45X, 46XY Mosaicism Presenting with Virillization in Puberty

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Disorders of sex development (DSD) are characterized by atypical development of chromosomal, gonadal, or phenotypic sex. 45X,46XY mosaicism is a type of sex chromosome DSD which presents with a wide heterogeneity of manifestations. We report the case of a 13-year-old phenotypically female who presented with clitoromegaly at puberty. Testosterone level was elevated on serology. Out of the 50 cells examined, 43 cells had Monosomy X while 7 cells had a normal male karyotype. She was managed by a multidisciplinary team. Due to the presence of Y chromosome, the solid nodular structure seen on the right gonad in magnetic resonance imaging and the pain caused by the phallus, Laparoscopic bilateral gonadectomy, salpingectomy and clitoroplasty were done after a shared decision making. Histopathology revealed Gonadoblastoma and Germ cell neoplasia-in-situ of the right gonad justifying timely removal. She was then maintained on estrogen for induction of secondary sexual characteristics.

Key words: Mosaicism, virilization

Introduction

Sexual differentiation starts around 6-7 weeks age of gestation when the chromosomal sex directs the bipotential gonads to develop to either testes or ovaries. In males, the expression of SRY upregulates genes responsible for testes differentiation. Conversely, in females, the uninhibited expression of WNT4 induce ovarian differentiation. Under the influence of SRY, the gonad develops into a testis containing spermatogonia, Leydig cells and Sertoli cells. Sertoli cells produce Anti-Mullerian Hormone which induces regression of the paramesonephric ducts. Leydig cells produce testosterone which support growth of the mesonephric ducts that will give rise to seminal vesicle, efferent ductules, epididymis and vas deferens. Some testosterone is converted into dihydrotestosterone which supports development of the prostate gland, penis, and scrotum. In the absence of SRY, as in females, the gonad develops into an ovary with oogonia and stromal cells. Since no testosterone is produced, the mesonephric ducts regress. Since there is no Anti-Müllerian Hormone, the paramesonephric ducts will persist and give rise to the oviducts, uterus, and upper 1/3 of the vagina. The urogenital sinus contributes to the formation of the bulbourethral glands and the lower 2/3 of the vagina.[1]

Disorders of sex development (DSD) are congenital conditions characterized by atypical development of chromosomal, gonadal or phenotypic sex.[2] It has been estimated that its individual incidence is approximately 1 in 4,500–5,500 newborns.[3] 45X,46XY mosaicism, the case of our patient, is the most common type of sex chromosome abnormality DSD with an incidence of 1 in 10,000.[4] There is no absolute management of these cases considering the variation of manifestations, their onset and patient preferences. Each case is different and reporting such cases can

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be of help to other practitioners too. Management of these cases is long-term involving a myriad of professionals working with the patient and his/her family. Sex assignment is challenging and the surgical approach has evolved over the years with increased emphasis on a conservative approach and the delay of irreversible surgery until adulthood however the risk for development of germ cell tumors should also be considered.[5]

Reported here is the timely multidisciplinary management of a patient with a sex chromosome DSD.

The Case

This is a case of a 13-year-old phenotypically female with no remarkable medical history who came in for an enlarging mass on the upper part of the vulva. Her grandmother first noted this around 4 years prior to consultation when she was assisting the patient with bathing. The patient was not bothered until a year prior when the mass started to be painful when compressed or touched. Also during this time, she was concerned that her height and breast development were not at par with her peers. Accompanied by her mother, they consulted a gynecologist who requested for a pelvic ultrasound revealing an infantile uterus, thin endometrium and bilateral gonadal streaks. Ultrasound of kidneys, ureters and bladder was unremarkable. Impression at this time was psuedohermaphroditism. Karyotyping was requested and the patient was then referred to our institution for further management.

On physical examination, vital signs were stable. Anthropometric measurements were plotted in the WHO/CDC charts. Her weight (36.15kg) was within the lower normal for age. She had short stature (144cms) which was at the 3rd percentile for age. Her BMI was normal at 17.02 kg/m^2. There were no acne nor neck masses. Hair distribution was normal. Abdomen was soft and nontender without palpable masses. Breasts and Pubic hair were Tanner stage I and II respectively (Figure 1). Her clitoris was enlarged and long measuring 5cm x 2cm x 1.5cm with no identifiable urethral meatus on both the ventral and dorsal areas. It was classified as Prader Stage III. A vestibule was noted when the labia majora and minora were separated. A urethral meatus was seen in the superior portion of the said vestibule. Under anesthesia, the vaginal canal was patent and measured 6 cm in length leading to a small cervix. There were no labial or inguinal masses (Figure 2).

