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Case Reports

***SRY*-negative in 46, XX Male Testicular DSD: A Case Report**

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Abstract

Background: The sex determination process requires distinct signaling pathways to generate either testis or ovaries from the same precursor structures, the primordial gonad. Deviations of this signaling mechanism may result in disorders/differences of sex development (DSD). The 46, XX testicular DSD is a rare genetic condition identified by a discrepancy between genetic and phenotypic sex caused sex reversal syndrome.

Case Presentation: We describe the case of a 5 years-old 46, XX boy with ambiguous genitalia. On physical examination he had severe hypospadias, bifid scrotum, micropenis and palpable bilateral testes. Cytogenetic analysis of patient reveals a 46, XX karyotype. Hormonal assay showed low level of FSH, LH and Testosterone and there was no evidence of Mullerian structures based on pelvic imaging. The histopathology of gonadal tissue showed a Leydig cell hyperplasia which gives the impression of Sertoli cell nodule. Polymerase chain reaction (PCR) analysis failed to identify the presence of *SRY* gene, therefore a diagnosis of 46, XX Testicular DSD with *SRY*-negative was established.

Conclusion: This report presents a rare case of *SRY*-negative 46, XX Testicular DSD in a boy with ambiguous genitalia which diagnosis was made by clinical, cytogenetic, histopathology and molecular analysis and psychological evaluation. This finding suggests that further advanced evaluation should be conducted to provide decisive diagnosis and genetic counseling for patients and their family.

Keywords: Disorders of sex development; ambiguous genitalia; 46 XX, *SRY* gene

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INTRODUCTION

46, XX testicular disorders/differences of sex development (DSD) is a rare genetic condition resulting from disruption in the genetic pathways underlying the development and differentiation of the gonad which identified by discrepancy among genetic and phenotypic sex.¹ This term was first published by la Chapelle et al in 1964 in a male patient clinically diagnosed as hypergonadotropic hypogonadism with 46, XX karyotype.² Estimated prevalence of this condition is 1 in 20.000 newborn males.³ A comprehensive approach in the management of 46, XX testicular DSD can be challenging thus required a systematic and multidisciplinary approach to establish the diagnostic.

Approximately 90% cases of 46, XX testicular DSD exhibit Y chromosomal material bearing the *SRY* gene, which is typically translocated during meiosis to the

distal portion of the short arm of X the chromosome or to an autosomal chromosome.^{4,5} Apart from the translocation of the fragment of Y chromosome containing *SRY* gene to the X chromosome or autosome, there were also several distinct hypothesis have been proposed for understanding the etiology of 46, XX sex reversal, including the possibility of a mutation of the gene triggering testis differentiation in *SRY* negative XX males and cytogenetically undetected mosaicism of Y chromosome limited to the gonad.⁶

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While *SRY* gene is considered to be the main regulatory factor that is responsible to the activation of a cascade of testis-determining genes, a number of other genes involved in gonadal differentiation needs to be identified. Therefore, among the rest of 10% cases of 46, XX Testicular DSD-*SRY* negative, further molecular testing are suggested to obtain the definitive diagnosis.^{5, 6} Here, we investigated the clinical presentation, cytogenetic analysis and molecular mechanism in a patient with 46, XX testicular DSD, who referred to our hospital for the evaluation of ambiguous genitalia.

CASE REPORTS

A 5-years-old boy was referred to genetic clinic at Diponegoro National hospital for evaluation of genital ambiguity. There no family history of ambiguous genitalia were seen in the family. There was no risk of endocrine disruptor chemical exposure such as pesticide, insect repellent and maternal exposures to cigarette smoke during pregnancy. The patient was born at term after an uneventful pregnancy, and there was no parental consanguinity. He has a healthy 3 years-old brother. On physical examination, weight and height of patient were 15.2 kg and 103.4 cm (50th centiles height-for-age and weight-for age) respectively with no developmental problem. The phallus length was 3.3 cm and the opening of the urethral meatus was located on scrotal region. The scrotum was bifid with small palpable gonad at the scrotal sac with volume 1 ml on the right side and 0.5 ml on the left side (Figure 1). The baseline serum concentration of hormonal analyses was as follows: serum levels of FSH: 0.81 mIU/mL (prepubertal normal range: 0.38-3.6), LH: <0.10 mIU/mL (prepubertal normal range: 0.7-2.0), and testosterone: <2.50 ng/mL (prepubertal normal range: <2.5-6.12) showed a normal range within his age. Pelvic ultrasound showed no structure like uterus, ovary and other Mullerian structures. A gonadal biopsy was performed, and specimens were obtained for microscopic analysis. The biopsy showed a Leydig cell hyperplasia which gives the impression of Sertoli cell nodule.

