Case Report
Unraveling of Diagnostic Odyssey in A Girl with Primary Amenorrhea: A case report

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Abstract
Primary amenorrhea may result from congenital abnormalities in the development of the gonads, genital tract, or external genitalia or from a disturbance within the hypothalamic-pituitary-ovarian axis. Gonadal dysgenesis is a disorder of sex development in which the diagnosis is based on the histology of gonads and is the main cause of primary amenorrhea. Optimal protocol of management for phenotypic female with 46, XY gonadal dysgenesis involves prophylactic gonadectomy at diagnosis.

Case Presentation: The patient was referred to our hospital at the age of 15 years old for primary amenorrhea. She was obese with no secondary sex signs. Gynecologic examination revealed a normal vagina and clitoris. Rectal Toucher examination revealed no internal genitalia structure. The laboratory data: FSH levels were above normal range, LH and testosterone levels were within normal range. In pelvic ultrasonography uterus and vaginal structure and testis were not visualized. Cytogenetic and AR gene analysis found a 46, XY karyotype and no pathogenic variants. On laparoscopy, Mullerian structure and Wolffian remnant structure were identified and biopsies were performed. Based on histopathological examination and immunohistochemical markers of the right and left gonad showed the impression of Malignant Mixed Germ Cell-Sex Cord Stromal Tumor. SRY gene examination was positive. Examination of other DSD gene analysis has not been done. A Second laparoscopy for gonadectomy and removal of Mullerian and Wolffian remnant structure were performed.

Conclusion: Chromosomal analysis should become the first line testing in primary amenorrhea followed by advanced molecular test. Multidisciplinary management is recommended for DSD cases.

Keywords: Primaryamenorrhea, Gonadal dysgenesis, Disorders of sexual development, Genetic mutation

INTRODUCTION
Primary amenorrhea is the failure of menses to occur by age 16 years, in the presence of normal growth and secondary sexual characteristics or failure of menses to occur by age 14 in the absence of normal growth and secondary sexual characteristic. Primary amenorrhea is most common caused by gonadal dysgenesis (approximately 30-40%), followed by Mullerian agenesis, and androgen insensitivity syndrome.1

The term disorders of sex development (DSD) are defined by “congenital conditions in which development of chromosomal, gonadal or anatomical sex is atypical.”2 DSD classification proposed by the multidisciplinary conference in Chicago 2005 are 46, XX DSD, 46, XY DSD and sex chromosomal DSD.3-4

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The overall incidence rate for DSD is 1 in 5,500, genetic deformities during sexual differentiation is the predominant cause. DSD are commonly correlated with genital ambiguity, that often caused physical and psychological problems. Patients with XY pure gonadal dysgenesis, XY mixed gonadal dysgenesis and XY androgen insensitivity syndrome known to be DSD in XY phenotypic females.Presentation and distinctive measures of XY pure gonadal dysgenesis are presence of female external and internal genitalia with 46, XY karyotype. The patients usually have no Turner stigmata and may present with uterus and oviducts and nonappearance of follicles in the gonadal line. The failure of gonad differentiation into functional testes in 46, XY pure gonadal dysgenesis DSD caused the reproductive tract formed into a female pathway. Mixed gonadal dysgenesis is identified in patients with a one sided testis (frequently intra-abdominal), a contra-lateral gonadal line, formation of pertinacious Mullerian duct with differing levels of undermasculinization. The great number of patients diagnosed with gonadal dysgenesis have an evident anomaly implicating X chromosome, although mosaicism also have been reported with Y chromosomes. Androgen insensitivity syndrome (AIS) is a rare X-linked disorders due to pathogenic variants in the androgen receptor (AR) gene (Xq11–q12). Molecular diagnosis was made by identification of pathogenic variants in the AR gene.

