Ambiguous genitalia in neonates: a 4-year prospective study in a localized area

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الأعضاء التناسلية الظاهرة المبهمة المعالم لدى الولدان: دراسة استباقية دامت 4 سنوات في منطقة محددة

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الخلاصة: تهدف هذه الدراسة للتعرف على الإمراضية المحتملة للأعضاء التناسلية الظاهرة المبهمة المعالم لدى 41 وليداً حُوِّلوا إلى مستشفى في مدينة الهفوف، في المملكة العربية السعودية. وكان أشيع الأسباب للأعضاء التناسلية الظاهرة المبهمة المعالم لدى المصابين النمط الصبغي 46 XX (وعددهم 14 مريضاً)، وفرط التنسج الولادي الكظري، واضطراب عام تشوهي. أما لدى المصابين بالنمط الصبغي 46 XY (وعددهم 18 مريضاً)؛ فقد كان أشيع الأسباب عيب التخليق البيولوجي لسبيل التستيرون، حتى مع الترافق باضطراب تشوه معمم. أما المرضى ذوي النمط الصبغي غير السوي، (وعددهم 3 مرضى)، وكان لدى واحد منهم ثلاثية الصبغي 18 (47 XX)، وقد مات بعد 3 شهور من ولادته، ولدى اثنين منهم أنماط مختلفة من متلازمة تيرنر الفسيفسائية. ولم يتم تحديد النمط الصبغي لدى 6 مرضى. وقد لوحظت السوابق العائلية الإيجابية للأعضاء التناسلية الظاهرة المبهمة المعالم لدى 4 مرضى.

ABSTRACT This study aimed to determine the possible etiology of ambiguous genitalia in 41 newborn infants at a referral hospital in Hofuf city, Saudi Arabia. In 46,XX karyotype patients (*n* = 14), congenital adrenal hyperplasia and general malformation disorder were the most common causes of genital ambiguity, while in 46,XY karyotype patients (*n* = 18), testosterone pathway biosynthetic defect was the most common cause even in conjunction with a generalized malformation disorder. In patients with abnormal karyotype (*n* = 3), 1 had trisomy 18 (47,XX) and died after 3 months and 2 had different types of mosaic Turner syndrome. The karyotype was undetermined in 6 patients. Positive family history of ambiguous genitalia was noted in 4 patients.

Ambiguïté sexuelle chez le nouveau-né : une étude prospective de quatre ans dans une zone circonscrite

RÉSUMÉ Le but de cette étude était de déterminer l'étiologie possible de l'ambiguïté sexuelle chez 41 nouveau-nés dans un hôpital de recours de la ville d'Hofuf, en Arabie Saoudite. Chez les nourrissons présentant un caryotype 46,XX (*n* = 14), l'ambiguïté sexuelle était le plus souvent due à une hyperplasie congénitale des surrénales et à une malformation générale, alors que chez les bébés ayant un caryotype 46,XY (*n* = 18), la cause la plus fréquente était une anomalie caractérisée par l'absence de biosynthèse de la testostérone, y compris lorsqu'elle était associée à une malformation générale. Parmi les nouveau-nés présentant un caryotype anormal (*n* = 3), l'un, souffrant de trisomie 18 (47,XX), est décédé à l'âge de trois mois, tandis que les deux autres enfants étaient atteints de différents types de syndrome de Turner en mosaïque. Le caryotype était indéterminé chez six nouveau-nés, et des antécédents familiaux d'ambiguïté sexuelle ont été constatés chez quatre nourrissons.

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Received: 16/08/07; accepted: 24/10/07

Introduction

The diagnosis of ambiguous genitalia in a newborn infant is an emergency that can be difficult to manage, not only because salt-wasting entities must be ruled out, but also due to the importance of gender assignment before psychological gender is established [*1*]. Particularly in less-developed areas of the Arab world with a legacy of tribalism, the situation is more complex socially and psychologically. Although hormonal, genetic, molecular and radiographic investigations are needed to determine the etiology, physical examination remains a key for diagnosis [*2*], particularly careful palpation to locate gonads at the genital folds or in the inguinal region. In most cases it is impossible to correlate the etiology of the disorder with the appearance of the external genitalia [*3*].

In this study, we aimed to determine the possible etiologies of ambiguous genitalia in newborn infants attending the main referral centre for Al-Ahsaa area in the Eastern Province of Saudi Arabia.

Methods

The study group comprised all recently born infants (in the first 4 weeks of age) who presented for endocrinologist evaluation in the Maternity and Children Hospital, Hofuf, over the period January 2003–December 2006. The hospital is the main referral centre for Al-Ahsaa area in the Eastern Province, which is one of the most populated areas in Saudi Arabia, and so the data may represent a profile of the problem of ambiguous genitalia in the whole country.

