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Disorders of Sex Development: Molecular Versus Clinical and Cytogenic Studies and Their Correlation with Diagnostic Outcome and Sex of Rearing in A Local Community

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ABSTRACT

BACKGROUND: *This study aimed to support clinical professionals in the initial evaluation and diagnosis of children with suspected DSD , to provide a framework to standardize laboratory and clinical practice, and to acquire more knowledge on the molecular mechanism of sex determination .*

METHODS: *This was a single center, prospective case review. A total of 42 patients with DSD were referred to our Pediatric Surgery Unit at the Maternity and Child Teaching Hospital . We examined Barr bodies and karyotype for all patients. PCR amplification was also performed for the detection of SRY and ALT1 gene loci on Y and X chromosomes, respectively. Statistical analyses were performed using SPSS version 20 computer software.*

RESULTS*: A pure 46XX karyotype was identified in 35.7% of cases, while a pure 46XY karyotype was identified in 50% of cases. The SRY locus identified by PCR was tested for its validity in predicting a pure 46XY DSD karyotype. The test was 100% sensitive and 85.7% specific. Among the group of male social sex, the mean internal masculinization score was significantly higher in those with a pure 46XY karyotype (5.9) compared with those with a pure 46XX and those with a mixed karyotype (3.5) .*

CONCLUSIONS: *The management of children with DSD and a lack of diagnostic facilities still remain major challenges in Iraq. Multicenter studies, including larger population sizes, using molecular genetic analyses to detect the actual incidence of genital anomalies and DSD in our*

community, are required to serve these patients and their families.

Key words : *DSD, polymerase chain reaction, karyotype, diagnostic outcome .*

BACKGROUND

Disorders of sex development (DSDs) are serious conditions with a worldwide incidence of 1:5000 live births. However, in Iraq, there are no accurate data on DSDs. DSD is an important topic in Islamic countries because of the strong psychological pressure they impose, such as stigmatization and social exclusion, leading to suicide in some cases. The generic term DSD was proposed and defined as congenital conditions in which development of chromosomal, gonadal, or anatomical sex is atypical [1-4]. This nomenclature, which is recommended instead of the word "intersex", referring primarily to external genital ambiguity, may be confusing in the clinical evaluation of patients.

DSD can serve as an "experiment of nature" to decipher the genetic and hormonal components of sex-specific gene expression in humans. Genetic and hormonal influences on sex extend beyond the reproductive tract, modifying body proportions, hair distribution, gender identity, sex-specific behavior, and many other features that also affect DSD patients clinically in multiple ways [5-7]. Improved understanding of the architecture of sex-specific gene expression may also contribute to better clinical assessment of DSD patients.

The presence or absence of the Y chromosome (SRY gene in particular) determines the sex in mammals. The sex-determining region of the human Y chromosome (SRY) encodes a testes-determining factor, which initiates male sex determination [8-11]. However, this does not exclude the possible effects of other sex genes on the Y chromosome. If these genes exist, they must be located adjacent to the pseudoautosomal boundary, close to SRY [8]. SRY is hypothesized to function as a transcription factor, triggering a cascade of gene interactions that induce the bipotential fetal gonads to develop into testes. This leads to Sertoli cell differentiation, with subsequent production of Mullerian inhibiting substance and regression of the Mullerian ducts [12,13]. Careful genetic analysis of cases with abnormal sexual development, presenting with chromosomal translocations or deletions/duplications, has resulted in the identification of many genes playing a role in sex determination [14]. Polymerase chain reaction (PCR)-based sex determination identified by the presence (male) or absence (female) of the SRY gene has already been described. This method needs to have an internal control to verify the absence of the SRY gene from the failure of PCR amplification [15]. In this study, we used the ATL1 locus as an internal control. The ATL1 is the sequence in the FMR1 (fragile X mental retardation 1) gene located on the long arm of the X chromosome [16].

The main objective of this study was to support clinical professionals in the initial evaluation and diagnosis of children with suspected disorders of sex development. We also aimed to provide a framework to standardize laboratory and clinical practice, and to review our experience to provide a simple PCR-based genomic DNA diagnostic method [17]. We evaluated the clinical variant, different patterns of presentation, methods of diagnosis of DSD and to acquire more knowledge on the molecular mechanism of sex determination in Al Qadisiya governorate, Iraq.

METHODS

This was a single center, prospective case review. A total of 42 patients with DSD were referred to our Pediatric Surgery Unit at The Maternity and Child Teaching Hospital from the 1st of January 2008 to the end of May 2012, and all their record

were analyzed. Diagnosis was set according to the appearance of external genitalia, cytogenetic analysis, and hormonal status Ascertainment of the gonadal sex and was made based on the inability to determine the sex of the child on a physical examination. The severity of the ambiguity (according to Sinnecker et al.'s classification and Ahmed et al.'s external masculinization score [EMS]) [18-19] , penile length (z score according to Gabrich et al.) and the degree of severity of virilization of female external genitalia were determined by applying the Prader classification [20-21] . A thorough clinical examination, including associated anomalies or dysmorphic features, was performed. Routine investigations were requested for all the patients. Barr bodies in buccal smear cells, karyotype (cytogenic) using two techniques with lymphocyte culture, chromosomal analysis from venous blood sampling, and 20 metaphase spreads were studied on the basis of the trypsin-Giemsa banding (GTG) technique (used in limited patients) because such an approach is only available in a special center in the capital and it is expensive. Accordingly and for selected patients we performed an abdominopelvic ultrasound scan, an abdominopelvic MRI and CT scan study, and a voiding cystourethrogram. We measured serum electrolytes and performed hormonal assays for all patients. Gonadal biopsies were carried out in selected cases .

We performed PCR amplification for the detection of SRY and ALT1 gene loci on the Y and X chromosomes, respectively, in all of the patients at the Medical Microbiology and Clinical Immunology Department of Al Qadisiya University. DNA extraction purification was performed by a genomic DNA mini kit from whole blood (Geneaid, USA). Each 35-ul PCR reaction comprised 5 ul of template DNA solution, 1.5 ul of 25 pmol each of SRY and ATL1 primers, 20 ul of PCR preMix (Bioneer, USA), and 12 ul of nuclease-free water. All PCR reactions were performed in a thermal cycler (Sprint Thermocycler IP 20, USA) at 94°C (2 min) for initial DNA denaturation, followed by 35 cycles of 94°C (15 sec) for DNA denaturation, 65°C (20 sec) for primer annealing, and 72°C (20 sec) for primer extension. A final extension of the cycle was performed at 72°C for 10 min for the SRY gene and the same PCR protocol was performed with a change in annealing temperature to 60°C for the ALT1 gene. The amplified PCR products were separated on 2.5% agarose gels and stained with ethidium bromide. They were visualized under ultra violet transillumination for the presence of a band size of 254 bp for SRY (Y chromosome) and a band size of 300 bp for ATL1 (X chromosome). The A100bp DNA ladder (Bioneer) was included in each run. The sequences of oligonucleotide primers were 5'-CAT GAA CGC ATT CAT CGT GTG GTC-3' and 5'-CTG CGG GAA GCA AAC TGC AAT TCT T-3' for SRY, and 5'-CCC TGA TGA AGA ACT TGT ATC TC-3' and 5'-GAA ATT ACA CAC ATA GGT GGC ACT- 3' for ATL1 [22]. Patients were usually co-managed by pediatricians, clinical immunologists, pathologists, and radiologists. Written informed consent was obtained from all the parents for publication of this article and accompanying images. The study was approved and authorized by the statistics and archives unit at Al Qadisiya Health Office, the Ministry of Health, Iraq. Data were translated into a computerized database. Expert statistical advice was sought. Statistical analyses were performed using SPSS version 20 software.