The scarcity of 46 XY GD patients with an incidence rate 1 in 100,000 in which most cases caused by genetic disorders. Patients with GD conventionally have mutations on the testis-determining gene SRY, and other genes such as WT1, DHH, NR5A1, SOX9, GATA4, FOG2/ZFPM2, and MAP3K1, as well as chromosomal adjustment including deletions of chromosome 9p and duplications of Xp22. After the diagnosis is established, immediate gonadectomy must be performed, because there is 30% risk of malignant gonadal line alterations in patients with 46, XY complete gonadal dysgenesis (CGD) at the age 40 years. Gonadoblastoma is the most common gonadal tumor in 46, XY CGD. Most dysgerminoma are gonadoblastoma associated with malignant germ cell tumors with an incidence of 50%-60% of cases. It is very important to be able to carry out a careful initial assessment and early identification, because there is a risk for malignancy alterations that can occur in early life and increases sharply with age even though the history of the origin of gonadoblastoma is unclear. Gonadal histology is the basis for establishing a diagnosis of gonadal dysgenesis which is a disorder of sex development. Preventive gonadectomy at diagnosis is the most ideal management protocols for phenotypic females with 46, XY GD. In this case report we present a case of primary amenorrhea with gonadal dysgenesis.

CASE REPORTS
The patient was referred at the age of 15 years old for primary amenorrhea. She was obese (Asian criteria), weighing 77.7 kg for a height 166 cm (BMI 28 kg/m²). Her pubertal status based on Tanner scale, was A1, M1, P1. Gynecologic examination revealed a normal vagina and clitoris. Rectal Toucher examination revealed no internal genitalia structure was found. The laboratory data: FSH levels 47.15 mIU/ml (normal range: 5.7-8.77 mIU/ml), LH levels 10.53 mIU/ml (normal range: ≤ 15.97 mIU/ml), testosterone level 17.15ng/dl (normal range: 14.12-48.99 ng/dl). In pelvic ultrasonography uterus and vagina structure were not visualized and no testes structure were identified. Approval from the medical ethics committee of Faculty of Medicine Diponegoro University and informed consent from the patients and her parents prior to their participation in this case report were obtained. Her karyotype was 46, XY. AR gene analysis was no mutation. On laparoscopy, Mullerian structure (uterus, fallopian tubes, and gonads) was identified (fig. 1), a vas deferens-like structure in the lateral pelvic wall was found and biopsies were performed. Based on histopathological examination and immunohistochemical profile of the right and left gonad showed seminiferous tubules containing Leydig cells and no visible epithelial layer of the ducal deferens with the impression of Malignant Mixed Germ Cell-Sex Cord Stromal Tumor. Mullerian structures were not confirmed. Immunohistochemical markers for gonadal germ cell tumors are positive for PLAP and CD117. Further analysis of DSD genes, SRY gene alleles was found. Examination of other DSD gene analysis has not been done. The patient was consulted to the urology department for a second laparoscopy, gonadectomy and removal of Mullerian/Wolffian remnant structure were performed. Gonadal dysgenesis with gonadoblastoma and Malignant Germ Cell Tumor was confirmed.

Figure 1. Laparoscopic of Mullerian structure. Left/Right streak gonad (arrow), left/right fallopian tubes (double arrow), uterus (up arrow).

DISCUSSION
Performing cytogenetic investigation procedures is a compelling reason for every patient experiencing hypergonadotropic hypogonadism related with external and internal genitalia in female even when there is no clinical characteristic of Turner syndrome, but it is difficult to gain karyotypes. This was done considering the significant proportion of XY patients among those with GD. Current research shows there is a high incidence of XY patients among those with GD where more than one third of the specimen has a chromosome structure, which shows the prominence of analyzing the karyotype of patients with this clinical feature.