A neonate was considered to have genital ambiguity when there was difficulty in determining the sex on initial view or when the external genitalia showed significant structural deviation from normal in an apparent male or female. The clinical genital features of concern were: clitoromegaly, vaginal absence or hypoplasia, urogenital sinus, significant micropenis, hypospadias, labioscrotal fusion, in addition to palpable/impalpable gonads or undescended testis/testes (if palpable). Apparent males with simple hypospadias or undescended testes (but with normal phallus) or borderline micropenis (but with palpable descended testes) were not considered ambiguous.

A detailed history was taken and physical examination and screening for pituitary hormones (thyroid stimulating hormone, adrenocorticotropic hormone (ACTH) and gonadotropins) were conducted. Diagnostic evaluation included serum biochemistry, other hormonal profile, chromosomal study and imaging studies. Hormonal profile included basal concentrations of 17-hydroxyprogesterone, cortisol, dehydroepiandrosterone, testosterone and dihydrotestosterone and, when necessary, stimulation tests by human chorionic gonadotropin (hCG) and ACTH. Chromosomal analysis was done on cultured lymphocytes, including sex-determining region Y (SRY) gene signalling by polymerase chain reaction. In addition, radiological imaging included abdominal and pelvic ultrasonography and, when necessary, genitogram and/or magnetic resonance imaging for anatomical localization and identification of gonads and internal genitalia. These investigations were done in the laboratories of King Faisal Specialist and Research Center, King Fahad Hofuf Hospital and the Maternity and Children Hospital. Intramuscular testosterone was tried in 7 patients assigned as males with 46,XY karyotype to assess phallic growth response.

According to the karyotyping, our cases were classified into 4 groups: 46,XY karyotype, 46,XX karyotype, ambiguous karyotype and undetermined karyotype. Sex assignment was matching with the karyotype, except in those patients who died soon after birth without time to carry out karyotyping or other tests.

Results

A total of 41 cases were reported to have ambiguous genitalia during the study period.

These included 14 patients with 46,XX karyotype, of whom 13 (92.9%) had enlarged clitoris and 1 (7.1%) had hypoplastic clitoris and vagina (Fraser syndrome); 6 patients had labial fusion (42.9%) (Table 1). No gonads were palpable in any of them. Congenital adrenal hyperplasia (CAH) was diagnosed in 5 patients as 21-hydroxylase deficiency (35.7%); 3 of these were salt-losers. Extragenital anomalies (always multiple) were reported in 4 cases (28.8%). These included cloacal exstrophy, imperforate anus, congenital heart disease, omphalocele, renal agenesis, skeletal anomalies and dysmorphism. A positive family history was reported in 3 patients, 2 of whom were sisters with congenital adrenal hyperplasia, while the third had a similarly affected cousin with isolated clitoromegaly. All patients were raised as females. Death was reported in 1 patient because of multiple congenital anomalies.

Eighteen (18) patients had 46,XY karyotype (Table 2). Micropenis was seen in 16 cases (88.9%); 9 of these had both testes undescended (50.0%) and 2 had undescended left testis (11.1%). Hypospadias was found in 4 patients (16.7%), 3 with penile type and 1 had penoscrotal type. Positive family history of undescended testes in several family members was reported in 1 patient. The hCG test was negative in 11 patients (61.1%). Extragenital anomalies were reported in 7 cases (38.9%). These included skeletal anomalies (congenital hip dysplasia and club foot), congenital lobar emphysema, renal anomalies, imperforate anus and dysmorphism. Three (3) syndromic cases were reported

Table 1 Clinical data and likely causes of ambiguous genitalia in 46,XX karyotype children (*n* = 14)

Clinical data and causes	No.	%
Clinical data		
Clitoromegaly	13	92.9
Labial fusion	6	42.9
Extragenital anomaly	4	28.6
Extremely ambiguous genitalia	1	7.1
Death	1	7.1
Positive family history	3	21.4
Causes		
Congenital adrenal hyperplasia (21-hydroxylase deficiency)	5	35.7
Generalized developmental malformation	4	28.6
Fraser syndrome	1	7.1
Congenital local genital defect	4	28.6

(Goldenhar syndrome, VATER association and Potter syndrome). Surprisingly, 1 patient proved to have pseudohypoaldosteronism supported by refractory electrolyte disturbances with normal corticosteroid pathway and high aldosterone. Death was reported in 2 patients because of Potter syndrome and hydrops fetalis. All cases were raised as males.

Three (3) patients had abnormal karyotype: all had ambiguous genitalia in addition to the relevant characteristic features of the chromosomal aberration; 1 patient had trisomy 18 (47XX) and the other 2 patients had different types of mosaic Turner (1 with SRY gene, 1 with no SRY signal detected). All were raised as females. The patient with trisomy 18 died after 3 months because of complex congenital heart disease.

The undetermined karyotype group included 6 patients who presented with severe ambiguous genitalia in association with severe lethal congenital anomalies (congenital cyanotic heart lesions, brain malformations, renal and/or respiratory anomalies) and death ensued soon after birth.

