Etiologic diagnosis of children with ambiguous genitalia in Jos University Teaching Hospital

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Abstract

Background: Ambiguous genitalia (AG) is a stigmatized condition with varied etiologies. The aim of this review is to describe the etiologic profile of children with AG in a developing country.

Methods: This is a retrospective review from January 2014 to December 2018 of children presenting with AG. Socio-demographic, clinical, ultrasonographic (USS), laboratory data including karyotype and diagnosis were retrieved and presented using descriptive statistics.

Results: Fifteen cases of AG were reviewed. The age range was 1 week to 14 years at presentation. Five children were 1-28 days, 3 each were between 1-11 months and 1- 5 years and 4 were aged ≥6 years. Four children had sibling with AG, 1 family each had a history of previous neonatal death and consanguineous family. Three children each had features of salt lost and clitoromegaly after clitoral reduction, and 2 were hypertensive. Two cases had palpable gonads. USS showed Mullerian structures only in 11 cases, wolffian structures in 2 and mixed picture in 2 children. Serum 17 hydroxyl progesterone (17 OHP) was elevated in 9 and normal in 4 and not available in 2 patients. Karyotype obtained were: 46XX in 6 cases, and one cases each of 45X (11), 46 XY (19) and 45, X [11]/46, XY. A diagnosis of 46 XX CAH (congenital adrenal hyperplasia) 21 hydroxylase deficiency subtype was made in 9 children, CAH 11 beta hydroxylase deficiency subtype was diagnosed in 2 children and 2 cases each of mixed gonadal dysgenesis and 46XY DSD were diagnosed.

Conclusion: 46XX DSD CAH is the commonest cause of AG in our locality. The etiology of AG will be better defined with a larger sample size and a comprehensive laboratory evaluation.

Keywords: Ambiguous, Genitalia, Congenital, Adrenal, Hyperplasia

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Introduction

Gender is an essential feature of the cultural and socio-political existence of humans. It is almost always assigned as male or female after the delivery of every newborn by assessing the external genitalia.[1] However, in some instances this dichotomy is blurred or ambiguous due to faulty sexual development which is a complex embryonic process, that involves the interactions of multiple genes, transcription factors and hormones that ensures synchrony of chromosomal, gonadal, genital sex in an individual.[2-4]

An aberration in sexual development that results in dissociation of chromosomal sex from gonadal sex and/ or phenotypic sex is termed Disorder of Sex Development (DSD) that most often presents with ambiguous genitalia.[1,5] This disorder affects about 1 in 4500 newborns but most importantly, it is a major psychologic stressor to parents.[6,7] Furthermore, an unguarded assignment of gender by the attending physician/ Health worker at birth may have significant long-term effect on the child or the parents. Also, a clear diagnosis may prevent death during early postnatal life from salt losing forms of Congenital Adrenal Hyperplasia (CAH) which is a common cause of DSD.[8]

Because of the complex process of sexual development, the work up of children with ambiguous genitalia is not only expensive but extensive and complex. Therefore, a step by step approach to decipher etiology at minimal cost is essential especially in resource limited setting.[9] An etiologic diagnosis will differentiate those in need of medical treatment in addition to the surgical repairs.[10] This study is a retrospective review of the etiologic profile of children seen with ambiguous genitalia at the Pediatric endocrinology clinic of a tertiary hospital in a developing world.

Methods

This study was retrospective review of patients managed in the Pediatric endocrinology unit of Jos University Teaching Hospital (JUTH), located in Lamingo, Jos, Plateau state in North central Nigeria who presented with ambiguous genitalia.

A child was considered to have ambiguous genitalia if: There is a male like phallus without palpable gonads or if the stretched penile length is less than 3 standard

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deviation for age, or if there is hypospadias. In the presence of the labio-scrotal fold (female like external genitalia) a child is considered to have ambiguous genitalia if it contains gonads or If there is clitoromegaly or If there is posterior fusion of the labio-scrotal fold.

Records of these patients seen between January 2014 to December 2018 were retrieved. Data retrieved included presenting features, features of virilization such as acne, early development of pubic hair. Other information retrieved included previous neonatal sibling dates, history of siblings with similar disorder and history of consanguineous marriage in parents, if the child was dehydrated or in shock at presentation, presumed diagnosis. The measured blood pressures were considered elevated if the reading is above the 95th percentile for age and sex and height. [11]

The results of the abdominal and pelvic ultrasonogragy (USS) which were performed by a radiologist with the aim of delineating if the internal sexual organs are feminine or masculine or mixed were retrieved.