The phenotype of female with a blind vaginal pouch and no uterus is a distinctive sign of Complete Androgen Insensitivity Syndrome (CAIS). The existence of testes with histology according to testicular differentiation, 46, XY karyotype, and nonappearance of Mullerian duct
remnants are needed to establish the diagnosis of AIS.\textsuperscript{17} Primary amenorrhea is highly prevalent complaint that requires medical support during puberty. The clitoris and labia minora are underdeveloped in some cases.\textsuperscript{9} The proband referred to our clinic by reason of primary amenorrhea. Based on the clinical evaluation supported by hormonal assay and cytogenetic analysis, the patients were clinically diagnosis had CAIS and the AR gene analysis were performed. The analysis was only covering 8 exons of AR gene, to determine whether mutations were in the intron or regulatory region, it should proceed to a more advanced examination using the whole gene sequencing. Jaaskelainen (2012) declared that there are a large number of diverse mutations in the AR gene related with AIS. Missense mutations are the majority of mutations and most affect the ligand-binding domain of the AR. Exon 1 novel mutations values will increase if this exon is orderly filtered for mutations. Several cases shown where there were AIS characteristics in vivo and in vitro but no mutations in the AR coding area can be found. There has never been a case with mutations that occurred in the AR promoter region even though this could happen theoretically.\textsuperscript{17} Listyasari, et al (2019) reported clinical, imaging, hormonal, and molecular analysis among girls with primary amenorrhea, confirmed have CAIS.\textsuperscript{18} The clinical findings of this patient with no secondary sex signs, and from the molecular analysis of this patient, however, revealed no pathogenic variants in all exon, and diagnostic laparoscopy identified Müllerian structure, consequently the diagnosis of CAIS is excluded.

In pelvic ultrasonography of the patient showed that uterus and vaginal structure were not visualised and no testis structure were identified. The facts provided by pelvic MRI are superior to ultrasonography in recognizing Mullerian structures.\textsuperscript{19}

The majority types of tumors found in patients with DSD are gonadoblastoma and dysgerminoma.\textsuperscript{20} It is a well-established association between the Y-derived chromosome materials and the development of gonadoblastoma. It is known approximately in 15 – 20% cases of XXY patients and 30% cases of patients with XY gonadal dysgenesis will develop gonadoblastoma.\textsuperscript{21} In this case reports, the patient was confirmed for gonadoblastoma and Malignant Germ Cell Tumor. Gonadoblastoma described as an in-situ germ cell neoplasm that has potential for developing malignant germ cell tumors such as dysgerminoma.\textsuperscript{22,23} Gonadoblastoma can be identified in gonadal tissue with undifferentiated or immature testis differentiation.\textsuperscript{4} It was hypothesized from scientist that several factors involved in the evolution of gonadoblastoma during the gonad differentiation process were identical to factors involved in the growth of dysgenetic gonads.\textsuperscript{24} At present there are no genes that act as oncogene and are involved in the development of the gonadoblastoma locus on the Y chromosome. Patients with sex reversal showing pathogenic variants in WT1 and SOX9 genes, 9p deletions can have this tumor.\textsuperscript{25,26}

Comprehension of DSD category, age, and level of individual pubertal status is needed to make an appropriate timing of gonadectomy that will become a prominent point in DSD managements. Individuals with 46, XY GD and germ cell tumors have an incidence rate approximately at 25-33\%.\textsuperscript{27,28} There was a strong recommendation to do an early gonadectomy at diagnosis.\textsuperscript{5} In this case, patient was 15 years old and based on the histopathological and immunohistochemical findings from the first laparoscopic biopsies showed malignancy. Evidence of gonadal germ cell tumors is confirmed by immunohistochemical marker staining using PLAP and CD117.\textsuperscript{29} Therefore we performed a second laparoscopy for gonadectomy and removal of the Mullerian and Wolffian structure. Gonadal dysgenesis with gonadoblastoma and Malignant Germ Cell Tumor was confirmed based on anatomic pathology result.

Patients and their families frequently feel pressure after the establishment of the DSD diagnosis. Management collaboration between psychological care and counselling by psychologist is needed other than appropriate medical and surgical care.\textsuperscript{30} As to our DSD patient, a comprehensive multidisciplinary team is essential in order to provide the best of care for patient with DSD followed by genetic counseling and psychological support for the patient, and her family members. In this patient, gender assignm

CONCLUSION

Chromosomal analysis should become the first line testing in primary amenorrhea. A comprehensive and multidisciplinary approach of DSD is paramount for clinical managements for primary amenorrhea patients. Genetic etiology such as mutation involving the testis-determining gene SRY and other genes involved in sex determination should be elucidated through advanced molecular analysis such as gene panel using massive parallel sequencing.

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REFERENCES


