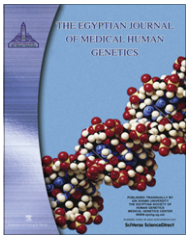
The Egyptian Journal of Medical Human Genetics (2012) **13**, 197–205

Ain Shams University

The Egyptian Journal of Medical Human Genetics

www.ejmhg.eg.net
www.sciencedirect.com



ORIGINAL ARTICLE

Profile of disorders of sexual differentiation in the Northeast region of Cairo, Egypt

Rabah M. Shawky [a,*], **Sahar M. Nour El-Din** [b]

[a] *Pediatrics Department, Ain-Shams University, Egypt*
[b] *Medical Genetics Center, Ain-Shams University, Egypt*

Received 26 September 2011; accepted 19 January 2012
Available online 27 April 2012

KEYWORDS

Sex differentiation;
Intersex;
Ambiguous genitalia;
Gonads;
Genital surgery

Abstract This retrospective study has been conducted to determine the frequency, types, clinical presentation and associated genomic errors in patients with sex differentiation errors and their relatives. The present study comprised of 908 index patients with sex differentiation errors who were registered at the Medical Genetics Center (ASUMGC), Ain Shams University. Out of 28,736 patients attending the center and 660,280 patients attending the Pediatrics clinic during the interval of 1966–2009. Our results showed that, the frequency among all patients attending the Pediatrics Hospital was 0.14%. Disorders of sex chromosome (Klinefelter syndrome and Turner syndrome) were the commonest, followed by mullerian dysgenesis. The commonest age of presentation was adolescence (>15–18 years) (36.56%), followed by patients aged 18 years or more (24.88%). In our study, 32.26% presented with primary female infertility, 27.86% adolescent girls presented with primary amenorrhea, 16.29% presented with male infertility, 10.35% presented with ambiguous genitalia at birth or soon afterward, 6.60% were females who presented with delayed 2ry sexual characters and short stature, 3.96% of our cases were boys who presented with microtestes and delayed 2ry sexual development and 2.75% presented with hirsutism. Central nervous system abnormalities were reported in 5.94% of our patients, ocular abnormalities in 4.29%, and cardiovascular system abnormalities in 2.86%. Three hundred and ninety-two multiple mutant genomic errors were defined

* Corresponding author. Address: 2 To Manbay St., Hammammat, Elkobba, Cairo 11331, Egypt. Tel./fax: +20 2 22585577.
E-mail addresses: shawkyrabah@yahoo.com (R.M. Shawky), sahar.gen79@yahoo.com (S.M. Nour El-Din).

Peer review under responsibility of Ain Shams University.
http://dx.doi.org/10.1016/j.ejmhg.2012.03.003

among relatives of index cases of DSD families, where definable errors represented 35.24% and non-definable errors represented 7.92%. Cytogenetic findings of various DSD showed that, 33.46% of cases with Turner syndrome phenotype had (45,X), and 64.89% were mosaic (45,X/46,XX). While, among the 130 studied cases with Klinefelter syndrome phenotype, 83.84% had 47,XXY. Out of 75 patients with ovotesticular DSD, 85.33% possessed a 46,XX chromosome complement. To conclude, sex determination and differentiation are sequential processes that involve genetic, gonadal, phenotypic and psychological sex. Disorders of sexual differentiation, or syndromes of intersexuality, result when errors occur at any of these steps. Establishing a precise diagnosis in DSD is just as important as in other chronic medical conditions with lifelong consequences.

1. Introduction

In the developing fetus, the genetic or chromosomal sex is determined at the time of fertilization. Thus a 46XX karyotype would normally give rise to a phenotypic female, and 46XY to a phenotypic male fetus. Differentiation of the gonads into either testes or ovaries determines the gonadal or hormonal sex and this occurs under the direction of an increasing number of identified genes and transcription factors which may be specific to either testicular or ovarian differentiation [1]. Gonadal hormone secretion determines the differentiation of the common genital tracts into either male (Wolffian) or female (Mullerian) internal tracts, and also the development of the external genitalia which results in the apparent or phenotypic sex. Subsequent development of the individual will result in the evolution of the psychological or behavioral sex, which is an important consideration in disorders of sexual development (DSD). The process of sex determination and phenotypic development begins as early as the 5th week of gestation and both internal and external genital differentiation are more or less complete by the end of the first trimester. Disorders of sex development arise as a result of a mismatch between the genetic, gonadal and phenotypic sex and are the result of early disruption in the programing of sex determination [2].

