Phenotypic Variation of 46,XY with Androgen Insensitivity Syndrome in Indonesia

Ratna Dewi Fitri Ayu Wijaya[1], Annastasia Ediati [2], Achmad Zulfa Juniarto[3]
[1]*Master Program of Biomedical Science, Faculty of Medicine UniversitasDiponegoro, Indonesia*
[2]*Faculty of Psychology, UniversitasDiponegoro, Indonesia*
[3]*Medical Biology and Biochemistry, Faculty of Medicine, UniversitasDiponegoro, Indonesia*

KEYWORDS
Phenotypic variation, AIS, EMS, Quigley scale, Height analysis

ABSTRACT:
Introduction: Androgen Insensitivity Syndrome (AIS) is a genetik disorder which has a resistance androgen receptor to the action of androgens resulting in impaired sexual development which results in phenotypic variations in 46,XY patients. Clinical examination of phenotypic variations by a medical professional will assist in establishing the diagnosis. The aims of this study is to identify phenotypic variations in AIS patients using the External Masculinization Score (EMS), quigley scale and height analysis.
Objectives: To Analyze phenotypic variations in 46,XY patients with Androgen Insensitivity Syndrome in Indonesia
Methods: A descriptive study was conducted on 30 patients from the Center for Biomedical Research (CEBIOR) Faculty of Medicine, Diponegoro University, Semarang from 2004-2019 who were identified as having AR gene mutations. Analysis of phenotypic variations was carried out based on EMS data, Quigley scale, and the patient's height at first admission.
Results: The average age of patients at first admission was 10.97 years, ranging from 1 month to 28 years. There were 6 CAIS patients and 24 PAIS patients. EMS for all patients was ≤9, whereas all CAIS patients had EMS of <3, and PAIS patients' EMS varied from 3 to 9. The quigley scale for all CAIS patients was ≥6, while the scale of PAIS patients varied from 2-5. The height of most CAIS and PAIS patients had normal category or corresponded to the average height of normal people at their age. At the same time, the height of 1 female adult CAIS patient and 1 boy PAIS patient had tall category, it means their height had above average height compared to the normal people in their age.
Conclusions: Phenotypic variations in 46,XY patients with CAIS in Indonesia are relatively uniform while PAIS is more diverse. EMS, quigley scale, and height analysis are appropriate initial screenings in physical examination to direct supporting examinations towards diagnosis.

1. Introduction

Androgen Insensitivity Syndrome (AIS) is a genetic disorder in which androgen hormone synthesis is normal, but there is resistance from the androgen receptor to androgen action, and it falls into the 46,XY DSD group with a spectrum of phenotypes that depends on residual androgen receptor activity, ranging from an entirely female phenotype to a male phenotype with infertility or undermasculinization.[1] There are three main classifications of AIS: Complete Androgen Insensitivity Syndrome (CAIS), Partial Androgen Insensitivity Syndrome (PAIS), and Mild Androgen Insensitivity Syndrome (MAIS).[2,3] Most AIS inheritance patterns are X-linked, although sporadic de novo variants have been identified. AIS is caused by pathogenic variants in the AR gene located on the long arm of the X chromosome (Xq11-12).[3]The clinical picture of AIS varies greatly depending on the severity of androgen resistance. Clinical variations in external genitalia in men can be observed and analyzed using several assessment tools, such as the quigley scale and EMS.[4]

EMS is a validated assessment system used to evaluate the level of masculinization in individuals with ambiguous genitalia.[4] EMS is focused on physical examination of the external genitalia, such as scrotal fusion, micropenis, location of the external urethral meatus, and gonad position, while the quigley scale is used to assess the development of the external genitalia in 46,XY DSD. Quigley scale consists of 7 levels between "completely masculine" to "completely feminine".[5]

AIS patients have a disorder of the androgen hormone which plays a vital role in male growth and bone density. A previous study found that the average height of adult AIS patients in Switzerland is higher than that of normal women and slightly lower than that of normal men.[6] Meanwhile, in Indonesia, there is no available data or research on the height of AIS patients