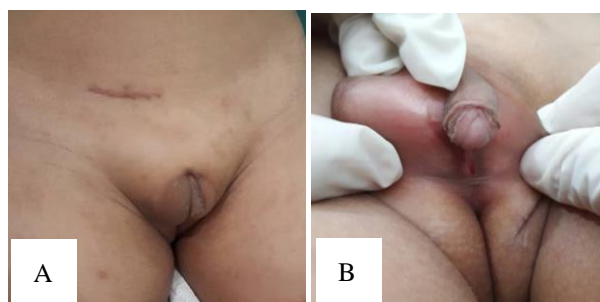


Figure 1. The external genitalia of patients.
A. Bifid scrotal with minimal scrotalization.
B. Noted palpable gonads located at scrotal sac with scrotal hypospadias.

Ethics approval was obtained by the Faculty of Medicine Diponegoro University Ethics Committee before conducting the study, and parents gave informed consent for this study (No.24/EC/FK-RSDK/I/2017). Cytogenetic analysis was identified a 46, XX karyotype. Polymerase chain reaction (PCR) and electrophoresis were performed using specific primers for *SRY*;

however, we could not find any band of amplified *SRY* materials. Therefore, the diagnosis of a 46, XX testicular DSD with *SRY*-negative was made.

The patient was raised by his parents in a religious Muslim family where male and female have distinctive roles and responsibilities. Initially the patient was raised as a male gender since birth. The parents raised confusion regarding their baby genital abnormalities which brought the family to visit clinician in their neighborhood then referred to our center. The dilemma of sex reassignment was raised when the diagnosis was made. After knowing that there was discrepancy between the results of chromosome analysis and phenotypic sex characteristics, the parents experienced confusion how to raise their child in the future. The counseling process including psychological evaluation was performed for the parents and also the patient. Upon discussing the possibilities clinical management for patient, the father considering gender reassignment, though our team suggest postponing gender adjustment and plan for genital reconstruction surgery until the child reach legal age and able to make own decision. Unexpectedly, during follow-up, it turns out that the father has changed the gender of the child into a female. Now the child wear female Muslim praying attire and sit with female at religious functions in accordance with the Muslim sharia.

DISCUSSION

A 46, XX testicular DSD can occur when gonadal differentiation process is impaired, especially due to alterations in the ovarian or testicular signaling pathways.⁷ In the process of gonadal differentiation, the *SRY* gene has a pivotal role for activating a cascade that influences testis-determining genes.⁸ Most of 46, XX testicular DSD patients have *SRY* gene translocated to the X chromosome or autosomal chromosomes.⁴ However, cytogenetic analysis of the patient in this report confirmed a 46, XX karyotype and molecular analysis of *SRY* gene in this case showed a negative result. Results of the present study were consistent with previous study by Li et al 2014 which states that there was two subgroups of 46, XX males, *SRY*-positive and *SRY*-negative.⁹ Considering the reports of *SRY* negative is rare, approximately only 10% from total cases of 46, XX testicular DSD, the etiology of testis formation in *SRY* negative cases remains unknown.^{9, 10}

Sex differentiation is regulated by complex molecular signaling pathways which lead to the development of the initially bipotential gonad as either a testis or an ovary. Two different pathways are being proposed as testicular tissue developed in the *SRY*-negative XX gonad: the elevated expression of pro-testis genes or inadequate expression of pro-ovarian genes.⁴ The *SOX9* gene, a pro testis-gene, is known to be responsible for the etiology of 46, XX testicular DSD with *SRY* negative cases through duplication of *SOX9* gene or its upstream promoter region.^{11, 12} In addition to *SOX9* gene, other pro testis-genes such as *SOX3*, *SOX10*, *DMRT1*, *CBX2*, and *FGF9* genes may contribute to *SRY* negative 46, XX Testicular DSD.^{4, 7} Furthermore, insufficient expression in pro-ovarian genes such as *WNT4*, *RSPO1* and *FOXL2* genes have been associated with 46, XX Testicular DSD.^{4, 7, 13} There are still uncharacterized genes causing DSD in this case. Whereas other candidate genes are being studied on

the basis of experimental models, the implication on clinical managements of unrevealing of the genetic etiologies of XX testicular DSD should be undertaken as the basis for genetic counselling for patient and family members. Utilization of WGS should be considered for detecting exome regions, promoter regions and variations in RNA and not covered by the exome kit. A customized microarray for DSD also could be considered to analyze and detect large deletions and duplications within DSD genes.