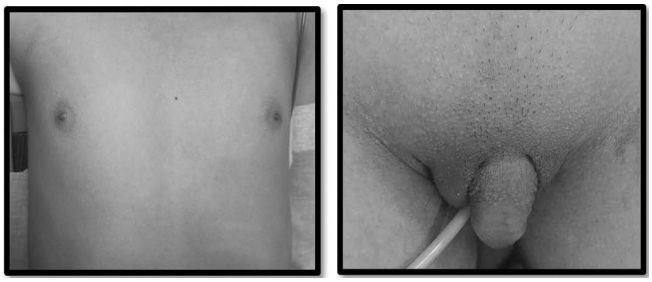

Figure 1. Sexual maturity ratings for breast and pubic hair

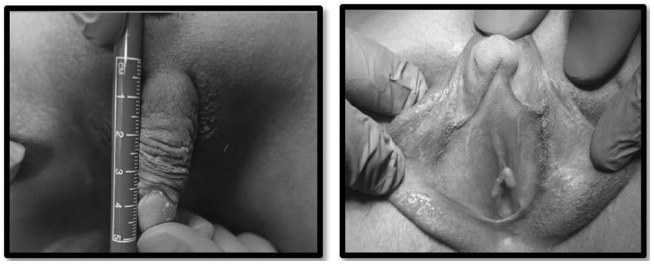

Figure 2. Phallic enlargement

The patient was referred to Pediatric Endocrinology, Adolescent Medicine, Pediatric Urology and Reproductive Medicine services. Comprehensive work-ups were done.

Magnetic resonance imaging of the lower abdomen revealed a small anteverted uterus measuring 2.0cm x 0.9cm x 1.7cm with a thin endometrium measuring 0.2 cm (arrow A). The cervix measured 1.7cm x 0.8cm x 1.4cm with no enhancing lesions (arrow B). The vagina was intact (arrow C) and a small phallic structure (arrow D) measuring 1.4cm x 1.3cm x 5.4cm with no definite testicular structures was identified (Figure 3). There was a solid nodular structure measuring 1.3cm x 1.3cm x 1.7cm seen on the right gonad. The left gonad was not visualized (Figure 4).

Blood work-ups were requested consisting of different hormonal assays. All values were within normal range except for an elevated testosterone value of 40 ng/dl. The patient's karyotype revealed a Mosaic Monosomy X with a normal male cell line. Out of the 50 cells examined, 7 cells showed loss of one sex chromosome (green circle) and 43 cells showed a normal male (red circle) karyotype (Figure 5).

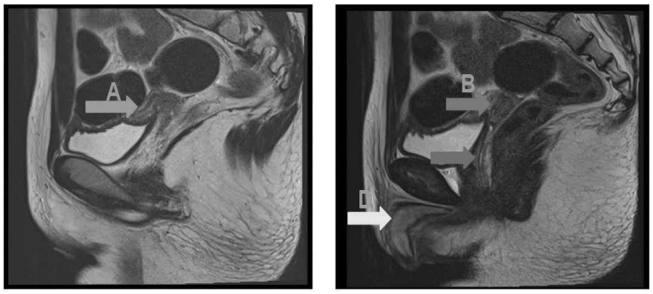

Figure 3. MRI of the lower abdomen (sagittal view)

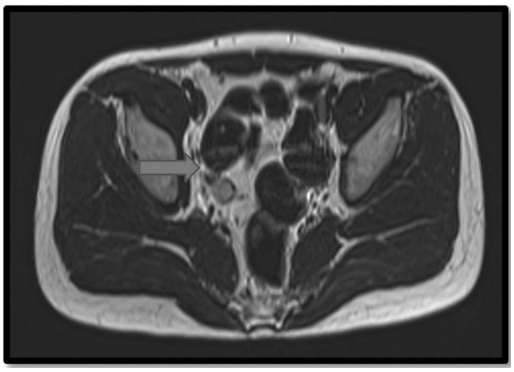

Figure 4. MRI of the lower abdomen (axial view)