The bone age usually assessed by a radiograph of the left wrist, distal forearm and the digits and interpreted using the Hand bone age atlas were retrieved. [12] Children with a chronologic age above 2 years for age were considered to have advance bone age.

Serum electrolyte assay results with focus on establishing the presence of hyponatremia (,130mmol/l) and hyperkalemia (5.5mmol/L) suggestive of a salt losing form of congenital adrenal hyperplasia were retrieved. The serum assay results of 17 hydroxyl progesterone were also retrieved and values greater than 200ng/dl were considered elevated. Finally, the karyotype results and final diagnosis were retrieved.

Diagnostic criteria: A child with ambiguous genitalia was considered a 46 XY DSD if there were palpable gonads in the scrotal sac or along the inguinal or labioscrotal folds or 46XX DSD if there were no palpable gonad.

A child with 46 XX DSD is diagnosed with Congenital adrenal hyperplasia the 21hydroxylase deficiency subtype if: There are features of over virilization, normal blood pressure, Mullerian structures on USS, advance bone age, elevated 17 hydroxyl progesterone with or without a 46 XX karyotype. A salt losing form of 46XX DSD CAH 21 OH type if the child is dehydrated hyponatremia and hyperkalemia were present with features of dehydration or shock

A child with 46 XX DSD was diagnosed with CAH probable 11 beta hydroxylase deficiency subtypes if the above features are present with hypertension and a low to normal 17 hydroxyl progesterone.

A mixed or partial gonadal dysgenesis was considered if a child with ambiguous genitalia had both Mullerian or wolffian structure on USS, a normal or low 17 OHP with mixed type karyotype picture like 45X (11)/46XY (19), 45, X [11]/ 46, XY [16]

A child with palpable gonads was considered to have 46XY DSD

Data Management: All data collected were presented using descriptive statistic and presented as proportions and ratio. Mean and standard deviation of continuous data were calculated for continues variable.

Results

15 children with ambiguous genitalia were seen during the 4 year of review. Their socio demographic variables are shown in Table 1. Of the 15 children with ambiguity, thirteen had no palpable gonads, two had palpable gonads: one patient had bilaterally descended gonads with bifid scrotal sac and hypospadias, while the other had unilaterally undescended testes with hypospadias.

Table 1: Socio-demographic profile of children with DSD

Variable	N	%
Age at presentation		
Neonatal (1-28 days)	5	33.3
1-11 months	3	20.0
1-5 years	3	20.0
Above 5 years	4	26.7
Residence		
Rural	3	20.0
Urban	12	80.0
Social class		
Upper	5	33.3
Middle	10	66.7
Lower	0.0	0.0
Birth order		
First born	9	60.0
Not first born	6	40.0
Families with a previous neonatal death		
Yes	1	6.7
No	14	93.3
Families with 2 affected siblings		
Yes	4	26.7
No	11	73.3
Consanguineous families		
Yes (cousins)	1	6.7
No	14	93.3

Table 2 showed that 3of 15 children had presented period with features suggestive of salt losing form of CAH (dehydration and hyperkalemia, hyponatremia.) Hyperpigmentation was reported in only one child. Four

children presented with clitoromegaly after an earlier clitoplasty, Hirsutism and premature pubarche was reported in 5 of 15 children who presented late.

Table 2: Clinical Presentation of children with ambiguous genitalia N=15

Variable	N	%
Genitalia		
Ambiguous without palpable gonads	13	86.7
Ambiguous with palpable gonads	2	13.3
Hyperactivity		
Yes	1	6.7
No	14	93.3
Hyperkalemia, hyponatremia, severe dehydration		
Yes	3	20.0
No	12	80.0
Hyperpigmentation		
Yes	3	20.0
No	12	8.0
Acne		
Yes	3	20.0
No	12	80.0
Clitoromegaly after clitroplasty		
Yes	4	26.7
No	11	73.3

The blood pressure was assessed in only 7 children out of which 2 children had elevated blood pressure. The median systolic blood pressure was 90mmHg, the first quantile(Q1) and third quantiles (Q3) were 80mmHg and 130 mmHg respectively.

Table 3: mean, median and inter quartile range of BP, serum sodium, potassium and 17 hydroxy progesterone.