2. Methods

This study scrutinized the patient records at the Center for Biomedical Research (CEBIOR) Faculty of Medicine, UniversitasDiponegoro, Semarang, from 2004 to 2019. Data were obtained from AIS

patients who had undergone a complete physical examination and chromosomal and hormonal analysis. There were 30 patients who were identified as having AR gene mutation. The inclusion criteria were patients who had result 46,XY of karyotyping, had an AR gene mutation, and were clinically diagnosed with AIS. If there was no AR gene mutation and the subjects had undergone genital surgery before, then they would be included in the exclusion criteria. Samples were taken by consecutive sampling. All patients from 2004 to 2019 who met the inclusion and exclusion criteria were picked as research samples. Patient data used as research subjects were collected and tabulated in a phenotypic tabulation consisting of EMS, Quigley scale, and body height analysis.
Data would be presented in tables and graphs. Variations in the subjects' phenotypes assessed using the EMS score, quigley scale, and height analysis would draw conclusions. The study was performed after receiving approval in the form of ethical clearance from The Health Research Ethics Committee, Faculty of Medicine UniversitasDiponegoro (study reference number 388/EC/KEPK/FK-UNDIP/X/2022).

3. Results

Table 1 shows the subject characteristics, including diagnosis, age, gender, quigley scale and EMS at first admission, while table 2 shows Z-score and it's category. There were 6 CAIS patients: 4 adults and 2 pediatric patients. Meanwhile, 24 PAIS patients consisted of 3 adults and 21 pediatric patients. All CAIS patients were female, while PAIS patients comprised 3 girls, 2 adult females, 18 boys, and 1 adult male.

Table 1.Subject's characteristics of *quigley scale* and EMS

Examination	**Sccore**	**CAIS**				**PAIS**			
		Paediatric		**Adult**		**Paediatric**		**Adult**	
		F (n = 2)	**M** (n = 0)	**F** (n = 4)	**M** (n = 0)	**F** (n = 3)	**M** (n = 18)	**F** (n = 2)	**M** (n = 1)
Quigley scale	1								
	2						6		1
	3					2	8	1	
	4					1	3	1	
	5						1		
	6			2					
	7	2		2					
External Masculinization Score	0	2		3					
	1								
	2			1					
	3					1	5	1	1
	4								
	4.5					1	1		
	5					1	1	1	
	6						6		
	7						3		
	8								
	9						2		

Table 2.Subject's characteristics of HAZ

Diagnosis	Gender	Age	< -3 SD	-3 SD s.d.<-2 SD	-2 SD s.d. +2 SD	+2 SD s.d. +3 SD	>+3 SD
			Severe stunting	*Stunting*	Normal	Tall	Severe Tall
CAIS	Female	Paediatric (n = 2)			2		
		Adult (n = 4)			3	1	
PAIS	Female	Paediatric (n = 3)			3		
		Adult (n = 2)			2		
	Male	Paediatric (n = 18)			17	1	
		Adult (n = 1)			1		

4. Discussion

There were 6 CAIS patients and 24 PAIS patients. EMS for all patients was ≤9, whereas all CAIS patients had EMS of <3, and PAIS patients' EMS varied from 3 to 9. Based on a previous study, patients with EMS <7 are considered to have ambiguous genitalia, as well as patients with an EMS <3 are associated with genetic causes of 46,XY DSD and recommended for genetic analysis screening, especially in patients who are AIS suspects.[4]

There were 4 important points for assessing the external genitalia: scrotal fusion, micropenis, Meatus Urethra Externa (MUE) position, and gonad position. In this study, All CAIS patients had 0 score on the scrotal fusion assessment point.[7]This shows that complete androgen hormone resistance occurred in CAIS patients, so all CAIS patients in this study (100%) had an abnormal scrotum shape (bifid scrotum). Meanwhile, 52.36% of male PAIS patients and 60% of female PAIS patients also had bifid scrotum. This result was in line with a previous study stating that the majority of 46,XY DSD patients had bifid scrotum.[8] Another study in the United States found that 2 out of 16 patients with bifid scrotums were confirmed to have AR gene mutations.[9]

