

Primary Testicular Failure: An Overview

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Introduction

Primary testicular failure (PTF) or hypergonadotropic hypogonadism refers to conditions where testes fail to produce sperm despite adequate hormonal support. Primary testicular failure is major cause of non-obstructive azoospermia and oligospermia. It affects approximately 1% of all men and 10% of those seeking fertility evaluations [1]. Non-obstructive azoospermia i.e. defect in spermatogenesis can be primary (testicular pathology) or secondary (pathology outside testes; mostly hypothalamo-pituitary axis or pre-testicular). The pre-testicular (hypogonadotropic hypogonadism) causes of azoospermia may be defined as extragonadal endocrine disorder originating in the pineal, hypothalamus, pituitary, or adrenals, which have an adverse effect on spermatogenesis through aberrant hormonal action. The testicular (hypergonadotropic hypogonadism/primary testicular failure) causes of azoospermia are primary defects of the testes. Primary testicular failure is classified into four distinct subtypes viz., sertoli cell only syndrome (SCOS), germ cell/maturation arrest (GCA/MA), hypospermatogenesis (HS) and tubular fibrosis (TF) according to histopathology/cytology findings although accurate categorization is only possible by multiple testicular biopsies/fine needle aspiration cytology (FNAC). However, often one may find differences in subtypes between testes viz., SCOS in one side and tubular fibrosis in other side or any other combinations and these cases are usually labeled as mixed group. In addition many patients may present as complete germinal cell aplasia in some tubules whereas complete spermatogenesis in adjacent tubules (focal germinal cell aplasia) or some tubules with sertoli cells only/hyaline sclerosis and other tubules with complete spermatogenesis or any combinations. This categorization should be considered as a description of histopathologic phenotypes of spermatogenic failure, and not as manifestations of disease entities. Sometimes, patients may present as hypospermatogenesis/maturation arrest initially and later as sertoli cell only syndrome/testicular fibrosis over a period of few years hence diagnosis may change with time. Sertoli cell only syndrome or germ cell aplasia applies to a testis, in which germ cells at any stage are absent, but the tubular architecture is not affected by fibrosis, and supporting cells continue to be present. Sertoli cells play a central role in development of a functional testis, and hence in the expression of a male phenotype. Sertoli cell only syndrome is a common finding of non-obstructive azoospermia. It is a histopathologic phenotype of spermatogenic failure and described first by Castillo et al. [2], in complete germ cell aplasia, the tubules are reduced in diameter, contain only sertoli cells and no other cells involved in spermatogenesis are present. The primordial germ cells either do not migrate from the yolk sac into the future gonads or do not survive in the seminiferous tubules. Germ cell aplasia can also be focal with a variable percentage of tubules containing germ cells, but even in these tubules, spermatogenesis is often have limited activity [3] and hence, should be categorized as hypospermatogenesis. Maturation/Germ Cell arrest is the interruption of normal germ cell maturation at a specific

cell stage including spermatogonia, spermatocyte or spermatid level. In hypospermatogenesis all the stages of spermatogenesis are present in some or all tubules, but they are reduced in number. Tubular fibrosis presents as thickening of peritubular membrane and hyaline deposition on basement membrane with absence of germ cells and sertoli cells.

Despite extensive efforts, cause of primary testicular failure in most (over 50%) cases is still unknown and reflects our poor understanding of the mechanism governing spermatogenesis. Hence, it is important to explore underlying etio-pathology to find out answers for managing these cases. This write up is an attempt towards this direction.

