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Case Report

Klinefelter Mosaicism 46, XX/47, XXY with Ovotestis- DSD

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Abstract

Klinefelter syndrome is a relatively common chromosomal condition affecting approximately 1 in 500-1,000 males. 46, XX /47 XXY Klinefelter Syndrome mosaicism is rare enough, resulting in a few cases described in literature. Variable phenotypes and clinical presentations such as gynecomastia, infertility, cryptorchidism, and disorders of sexual development (DSD) are associated with this karyotype presentation. The association of Klinefelter syndrome mosaicism 46 XX/47 XXY and OT DSD is a rare feature. We report the case of a 34-year-old man who presented for semen analysis and karyotyping in our unit. The patient had bilateral gynecomastia and absence of facial hair. Penile length was 4,5 cm with an external meatus located on the posterior face of the phallus, characterizing a posterior hypospadias. Testis was palpable in the right hemiscrotum, but the left hemiscrotum was empty. Ultrasonography revealed the presence of the left gonad located in the left iliac fossa, while the right gonad in the scrotum had testicular morphology according to ultrasound exam. Chromosomal analysis revealed 46, XX/47, XXY mosaicism, and semen analysis an azoospermia. Our patient underwent surgery because of the risk of malignancy, and histopathologic examination of the left excised gonad confirmed the structure to be an ovotestis. The biopsy of the right gonad, realized for eventual cryopreservation, revealed atrophic seminiferous tubules and a pseudo tumoral aspect of Leydig cells with hyperplasia without atypia. Personalized approach and multidisciplinary care are needed to get a diagnosis, resolve sex reassignment, and improve the quality of life of the patient. In that feature, the percentage of XX cells could play a role on phenotype, particularly on Müllerian structure persistence, but also on a relative increased risk of malignancy degenerescence compared to other cases of OT-DSD.

Keywords

KS Mosaicism, Ovo testis (OT)-DSD, Percentage of 46, XX Clone Cells, Germ Cell Tumor

1. Introduction

Klinefelter syndrome (KS) is a relatively common chromosomal condition affecting approximately 1 in 500–1,000 males [1]. In some cases, individuals with Klinefelter syndrome can present with a mosaic karyotype, where a portion of their cells have the typical 47, XXY constitution, while others present formula constituting another clone [2]. 47 XXY/46 XX mosaicism is rare enough, resulting in a few cases described in literature [3]. This feature has variable phenotypes and clinical

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presentations such as gynecomastia, infertility, cryptorchidism, and disorders of sexual development (DSD), defined in the Chicago Consensus of 2006, as congenital conditions in which the development of chromosomal, gonadal, and anatomical sex is atypical [4]. Less than 20 cases of association of 46, XX / 47, XXY and OT DSD have been reported in the literature. We report in this study a case of this association and a review of all the cases reported in the literature, highlighting the different phenotypes and OT presentation. We are also discussing diagnostic challenges in that feature and potential implications for management and patient.

2. Case Report

We report the case of T. B., a 34-year-old man, married, who presented in our unit of cytogenetics and reproduction for a semen analysis and a karyotype. The patient was followed in urology for cryptorchidism and hypospadias. However, the management of a 3-year primary infertility was its main request.

2.1. Physical Examination

After a physical examination of the patient, we recorded a normal BMI with a height of 183 cm and a weight of 72 kg. The patient presented with bilateral gynecomastia. The examination of the external genitalia revealed a penile length of 4 cm with an external meatus located on the posterior face of the phallus, characterizing a posterior hypospadias. Testis was palpable in the right hemiscrotum, but the left hemiscrotum was empty. The systemic examination was unremarkable, and a male distribution of the pilosity was noted, even if the patient had no beard. (Figure 1)