RESULTS

A total of 42 patients met the criteria for DSD. There was a wide variation in age at presentation, ranging from 1 month to 19 years. There was no obvious or statistically significant difference in the mean age between males and females (7 and 6.8 years, respectively) (**Table 1**). A social label of male sex was observed in 52.4% of the study sample (**Table 2**). Congenital adrenal hyperplasia (CAH) was the underlying cause in 13 of 42 patients, 11 of whom showed a 46XX karyotype

(CAH, 21OH-deficiency in 8 patients, and 11ß-OH deficiency in 3 patients) **Figures 1 and 2** show examples of these clinical varieties. Two cases were 46XY, and both had 3β-hydroxylase deficiency (CAH, 3B-OH). The clinical diagnosis was based on the peripheral blood karyotype (**Figure 3**). A pure 46XX karyotype was identified in 35.7% of cases, while a pure 46XY karyotype was identified in 50% of cases. The remaining 14.3% of DSD cases had a mixed chromosomal karyotype (**Table 3**).

The SRY locus identified by PCR was tested for its validity in predicting male social sex. The test was 95.5% sensitive and 85% specific. **Figures 4, 5 and 6** show a series of results tested conventional PCR. The proportion of false negative tests was 4.5%. Therefore, a negative test result can exclude a male social sex with 94.4% confidence (negative predictive value [NPV]=94.4%) in a clinical context with an almost equal odds pretest probability of 52.4% for being male. A positive test result can establish a male social sex with 87.5% confidence in a clinical context with an almost equal odds pretest probability of 52.4% for being male. The overall accuracy of the test was 90.5%, with a high level of agreement beyond chance between the test and male social sex. No significant disagreement was observed between the test and social sex .The SRY locus identified by PCR was tested for its validity in predicting a pure 46XY DSD karyotype. The test was 100% sensitive and 85.7% specific. The proportion of a false negative test was 0%. Therefore, a negative test result can exclude a 46XY karyotype with 100% confidence (NPV=100%) (**Tables 4 and 5**). A positive test result can establish the presence of pure 46XY DSD, with a 87.5% confidence in a clinical context with an equal odds (50:50) pretest probability of having such a karyotype.

Barr bodies were identified by buccal smear and tested for validity in predicting female social sex. The test was 55% sensitive and 100% specific. The proportion of false negative tests was 45%. Therefore, a negative test result can exclude a female social sex with 71% confidence (NPV=71%) in a clinical context with an almost equal odds pretest probability of 47.6% for being female. A positive test can establish a female social sex with 100% confidence in a clinical context with an almost equal odds pretest probability of 47.6% for being female. The overall accuracy of the test was 78.6%, with a low level of agreement beyond chance between the test and female social sex (Kappa statistic=56%). A statistically significant disagreement was observed between the test and social sex (P for McNemar's test=0.004) (**Table 6**).

Among those with a male social sex, 81.8% had a pure 46XY karyotype and the remaining 18.2% had a mixed chromosomal type. **Figures 7 and 8** show examples of these cases. Among those with a female social sex, 75% had a pure 46XX karyotype, while 15% had a pure 46XY karyotype, and the remaining 10% had a mixed chromosomal type. **Figures 9 and 10** show two rare clinical varieties of DSD with 46XY and a mixed chromosomal type, respectively. There was a statistically significant association between social sex and karyotype classification **(Table 7**). The mean (±SD) age at presentation was slightly higher for the mixed chromosomal type (8.7 ± 8.1 years) than for those with pure 46XX and 46XY (6.9 and 6.4 years of age, respectively). However, the observed differences were too small to be statistically significant (**Table 8**).

The mean masculinization scores (internal, external, Prader, and total scores) were significantly higher in those with a male social sex than in those with a female social sex. Social sex had a strong positive effect on the masculinization score (as shown by a Cohen's d >1). The Prader score was the most affected by social sex compared with the IMS and EMS (**Table 9**). Among those with a male social sex, the mean IMS was significantly higher in those with a pure 46XY karyotype (mean, 5.9) than in those with a pure 46XX karyotype and those with mixed karyotype (mean, 3.5). The mean EMS was not significantly different between those with a pure

46XY karyotype (mean, 5.4) and those with a mixed karyotype (mean, 7.0). The mean Prader and total scores were not significantly different between the two karyotypes (**Table 10**). Among female social sex, the three masculinization scores showed a moderately strong positive linear correlation. Among females, the IMS, EMS, and Prader score tended to behave in a similar manner. Therefore, when masculinization is high externally, it is also expected to be high internally. Among male social sex, there was no correlation between the three scores. Among males, there was discordance between the magnitude of internal and external masculinization. Therefore, when the external score was high, the internal score tended to be low and vice versa**.** We could identify a clinical criteria with parameters selected masculinization scores when used as a test to predict pure 46XY karyotype cases with 100% sensitivity for the EMS (P=0.008), 95.2% for the IMS (P <0.001), and 100% for the Prader score (P <0.001) (**Table 11**), and the trade-off sensitivity and 1-specificity the selected masculinization scores (**Figure 11,** the validity of the ROC curve when used a test to analyze these scores and to predict a pure 46XY karyotype among DSD cases) .

Table 1: Mean age by social sex.

	Social sex		
	Female	**Male**	**P (t-test)**
Age (years)			0.96[NS]
Range	(0.3 - 19)	(0.1 - 19)	
Mean	7.0	6.8	
SD	7.6	7.6	
SE	1.69	1.61	
N	20	22	

Table 2: Frequency distribution of the study sample by social sex.

	N	**%**
Social sex		
Female	20	47.6
Male	22	52.4
Total	42	100.0

Table 3: Frequency distribution of the study sample karyotype.

	N	**%**
DSD classification by karyotype		
46XX DSD	15	35.7
46XY DSD	21	50.0
Mixed chromosomal type	6	14.3
Total	42	100.0

Table 4: Validity parameters obtained by PCR for the SRY locus in predicting a pure 46XY karyotype.