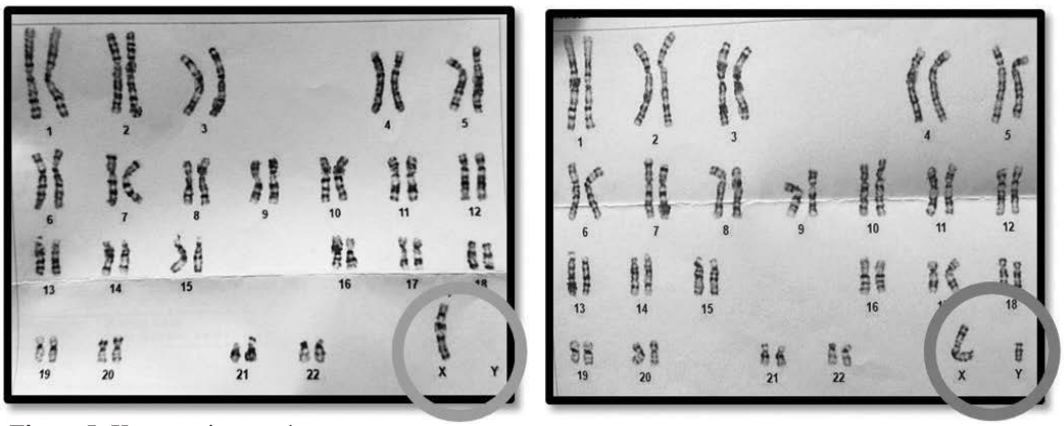

Figure 5. Karyotyping result

Table 1. Hormonal assay results

Test	Patient's Value	Reference
Estradiol	39.5 pg/ml	9 – 350 pg/ml
Testosterone	40 ng/dl	10-20 ng/dl
Luteinizing Hormone	6.6 mIU/ml	2 -15 mIU/ml
Follicle Stimulating Hormone	3.5 mIU/ml	1.5 – 9.3 mIU/ml
17- OHP	0.58 ng/ml	0.05 -2.0 ng/ml
DHEA-S	135.50 ug/dl	8.6 – 169.80 ug/dl
TSH	3.25 uIU/mL	0.27 – 4.20 uIU/mL
T4	101.5 nmol/L	59 – 154 nmol/L
T3	2.6 nmol/L	1.3 – 3.10 nmol/L
Anti-mullerian Hormone	1.3 ng/ml	
LDH	247 U/L	120 – 246 U/L
AFP	< 1.3 ng/ml	< 8.10 ng/ml
B-HcG	< 2 mIU/ML	< 2 mIU/ML

The patient's computed bone age is at par with the chronologic age according to Greulich and Pyle atlas. (Figure 6).

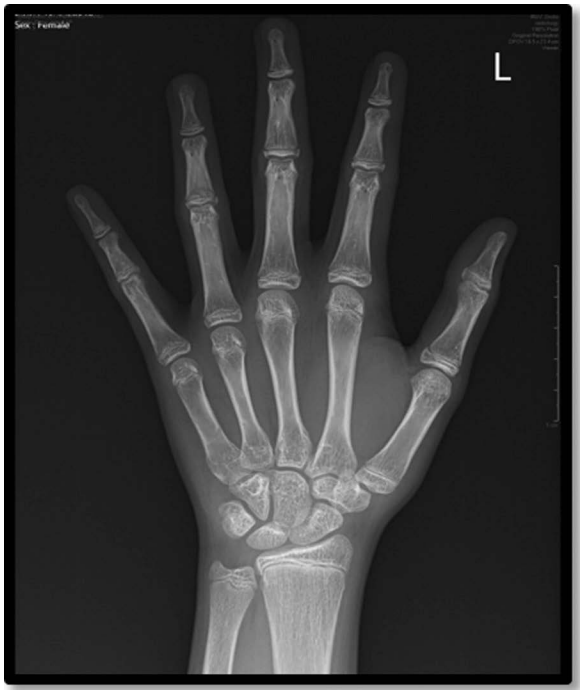

Figure 6. X-ray for the left wrist, hands and fingers for bone aging

With all the tests and diagnostic procedures done, working impression was a 45X, 46XY mosaicism. In a medical and family conference attended by the Pediatric Endocrinology, Adolescent Medicine, Urology and Reproductive Medicine services, the patient and her family expressed her desire to continue being identified as a female. Hence, after explaining the risks, complications as well as informed consent, a laparoscopic bilateral gonadectomy, salpingectomy and clitoroplasty under general anesthesia was contemplated.