	SBP	DBP	Na	K	17 OHP
Mean	95.6(27.8)	54.6(20.6)	136.1 (6.2)	5.1(1.1)	1321,8(1378)
Median	95	52	136	4.7	930
Q1	80	30	130	4	186.4
Q3	130	80	142	6	2169.5
IQR	50	50	12	2	1983.2

Abbreviation: SBP = systolic blood pressure; DBP= diastolic Blood pressure; Na= serum sodium, K= serum potassium, 17)HP= 17 hydroxyl progesterone.

The abdominopelvic ultrasonography of the thirteen patients with non-palpable gonads showed Mullerian structures derivatives only in 11 children, both Mullerian and wolffian structure derivatives were seen in 2 children. Wolffian structure derivatives only were also reported in 2 patients with palpable gonads

Serum assay of 17 hydroxyl progesterone obtained in the 13 children with suspected 46XX DSD because they have no palpable gonads. The median 17 OHP was 930ng/dl with a Q1 and Q3 of 186.4 and 2169.5 ng/dl respectively. 17 OHP was elevated (above 200ng/dl) in 9 children and within normal in 4 children.

Of the 15 children Karyotyping was obtained in 8(eight) of 15 (fifteen) children.

Table 4 summary of patient's clinical features

S/N	Age	BP	Na+	K	17OHP	USS	Bone Age	Karyotype	Diagnosis
1 MH	3/52	-	135	4.6	298	F	-	Nil	CAH
2. VO	1/52	-	130	6.0	2138	F	-	46XX	CAH
3. GO	3/52	-	135	4	191.6	F&M	-	45X (11)46XY(19)	MCD
4. HI	3/52	-	125	6.8	4395.6	F	-	46XX	CAH
5. ES	3/52	-	136	4	Nil	M		Nil	46XY DSD
6. JP	11/12	-	137	6.6	600	F		46XX	CAH
7. B	8/12	-	130	4.5	94	F&M		45X(11) 46XY(16)	MCD
8. KN	7/12	-	138	4	Nil	M		Nil	46XY DSD
9. OJ	1yr 9/12	80/30	130	7.1	930	F	4	46XX	CAH
10. MO	16/12	102/52	148	4.6	181.1	F	6	Nil	CAH
11. NB	6year	95/50	132	4.0\	1386	F	9	Nil	CAH
12. NY	2 years	60/30	142	5.3	1208	F	5	Nil	CAH
13. AS	6year	140/80	142	5.6	98	F	9	46XX	CAH
14. SU	14 year	130/80	138	5	3462	F	18	46XX	CAH
15. BT	7 years	90/60	144	4.7	2201	F	11	Nil	CAH

Abbreviation: SEC= socioeconomic class, BP = blood pressure, Na= sodium, K = potassium, 17OHP= 17 hydroxyl progesterone, USS = ultrasonography of the abdomen and pelvis, F= female Mullerian structure, M= male wolffian derived structure, F & M= Female and male structural derivative

The karyotype was 46XX in 5 of 9 children with elevated 17 OHP, the remaining four children with elevated 17OHP were unable to pay for karyotyping.

The karyotype was obtained in 8 children, 7 children were unable to pay for karyotype. The karyotypes obtained were 46XX in 6 children and 46XX, 45X (11), 46XY (19) in one patient and 45, X [11]/46, XY [16] in the last patient.

The diagnosis was 46XX CAH the 21OHP type were confirmed in 5 children who had Mullerian structures, elevated 17OHP and a 46XX karyotype. 4 children had features of androgenization, advanced bone age elevated 17 OHP and were also diagnosed with 21OHP deficiency.

The diagnosis of probable 46XX CAH 11beta subtype was made in 2 patients with elevated blood pressure, only Mullerian structure on USS, features of androgenization, normal 17 OHP , a sibling with similar disorder in one of the patient that was unable to obtained a karyotype and a 46XX karyotype in the other patient.
Two patients with ambiguous genitalia, combined Mullerian and wolffian derivatives, normal 17 OHP had 46XX, 45X (11), 46XY (19) in one patient and 45, X [11]/46, XY [16] in the second patient were diagnosed with mixed/partial gonadal dysgenesis. The two children with palpable gonads and wolffian structures were not evaluated further and were suspected of having 46XY DSD.

Discussion

This study reviewed fifteen patients with ambiguous genitalia with focus on the etiologic work up in a limited resource setting, like other congenital disorders establishing an etiologic diagnosis is frequently challenging and requires expertise and an experienced multi-disciplinary team, extensive imaging, hormonal and genetic work up.