	Pure 46XY DSD karyotype		
SRY locus PCR	**Negative**	**Positive**	**Total**
Negative	18	0	18
Positive	3	21	24
Total	21	21	42

Sensitivity=100%
Specificity=85.7%
PPV=87.5%
NPV=100%
P (Macnemar) = 0.25[NS]
Observed agreement (Accuracy)=92.9%
Kappa=0.86 P<0.001

Table 5: Validity parameters obtained by PCR for the SRY locus in predicting male social sex.

	Social sex		
SRY locus PCR	**Female**	**Male**	**Total**
Negative	17	1	18
Positive	3	21	24
Total	20	22	42

Sensitivity=95.5%
Specificity=85%
PPV=87.5%
NPV=94.4%
P (Macnemar) = 0.63[NS]
Observed agreement (Accuracy)=90.5%
Kappa=0.81 P<0.001

Table 6: Validity parameters of Barr bodies identified by buccal smear in predicting female social sex.

	Social sex		
Buccal smear (Barr body)	**Male**	**Female**	**Total**
Negative	22	9	31
Positive	0	11	11
Total	22	20	42

Sensitivity=55%
Specificity=100%
PPV=100%
NPV=71%
P (Macnemar) = 0.004
Observed agreement (Accuracy)=78.6%
Kappa=0.56 P<0.05

Table 7: Association between social sex and karyotype.

	Social sex			
	Female		Male	
	N	%	N	%
DSD classification by karyotype				
46XX DSD	15	75.0	0	0.0
46XY DSD	3	15.0	18	81.8
Mixed chromosomal type	2	10.0	4	18.2
Total	20	100.0	22	100.0

P (Chi-square) <0.001

Table 8: Mean age by karyotype.

	DSD classification by karyotype		
	46XX DSD	46XY DSD	Mixed chromosomal type
Age (years)			
Range	(0.3 - 19)	(0.3 - 19)	(0.1 - 16)
Mean	6.9	6.4	8.7
SD	7.7	7.4	8.1
SE	1.99	1.62	3.30
N	15	21	6

P (ANOVA) =0.82[NS]

Table 9: Difference between male and female social sex in mean masculinization scores.

	Social sex		
	Female	Male	P (t-test)
EMS (external masculinization score)			0.002
Range	(1 - 9)	(2 - 9)	
Mean	3.7	5.7	
SD	1.9	1.9	
SE	0.42	0.41	
N	20	22	
Cohen's d effect size of being a male compared to female = 1.05			
IMS (internal masculinization score)			<0.001
Range	(3 - 9)	(2 - 9)	
Mean	3.7	5.5	
SD	1.6	1.6	
SE	0.35	0.34	
N	20	22	

Cohen's d effect size of being a male compared to female = 1.13			
Prader score of female genital verilization			<0.001
Range	(0 - 4)	(1 - 5)	
Mean	1.4	3.3	
SD	0.9	1.3	
SE	0.20	0.27	
N	20	22	
Cohen's d effect size of being a male compared to female = 1.73			
Total score of masculinization			<0.001
Range	(6 - 18)	(9 - 19)	
Mean	8.8	14.4	
SD	3.8	2.8	
SE	0.85	0.60	
N	20	22	
Cohen's d effect size of being a male compared to female = 1.7			

Table 10: Differences in mean masculinization scores between the three classes of karyotype among male social sex.

	DSD classification by karyotype		
Males only	**46XY DSD**	**Mixed chromosomal type**	**P (t-test)**
EMS (external masculinization score)			0.14[NS]
Range	(2 - 9)	(5 - 8.5)	
Mean	5.4	7.0	
SD	1.9	1.6	
SE	0.45	0.79	
N	18	4	
IMS (internal masculinization score)			0.004
Range	(2 - 9)	(2 - 5)	
Mean	5.9	3.5	
SD	1.3	1.3	
SE	0.31	0.65	
N	18	4	

Prader score of female genital verilization			0.65[NS]
Range	(1 - 5)	(2 - 4)	
Mean	3.3	3.0	
SD	1.4	0.8	
SE	0.32	0.41	
N	18	4	
Total score of masculinization			0.48[NS]
Range	(9.5 - 19)	(9 - 15.5)	
Mean	14.6	13.5	
SD	2.8	3.0	
SE	0.67	1.51	
N	18	4	

Table 11: Linear correlation coefficients between the three elements of masculinization scores stratified by social gender.

	EMS (external masculinization score)	**IMS (internal masculinization score)**
Female		
IMS (internal masculinization score)	r=0.594 P=0.006	
Prader score of female genital verilization	r=0.739 P<0.001	r=0.589 P=0.006
Male		
IMS (internal masculinization score)	r=-0.078 P=0.73[NS]	
Prader score of female genital verilization	r=0.131 P=0.56[NS]	r=0.123 P=0.59[NS]

