ORIGINAL RESEARCH PAPER

Genetics

KEY WORDS: Swyer syndrome, primary amenorrhea, gonadal dysgenesis, complete gonadal dysgenesis, streak gonads.

CYTOGENETIC TESTING IN INDIVIDUALS WITH A TYPE OF HYPOGONADISM LEADING TO DISTURBANCES IN SEXUAL DIFFERENTIATION: XY FEMALES.

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ABSTRACT

46,XY complete gonadal dysgenesis is a condition that affects sexual development and is determined by an individual's karyotypic make-up. One X and one Y chromosome in each cell, normally found in males, are seen here in females, with typical external female genitalia. Streak gonads are present and as there are no sex hormones being produced, these cases present with primary amenorrhoea. Several gene mutations may be responsible for this presentation where individuals with a male karyotype show the presence of mullerian structures. It is essential to identify these cases because the non-functional gonads have to be surgically removed to avoid risk for malignancy. Also, hormone replacement therapy can be given to identified individuals to help produce pubertal changes including menstruation. We have reported here, cases of Swyer syndrome with partially developed breast, with Turner's-like phenotype, normal to underdeveloped uterus and streak gonads/unilateral dysgenetic testis.

INTRODUCTION

An individual's chromosome complement decides the overall including the sexual development of the person. Sometimes however the chromosomes don't match the phenotypic presentation of the patient. Individuals presenting with hyper-gonadotrophic hypogonadism upon investigation sometimes reveal typical female external genitalia like the vagina with uterus, fallopian tubes (normal mullerian structures) but with XY sex chromosomes. These cases are seen to exhibit Swyer's syndrome, which though a congenital condition, persons involved do not show any outward symptoms until their teens when they present with primary amenorrhoea (Swyer, 1955).

Functional gonads are replaced by streak gonads which may become cancerous at some point (Piazza and Urbanetz, 2019). About 30% of XY females develop gonadal tumors from the cells that would form the testes / ovary. These tumors may form at any age even before the syndrome is detected. It is essential that these individuals are identified and the residual gonadal tissue is removed to avoid this risk.

Streak gonads are not able to produce the sex hormones and hence there is no development of secondary sexual characteristics in the individual. Adequate hormonal, ultrasound and chromosomal work-up of the individual is required for proper understanding of the disorder. Chromosome testing is important so that they can be offered hormone replacement therapy around puberty in order to induce menstruation and for the enlargement of breasts and uterus. Often, this is thought of as transient delay of growth and pubertal processes. Even in the cases where the condition is transient, early and appropriate work-up helps to rule out chromosomal anomalies and offer short-term treatment to augment growth and achieve sexual milestones. Here, we share our findings of some cases that were referred to us with primary hypogonadism/Turner's like phenotype with amenorrhoea, for chromosomal work-up.

MATERIALS AND METHOD

For cytogenetic analysis, the method described by Seabright et al (1971) was followed. Briefly, 2 ml peripheral blood sample was collected from the patient in a sodium heparin vaccutainer. Lymphocyte culture was done in RPMI 1640 medium to which peripheral blood and PHA was added and incubated at 37°C for 72 hours. Cell harvesting was done by adding colchicine and then hypotonic treatment of the pellet. After fixation using 3:1 methanol-acetic acid, G-banded metaphases were prepared for analysis and chromosomal study was performed. According to the guidelines of the International System for Human Cytogenetic Nomenclature (ISCN, 2016) karyotyping was done and numerical as well as structural abnormalities were recorded. None of our cases with the Y chromosome had mosaicism. Patients identified with chromosomal abnormalities were given post-test genetic counseling for appropriate management in each case along with case discussion with the referring clinician.

Case Presentation

The cases were mostly referred for primary amenorrhoea and delayed puberty. There was no other relevant past medical or family history in any of the cases. Ultrasound report showed hypoplastic /normal uterus with bilateral streak gonads. Some of the cases had partially developed breasts, and these were sent for testing for Turner's phenotype. The individual's ages ranged from 12-19 years of age. Biochemical testing ruled out any enzymatic deficiencies that could lead to primary amenorrhoea. Karyotypic findings were immediately discussed with the referring physician to prioritize an early prophylactic gonadectomy given the high-risk for malignancy. Evaluation of family members was also recommended to know if this was a sporadic finding or not.

DISCUSSION

Several factors along the hypothalamic-pituatary-gonadal axis have to function normally for gonadal development and the production of sex steroids. There are several genes involved along this pathway; mostly studied are the ones involved in the normal sexual development of the individual. Mutational changes in these genes can disrupt critical processes in a growing fetus, thus presenting with phenotype-chromosome mismatches. Very often, mutations in the sex determining factor (SRY) gene on the Y chromosome are responsible for the condition (Lim et al, 1998). The undifferentiated gonads fail to develop into testes in the XY fetus and hence there is no testosterone or anti-mullerian hormone. Wolfian ducts fail to develop so there are no male internal organs. Mullerian ducts develop (lack of anti-mullerian hormone) into normal internal female organs.