Disorders of sex development (DSD), previously referred to as intersex disorders, comprise a variety of congenital diseases with anomalies of the sex chromosomes, the gonads, the reproductive ducts and the genitalia. The term 'disorders of sex development' (DSD) is now proposed to define congenital conditions in which a disharmony between chromosomal, gonadal and anatomical sex exists [3]. A new classification system for the causes of DSD has been proposed based on the karyotype. This terminology, however, also includes the term "sex" in the description of the specific developmental abnormality with the inevitable associated connotation [4]. DSD is loosely classified into four groups on the basis of histological features of the gonadal tissue: XX-DSD with two ovaries (female pseudohermaphroditism), XY-DSD with two testicles (male pseudohermaphroditism), ovotesticular DSD with both ovarian and testicular tissue (true hermaphroditism) and gonadal dysgenesis either mixed (a testis and a streak gonad) or pure (bilateral streak gonads) [2].

Sex differentiation is comprised of many steps. Problems associated with sex differentiation, or syndromes of intersexuality, occur when errors in development take place at any of these steps. Problems can arise at fertilization when chromosomal sex is established. For example, girls with Turner syndrome have a 45,XO karyotype and boys with Klinefelter syndrome have a 47,XXY karyotype. It is also known that some women have a 46,XY or 47,XXX karyotype and some men have a 46,XX or 47,XYY karyotype [5]. Disorders of sex differentiation can occur when a bipotential gonad is incapable of developing into a testis or an ovary. The inability to develop testes may occur if a gene such as SRY is absent or deficient. When this is the case, a 46,XY fetus will not receive the SRY signal to develop testes despite the presence of a Y chromosome. Additionally, 46,XY fetuses may begin to develop testes, but this development can be thwarted, and subsequently Mullerian-inhibiting substance (MIS) and androgen production may be absent or diminished [6].

Intersexuality can also result as a consequence of problems related to Mullerian or Wolffian duct development. For example, MIS secretion accompanied by the absence of androgens or the inability to respond to androgens can result in a fetus lacking both male and female internal duct structures. In contrast, the absence of MIS accompanied by androgen secretion can result in a fetus possessing both male and female internal duct structures to varying degrees [7].

Children born with deviations from normal development of the sex organs can be expected to grow up successfully and to lead enriched lives [8]. However, their problems must be considered carefully. In cases of abnormal sex differentiation, efforts should be made to determine the reason for the abnormality as treatment may vary according to the cause of the disorder [9].

Herein, we conduct a retrospective study to determine the frequency, types, clinical presentation and associated genomic errors in patients with sex differentiation errors and their relatives who were registered at Medical Genetics Center, in the Pediatrics Department, Ain-Shams University, Cairo, Egypt, in the period, 1966–2009. This hospital has a high standard of healthcare, so patients from nearly all governorates of Egypt attend this hospital to receive good health care.

2. Patients and methods

The present study comprised of 908 index patients with sex differentiation errors who were registered at the Medical Genetics Center (ASUMGC), Ain Shams University, out of 28,736 patients attending the center and 660,280 patients attending the Pediatrics clinic during the interval of 1966–2009.

All the patients were referred to the Medical Genetics Center for diagnosis, therapy and genetic counseling. Index cases were subjected to the following studies: Detailed history taking including personal history, age, sex, birth origin (paternal and maternal), treatment history (drugs or operations), perinatal history, age at presentation, main complaints and sex of rearing.