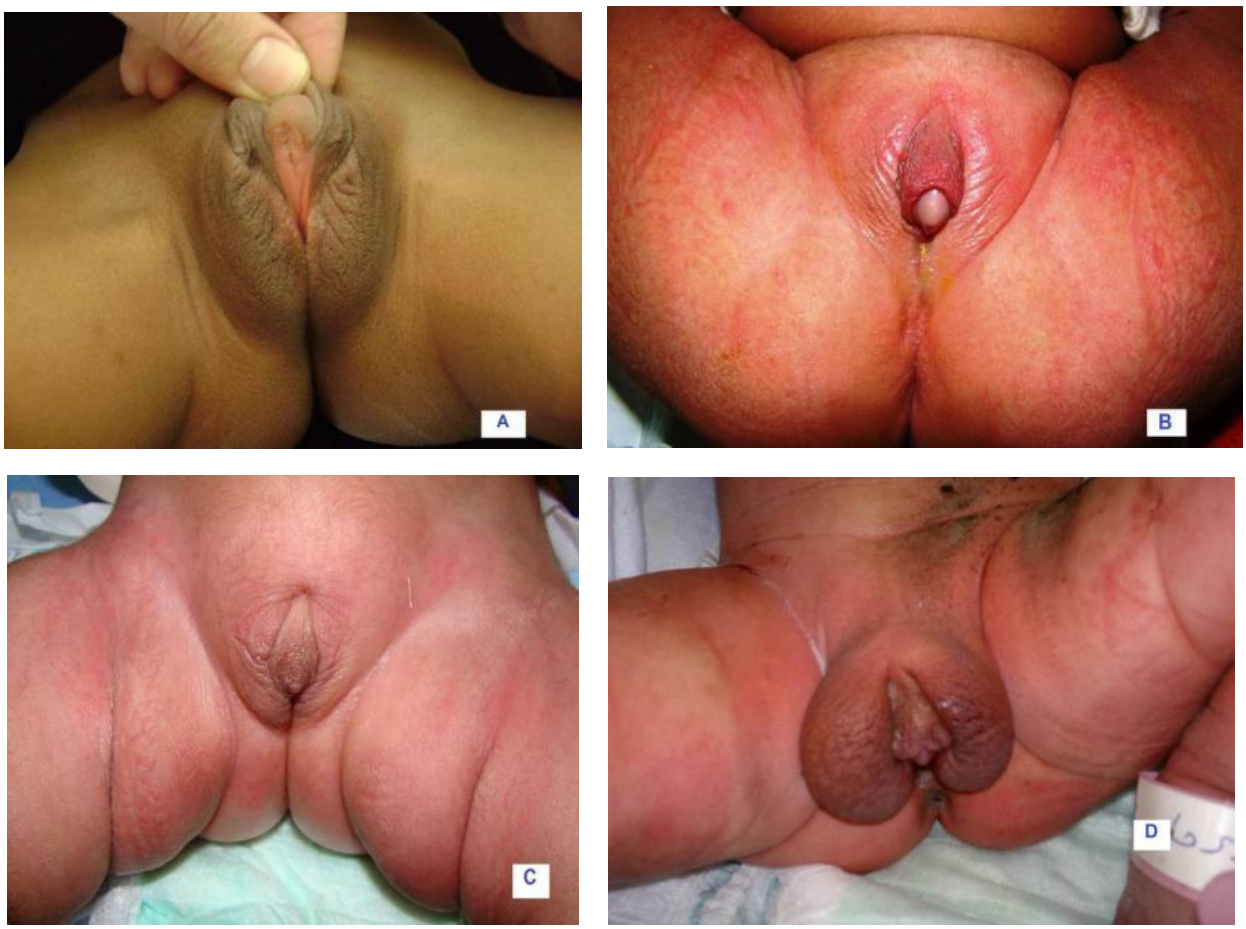

Figure 1 : A,B,C and D ,different cases of CAH ,all female with 46XX karyotype , ATL1 positive and SRY negative in all these cases.

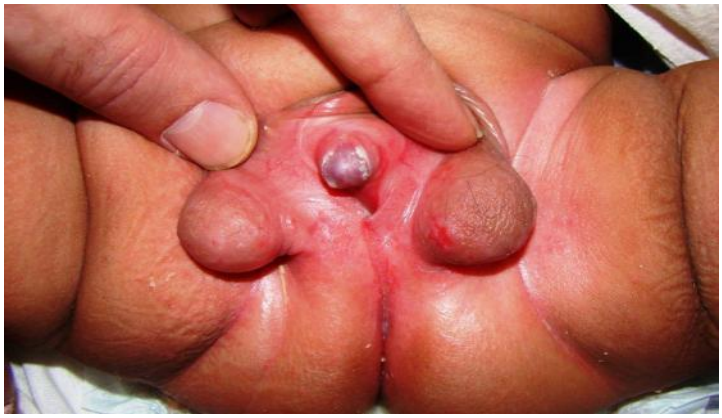

Figure 2 : Congenital Adrenal Hyperplasia one of two siblings with type 3B-OH deficiency, simple virilizing , penoscrotal hypospadias bifid scrotum and bilateral descended gonads were palpable in the scrotum ,46XY and positive SRY .

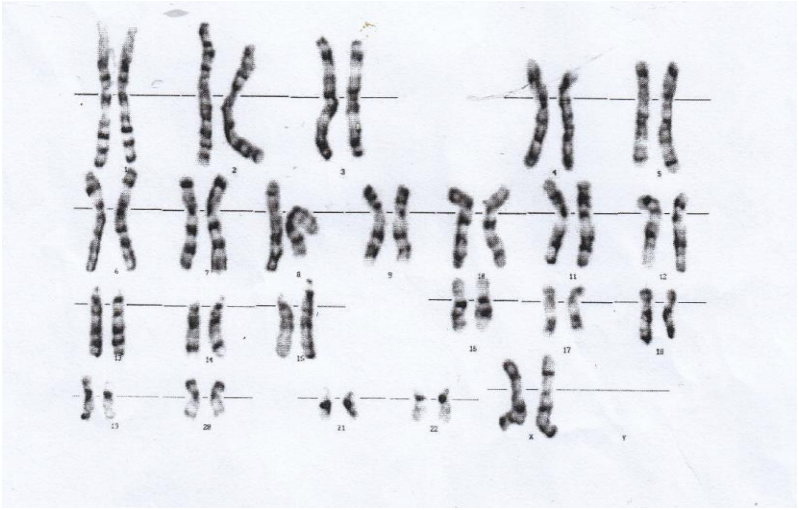

Figure 1 : Twenty metaphase spreads were studied on the basis of Trypsin and Giemsa produce G-banded chromosomes (GTG) technique at 450 band resolution; revealing 46,W. pattern (Analysis Method: 20 metaphase spreads, GTG technique, 500 band resolution).

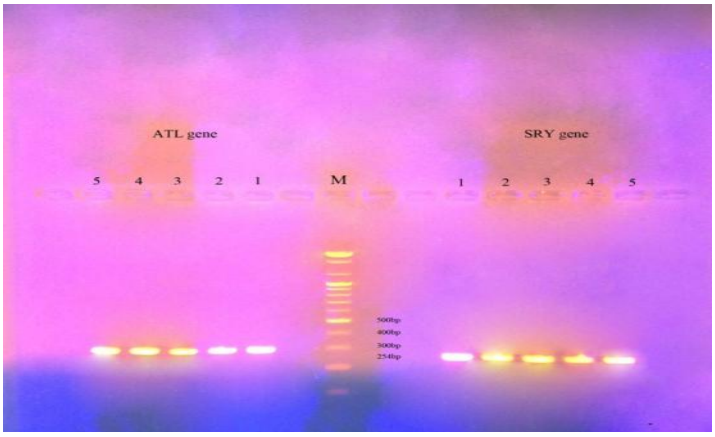

Figure 2 : Agarose gel electrophoresed of conventional PCR products for ATL1 and SRY specific primers. Lane M: Gene ruler 100 bp ladder; Lane 1 to lane 5 showing band sized 300 bp for positive ATL1 (X chromosome) and band sized 254 bp for positive SRY (Y chromosome).