While genes involved in DSD have received a considerable amount of attention, the aetiology of DSD is still largely unknown. Research on environmental factors has been extensively conducted to elucidate the cause of DSD. The exposure to endocrine disrupting chemical (EDC) may be responsible for a variety of phenotypes in DSD.¹⁴ Environmental estrogens that resemble estradiol and bind to estrogen receptor are the most observed EDC, for instance presented in pesticide, insect repellent, bisphenol A, prescription estrogens such as diethylestilbestrol and ethynyl estradiol.^{15, 16} Hypospadias prevalence also seemed to be higher in areas of intensive pesticide use or in agricultural areas.¹⁷

DSD is commonly linked to a higher risk of developing a germ cell tumor.¹⁸ In this report, our patient was diagnosed with a 46, XX DSD with testicular Leydig cell hyperplasia. Leydig cell hyperplasia, a neoplasia with non-germ cell origin, is low in the general population with incidence 5-10% of testicular neoplasm.¹⁹ They are considered to be under-reported in the literature, with only a limited number of reported case studies. A case report by Kim et al., identified a Leydig cell hyperplasia in an infertile male with *SRY*-negative 46, XX testicular DSD.²⁰

The mechanism of neoplasm on Leydig cell hyperplasia have not yet been elucidated. One hypothesis is a dysfunctional negative feedback regulation of the hypothalamic–pituitary–gonadal axis, leading in increased plasma levels of LH inducing Leydig cell hyperplasia.²¹ In this patient, a failure of gonadal function may cause a malfunctioning negative feedback mechanism of gonadotrophins action that contribute to increased levels of LH and proliferation of Leydig cell.

There are no reports of recurrence or metastasis in prepubertal children with Leydig cell hyperplasia have been reported.²² Nevertheless, periodic follow-up with clinical evaluation and imaging is advised related to this rare condition.²³ The patient was recommended for regular follow-up and no additional medical care or surgical decision have yet been made.

The basis for establishing gender assignment in a baby with ambiguous genitalia shall require a comprehensive evaluation where applicable, such as physical examination, genetic analysis, psychologic evaluation, surgical option and potential for fertility.²⁴ Our multidisciplinary team promotes the exchange of information between the family and the panels of doctors. Parents are informed with the differential diagnosis, details about molecular study, hormonal assay and psychological support. Due to stigma and sensitive issue regarding DSD, mental health and quality of life of parental and patient are often adversely affected by this condition amidst the patient may experience gender dysphoria during puberty. We conducted a

psychological evaluation to inform the family regarding the process of sexual differentiation and how DSD may develop during fetal development. We also offered encouragement and support for parents to accept their baby's condition and reassurance that all team members are working together to provide the best possible care for the patient.

However, a recognition to parental background and expectations, social conditions, ethnic, cultural or religion factors also important to take into consideration.²⁵ In this case, the parents decide for themselves to change the gender of their child without further discussion with our team. The spontaneous sex reassignment by parents is unavoidable since parental attitude toward child rearing based their believe and limited parental understanding of the condition. This finding in accordance with a study by Ediat et al showed that gender change are especially apparent in patients who are struggling to make ends meet or in areas where there is insufficient medical knowledge and assistance.²⁶ After being informed that the parents did not acknowledge the recommendations of the team's advice about the gender assignment and decide to made a gender reassignment for the patient, our strategy was to do regular evaluation for this family. The family continues to be seen in follow-up care to monitor growth and development, educate their child about her condition at developmentally appropriate times and general assessment of quality of life for the child and the family. Any medical surgery program and initiation of hormonal treatment will be postponed until the child can participate in decision making process.

The present case has had several limitations. Immunohistochemical staining related to the development of germ cell tumor such as OCT3/4, TSPY, Inhibin-alpha and Ki-67 labelling index for expression of antigen-positive nuclei has not been performed. In addition, endocrine investigations on the hCG stimulation test have not been established, which preclude us from furthering our understanding of the gonadal function in this patient.

CONCLUSION

In summary, we are presenting a rare case of *SRY*-negative 46, XX testicular DSD in a boy who present with ambiguous genitalia in early childhood. Our patient has presenting with undermasculinisation which characterized by severe hypospadias, micropenis and bifid scrotum. This case report has emphasized that integrating physical examination, cytogenetics, hormonal study, imaging, histopathology analysis, molecular investigation and psychological evaluation are paramount for establishing the diagnosis. Since the process of sex determination in human has not yet completely understood, an extensive research is necessary for making advances in this field that may provide valuable information for the diagnosis and clinical management of DSD. In recognition of gender development that remaining as a complex process, we promoted a patient-centered care including psychological evaluation that pay attention to the patient's and family's preferences and beliefs. This approach would provide the best highest quality care for patients with DSD and their families.

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