Etiologies

Primary testicular failure can result from a variety of congenital or acquired disorders, including chromosomal (Klinefelter syndrome, Y chromosome microdeletions, etc), single gene mutations, cryptorchidism, varicocele, etc. Klinefelter Syndrome is the most common known cause of primary testicular failure [4,5]. It affects approximately 1 in 1000 males [6] and is characterized by extra sex chromosome (X). Although an extra X chromosome (47,XXY) is the most common form, some men with Klinefelter syndrome have a greater number of X chromosomes or mosaicism (48,XXX, 46,XY/47,XXY) [7]. Infrequently, a 46,XX males, resulting from translocation of the testis-determining gene (SRY) to an X chromosome, also have klinefelter syndrome phenotype [8]. The phenotype varies with the number of extra X chromosomes, and possibly also with the number of trinucleotide CAG repeats on the androgen receptor gene (a polymorphism); as the length of the repeat sequence increases, androgen receptor activity decreases. A longer CAG repeat sequence has been associated with taller stature, lower bone mineral density, gynecomastia, and decreased penile length [9]. Men with klinefelter syndrome generally have small, firm testes (depending upon whether prepubertal or post pubertal atrophy), resulting from damage to both seminiferous tubules and leydig cells. Serum concentrations of FSH and LH are elevated and testosterone levels are decreased to varying extent. Affected men have severely reduced sperm counts and are under-virilized [7,10]. Cryptorchidism is more common in men with klinefelter syndrome and causes more severe testicular damage [11]. Later in life, they have an increased risk for breast cancer, germ cell tumors, varicose veins and diabetes mellitus [12]. Other chromosomal abnormalities associated with primary gonadal failure include the 45,X/46,XY karyotype (mosaicism), causing a syndrome characterized by short stature and other features of Turner syndrome [13]. Because the testes may be streaks, dysgenetic, or normal, the phenotype varies from female to male. In those with a streak and a dysgenetic testis (mixed gonadal dysgenesis), the risk of gonadoblastoma is increased (approximately 20%), and gonadectomy is therefore indicated.

Microdeletions of the long arm of the Y chromosome are now recognized as a relatively common cause of primary testicular failure

(severe oligospermia and azoospermia), affecting up to 20% of men with infertility [14]. Most microdeletions are mapped to the Yq11 region (named azoospermia factor, or AZF), which contains three regions, AZFa, AZFb, and AZFc. Deletions of the AZFa or AZFb typically result in azoospermia. Mutations in the AZFc region cause infertility of varying severity, ranging from oligospermia to azoospermia and are the commonest microdeletions in humans [15]. The DDX3Y and USP9Y genes located in the AZFa region and have important role in spermatogenesis. Deletions of these genes are consistently observed with azoospermia [16,17]. Y chromosome microdeletions also have been observed in men with cryptorchidism, varicocele and obstructions of the vas deferens [18].

In our own study on 164 apparent idiopathic primary testicular failure cases we could find out underlying cause in about 21% cases (8.5% sex chromosomal abnormality, 11.6% Yq microdeletion i.e., AZFa,b,c and 0.6% combined sex chromosome abnormality as well as Yq microdeletion). When we dissected out in relation to subtypes we find different frequency of detectable causes (chromosomal and Yq microdeletion). We find underlying causes maximum (45%) with testicular fibrosis and minimum (8.5%) with germ cell/maturation arrest groups. Detectable cause was found in 22% cases (13.2% sex chromosomal abnormality and 8.8% Yq microdeletion) of sertoli cell only syndrome, 8.5% cases (0% sex chromosomal abnormality and 8.5% Yq microdeletion) of maturation arrest, 21% cases (0% sex chromosomal abnormality and 21% Yq microdeletion) of hypospermatogenesis, 31.5% cases (10.5% sex chromosomal abnormality and 21% Yq microdeletion) of mixed group and 45% cases (27.3% sex chromosomal abnormality, 9.1% Yq microdeletion and 9.1% combined) of testicular fibrosis. When we analysed our result according to subtypes, we have found few chromosomal abnormality in hypospermatogenesis and maturation arrest cases. We have found Yq microdeletion in all subtypes and no specific type of deletion with any specific sub types (ongoing work).

Normal male sexual differentiation and spermatogenesis require both normal androgen production and normal androgen receptors. The androgen receptor plays an important role in the differentiation of spermatids and their release from the seminiferous epithelium. The number of trinucleotide CAG repeats in exon 1 of the androgen receptor gene is inversely correlated with its transcriptional activity [9]. A meta-analysis with 33 published studies revealed that men with spermatogenic disorders had longer CAG repeat lengths [19]. Similarly disorders of estrogen synthesis or action also associated with spermatogenic defect. Impaired spermatogenesis has been observed in mice and in men lacking a functional estrogen receptor alpha [20,21]. In mice inactivating mutation in the aromatase enzyme also causes spermatogenic defect [22]. FSH receptor gene mutation too affects spermatogenesis [23]. Men with myotonic dystrophy (an autosomal disorder associated with impaired motor function, cataracts, premature balding, mild mental retardation, and hypogonadism) also exhibit abnormal spermatogenesis [24]. Mutations in the SYCP3 gene (involved in regulation of the synapse between homologous chromosomes during meiosis) have been implicated as a potential cause of spermatogenic defect [25]. Others genes viz., DAZL (an autosomal homolog of the DAZ, deleted in azoospermia, gene) [26,27], PRM1 and PRM2 (protamines involved in chromatin compaction), TNP1 and TNP2 (transition nuclear proteins) and USP26 (deubiquitinating enzyme family) also are associated with spermatogenic defects [25]. We are working on primary testicular failure on various etiological aspects, including genotype phenotype co-relation using array CGH. Our initial findings indicates association