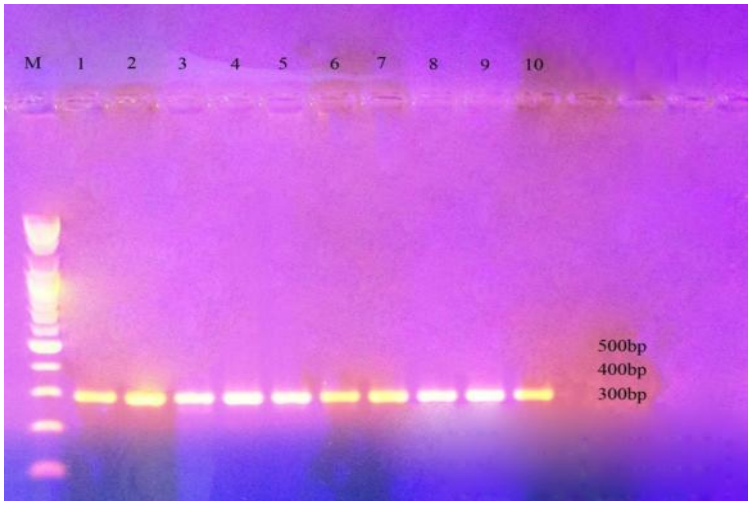

Figure 5 : Agarose gel electrophoresed PCR products showing band sized 300 bp for ATL1 specific primers (X chromosome). Lane M : Gene ruler 100 bp ladder; lane 1: health male control ; from lane 2 to lane 10: positive ATL1.

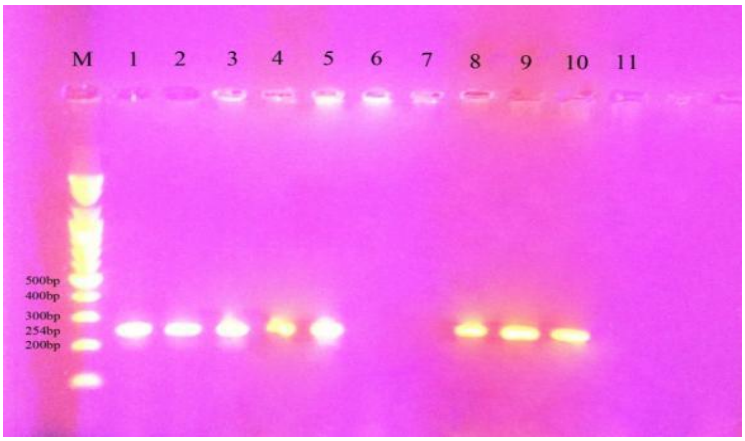

Figure 6 : Agarose gel electrophoresed PCR products showing band sized 254 bp for SRY specific primers (Y chromosome). Lane M : Gene ruler 100 bp ladder; lane 1: health male control, lane 6, lane 7: SRY negative; lane 2; lane 3; lane 4; lane 5; lane 8; lane 9; lane 10 : SRY positive.

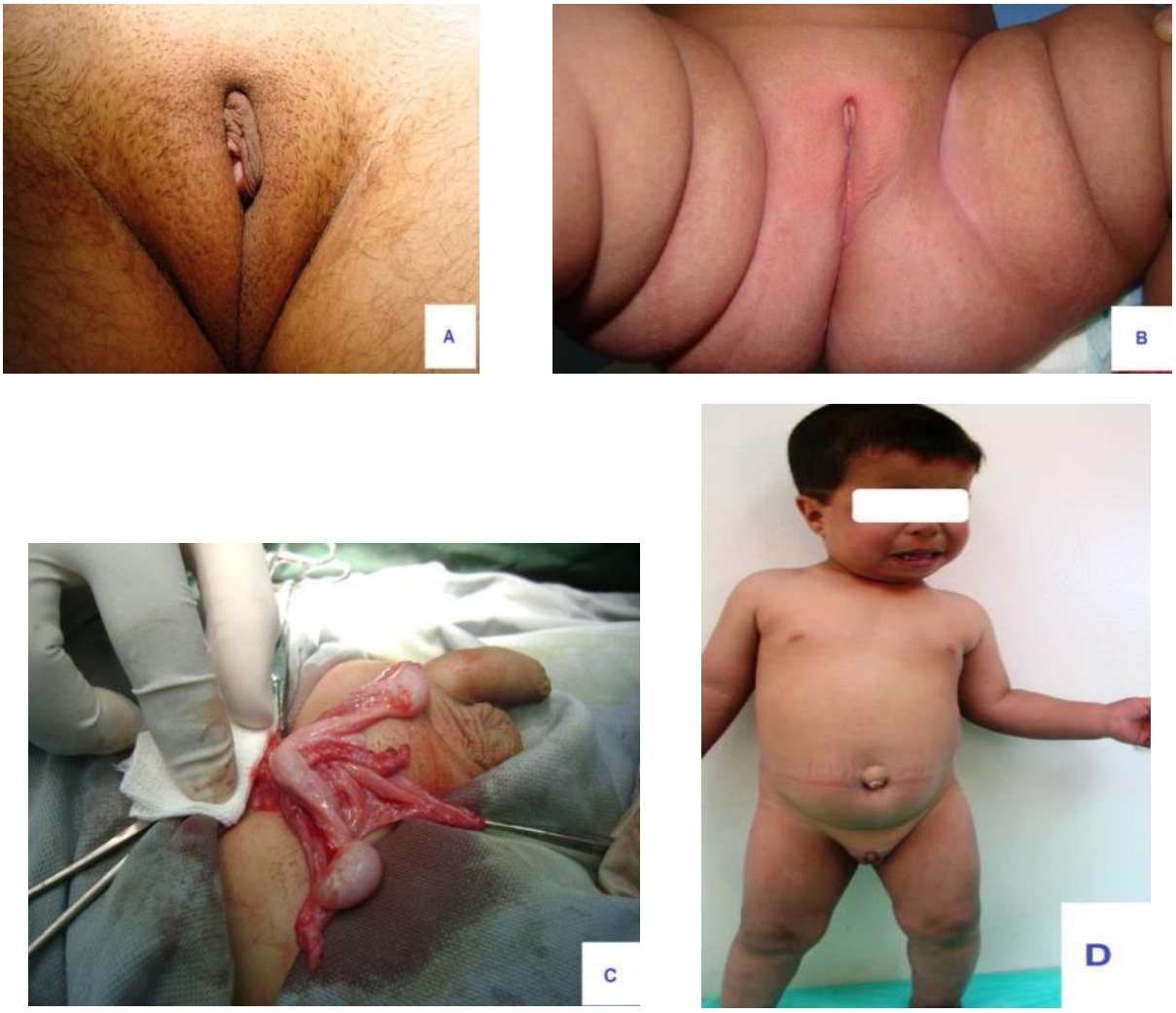

Figure 7 : A-Partial androgen insensitivity syndrome clitoromegaly, and incomplete puberty . **B-** Six months infant with vanishing testes syndrome , microphalus. **C-** persistent Mullerian (paramesonephric) duct, right both testis , presence of Mullerian structures , right inguinal hernia . **D-** Micropenis ,bilateral cryptorchidesim) syndrome manifestation . **A,B,C** and **D** ,46XY karyotype and SRY positive .