The second point of EMS is micropenis, a medical diagnosis based on the length of stretched penis (< -2.5SD).[10] The micropenis assessment points in EMS have a range of scores of 0 to 3. A score of 0 means micropenis, while a score of 3 means the length of the penis is within the normal range. All female CAIS and PAIS patients (100%) had micropenis, while 68.42% of male PAIS patients had micropenis. Based on these data, micropenis is a pathognomonic sign in AIS patients, especially in female CAIS and PAIS patients. A previous study stated that micropenis was proven to be associated with AR gene mutations, even with or without other symptoms of undermasculinization.[11] It shows the importance of micropenis screening in EMS at the beginning of the examination in DSD patients.

The MUE position is normally at the distal of the penis (coronal end). If the MUE is below it, it is called hypospadias.[12] The MUE position on EMS is grouped into 4 categories with different score values. In this study, all CAIS patients had a score of 0 on the MUE position assessment point. It shows that all CAIS patients had hypospadias perineal. Male PAIS patients (84.21%) and female PAIS patients (80 %) also had perineal hypospadias, so perineal hypospadias was one of the pathognomonic signs in AIS patients. Based on a previous study, perineal hypospadias had been proven to be associated with AIS, which is usually accompanied by other phenotypes of external genital abnormalities such as cryptorchidism and micropenis.[13] Another study discovered that 3% of 292 Caucasian boys with isolated hypospadias had an AR gene mutation.[13] This is in line with the results of this study, which found that all CAIS and PAIS patients had hypospadias, while perineal hypospadias was one of the dominant phenotypic variations in both CAIS and PAIS patients.

The majority of CAIS patients (83.33%) had no palpable gonads on physical examination. Meanwhile, the majority of female PAIS patients (60%) and male PAIS patients (84.21%) had the gonads palpated in the scrotum. Gonads that are not palpable or cannot be felt in the scrotum are called undescended testicles or cryptorchidism.[14]In CAIS and PAIS patients, cryptorchidism occurs primarily due to insensitivity to androgen hormones. Increased prevalence of cryptorchidism among boys with diseases or syndromes associated with congenital reduction in androgen secretion or action, such as patients with AIS.[15]

Based on the results of this study, CAIS patients had EMS of <3 while PAIS patients had varied EMS from 3 to 9. Several dominant phenotypes were found in CAIS and PAIS patients. CAIS patients had bifid scrotum, micropenis, perineal hypospadias, and non-palpable gonadal position as the dominant phenotypes. Meanwhile, both male and female PAIS patients had the same phenotype with CAIS, except for the position of gonads; the majority of gonad positions were in the scrotum. Male and female PAIS patients were difficult to differentiate. However, this study discovered that all female PAIS patients had micropenis while male PAIS did not.

The greater value of the Quigley scale means a more feminine external genitalia appearance.[5] Based on these data, the Quigley scale in four patients with CAIS was 7, while the scale of the other two patients with CAIS was 6. In patients with PAIS, the Quigley scale varied from 2 to 5. In women with PAIS, three patients had a Quigley scale of 3, and two patients had a Quigley scale of 4. Meanwhile, in the men with PAIS group, seven patients had a Quigley scale of 2, eight patients had a Quigley scale of 3, three patients with a Quigley scale of 4, and one patient with a Quigley scale of 5.

The first six levels of the Quigley scale are differentiated based on the degree of masculinization of the genitals. Quigley describes the scale as depicting "severity" or "damaged masculinization." Grade 1 is indicated when the external genitalia are fully masculine. This study associated that situation with MAIS. Grades 6 and 7 are indicated when the external genitalia are fully feminine. This study associated these with CAIS. Levels 2 to 5 measure four degrees of increasingly feminine genitals related to PAIS. Grade 7 is indistinguishable from grade 6 until puberty and, thereafter, differentiated by the presence of secondary terminal hair. The presence of secondary terminal hair indicates grade 6, while grade 7 is indicated if there is no terminal hair. Based on the results of this study, it can be concluded that the Quigley scale of all CAIS patients was ≥6, and the Quigley scale of PAIS patients, both male and female, varied from 2 to 5.

