bead as each deed has the unique property of optical density which is most common in fluorescent colour[99]. In the past few years, researchers have evolved new cytogenetic techniques. A new technique known as comparative genomic hybridization provides an alternative means of genome-wide screening for copy number variation[100]. It uses two genomes (test and control) which are differentially variable and competitively hybridized to metaphase chromosomes.

AZF gene is located on the Y chromosome of the *AZF* region. Deletion in this region may not produce sperm. There are two

regions AZF Ⅰ (AZFa), AZF Ⅱ (AZFb and AZFc). Micro deletion in that may produce azoospermia and severe oligospermia. Due to the highly variable length of the Y chromosomes, the presence of *AZF* gene was suspected at that time by the researchers. The active AZF chromatin is visible during the decondensation of the Y chromosome in the nuclei of spermatogonia before it pairs with the X chromosome forming the X-Y chromatin structure in the spermatocyte of nuclei[101].

9.1. The AZFa region

The *AZFa* has about 400-600 kb of DNA and is located in the proximal portion of the interval of the Y chromosome[102]. *AZFa* encodes two protein entities: USP9Y and DDX3Y.

USP9Y: This protein belongs to ubiquitin-specific proteases and member of the C-19 cysteine peptidase family[103]. USP9Y is ubiquitously expressed in adult and embryonic tissues and shares 91% identity with its X-homologue. The role of USP9Y is noted in the regulation of protein turnover during spermatogenesis. This protein is majorly responsible for the azoospermia and the cause of AZFa deletion phenotype[104].

DDX3Y: The protein encoded by *AZFa* is a member of the DEAD-box family which has nine conserved motifs including the DEAD motif and these are thought to involve adenosine triphosphate binding hydrolysis, RNA binding and the formation of intermolecular interaction[105]. Mutations in this gene result in male infertility, a reduction in germ cell numbers and can result in Sertoli-cell only syndrome.

9.2. The AZFb region

The *AZFb* spans around 1-3 Mb of DNA and is found on the distal portion of deletion interval 5 to the proximal end of deletion interval 6. The *AZFb* gene content reflects different sequence types having single gene mapping with ampionic gene families. *AZFb* region has a total of 5 different single-copy transcriptions.

KDM5D: This is the male-specific histone protein KDM5D (demethylase lysine-specific demethylase 5D) present on the Y chromosome.

EIF1AY: AZF gene in testicular biopsies of azoospermic men revealed a lack of EIF1AY expression, which can sporadically contribute to azoospermia.

RPS4Y2: It is Y-linked ribosomal protein-coding gene that is evident to restrict the gene expression in the testis and prostate. The gene expression of *RPS4Y2* is highly expressed in testicular biopsy containing the germ cells[106].

CYorf15A and CYorf15B: The CYorf15A and CYorf15B sequences have X homolog that belongs to the family of taxian group and had been of ten correlated or linked to the transcriptional regulation of osteoblasts[107].

CYorf15B: Multiple gene copies.

XKRY: Heat shock transcription factor (*HSYF*) has been mapped in AZFb region on the Y chromosome whose deletion results in severe male infertility. HSFY belongs to the heat shock factor family that is implicated in spermatogenesis both in animals and humans. *HSPA2* gene (the homologue of the murine hsp70.2) is significantly expressed in testis with normal spermatogenesis, whereas wake expression is detected in testis with abnormal spermatogenesis, and no expression is seen in Sertoli cell-only syndrome.

PRY: It has two functional units known as b1 and b2 or also known as PRY and PRY2; these both are testis-specific. Men with abnormal semen parameters have increased PRY levels in ejaculated sperm; this indicated the connection between expression and defective spermatogenesis[108].

RBMY1A1: The *RBMY* gene family is found on the Y chromosome of all mammals, and microdeletions are strongly associated with infertility in men. RBMY1A1 is supposed to be RNA binding domain that closely resembles two resemble RNA binding proteins: *YRRM* (for this reason this gene is implicated in azoospermia), and hnRNP (glycoprotein as well as autoantigen)[109].

9.3. The AZFc region

AZFc is located at the distal part of the deletion interval 6 of the Y chromosome and spans nearly 3.5 Mb of euchromatin. In idiopathic azoospermia, deletion of *AZFc* is common. There are 8 family members of the AZFc gene respectively: *BPY2*, *CDY*, *DAZ*, *CSPG4LY*, *GOLGAZLY*, *TTY3*, *TTY4* and *TTY7*[110].