Intraoperatively, the uterus appeared small and flattened (Figure 7). Both fallopian tubes were grossly normal. The right gonad appeared cystic and measured 1.5cm x 1cm x 1cm (Figure 8). The left gonad was small and appeared streak measuring 0.8cm x 0.3cm (Figure 9). The rest of the abdominal organs appeared grossly normal. Clitoroplasty was done by the urology service (Figure 10).

Histopathology showed Gonadoblastoma, Germ Cell Neoplasia in Situ, Nontumoral seminiferous tubules with Sertoli-only pattern with negative surgical cell lines of resection on the right gonad (Figures 11 & 12) and Ovarian stromal streak for the left gonad (Figure 13). Both tubes were histologically unremarkable.

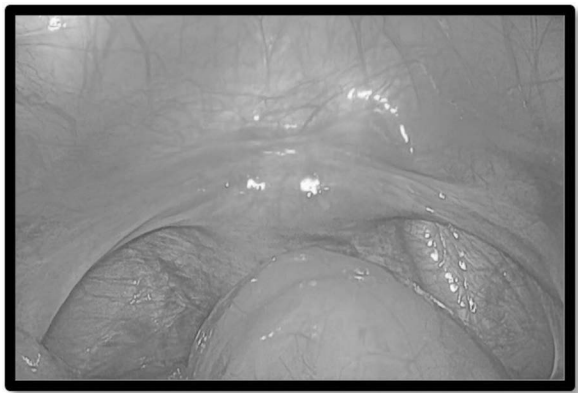

Figure 7. Infantile uterus on laparoscopy

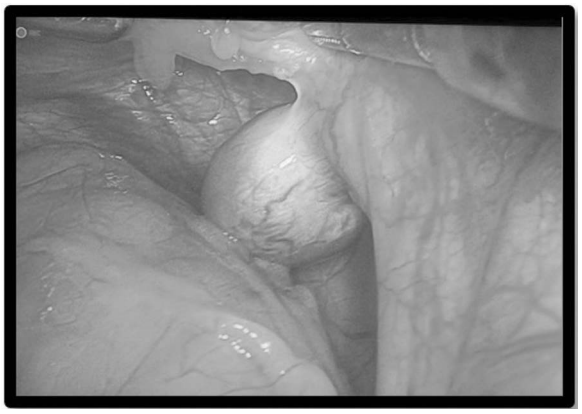

Figure 8. Right gonad on laparoscopy

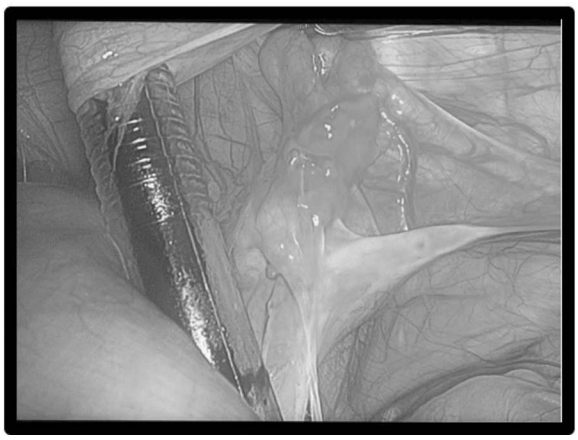

Figure 9. Left gonad on laparoscopy

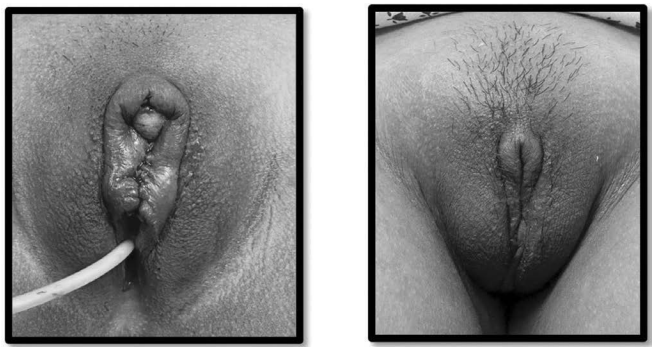