- Index pedigree design taking into consideration, the positive family history of similarly affected cases and their relation to the index patient, positive parental consanguinity, multiple mutant genomic errors in patients and their relatives.
- Thorough clinical examination was made and recorded for each patient and their families, including genital and somatic examinations, anthropometry, assessment of pubertal stage, hypertension, associated anomalies or dysmorphic features. Criteria suggesting DSD included overt genital ambiguity, apparent female genitalia with clitoromegaly, posterior labial fusion or inguinal/labial mass, and apparent male genitalia with non-palpable testis, micropenis, isolated perineal hypospadias or mild hypospadias with undescended testes [10]. Genital examination in males included examination of testes for number, site, volume using the Lambert equation (length [L] × width [W] × height [H] × 0.71). This formula provides a superior estimate of testicular volume [11]. Examination of the penis for length, and site of external urethral meatus, and examination of scrotum were also done. Female genital examination, included examination of labia and clitoris for size, vaginal opening, and urethral opening. Assessment of primary and secondary sex characteristics (pubic hair and breasts in females and pubic hair in males) using the sex maturity ratings (SMRs) [12].
- Biochemical assessment, assay of serum 17-hydroxyprogesterone, testosterone, estradiol, DHT, androstenedione, FSH, LH, urine steroid analysis.
- Abdomino-pelvic ultrasound imaging to evaluate ambiguous genitalia and anomalies of the pelvic organs.
- Cytogenetic study of relevant cases by peripheral blood lymphocytic culture. Giemsa trypsin banding (GTG) of metaphase chromosomes was performed using standard methodology [13].
- The parents were then counseled about the nature of their child's abnormality.
- DSDs in our study, were classified according to the chromosomal study, the gonadal histology and the phenotype.

3. Results

3.1. Occurence rate

Out of 28,735 patients, registered at Medical Genetics Center, during the period of the study, 908 patients (3.15%) clinically confirmed the diagnosis of sex development disorders (DSD) in the Genetics clinic. The frequency among all patients attending the Pediatrics Hospital was 0.14%. The types of DSD detected in our study were presented in Table 1. Disorders of sex chromosomes (Klinefelter syndrome and Turner syndrome) were the commonest, followed by mullerian dysgenesis, 46,XX DSD (congenital adrenal hyperplasia, androgen secreting tumor), severe hypospadias, ovotesticular DSD, gonadal dysgenesis and 46,XY DSD (Table 1).

3.2. Epidemiological profile

As regards, the sex of rearing 548 cases (60.35%) was males, and 360 cases (39.64%) were females. All patients were of Egyptian nationality. Consanguineous marriage was reported among parents of 504 patients (55.50%). Two hundred and forty-eight patients (27.31%) had a positive family history of the same DSD. Lower Egypt was the commonest parental birth origin, followed by upper Egypt and Cairo (Table 2). The ages of our cases ranged from 1 month to 28 years old, the commonest age of presentation was adolescence (>15–18 years) (36.56%), followed by patients aged 18 years or more (24.88%) (Table 3).

Table 1 Frequency & types of disorders of sex development (DSDs).

Type of disorder	No.	%
Sex chromosome DSD	375	41.29
Klinefelter syndrome	130	14.31
Turner syndrome	245	26.98
Dysgenetic DSD	74	8.14
Gonadal dysgenesis	74	8.14
Ovotesticular DSD	75	8.25
46,XY DSD	66	7.26
CAIS[a]	40	4.40
PAIS[b]	26	2.86
46,XX DSD	92	10.13
CAH[c]	78	8.59
Androgen secreting tumor	14	1.54
Mullerian dysgenesis	138	15.19
Severe hypospadias	88	9.69
Total	908	100.00

[a] CAIS = Complete androgen insensitivity syndrome.
[b] PAIS = partial androgen insensitivity syndrome.
[c] CAH = Congenital adrenal hyperplasia.

3.3. Clinical presentations of DSD

The variability in the manifestation of DSD covers a spectrum ranging from normal external female and male phenotypes to ambiguous genitalia. In our study, 293 patients (32.26%) presented with primary female infertility, 253 (27.86%) adolescent girls presented with primary amenorrhea, 148 cases (16.29%) presented with male infertility, 94 cases (10.35%) presented

Table 2 Epidemiological profile.