In previous cases in Africa, an incidental diagnosis of CAIS was found in a patient of puberty age with complaints of inguinal hernia and primary amenorrhea where the patient had a Quigley scale score of 7 and was born with a female phenotype without signs of masculinization of the genitals.[16] In another case in Indonesia, there were 5 female patients from three different families with a history of inguinal hernia and a Quigley scale ranging between 6 and 7 who were proven to have AR gene mutations and diagnosed with CAIS.[17]Based on the results of this study and previous case findings, it can be concluded that the Quigley scale assessment is essential as an initial screening for possible diagnosis. Any delay in diagnosing AIS in patients is a grave concern, considering that determining gender will significantly influence the patient's future and quality of life

Phenotypic variations in CAIS and PAIS patients also manifest in height since the function of the androgen hormone regulates height growth, too, especially in males.[18] Height analysis in this study differentiated between CAIS and PAIS patients, and they were grouped into 2 age groups: pediatrics and adults. There were 2 pediatric CAIS patients, 4 adult CAIS patients, 21 pediatric PAIS patients consisting of 3 girls and 18 boys, and 3 adult PAIS patients comprising 2 female and 1 male. Age grouping and gender data collection at the beginning of the examination were applied to determine the curve used to compare the Z-score based on age according to gender or Height for Age (HAZ).Height analysis was determined using the national reference curve for Indonesian pediatric growth.[19]

Z-score in majority of CAIS and PAIS patients had normal value, which means the patient's height corresponded to the average height of normal people of their age, while the height of 1 adult female CAIS patients and 1 boy PAIS patient was tall that means their height was above the average height of normal people in Indonesia. In this study, most of subject had varied Z-scores, but still in the normal category. Previous research in the United States discovered 22 CAIS patients and 6 female

PAIS patients who had an average height of 174 cm. This average height is higher than that of the normal adult female in America (162.3 cm).[20] Meanwhile, in Denmark, the height of adult male AIS patients is in the normal category or correspond according to the height of a normal adult male.[21]This is in line with this study, where most of AIS tend to have normal height and a few patients have taller than the normal people at their age since the levels of androgen hormones in the bodies of AIS patients remain greater than those of the normal females at the same age.

Androgens are essential for bone development and maintenance of bone mass. Androgens are known to stimulate the growth of long and radial bones, thereby increasing the size of the cortical bones. Long bones grow through endochondral bone growth and epiphyseal plates, while radial bones grow through periosteal apposition. Cartilage cells, especially chondrocytes, proliferate and differentiate under the regulation of various endocrines, such as GH, IGF-I, TGF-beta, and vitamin D metabolites. When puberty begins, androgens and estrogens stimulate endochondral bone development. At the end of puberty, epiphyseal plate growth begins to close and is primarily mediated by estrogen via estrogen receptors through the aromatization of androgens into estrogen[22].

Bone Mass Density (BMD) in CAIS patients was lower due to a combination of bone resistance to androgen action and estrogen deficiency. The final height in CAIS patients was above the average height of normal females due to the growth action of the Y chromosome growth-control gene (GCY). Previous research noticed that newborns with CAIS had the same average length as the normal ones. This suggests that postnatal factors affect the height of individuals with AIS.[23]

The endocrine profile in AIS patients is consistent with androgen resistance characterized by an increase in serum testosterone levels associated with an increase in LH, indicating a decrease in the negative feedback of androgen hormones on the anterior pituitary, while FSH levels are usually normal. In the post-puberty period, estradiol levels can be normal or slightly increased for male patients. At the hormonal level, no specific differences can be observed, in contrast to the phenotype between CAIS and PAIS patients. Androgens have a direct effect on AR and an indirect effect on the estrogen receptor (ER) through aromatization. In addition, androgens can stimulate growth hormone secretion and indirectly influence circulating insulin-like growth factor 1 (IGF-1) through peripheral and central aromatization. Therefore, children with AIS may show different growth patterns than normal children due to the influence of many mechanisms.[22,23]