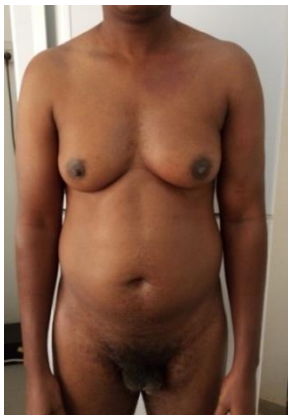


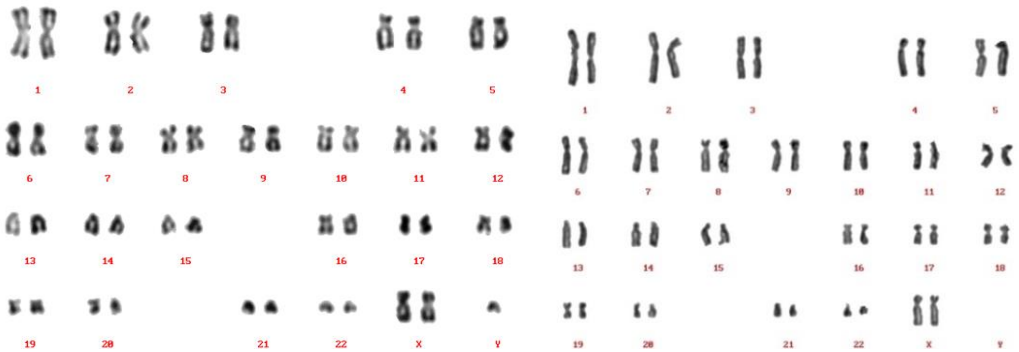
Figure 1. *Patient with bilateral gynecomastia and empty left hemi-scrotum.*

2.2. Ultrasound

The ultrasonography revealed the presence of a gonad in the pelvis located in the left iliac fossa. In the left hemiscrotum, the gonad presented testis echostructure with a diameter of 25 mm.

2.3. Karyotyping

A blood sample in a heparinized tube was used to perform a lymphocyte culture. After administration of colchicine 50 minutes before the end of culture and then hypotonic shock, thermal denaturation (RHG) (reverse banding by heating and Giemsia staining) was performed. The establishment of the karyotype and its interpretation according to the ISCN 2016 guidelines [5], made it possible to establish the formula mos 46, XX [40] /47, XXY [10]. Ninety-five metaphases were analyzed, and we found a percentage of 80% of XX cells and 20% of XXY cells and (Figure 2).



A B

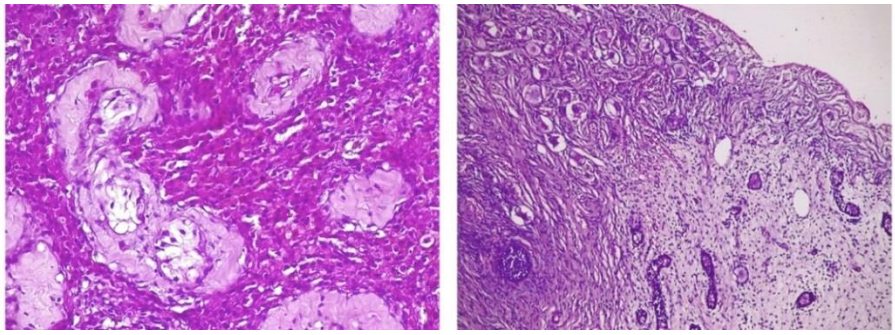
Figure 2. *Mos 46, XX [40] /47, XXY [10]. 2A: clone of XX cells and 2B: Clone of XXY cells.*

2.4. Semen Analysis

The patient had azoospermia, which was confirmed by a second examination of the pellet of the sperm sample obtained after centrifugation.

2.5. Histology

After counseling concerning gonadoblastoma risk, the patient was on demand for resection of the pelvic gonad and also a mastectomy, and he reaffirmed male sexual orientation. The histology of the resected gonad determined the presence of an ovotestis (Figure 3A). A biopsy of the right gonad was proposed for eventual cryopreservation of gametes and revealed the presence of a testicular parenchym with an atrophy of seminiferous tubules and pseudotumoral hyperplasia of Leydig cells. (Figure 3B)



A B

Figure 3. *3A Right gonad: HE (x400) hyalinized seminiferous tubules with Leydig cell hyperplasia without atypia giving pseudotumoral aspect 3B: left gonad HE (X400) seminiferous tubules coexisting with follicles in the same tissue realizing an ovo-testis.*

3. Discussion

3.1. Cases Reported in the Literature

The most frequent mosaic form for Klinefelter syndrome is 46, XY/47, XXY affecting about 10 % of cases of KS mosaicism [6]. The 46, XX /47, XXY mosaic karyotype is particularly rare and the current literature suggests that the prevalence of 46, XX /47, XXY mosaic Klinefelter in individuals with DSD is relatively low. Mazen et al. [7], in 2021, reported a rate of 1.5% among a cohort of 226 Egyptian patients, while Pattemshetty et al. [6] described a lower prevalence of 0.08% in their study.