between CNVs of PAR 1 and 2 with testicular maturation arrest in approximately 30% cases (ongoing work) besides possible association with heavy metals like manganese, cadmium, lead, and nickel in testicular maturation arrest [4,28].

Cryptorchidism/failure of testicular descent, an androgen-dependent process, is one of the major causes of spermatogenic failure. It is commonly seen with Kallmann syndrome, androgen resistance, and defects in testosterone synthesis. Cryptorchidism can be unilateral or bilateral and, in either case, is associated with impaired spermatogenesis and an increased risk for developing testicular tumors. Varicocele results from dilation of the pampiniform plexus of the spermatic veins. They are more prevalent in infertile men (up to 30%) and are 10 times more commonly found on the left side than on the right side, probably because the left spermatic vein is longer and joins the left renal vein at a right angle [29]. Although, increased testicular temperature, delayed removal of local toxins, hypoxia, and stasis are viewed as the mechanisms likely responsible for the association between varicocele and infertility, however no causal relationship has been established yet [30]. Mumps orchitis is also recognized as a cause of primary testicular failure. Although rarely implicated in prepubertal ages, it is responsible in adult men. The mechanism may involve damage to the germinal epithelium, ischemia, or immune dysfunction [31]. Drugs that can adversely affect spermatogenesis are alkylating agents (e.g., cyclophosphamide, chlorambucil, etc), anti-androgens (e.g., flutamide, cyproterone, spironolactone, etc), androgens (high dose) and anabolic steroids [32]. Doses of radiation as low as 0.015 Gy (15 rads) can suppress spermatogenesis and doses above 6 Gy generally cause permanent azoospermia and infertility [33]. Environmental exposures that may act as gonadotoxins include heat, smoking, heavy metals, [4,28] organic solvents, and pesticides. Chronic illness such as chronic renal insufficiency, cirrhosis of liver, etc can also result in primary testicular failure.

Biomarkers

Although diagnosis of PTF is only possible through testicular cytology or pathology however one can predict the condition by analysis of biomarkers. In the workup of azoospermia, FSH is the classical endocrine parameter to discriminate testicular impairment of spermatogenesis from obstructive disorder. Several studies confirm that FSH level is a valuable predictive marker of the histological picture of the testis [34] but a wide overlap between values in normal and reduced spermatogenesis limits its diagnostic accuracy [35]. Human FSH is a glycoprotein consisting of α and β subunits. The β -subunit of FSH is specific. This confers both immunological and functional specificity. FSH promotes gametogenesis in the gonads.

Inhibin B is heterodimeric polypeptide hormone. Inhibin B is the major circulating inhibin in men [36]. It selectively suppresses the secretion of the FSH and has local paracrine actions on the gonads. It is produced by the sertoli cells of the testes in the male. Its primary role appears to be in the regulation of gametogenesis via negative feedback on the production of FSH. In the prepubertal testis it is predominantly produced by the Sertoli cells, while the site of production in the adult testis is still controversial, in particular with abnormality however, principally by the Sertoli cells [36]. Inhibin B production varies during life. There is an inhibin B peak in serum shortly after birth (coincide with increased serum FSH), probably reflecting the proliferating activity of the sertoli cells [37]. Afterwards, inhibin B level decreases and remains low until puberty, when it rises again, first as a

consequence of FSH stimulation and then as a result of the combined regulation of FSH and spermatogenesis [37]. In adult male, serum level of inhibin B is stable throughout life [38]. Inhibin B expression and secretion in men is positively correlated with Sertoli cell function and number, sperm number and spermatogenic status and negatively correlated with FSH [39]. Men with hypospermatogenesis and spermatogenesis arrest may have lower levels of inhibin B. Men with sertoli cell only (SCO) syndrome have extremely low levels of inhibin B [34,37]. Suppression of spermatogenesis by exogenous testosterone or chemotherapy decreases serum inhibin B. It is a good marker of spermatogenesis and may offer an improved diagnosis of testicular dysfunction [34,40]. Based on these observations, it has been suggested that inhibin B could be a good marker for spermatogenesis [41].