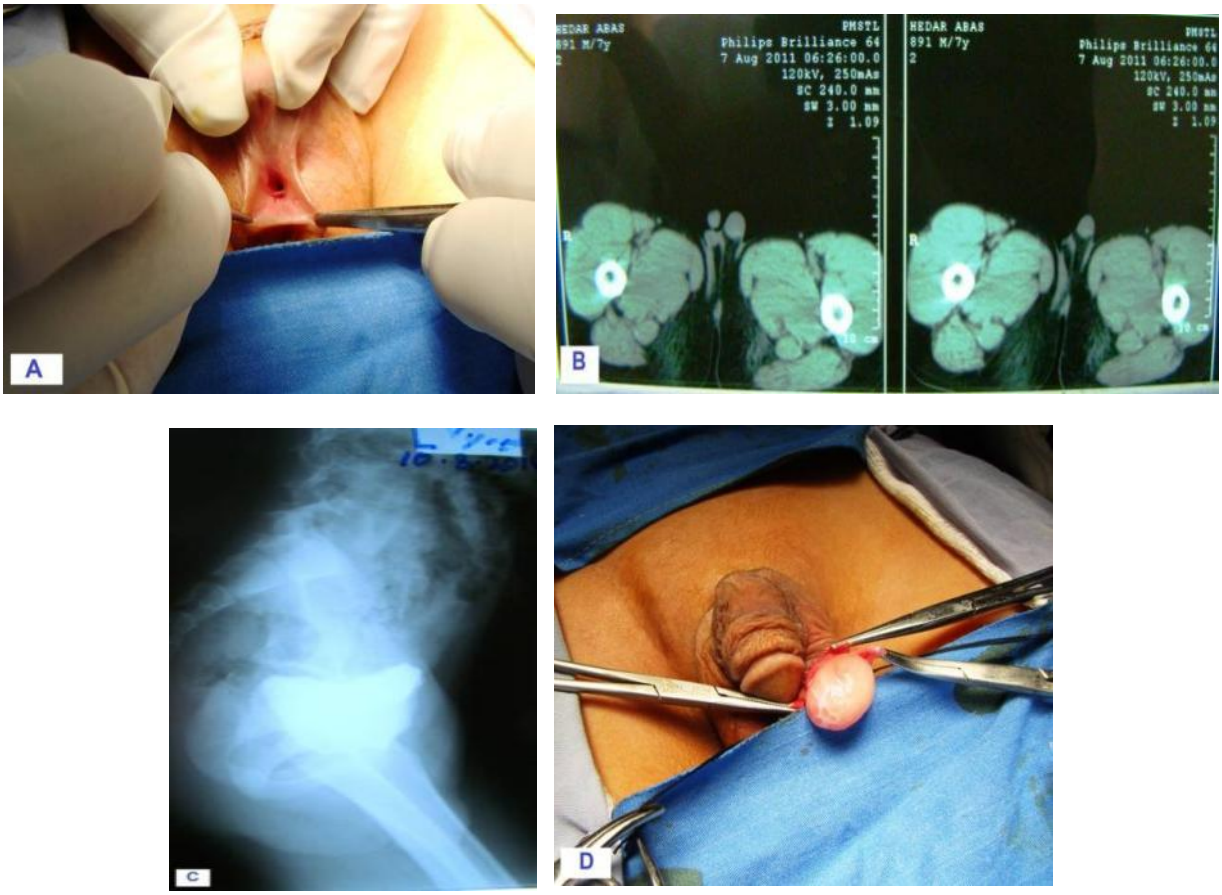

Figure 8 : True hermaphrodite **A-** penoscrotal hypospadias . **B-** CT scan study left hemiscrotum with gonad. **C**-Voiding cystourethrogram VCUG revealed evidence of small remnant of urogenital sinus **D**-per exploration for biopsy which revealed ovotestis ,46XY karyotype and SRY positive .

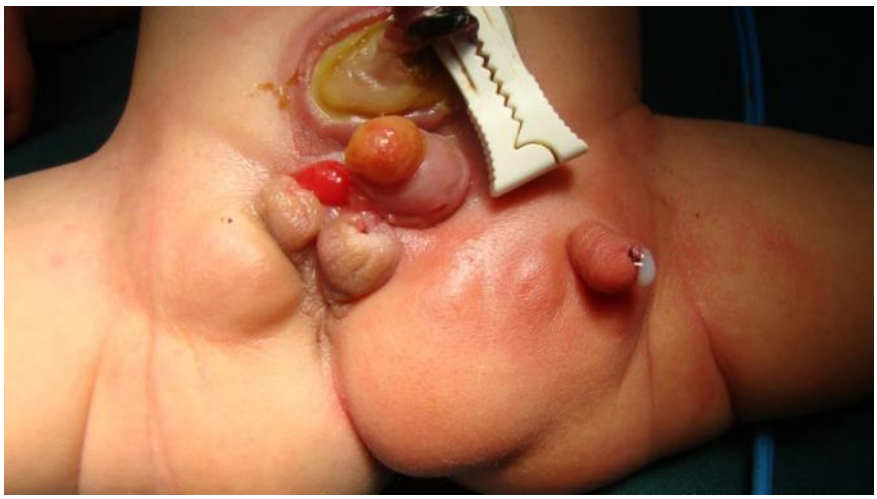

Figure 9 : A neonate with cloacal variant, sharing both vagina and small penis attached parasitic twin ,46XX karyotype with negative SRY.