In this study, CAH accounted for over three quarters of patients presenting with ambiguous genitalia. Studies in most Nigerian series have reported similar findings with CAH being the commonest cause of ambiguous genitalia as it accounts for a quarter to about three quarter of cases. [13-17]. This suggest that a workup to exclude CAH in children with genital ambiguity is necessary. These work up as demonstrated in our study requires a focus history that can give clues to features of androgenization such as acne, clitoromegaly, clitoromegaly may occur even after clitoral reduction in children who are not place on medical therapy, this was reported in four of our patients. Parental consanguinity was reported in only one of our patients, this is similar to other local Nigerian studies. [13,17] but contrast to reports from Asian countries where about half to three quarters of children with CAH have parents that are consanguineous. [18-19] This may suggest that the CAH in Nigerian series may results from new mutation, however this assertion can only be proven if genetic mapping is available.

Five patients (thirty three percent) in this current review presented during the neonatal period. The report in Ife and Benin found that all their patient presented after the neonatal period. [15,20] A study in Egypt similarly, found that majority of Patients presented during adolescent and adulthood and those presenting early, during the neonatal age, often have severe ambiguity.[18] Late presentation is a problem in most centers in Africa and may only be overcome when newborn screening for CAH is introduced as part of general newborn care. [11-14]. Reasons for late presentation of patients was not access in this study but few patients had clitoral reduction and represented later suggesting a need for health education across the varied categories of health workers. [21].

About one third of the CAH patients in this review presented with vomiting, diarrhea and increasing skin pigmentation, presences of hyperkalemia and hyponatremia. This presentation is in keeping with a salt losing form of classic CAH which accounts for about 30 percent of classic CAH in most Nigerian series but differ significantly from the seventy five percent reports from Europe. [21] The reasons for this variability may not be answered now because this study and other Nigerian study lacks the power for generalization. But other reasons may include: a low suspicion of CAH among health workers, the likelihood of male CAH with salt losing form to be missed and probably die unnoticed because their external genitalia are not ambiguous. This assertion is further supported by the fact that in our clinic registry only one male child has so far been diagnosed with CAH. Theoretically, this is a small proportion as 50% of CAH patients ought to be males because the genetic mutation associated with the enzymopathy causing CAH is transmitted by an autosomal recessive pattern of inheritance [1]

This study is comparable to that in Lagos where these group of children were second commonest cause after 21 hydroxylase deficiency. [13] In this study two children had CAH with hypertension. This proportion is lower compared to a study in Ile Ife south west Nigeria were about 50% of children with ambiguous genitalia were reported hypertensive. [16] This difference may suggest genetic variability of the sub class of CAH, or may not exist as most of our neonatal and infants blood pressure was not assess.

Karyotyping is an essential tool in diagnosis of DSD and helpful in diagnosis. Its availability is vital in establishing an etiologic diagnosis in any child with DSD and cannot be substituted with bar bodies, SRY genetic analysis have significant limitation. [1,15]. One of child with

ambiguous genitalia, non-palpable gonads, predominant Mullerian structures and abdominal gonads which were only visualized by laparoscopy had 46X (19), 46XY (11) karyotype. Such mixed chromosomal disorder might be miss diagnose without a karyotype as bar bodies may be present and SRY genetic analysis maybe present. However, in this study only about half of the review patient had karyotyping. The remaining half were limited by cost as most Nigerians pay out of pocket for healthcare. It is important that physicians look critically into ways of overcoming this limitation as a karyotype provides vital information in care and management.

There were two patients with suspected 46XY DSD in this study. In a study by Ekenze et al [16] about forty percent of 39 patients with DSD were considered as 46XY category. this study may have overestimated these cases because a karyotyping was not done.

Conclusion

46XX DSD are commonest cause of ambiguous genitalia with CAH as the commonest causes of ambiguous genitalia; other causes were mixed chromosomal disorder and 46XY DSD. The full understanding of etiology of ambiguous genitalia in our environment will be clearer as more and more children with genital ambiguity have access to appropriate full clinical and laboratory evaluation. This study is limited by a small sample size; it is retrospective in nature. Furthermore, more needs to be done in terms of laboratory evaluation for example none of our patient had a genetic analysis to identify the type of mutated enzymes Congenital Adrenal

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