Figure 10. External genital appearance after clitoroplasty

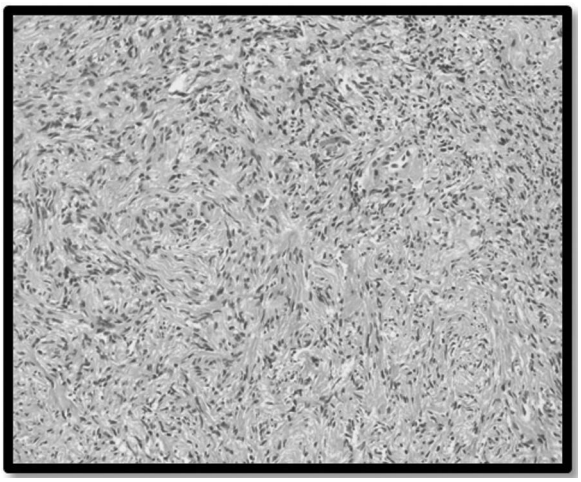

Figure 13. Low power view of a section of left gonad comprised of fibroblast-like stromal cells arranged in storiform pattern

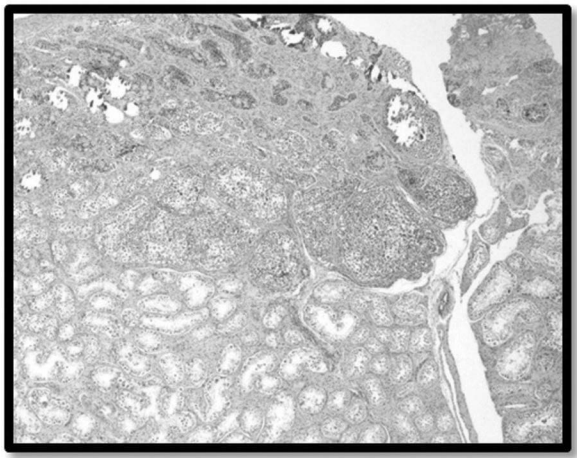

Figure 11. Scanner view of testicular tissue with a 0.4 cm focus of rounded nests and sheets of cells in the periphery

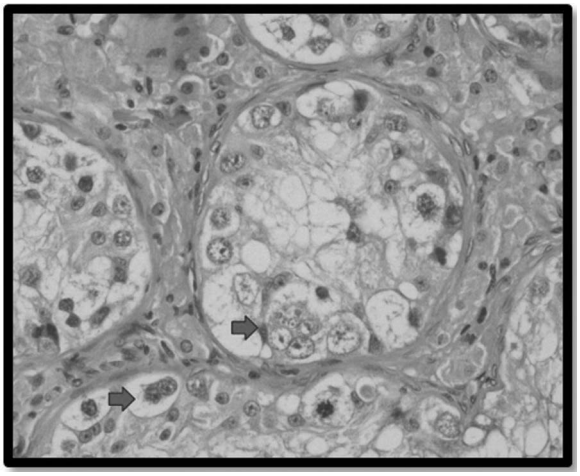

Figure 12. High power view of a section from right gonad showing larger cells with enlarged nuclei and nuceloli and clear cytoplasm resembling Germ Cell Neoplasia-In-Situ

Postoperatively, she followed up after 2 weeks free from pain with improved disposition. She expressed relief that the surgery was timely done and that she was very satisfied with the appearance of her genitalia. She was then started on conjugated equine estrogen 0.3 mg once a day. After 3 months, secondary sex characteristics improved (Figure 14).