The 46, XX /47, XXY mosaic karyotype has been associated with a range of clinical presentations, including ambiguous genitalia, micropenis, cryptorchidism, and ovotestis. Ovotestis is defined by the presence of ovarian and testicular tissue within the same gonad [3]. It is characterized by the presence of both ovarian tissue with the presence of primordial follicles and testicular tissue containing seminiferous tubules, in the same individual. These tissues may be present together unilaterally or bilaterally in the same gonad(s) or separately with ovarian tissue on one side and testicular tissue on the other, also known as lateral OT-DSD [8].

Tangshewinsirikul et al. [2] reviewed in 2020 a total of 19 cases of 46,XX /47,XXY KS mosaicism from 1969 and among patients with available data, OT-DSD was present in most cases (11 cases over 19). Since then, to our knowledge, six cases of association of 46, XX /47, XXY mosaic Klinefelter et OT have been reported. In Table 1, cases reviewed in the literature are presented.

Table 1. *Clinical presentation and percentage of XX clone cell of patients with 46, XX/47, XXY mosaicism and OT DSD.*

#	Author	Age	Percentage XX clone in PB	Sexual phenotype	OT DSD Side Right/Left and Situation (Cryptorchidism)	Gynecomastia And Müllerian structures	Assigned sex
1	Our present case	34 y	80%	Phallus Hypospadias	OT/T A/S	Gynecomastia	Male
2	Tiffany Guess et al [3]	13 d	83,3%	Genital ambiguity	OT NA	NA	Female
3	Tiffany Guess et al, 2024 [3]	6 y	70%	NA	NA	NA	Male
4	Tiffany Guess et	15 y	70%	NA	NA	NA	Male

#	Author	Age	Percentage XX clone in PB	Sexual phenotype	OT DSD Side Right/Left and Situation (Cryptorchidism)	Gynecomastia And Müllerian structures	Assigned sex
	al, 2024 [3]						
5	Tiffany Guess et al, 2024 [3]	15 y	86,7%	NA	Germ cell tumor	Gynecomastia	Male
6	Mohamed Hssaini et al [9]	6 m	54%	Genital ambiguity	O/T A/I	Uterus vagina	Male
7	Tanshewinsirikul et al [2]	27weeks	71%	Genital ambiguity Hypospadias	T/OT I/A	Uterus Fallopian tubes Vagina	Male
8	Butler et al [10]	4m	62%	Genital ambiguity Hypospadias	T/O S/A	Uterus Fallopian tubes Vagina	Male
9	Ozsu et al [11]	5m	66	GA hypospadias	T/O S/I	Uterus Fallopian tubes	Male
10	Mao et al [12]	1,7 y	NA	Genital ambiguity	OT/T A/I	Uterus Fallopian tubes Vagina	Male
11	Kanaka-Gantenbein et al [13]	13	70%	Phallus	T/O S/I	Gynecomastia Uterus Fallopian tubes Vagina	Male
12	Bergmann et al [14]	14	50%	Genital ambiguity Hypospadias	OT/OT I/I	Gynecomastia Uterus Fallopian tubes Vagina	Male
13	Isguven et al [15]	14,5	85%	Phallus hypospadias	T/OT S/S	Gynecomastia Uterus Fallopian tubes Vagina	Male
14	Chouhan et al [16]	15	87%	Phallus	OT/O S/S	Fallopian tubes Vagina	Male
15	Talreja et al [8]	16	80%	Phallus	T/O S/A	Gynecomastia Fallopian tubes Vagina	Male
16	Torres et al [17]	16	72%	Genital ambiguity Hypospadias	OT/O S/S	Fallopian tubes Vagina	Male
17	Perez et al [18]	16	62 %	Genital ambiguity Hypospadias	T/O	Uterus	Male
18	Mohd et al [19]	12	88%	Genital ambiguity	O/T	NA	Male

PB: Peripheral blood; NA: not available; I: Inguinal canal; S: scrotum; A: abdominal

In Guess et al.'s study [3], taking into account 34 patients with 46, XX /47,XXY mosaicism, Ovotestes were present in 12% of individuals (4 cases). In that cohort, patients with ovo testis had a significantly higher percentage of XX cells and

one of them were assigned female at birth. (case # 2). A case of malignant degenerescence was recorded with germ cell tumor. (case # 5).

According to Tangshewinsirikul [2], the 46, XX/47, XXY mosaic KS should be treated more toward OT-DSD, rather than a simple mosaic KS because in his review, 85% of patients with mosaicism 46, XX /47, XXY had OT DSD. Nevertheless, Tiffany Guess et al, [3], in 2024 found only a percentage of 12% OT-DSD in her series concerning 34 patients diagnosed with this mosaicism across 14 institutions.