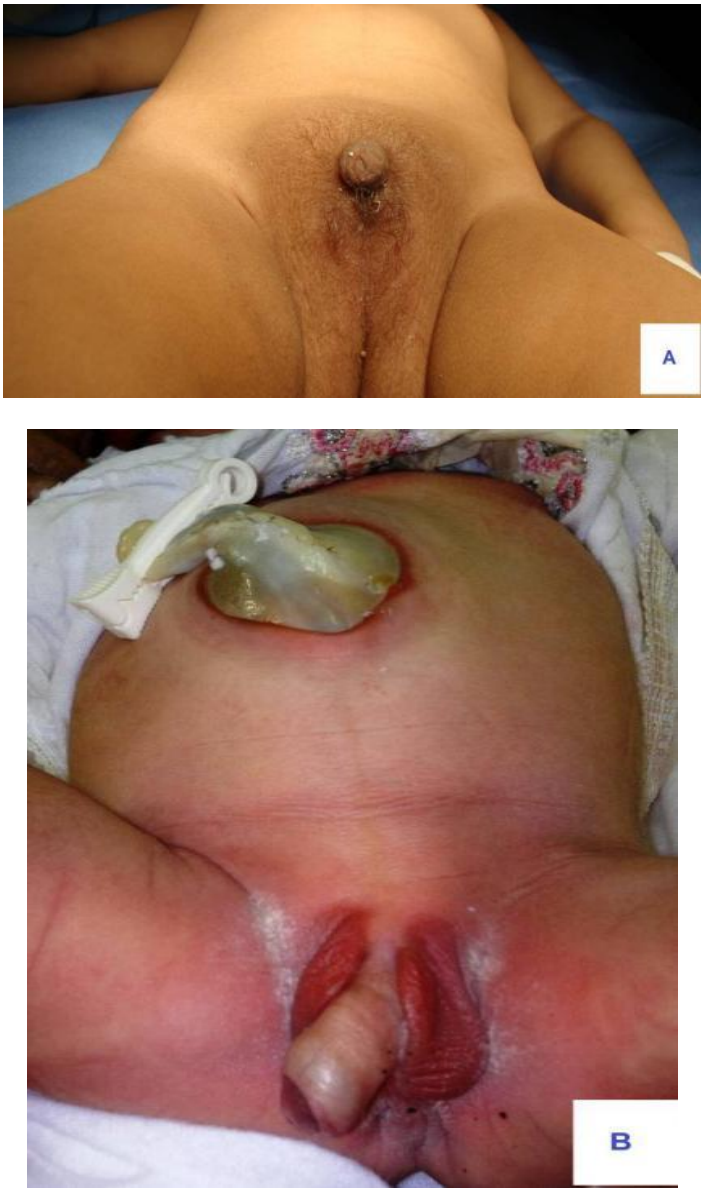

Figure 10 : A- partial virilization and ambiguous genitalia , dysgenetic testicle on one side and a streak gonad on the other , DSD at 11 months of age with chimeric 45X/46XY and PCR revealed positive SRY . **B**- Turner syndrome ,omphalocele, imperforate anus,complete gonadal dysgenesis , 45XO karyotype and negative SRY.

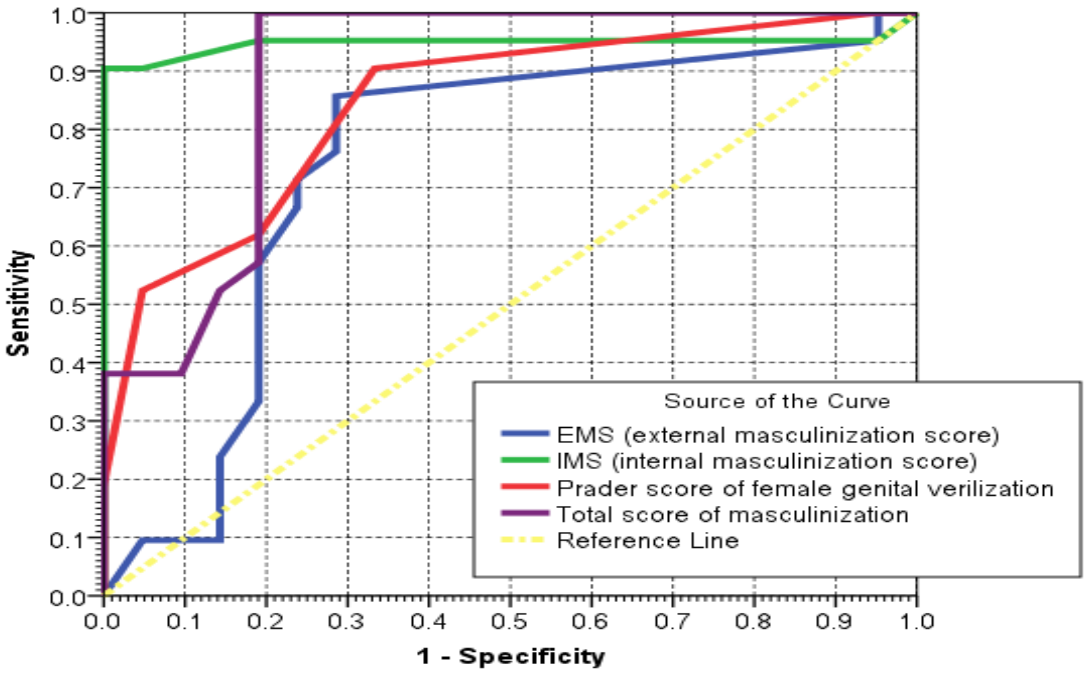

Figure 11: ROC curve showing the trade-off between sensitivity (rate of true positive) and 1-specificity (rate of false positive) for the selected masculinization scores when used as a test to predict a pure 46XY karyotype among DSD cases.

DISCUSSION

The birth of a healthy, normal neonate is the expectation of every parent, but sometimes identification of sex is difficult at birth when the neonate is born with ambiguous genitalia. Age of presentation of DSD will depend upon the degree of dysfunction caused [23]. In our study, the ages of our patients ranged from 1 month to 19 years, and the most common age of presentation was adolescence. Consistent with our results, Hashem et al. [24] reported that adulthood was the most common age group in patients with genitogonadal differentiation errors among Egyptians. Dessouky et al. [25] studied 314 cases presenting with intersex problems. They reported that the ages at presentation ranged with a mean of 5.5 years. This suggests that diagnosis of patients with DSD is usually delayed in our local community. However, Hughes et al. [1] reported that DSDs are typically diagnosed at birth in infants with ambiguous genitalia, while disorders associated with phenotypic males and females may be diagnosed much later.

Our genetic study was divided into two main categories: karyotyping and molecular study. The peripheral karyotypes of sex chromosome DSD show marked variation. In our study, 15 patients (35.7%) were 46,XX and 21 patients (50.0%) were 46,XY. The limitations of our data collection may have led to estimation of less than the usual variation expressed by other authors. Sema et al. reported that 24% of patients had 46,XX DSD and 45% had 46,XY DSD [26]. With regard to the peripheral karyotypes, our results are consistent with other studies [27,28,29]. Individuals requesting cytogenetic tests need to be aware of the risk of errors. Incorrect diagnosis may result from the following: (1) mix-up of samples in transportation or in the lab; and (2) technical errors in different parts of the test. In addition, this test does not rule out the possibility of monogenic disorders, micro deletions, micro duplications, subtle chromosomal rearrangements, and low-level mosaicism.

In the 46,XX DSD group, the most common condition was CAH due to 21OH-deficiency, a finding compatible with its worldwide incidence

of 1:14 000 live births [27]. Males with classic CAH may not be diagnosed clinically at birth because they do not have genital ambiguity, except for scrotal hyperpigmentation and phallic enlargement. In an epidemiological study, the incidence of ambiguous genitalia in neonates was identified as 1:5000 births, and the most common diagnosis was CAH, followed by androgen insensitivity syndrome (AIS) and mixed gonadal dysgenesis [30]. In our study, karyotyping was an important early test using cultured leucocytes to determine the actual genetic sex of the patient. In the current study, only 73% of normal female cells contained an identifiable Barr body. Specimens for buccal smear should not be obtained during the first week of life or during adrenocorticosteroid or estrogen therapy, because these situations falsely lower the incidence of sex chromatin Barr bodies. Certain artifacts may be confused with nuclear Barr bodies. Poor slide preparations may obscure the sex chromatin mass and lead to a false negative appearance.