Anti-mullerian hormone (AMH) is a glycoprotein. AMH belongs to the transforming growth factor- β (TGF- β) super family. It is involved in the regulation of tissue growth and differentiation. AMH is produced by the sertoli cells of testis in the male, and by ovarian granulosa cells in the female. During embryonic development in male, secretion of AMH from testicular Sertoli cells is essential for the regression of the Mullerian ducts, and thus the normal development of the male reproductive tract. In the male, secretion of AMH by the Sertoli cells commences during embryogenesis and continues throughout life. AMH blood concentration decreases dramatically during puberty [42] and persists at very low values in adults [43]. Very little is known about the function of AMH in postnatal life; it has recently been shown that it controls Leydig cell proliferation and steroidogenic function [44] and that it may be related to germinal cell proliferation [45]. AMH is expected to be high in cases of sertoli cell immaturity as well as with conditions like sertoli cell hyperplasia or tumor. Maymon et al. [46] have observed immature sertoli cell status with high AMH in early MA (pre-meiotic) and SCOS. However, low AMH is observed most often with non-obstructive azoospermia in particular SCOS and TF [4,5,47]. Low AMH reflects a primary alteration in Sertoli cell function or number that may leads to spermatogenic arrest. Finding of normal as well as low AMH suggests etiologic heterogeneity and/or different stages (early with normal value and advanced with low value) of same disease.

Lactate is produced by sertoli cells and used by germ cells as an energy substrate [48]. Sertoli cells have enormous capacity of converting glucose into lactate. Round spermatids and pachytene spermatocytes are dependent on lactate [49], whereas ejaculated spermatozoa can use glucose or fructose as substrates [50]. Lactate could therefore be an important intermediate for the regulation of the survival of pachytene spermatocytes and round spermatids. One should expect low value of lactate in late maturation arrest (post meiotic; round stage). A high value of lactate indicates either non-utilization of lactate by germ cells as with SCOS or sertoli cell dysfunction. However, in our experience [4] with studies on lactate in PTF is more complex and difficult to find any co-relation although low value is seen in all subtypes in approximately 40% of cases (ongoing work).

We are working with several biomarkers and found inhibin B as best predictor/marker of primary testicular failure, in all subgroups excepting SCOS where FSH seems marginally better marker [34]. We did not find any advantage of adding AMH as another marker over FSH and inhibin B and recommend FSH and inhibin B combination should be used to assess PTF cases. Seminal lactate as a marker of PTF seems complex and no co-relation was detectable. Seminal lactate does not provide any important inputs. Furthermore, we did not find any

differences in AMH, Inhibin B and seminal lactate with or without chromosome abnormality or Yq microdeletion. The classic predictors of spermatogenesis are testicular size, semen analysis, FSH level and testicular histology. However, in our experience we have found frequently contradicting findings viz., small testicular size with better seminal parameters or SCOS with occasional sperms on semen. We have observed lower predictive value of FSH with MA as well as HS. The FSH value is often normal in these subgroups. It is also observed in hypospermatogenesis [35]. Inhibin B seems a better predictor in these situations. It can discriminate between complete absence of germ cells and less severe disturbances of sperm production in the testis. This is also supported by observation of more accurate prediction of the presence of testicular spermatozoa in nonobstructive azoospermia with the level of serum inhibin B [51,52]. Different views exist in the literature between FSH and inhibin B with spermatogenesis. In infertile patients with primary gonadal failure, inhibin B decreases and FSH increases. In general, there is good correlation with the degree of spermatogenic damage; arrest at the earlier stages having the lowest inhibin B levels viz. Sertoli cell only syndrome. Serum inhibin B is more accurate than serum FSH in predicting the presence of testicular spermatozoa in nonobstructive azoospermia [51]. However, there are reports claiming FSH with a higher predictive value than inhibin B [53]. Similarly, reports also claimed that combination of the two or inhibin B/FSH ratio [53] is better predictor for the presence of spermatogenesis. Tuttelmann et al. [54] also have found no significant differences in serum AMH levels between controls and men with oligozoospermia, confirming that serum AMH is not of diagnostic significance in men with impaired spermatogenesis. But, others [47] observed significantly lower serum AMH levels in non-obstructive azoospermia than in obstructive azoospermia. It is possible that other, still unrecognized factors take part in the complex interplay between FSH, inhibin B, AMH, Lactate and spermatogenesis. Normal testicular spermatogenesis is controlled by gonadotrophins and testosterone. The effects of these are modulated by endogenous factors such as estrogens, inhibins, etc. Estrogen is involved in the negative feedback effects of testosterone on brain and controls pituitary gonadotropin secretion. It is important for the delicate balance of the hypothalamo-pituitary-testis axis in male. Nevertheless the role of estrogen in male is still a matter of debates even though there is a growing body of evidence suggesting that estrogen play a role via their specific receptor (ER α and ER β) that are present throughout the genital tract besides effect on gonadotropin secretion. In our experience estrogen play role in some cases of early maturation arrest as evidenced by elevated level of estradiol (ongoing work).