3.2. Algorithm of Tests and Challenges

To establish a diagnosis, the first line test is karyotyping to be able to classify the DSD according to the Chicago consensus. as follows: 1) DSD sex chromosomes; 2) DSD 46, XX and 3) DSD 46, XY. Despite this classification, some conditions do not fit exactly into a specific diagnostic category or may belong to more than one category, such as cases belonging to DSD chromosomes but both included in KS mosaicism and OT DSD. [20]

According to Houk et al [21], the algorithm of the genetic study of DSD, implies then fluorescence in situ hybridization analysis for regions of the Y in order to identify, SRY translocation or research of presence of SRY gene. However, the overall positivity rate after searching for the SRY gene would only be between 10% and 35% [22]. Indeed, it would be possible for certain individuals that the determination of SRY is negative in the blood while the gene would be present and expressed in the testicle, as in the case of tissue mosaicism. [23].

Thus, Garc á-Acero et al. [24] suggest that patients should have studies of molecular biology and cytogenetics in gonadal tissue, given the possibility of mosaicism as well as changes related to epigenetic modifications.

The next step, according to guidelines [21] would be an evaluation of specific genes involved in the gonadal development (SRY, SF-1, WT1, CYP21, SOX9, DAX among others) by traditional molecular research methods, including Sanger sequencing combined with multiplex ligand dependent probe amplification.

The result could help to identify genetic variants and explain the phenotype. However, research of deletion and duplication by CGH array or snp array is less practiced and has identified causality, in nearly 50% of cases [25], In Hssaini et al study [9], for example, molecular analysis revealed no mutations in the related genes investigated in his study.

J Leonard et al [26], after a cfDNA (fetal DNA) screening for prenatal sex determination even reported a case, concerning erroneous first diagnosis of a 46, XX fetus with a postnatal infirmation using a genome-wide SNP array showing instead a 46, XX/47, XXY mosaicism with uniparental isodisomy and genital atypia.

Karyotype in DSD remains the first line test in DSD diagnosis. The availability of new techniques such as single-cell sequencing could change classical diagnostic algorithm and improve the diagnosis. After the genetic approach, epigenetic, metabolome, and transcriptome are also techniques that could allow a better understanding of mechanisms explaining the DSD issue [25]. Thus, each case seems to be so unique that, after karyotyping, the test algorithm has to be adapted for a personalized approach, considering patient phenotype and the new available tests with their advantages and their pitfalls.

3.3. Potential Implications for Management and Patients

Sex alignment may request patient majority or puberty. In our series of reviewed cases in the literature, all cases were assigned as male except one. Müllerian structures were present for quasi all cases. The majority benefit from a surgery to remove uterus and breast, so was the case of our patient and only one case was assigned female and it was at birth. A multidisciplinary approach is needed with psychological care to assess better quality of life fort his patients.

Concerning fatherhood, we did not find report of successful reproduction in 46, XX/47, XXY, whereas spontaneous fertility of KS has been described, even if it is rare. Given the absence of spermatogenesis commonly observed in the testicular histology of adult individuals with 46, XX/47, XXY, the chances of spontaneous fertility and effective ART seem to be modest. [27]

There have been studies reporting the low risk of malignancy in patients with the ovotesticular disorders (between 2.6% and 4.6%) compared to other DSD [8]. However, a greater risk for tumor development in 46, XX/XXY compared with patients with 47, XXY is attributed to an increased estradiol-to-testosterone ratio due to the presence of two X chromosomes with, in 15% of cases, a ‘’gene escape X-inactivation’’ on the additional chromosome [28].

4. Conclusion

46, XX/XXY KS mosaicism with OT DSD is a rare association including multiple phenotypes. In that feature, percentage of XX clone could play a role in the phenotype as persitant of müllerain structure but also a relative increased risk of malignancy degenerescence. Sex aligment requests a multidisciplinary approach. The quick evolution of disposable tests has to be considered for an adapted algorithm of management of these patients. Karyotype remains a gold exam in the establishment of the diagnosis but requires analysis of a large number of cells to not miss the cases of mosaism. New technologies, such as single-cell sequencing, could in the future, change the algorithm of recommended tests in that pathology.

Abbreviations

KS	Klinefelter Syndrome
OT	Ovo-testis

PB	Peripheral Blood
NA	Not Available
I	Inguinal Canal
S	Scrotum
A	Abdominal

Ethical Considerations

Our patient received explicit information regarding the scientific disclosure of the results and images. Thus, written consent by the patient for publication of this case report and any accompanying images was obtained.

Conflict of Interest

The authors declare no conflicts of interest.

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