Consanguineous marriage was reported among 78.57% of parents of our patients compared with 35.3% of consanguineous marriage among Egyptian populations (Muslim community) [31]. Conditions, such as CAH, which are perpetuated by autosomal recessive inheritance, are likely to affect multiple family members and to occur with greater frequency in countries where consanguinity is common. This occurrence is a reflection of multiple sibling involvement of a common autosomal recessive disorder in this community. Saedi et al [32] showed a high rate of parity and consanguineous mating among other Muslim communities, in the Saudi population.

Laparoscopy and mini laparotomy in the evaluation of children with ambiguous external genitalia are important, especially in developing countries [3]. This is because there is usually a challenge in determining the genetic sex of children, especially in developing countries where karyotyping and sophisticated imaging facilities are not available or require highly trained personnel. Therefore, these approaches in a few cases are performed in another center. The lack of a gonadal biopsy is mostly due to parental refusal, which prevents obtaining certain specific possible diagnoses and more certainty about the DSD type. Therefore, the cooperation and recognition of parental acceptance as a fundamental determinant of the success of any management strategy in the care of intersex children is critical [34]. Definition of 45,X/46,XY complete gonadal dysgenesis is the presence of bilateral streak gonads with a normal female phenotype. For diagnosis of 45,X/46,XY partial gonadal dysgenesis, bilateral dysplastic testes are required. There is a lot of confusion about the nomenclature for patients with gonadal dysgenesis [35,36].

When the external genitalia are ambiguous, the neonate is either a virilized girl or an undervirilized boy. The degree of ambiguity is defined using the Prader classification where the appearance of external genitalia is described on a scale of stages 0 to 6 [37]. Stage 0 is normal external genitalia of a girl and stage 6 is the appearance of a normal boy. The EMS, which is based on external genital features, is also used to describe the degree of ambiguity in an undervirilized boy [38]. The extent of masculinization in our patients varied considerably from slightly decreased to complete intersexuality of the external genitalia. The median EMS (3.7) for females and those patients reared as males was significantly higher than in female patients . This value was the same in our study when we analyzed the IMS. We consider that external genitalia should be assessed by the degree of virilization, as described by Prader [21]. In our community, gender is bipolar, with only males and females being recognized. Recent activism by certain groups in the developed world, fuelled by increasing numbers of transsexuals, has led to pressure towards the acceptance of a neutral gender, which is neither male nor female. The presence or absence of a penis is the paramount differentiating feature. Some communities use the degree of scrotal development as the main gender

determinant [39]. If not carefully examined, features, such as a micropenis or clitoromegaly, may lead to an inappropriate gender assignment, with far-reaching life-long psychosocial and psychological effects. Although scoring systems, such as the Prader scoring system for DSD, and modifications of this score, may provide an integrated summary of genitalia [40], these scoring system are not sufficiently discriminate for the full variations of spectrum of external genitalia. The EMS may provide a good scoring value for scrotal fusion, microphallus, the location of the meatus, and the location of the gonads, and may be a more discriminate and objective method for describing the external appearance [19]. We incorporated the EMS, IMS, and Prader score into the initial clinical and anatomical evaluations. We believe that such a scale remains descriptive, so there is a need for a more accurate anatomical classification of children with genital ambiguity and urogenital sinus abnormalities. The degree of variation of external genitalia from the norm does not always correlate with the type of DSD.

The study demonstrated the usefulness of markers of X and Y chromosomes in determining the sex of individuals with ambiguous genitalia. This study showed all complements of 46,XX DSD without any cytogenetic evidence of the Y chromosome and the proportion of false negative tests was 0%. Therefore, a negative test result can exclude a 46XY karyotype with 100% confidence (NPV=100%). Although a minority of individuals have been found to be positive for the SRY sequence [41], the majority of 46XX true hermaphrodites are negative for the Y-DNA sequence, including the SRY sequence [42]. However, there is no report of a Y-specific SRY sequence in female pseudohermaphrodites. In our study, only one case was compatible with this finding. The SRY gene was detected in all patients confirmed with pure XY DSD in spite of the variance in clinical diagnosis. In one case although there were two X chromosomes and a SRY sequence, there was no evidence of either testes or ovaries, indicating a disturbance in the cascade of gonadal differentiation. Another case no gonads were detected however, cytogenetic and molecular studies showed an XY constitution and SRY sequence. These findings indicate the possibility of a mutation or deletion in the SRY sequence, which might have failed to trigger the development of testes. In another patient, we detected the presence of the SRY gene in our patient with a male phenotype and 45X/46XY karyotype. This patient may carry the SRY gene because of X to Y translocation (normal testicle in one side). Patients with pure gonadal dysgenesis have bilateral streak gonads that fail to differentiate. Analysis of these patients and animal models led to the discovery that the SRY gene is located on the distal short arm of the Y chromosome and to the detection on autosomes of SRY homologues, such as the SRY homeobox gene SOX9 [43]. These patients have characteristic bilateral streak gonads, which are small and fibrotic, without typical germ-cell or supporting-cell morphology of testes or ovaries [44]. Although it is now clear that the SRY gene plays a central role in triggering the formation of the testis from undifferentiated gonads, it has been suggested that other genes located either on the X chromosome or autosome may be involved in testicular differentiation [45,46].

LIMITATIONS

There are some limitations to our study because of the relatively low incidence of DSD. Only 42 patients were identified over the 4-year period of this study. In addition, our findings are from a small-scale study in one state in a local community and we would caution against any generalization. Our review was limited to those cases documented at our single center. We are attempting to engender greater transparency and safety through the use and enrollment of the data in our study. In this context, the validity parameters (the sum of the EMS, IMS and Prader score) will add to the already available information on the probability of having DSD (depending on clinical criteria in combination

with other laboratory tests), and ***are*** not a substitute for clinical assessment.

CONCLUSION

Multicenter studies, including larger population sizes, using molecular genetic analyses to detect the actual incidence of genital anomalies and DSD in Iraq are required. However, our approach with limited experience using PCR analysis for these patients could be used for accurate gender detection. Karyotyping is systematic, while PCR analysis of the SRY gene provides information about the presence of a Y chromosome within 1 to 3 days. We hope to apply SRY gene molecular studies as a routine part of investigations of DSD (this test was confirmed by a formal karyotype in our study). Genetic counseling is also a crucial part in the management of DSD cases for both the patient and the family. DSD is not uncommon in our community. Increased awareness, a detailed history, and a careful physical examination, including good knowledge about the clinical scoring system coupled with appropriate laboratory and radiological investigations, will aid in early diagnosis and avoid serious conflictions. We suspect that some of our colleagues in developing countries have had a similar experience, and are hopeful that health care policy makers pay more attention to this sensitive area.

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