Phenotypic variations in CAIS patients in Indonesia were relatively uniform, while those of PAIS patients were more diverse. EMS of all patients was ≤9, whereas all CAIS patients had a total EMS of <3 and PAIS patients varied from 3 to 9. The Quigley scale of all CAIS patients was ≥6, while it varied from 2-5 for the PAIS patients.Individuals 46,XY with bifid scrotum, micropenis, perineal hypospadias, and non-palpable gonads were suspected of having CAIS. In contrast, if this phenotype was accompanied by palpable gonads in the scrotum, then they would be suspected of being PAIS. Male and female PAIS were difficult to differentiate, but in this study, all female PAIS had micropenis, while not all male PAIS had it. Another dominant phenotype in AIS patients was that the average height of both CAIS and PAIS patients was in the normal category or above the average height of the normal people

From this study, we know that genetic counseling was very needed. Communication and support are the main keys in genetic counseling, especially when test results are first announced because information about inheritance or inheritance patterns will cause feelings of guilt in parents.[24]Moreover, the basic principles of genetic counseling also refer to patient autonomy and privacy where a genetic counselor must give space to the patient and family to decide for themselves the steps to be taken and maintain the patient's privacy.[25]

Genetic counseling in Indonesia is still commonplace because of the lack of public awareness regarding its importance for the future of patients and families. The role of genetic counseling is very important as a space for consultation and a bridge for patients and families in finding out about the disorder they are suffering from and determining next steps, because the the aim of therapy in DSD' patients was to improve the patient's quality of life.

Refrences

1. Hughes IA, Davies JD, Bunch TI, Pasterski V, Mastroyannopoulou K, Macdougall J. Androgen insensitivity syndrome. *The Lancet*. 2012;380(9851):1419-1428. doi:10.1016/S0140-6736(12)60071-3
2. Juniarto AZ, Van Der Zwan YG, Santosa A, et al. Application of the new classification on patients with a disorder of sex development in Indonesia. *Int J Endocrinol*. 2012;2012. doi:10.1155/2012/237084
3. Liu Q, Yin X, Li P. Clinical characteristics, AR gene variants, and functional domains in 64 patients with androgen insensitivity syndrome. *J Endocrinol Invest*. 2023;46(1):151-158. doi:10.1007/s40618-022-01894-4
4. Su R, Adam MP, Ramsdell L, Fechner PY, Shnorhavorian M. Can the external masculinization score predict the success of genetic testing in 46,XY DSD? *AIMS Genet*. 2015;02(02):163-172. doi:10.3934/genet.2015.2.163
5. Quigley CA, Bellis A De, Marschke KB, El-Awady MK, Wilson EM, French FS. Androgen receptor defects: Historical, clinical, and molecular perspectives. *Endocr Rev*. 1995;16(3):271-321. doi:10.1210/edrv-16-3-271
6. Zachmann M, Prader A, Sobel EH, et al. Pubertal growth in patients with androgen insensitivity: Indirect evidence for the importance of estrogens in pubertal growth of girls. *ObstetGynecolSurv*. 1987;42(7):455-456. doi:10.1097/00006254-198707000-00017
7. Ahmed SF, Cheng A, Dovey L, et al. Phenotypic features, androgen receptor binding, and mutational analysis in 278 clinical cases reported as androgen insensitivity syndrome. *Journal of Clinical Endocrinology and Metabolism*. 2000;85(2):658-665. doi:10.1210/jc.85.2.658
8. Chaerunisa Ananda P, Faradz SM, DewiAriani M, Faradz SM. FENOTIP PADA KELAINAN GONADAL DISGENESIS 46, XY. 2016;5(4):1482-1492.
9. Swartz JM, Ciarlo R, Denhoff E, et al. Variation in the clinical and genetic evaluation of undervirilized boys with bifid scrotum and hypospadias. *J Pediatr Urol*. 2017;13(3):293.e1-293.e6. doi:10.1016/j.jpurol.2017.01.004
10. Hatipoğlu N, Kurtoğlu S. Micropenis: Etiology, diagnosis and treatment approaches. *JCRPE Journal of Clinical Research in Pediatric Endocrinology*. 2013;5(4):217-223. doi:10.4274/Jcrpe.1135
11. Bhangoo A, Paris F, Philibert P, Audran F, Ten S, Sultan C. Isolated micropenis reveals partial androgen insensitivity syndrome confirmed by molecular analysis. *Asian J Androl*. 2010;12(4):561-566. doi:10.1038/aja.2010.6
12. Baskin L. What is hypospadias? *ClinPediatr (Phila)*. 2017;56(5):409-418. doi:10.1177/0009922816684613
13. Kalfa N, Philibert P, Werner R, et al. Minor Hypospadias: The “Tip of the Iceberg” of the Partial Androgen Insensitivity Syndrome. *PLoS One*. 2013;8(4). doi:10.1371/journal.pone.0061824
14. Kolon TF, Herndon A, Baker L. Evaluation and Treatment of Cryptorchidism: AUA Guideline. Published online 2014.
15. Rodprasert W, Virtanen HE, Mäkelä JA, Toppari J. Hypogonadism and Cryptorchidism. *Front Endocrinol (Lausanne)*. 2020;10. doi:10.3389/fendo.2019.00906
16. Scott E, Greenberg T, Arndt S, Ramsay M, Shires R. *Complete Androgen Insensitivity Syndrome in a Black South African Family: A Clinical and Molecular Investigation*. 6, 665 (2006). doi:10.4158/EP.12.6.664
17. Listyasari NA, Robevska G, Santosa A, et al. Genetic Analysis Reveals Complete Androgen Insensitivity Syndrome in Female Children Surgically Treated for Inguinal Hernia. *Journal of Investigative Surgery*. 2021;34(2):227-233. doi:10.1080/08941939.2019.1602690