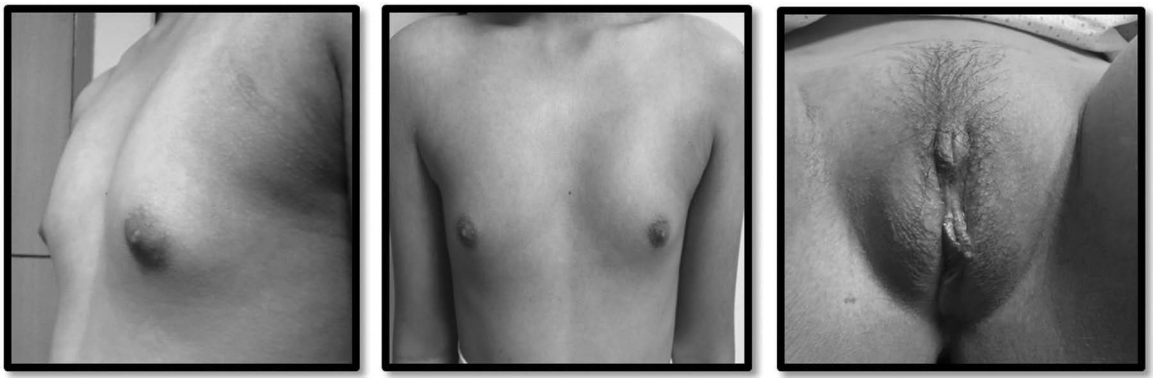

Figure 14. Breast and pubic hair Tanner stage III after 3 months of estrogen use

Discussion

Disorders of sex development (DSD) results in abnormal development of internal and external genitalia.[2] Applying the definition to our patient, she has a karyotype of 45X,46XY, her right gonad is a testis while her left gonad is an ovarian streak, her internal genitalia are composed of Mullerian duct derivatives however she presented with clitoromegaly and delayed secondary sexual characteristics. This qualifies her as having a disorder of sex development.

The Lawson Wilkins Pediatric Endocrine Society (LWPES) and the European Society for Pediatric Endocrinology (ESPE) Consensus Group classified in 2006 DSDs into 3 main categories namely: 1) 46 XX DSD, 2) 46 XY DSD and 3) Sex Chromosome DSD to which the index patient belongs.[6]

Sex chromosome mosaicism is characterized by the presence of two or more genetically different cell lines. In the case of our patient, she expressed a monosomy X cell line in addition to a cell line including complete or partial Y chromosome material. Anaphase lag or early embryonic mitotic non-disjunction are considered the major underlying mechanisms producing the 45, X cell line in an otherwise diploid 46,XY fetus, likely due to a structural abnormality of the Y chromosome or due to the small size of it.[7] These patients present substantial variations in genital phenotype, in a continuum from typical female through varying degrees of genital ambiguity to typical male genitalia. According to Farrugia, there appears to be no correlation between the percentage of mosaicism and genital abnormality.[5] Females are diagnosed earlier with a median age of 13.3 years old while males are diagnosed later at 29.1 years old according to a Danish retrospective study.[8] The combination of a unilateral testis, contralateral streak gonad and persistent Mullerian derivatives was previously called mixed gonadal dysgenesis (MGD), a common diagnosis given to 45,X/46,XY mosaic infants with genital anomalies. However, using this term as a diagnosis is no longer recommended as most published series of MGD have included patients with 46,XY as well as mosaic karyotypes. The consensus statement has since re- classified patients with 45,X/46,XY mosaicism and genital anomalies (sex chromosome DSD) and 46,XY patients with 'partial gonadal dysgenesis' (46,XY DSD) into two separate categories.[5] 45X,46XY mosaicism is opposed to Ovotesticular DSD which is a rare condition characterized by mixed ovarian and testicular tissue which may include bilateral ovotestes or an ovotestis and a contralateral ovary or testis. Majority of patients with Ovotesticular DSD has a 46,XX karyotype.

Patients with 45,X/46,XY mosaicism and its variants may be associated with other anomalies, namely cardiac and renal. Fortunately, our patient did not present with any of these.

In a recent study by Ren and others, basal LH value of 1.32 IU/L and LH/FSH ration of 0.34 has a high sensitivity of detecting puberty.[9] Since our patient's values are above these (6.6 IU/L and 1.8), we can conclude that puberty has indeed began. This elevated LH levels will act on the Leydig cells to produce testosterone. Levels above 20 ng/dl (our patient's value is 40ng/dl) indicate gonadal awakening. Hence, in 45X, 46XY mosaic patients, the functioning testis is the source of testosterone which is responsible for the virilization in puberty. Estrogens, on the other hand are generated mainly by the action of aromatase which converts testosterone to estradiol and androstenedione to estrone which explains the estradiol level of 39.5 pg/ml despite the nonfunctional ovarian streak.[10]

Genetic testing plays an important role in the evaluation of a patient with a possible DSD because knowing the genetic etiology improves the ability to predict the patient's phenotype, clarifies recurrence risk, and can be utilized in medical decision-making. This was not done in our patient due to financial constraints.