The syndrome may be inherited or due to a *de novo* mutation in the sperm cell or egg of that individual. 15-20 percent of

women with Swyer's karyoype have a mutation in the SRY gene resulting in the failure of testes formation. Mutations in several other genes in addition to SRY gene may also be contributing to this condition, given that the father from whom the faulty Y chromosome was inherited, did not present with this condition; other changes genetic or epigenetic, may be additionally required to develop this phenotype. For instance, the individuals with SF1 mutations may have adrenal insufficiency which should be investigated and treated if present, or NROB1 gene mutations on the X chromosome or mutations in the Map3K1 gene.

A XY karyotype can result from complete androgen insensitivity syndrome but the absence/underdevelopment of breasts and presence of uterus and pubic hair exclude this possibility. It is then that the diagnosis of pure XY gonadal dysgenesis is made. Familial studies were not performed due to inaccessibility/lack of awareness in cases; therefore, there is a likelihood to have missed out information on whether there is more than one member with gonadal dysgenesis in the family, siblings with genital ambiguity etc. There could be cases of XY gonadal dysgenesis that may result from an X-linked recessive or male-limited autosomal dominant gene. Further molecular testing for alterations in specific genes like DAX1, SOX9 mutations, duplication in 1p (p22.3-p32.2), deletions in 9p24.3 at the DMRT1 locus, deletion in 10q, 2q can give the pathogenesis of the XY sex reversal in these individuals (Koopman, 1999; Boyer et al, 2002; Hanley et al, 2000). There may be non-genetic factors like hormonal treatment around conception and pregnancy that may contribute to this condition.

From our study, 6/2000 individuals over a period of two years of testing referred for various cases of infertilities, reported with presence of mullerian structures but presented with primary amenorrhoea, streak gonads and were found to have a XY chromosome complement. Complete personal and medical history of the patient was recorded. Physical examination and investigations including MRI, ultrasound and hormone/enzyme studies were done to reach a clear diagnosis of the case presented for evaluation. Cools et al.(2011) suggested that the GBY gene (Y chromosome) encoding a testis specific protein may have an oncogenic function. Even cases with Turner's like appearance, where the Y chromosome was seen, the risk for gonadoblastoma is predicted (Brant et al., 2006). Our patients and clinicians were appropriately kept informed since there is a high risk for malignant transformation, and an early gonadectomy is recommended. These tumors show up in the second decade of life but may develop at a much earlier age also (Matsumoto et al., 2014).

Individuals should be tested cytogenetically for their chromosome complement for appropriate clinical management plan. Attention can also be paid to atypical intra- or extra-oral dental findings in some individuals, warranting rehabilitation (Lele and Lakade, 2014). Apart from prophylactic gonadectomy to remove the risk of residual tissue becoming cancerous, HRT can be planned to induce menstrual flow. Though the persons are infertile, they can bear a child through the implantation of donated eggs. Cytogenetic testing guided clinical application of HRT will play an important role in preventing osteoporosis later on, in life, in addition to coronary heart disease (Baker and Schillings, 2012). Lastly, reporting the condition should be accompanied by psychological counseling for the affected individual along with their family members, which will go a long way in the social rehabilitation of these individuals.

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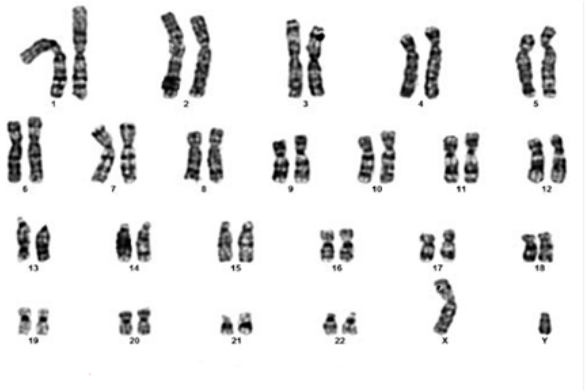
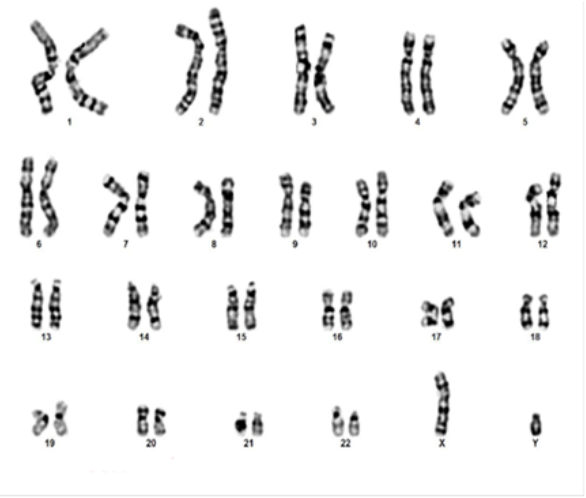
Figure 1:



Legend: A 17-year-old girl was referred to our clinic for evaluation of primary amenorrhea and delayed puberty. FSH - 11.77 mlu/mL , LH - 20.69 mlu/mL , prolactine - 10.70 ng/ml, uterus seen on ultrasound, karyotype obtained showed a Male karyotype with 46,XY.

Figure 2:



Legend: A 18-year-old girl was referred for evaluation of Turner's? with uterus seen and under-developed breasts; Karyotype obtained showed a male karyotype with 46,XY.

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