18. Zemel BS, Katz SH. The contribution of adrenal and gonadal androgens to the growth in height of adolescent males. *Am J PhysAnthropol*. 1986;71(4):459-466. doi:10.1002/ajpa.1330710409
19. Pulungan AB, Julia M, Barubara JR, Hermanussen M. Indonesian National Synthetic Growth Charts. *Acta Scientific Paediatrics*. 2018;1(1):1-15. https://www.researchgate.net/publication/326972272_Indonesian_National_Synthetic_Growth_Charts
20. Hellmann P, Christiansen P, Johannsen TH, Main KM, Duno M, Juul A. Male patients with partial androgen insensitivity syndrome: A longitudinal follow-up of growth, reproductive hormones and the development of gynaecomastia. *Arch Dis Child*. 2012;97(5):403-409. doi:10.1136/archdischild-2011-300584
21. Chen JF, Lin PW, Tsai YR, Yang YC, Kang HY. Androgens and Androgen Receptor Actions on Bone Health and Disease: From Androgen Deficiency to Androgen Therapy. *Cells*. 2019;8(11). doi:10.3390/cells8111318
22. Chen JF, Lin PW, Tsai YR, Yang YC, Kang HY. Androgens and Androgen Receptor Actions on Bone Health and Disease: From Androgen Deficiency to Androgen Therapy. *Cells*. 2019;8(11). doi:10.3390/cells8111318
23. Batista RL, Costa EMF, Rodrigues A de S, et al. Androgen insensitivity syndrome: A review. *Arch EndocrinolMetab*. 2018;62(2):227-235. doi:10.20945/2359-3997000000031
24. Yamaguchi M, Sameshima H, Ikenoue T. Genetic diagnosis and genetic counseling for androgen-insensitivity syndrome: A report of three cases. *Journal of Obstetrics and Gynaecology Research*. 2014;40(3):723-727. doi:10.1111/jog.12249
25. Yang M, Kim JW. Principles of genetic counseling in the era of next-generation sequencing. *Ann Lab Med*. 2018;38(4):291-295. doi:10.3343/alm.2018.38.4.291