DAZ gene, the family member of RNA binding protein that is preserved in all metazoans, controls meiosis. Mutation in the *DAZ* gene is proved disastrous for haploid gamete production, so it is important for spermatogenesis. Hence, special attention is needed because a mutation in the *DAZ* gene can cause azoospermia/oligospermia through Yq microdeletion. Mutation in each component of *DAZ* may have variable effects. Mutation in *DAZ2*, *DAZ3*, *DAZ4* is found in both fertile and sterile means as described in the family tree which may be inherited from father to son[111].

9.4. Other functions of AZF gene

It has been detected that other than testis several transcripts of the *AZF* gene were present in multiple tissues in varying quantities[112]. Among these, three protein-coding genes of *AZFa* (*USP9Y*, *DDX3Y*, *UTY*) and *AZFb* (*HSFY*, *KDM5D*, *EIF1AY*) are nearly found in all tissue[113]. There are many genes of *AZFb* and *AZFc* loci that seem to be expressed exclusively in the testis except for *RPS4Y2*, *DAZ*, *DDX3Y* and *CDY2A*. *RPS4Y2* was expressed in the prostrate, *DAZ* was expressed in the stomach, *DDX3Y*

was expressed in the stomach, and *CDY2A* was expressed in the epididymis[114]. The widespread detection of the transcripts of the *AZF* genes is also backed by the presence of the corresponding proteins in these tissues. The abundance of *AZF* gene mRNA in many somatic tissues is almost comparable to that of testis, proving that these are regulated[115].

This multi-tissue expression of *AZF* genes is categorical and not due to the misalignment of the sequences to their autosomal homologous transcripts as none of these genes was detected in female tissues including the ovary and the reproductive tract[116]. Furthermore, none of the *AZF* gene transcripts was detected in the adipose tissue, smooth muscles and the parathyroid gland, irrespective of sex.

10. Conclusions

The result of mutation is a change in the product of the amino acid sequence from protein metabolism. Therefore, the result of such a type of mutation is harmful and not at all favourable for the cell as a unit to perform the function. When mutation takes place, it alters the protein which may play an important role to perform the function, resulting in a serious medical condition or termed as a genetic disorder. One such example is Yq microdeletion. The Y chromosome incorporates the important gene that is responsible for the development of the testis and the regulation of spermatogenesis. There are many ampliconic and palindromic sites making them liable to self-recombination at the time of spermatogenesis that is they are susceptible to intrachromosomal mutation. The result of such a mutation leads to male infertility.

11. Future prospects

In this article, we have tried to focus on the prevalence of male infertility along with the tool to diagnose the newly undiagnosed case of male infertility through the medical/surgical history, lab investigations which include semen analysis, serological investigation (thyroid profile, lipid profile, serum testosterone), urine examination and histological examination. Moreover, focus has been made to diagnose the unknown cause of male infertility through genetic study. Y chromosome microdeletion is one of the major areas to work with and an attempt has been made to explore the methods regarding the genetic aspect of male infertility. These various methods like FISH technique, PCR, DNA sequencing, whole-genome sequencing are some of the genetic tools to detect the mutation/microdeletion. In this review, mainly the Y chromosome microdeletion has been detected by PCR method with the help of designed primer which detects the microdeletion in the Y chromosome. A walk-over on the Y chromosome is done with the help of a designed primer that detects the microdeletion on the specific area on the Y chromosome. From the future perspective point of view, only 10 exons have been designed and used for the detection of microdeletion which can also be called a hotspot area as frequent microdeletion; now some more areas can be selected for the detection of microdeletion by designing a greater number of exons for detecting the new area of microdeletion. The Prakriti concept of human constitution-wise personalized therapy under Indian Ayurvedic system of diagnostics and therapeutics is gaining interest in the recent decade with scientific evidence of correlation with the science of genomics[117-120]. This area may also be explored further with the integration of contemporary science with ancient wisdom.

Conflict of interest statement

The authors declare there is no conflict of interest.

Authors' contributions

Awanish Jaiswal and Anurag Pandey conceived the idea and wrote the manuscript. Mamta Tiwari, Akhtar Ali and Rohit Sharma edited and proofread the document. The entire team approved the submission of the final manuscript.

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