Evaluation

The evaluation of a patient with azoospermia is performed to determine the etiology, prognosis, treatment options and counseling. The diagnosis of azoospermia is made when no spermatozoa can be detected on high-power microscopic examination of centrifuged (for 15 minutes at a centrifugation speed of 3000 g or greater) seminal deposits on at least two occasions at an interval of 3 months (preferably). The initial important evaluation to determine cause and type should include fertility history, mumps, cryptorchidism with or without surgical correction, genital trauma or history of inguinal surgery, genital infections such as filariasis/tuberculosis, gonadotoxin exposures such as radiation therapy/chemotherapy or heat exposure and current medications. Family history of cystic fibrosis is also to be obtained. Physical examination should include secondary sex character (body habitus, hair distribution, bearded, moustache, sideburn and

gynecomastia, etc), body mass index, testis (size and consistency), epididymis (nodule/cyst/varicocele), vasa deferentia (present/absent), etc. The initial hormonal evaluation should include measurement of serum testosterone, prolactin and FSH levels and later inhibin B and/or AMH. One ultrasound with doppler study should be advised to exclude varicocele. Finally a FNAC or testis biopsy is required to confirm and subtype diagnosis.

Management

In general, primary testicular failure of genetic/idiopathic origin is associated with very poor fertility even with assisted reproductive technologies. Otherwise management of primary testicular failure is related to avoiding risk factors as preventive measure and/or early diagnosis along with early appropriate management. Universal MMR vaccination will minimize mumps orchitis related primary testicular failure. Early management of varicocele and/or cryptorchidism may result in restoration of fertility. The risks arising from chemotherapy, radiation or surgery may be preventable by judicious thinking and adopting preventive measures (care during surgery, prior cryopreservation of gamete/gonadal biopsy, protective measures during radiotherapy). Genetic abnormalities associated with the disorder are usually inherited and transmitted to offspring (often requires assisted reproduction), hence genetic counseling should be provided to patient once genetic etiology is detected. Recent studies suggest that many of the idiopathic PTF have genetic basis. It is time to investigate idiopathic PTF cases at genomic and epigenomic levels to find out the underlying causes. In azoospermic men with focal spermatogenesis or hypospermatogenesis or late maturation arrest, pregnancies can be achieved with testicular sperm extracted and injected into mature oocytes by intracytoplasmic sperm injection (ICSI). In vitro spermatogenesis from testes biopsy from these cases may result in pregnancy and live birth in near future. Currently there is no therapy for primary testicular failure with complete germ cell aplasia. However, in future predictive genomic medicine will help in identifying individual who are at risk for future PTF and will give time in planning to counter future problem through gonad cryopreservation/gamete cryo-banking. Most genetic etiology cases presented a normal spermatogenesis in younger age group however, spermatogenesis decreases rapidly in few years indicating accelerated programmed death. In future predictive medicine approaches will identify these cases and appropriate preventive measures may be instituted before complete testicular failure.

Conclusion

Primary testicular failure is a heterogeneous group and one should work/analyze selectively (viz., SCOS or HS or MA) as underlying causes are different in different subtypes. We think there is an urgent need for more study in each subtypes at genomic, epigenomic and proteomic level to find out underlying etiologies. Furthermore, we suggest that a different approach may be required to study cases of germ cell arrest/maturation arrest, as detectable cause is mostly unknown with this sub types (our initial findings suggest copy number variation of pseudo autosomal region 1 and 2 in approximately 30% cases). This initial information should excite more specific work to explore underlying pathophysiology and pathways that in future could help in understanding and managing primary testicular failure.

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