Comprehensive management includes surgery, hormone therapy and counselling. Sex assignment can be challenging. Decades ago, the recommendation is to do early gonadectomy and assignment of female sex. Presently there is an emphasis on a more conservative approach involving delay of irreversible surgery until adulthood when the patient can decide for himself. Factors to consider include long-term likely gender identity, urological and sexual function, genital appearance, the possible need for endocrine replacement therapy to induce puberty and throughout adult life; and any capacity for future fertility. All these factors are in addition to the malignancy risk associated with the condition, which has made gonadal management increasingly

challenging particularly to those raised as males[5]. Patients with segments of the Y chromosome, are at increased risk for germ cell tumors including gonadoblastoma which is a neoplasm comprised of a mixture of germ cells and elements resembling immature granulosa or Sertoli cells with or without Leydig cells or lutein-type cells in an ovarian type stroma. Gonadoblastoma has an increased prevalence of 15% to 40% in patients with 45,X/46,XY mosaicism[11]. In addition, the ectopic position of the dysgenetic testis adds to this since the prevalence of germ cell tumors in simple cryptorchidism is 4-10 times the normal prevalence of 6-11 per 100,000. Hence, gonadectomy should be done. Genital reconstruction in the form of clitoroplasty is proposed among 45X,46XY mosaicism patients who plan to continue as females followed by low dose estrogen therapy for induction of secondary sexual characteristics which was exactly what was done in our index case. After thorough counselling and explanation of risks and complications, shared decision making concluded that the patient will continue as a female and she expressed her relief that germ cell neoplasia in situ was caught early and she is very much satisfied with how her breasts and external genitalia appeared on her follow- up check-ups. For patients who wish to continue as a male, the decision to remove the located abdominal gonad is based on the high incidence of malignancy in an intra-abdominal gonad. Gonadectomy with a hysterectomy followed by scrotal fusion and contralateral orchidopexy is usually done. Monitoring for malignancy in an undescended testis may be difficult, but a repositioned testis can be monitored easily by self-examination. Giving testosterone can also be considered to induce secondary sex characteristics if with suboptimal levels of endogenous testosterone.[5]

Hormone therapy is given to establish and maintain secondary sex characteristics, achieve adult height, optimize bone health as well as sexual maturation.[10] Treatment should be started with low doses and slowly increased on the basis of pubertal stage progression and bone age maturation until the achievement of adult doses. Progestins need to be added after 12–24 months of estrogen mono treatment or at the occurrence of menarche in girls with a uterus for 10–14 days/month to induce menses and avoid endometrial hyperplasia. Our patient has been given conjugated equine estrogen at a dose of 0.3 mg per day. After 3 months, sexual maturity ratings improved. Breast and Pubic Hair were Tanner Stage III on reassessment which were previously Stage I and II respectively.

Children and adolescents with DSDs usually experience more stress and are at high risk of emotional problems affected by genetic factors, psychological factors, and social environmental factors. If there is no reasonable way to deal with their feelings, they may gradually develop serious emotional problems. The role of psychiatrists/psychologists are to establish cognitive status to assess ability to participate in decision-making, provide for the mental health needs of parents and assessment of parent/child relationship and facilitation of healthy parent/child relationship. Appropriate interventions would be necessary to prevent worse outcomes of social functioning impairment for these children. It is important to put emphasis on emotional stability in children with DSD in order to detect anxiety-related emotional disorders early.[12]

Summary and Conclusion

Any dysmorphic genital or physical feature would benefit from early consultation with the appropriate specialist and a thorough work-up to maximize ideal management and avoidance of incomplete/suboptimal treatment. Referral to tertiary centers that can offer adequate multidisciplinary care is pivotal in rare cases such as DSDs. Psychological and genetic counselling play equally important roles as surgery and medical management in terms of preventing depression and anxiety, orientation on long term aspects of each DSD and even inheritance patterns. The family plays an integral role in the management of these disorders. Their support and influence to the psychosocial health of a child with DSD cannot be underestimated.

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