Treatment strategies were planned according to the sex of raising and genital morphology and associated clinical features. These included repeated courses of hCG injections and orchidopexy for undescended testes, reconstructive surgery for hypospadias, clitoromegaly and vaginal hypoplasia and hormonal replacement in patients with congenital adrenal hyperplasia.

Table 2 Clinical data and likely causes of ambiguous genitalia in 46,XY karyotype children (*n* = 18)

Clinical data and causes	No.	%
Clinical data		
Micropenis	16	88.9
Undescended testes (both)	9	50.0
Extragenital anomaly	7	38.9
Hypospadias	4	22.2
Undescended testis (single)	2	11.1
Death	2	11.1
Causes		
Generalized developmental malformation, with testosterone biosynthetic defect	4	22.2
Generalized developmental malformation, with normal testosterone synthetic pathway	2	11.1
Syndromic:		
Goldenhar syndrome	1	5.6
VATER association	1	5.6
Potter syndrome	1	5.6
Congenital local genital defect, with testosterone biosynthetic defect	5	27.8
Congenital local genital defects, with intact testosterone synthetic pathway	5	27.8
Androgen insensitivity syndrome	1	5.6

Discussion

Approximately 1 in 2000 children globally is born with an intersex condition [4]. Great care should be always taken in the declaration of sex, which is guided by the etiology, the anatomic condition and family considerations. The last is a reflection of the cultural background. Once the appropriate sex assignment has been made, the next critical step is treatment and, if needed, reconstructive surgery in a timely fashion [5].

In Saudi Arabia, literature on ambiguous genitalia is scarce [6,7], creating a need for further exploration of the problem in this community. In our study, in spite of detailed diagnostic workup, a definitive etiologic diagnosis was made in less than one-third of the cases, including females with congenital adrenal hyperplasia, syndromic patients and patients with abnormal karyotype. Otherwise, causes of ambiguity in patients with 46,XY karyotype were, in order of frequency: testosterone biosynthetic defect (negative hCGT stimulation test response), part of generalized developmental malformation, congenital local genital defect or peripheral androgen insensitivity (suggested by high testosterone and dihydrotestosterone levels); however, there was a high degree of overlap between testosterone biosynthetic defect and other causes in half of

these patients. Lack of gonadal biopsy in our patients, mostly due to parental refusal, prevented our reaching certain specific possible diagnoses such as gonadal dysgenesis or Leydig cell aplasia/hypoplasia. In patients with 46,XX karyotype, CAH was the most common cause; other causes were considered as either a part of generalized developmental malformation or congenital local genital defect.

Ambiguity in patients with undetermined karyotype was suspected to be secondary to severe malformation disorder or unknown chromosomal disorder. Genetic predisposition, which was suspected in 3 cases with 46,XX karyotype and 1 with 46,XY karyotype, was not, however, a contributing factor despite the high incidence of consanguinity in the patients' parents (29.3% first degree and 36.6% second degree).

Our data may differ from other studies in different parts of the world [*3,8–10*], at least in the absence of true hermaphroditism and in the differing order of the top causes of genital ambiguity. However, it is important to remember that our study was hospital-based and age-limited, which might contribute to differences. Nimkarn et al. found that all female pseudohermaphrodites in their study had CAH, and the most frequent cause of male pseudohermaphroditism was androgen insensitivity or 5-alpha reductase deficiency (46.8%) followed by gonadal dysgenesis (19.1%) [*11*]. In another study, which included true and pseudohermaphrodites, sex chromosome abnormalities represented the majority of causes of intersex ,followed by CAH and androgen insensitivity disorders [*8*]. On the other hand, Forest et el. agreed with our study when they reported that 50% of pseudohermaphrodites had no etiological diagnosis [*12*]. In a Saudi study done in the 1990s (1989–99), congenital developmental defects were the most common etiology for ambiguous genitalia followed by CAH (mostly 21-hydroxylase deficiency) [*6*].

There are several literature reports of mosaic Turner patients associated with ambiguous genitalia secondary to the presence of SRY genes [*13,14*]. In our study, we reported 2 cases of mosaic Turner genotype, 1 of them had the SRY gene while in the other case no SRY signal was detected; this last case was considered as a unique variety and, to our knowledge, this is the first report of ambiguous genitalia in mosaic Turner genotype with no Y chromosome (mosaic 45,X[11]/46,X,idic(X)(q11)[9]).

Conclusion

In 46,XX karyotype patients, CAH followed by general malformation disorder were the most common causes of ambiguous genitalia in more than two-thirds of patients while in XY karyotype patients, testosterone pathway biosynthetic defects was the most common cause even in conjunction with a generalized malformation disorder. Mosaic Turner syndrome was a not uncommon cause for ambiguous genitalia.

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