Variable	No	%
Sex of rearing		
Male/female	548/360	60.35/39.64
Nationality		
Egyptian	908/908	100
Consanguinity		
+ve/−ve	504/404	55.50/44.49
Family history		
+ve/−ve	253/655	27.86/72.13
Parental birth origin[a] *(P/M)*		
Cairo	197/236	21.69/26.00
Lower Egypt	461/435	50.77/47.90
Upper Egypt	250/237	27.54/26.10
Total	908	100.00

[a] *(P/M)* = paternal/maternal.

Table 3 Age distribution of index cases.

Age	No.	%
Neonatal (1 month)	79	8.70
Early infancy (1 month to < 1 yr)	52	5.72
Late infancy (1–2 years)	57	6.27
Early childhood (2–6 years)	65	7.15
Late childhood (6 to <12 years)	51	5.61
Prepubertal (12 to <15 years)	46	8.37
Adolescence (>15 to 18 years)	332	36.56
18 years or more	226	24.88
Total	908	100.00

with ambiguous genitalia at birth or soon afterward, 60 patients (6.60%) were females who presented with delayed 2ry sexual characters and short stature, 36 of our cases were boys (3.96%) who presented with microtestes and delayed 2ry sexual development and 25 cases (2.75%) presented with hirsutism (Fig. 1,Table 3).

3.4. Other medical associations

Central nervous system abnormalities were reported in 54 (5.94%) of our patients, cardiovascular system abnormalities in 26 cases (2.86%), ocular abnormalities in 39 cases (4.29%), special sense defect in 7 patients (0.77%), endocrinal errors in 20 cases (2.20%), osseo-chondral defects in 10 patients (1.10%), musculoskeletal abnormalities in 13 patients (1.43%), dermatological errors in 28 cases (3.08%), renal troubles in 8 cases (0.88%) and gastrointestinal tract abnormalities in only 2 (0.22%) of our cases (Table 4).

3.5. Multiple mutant genomic errors among relatives

Three hundred and ninety-two multiple mutant genomic errors were defined among relatives of index cases of DSD families, where definable errors represented 35.24% and non-definable errors represented 7.92% (Table 5).

3.6. Cytogenetic findings of various DSD

In the 245 studied cases with Turner syndrome phenotype, 82 patients (33.46%) had (45,X) and 159 patients (64.89%) were

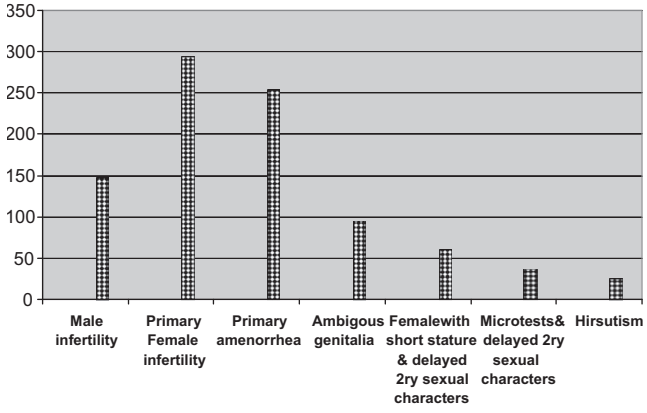


Figure 1 Clinical presentations of index cases.

Table 4 Associated errors expressed among index cases.

Associated genomic errors	No.	%
CNS[a]	54	5.94
Mental retardation	13	3.41
Seizures	23	2.53
CVS[b]	26	2.86
Congenital heart disease	11	1.21
Coronary heart disease	9	0.99
Varicosities of L.L	6	0.66
Ocular	39	4.29
Microphthalmia	8	0.88
Anophthalmia	7	0.77
Enophthalmia	5	0.55
Squint	13	1.34
Myopia	6	0.66
Special sense	7	0.77
Conductive hearing loss	7	0.77
Endocrinal	20	2.20
NIDDM	7	0.77
Goiter	9	0.99
Obesity	4	0.44
Osseo-chondral	10	1.10
Hypochondroplasia	10	1.10
Musculoskeletal	13	1.43
Flexion contracture of limbs	13	1.43
Dermatological	28	3.08
Cutaneous albinism	13	1.43
Xeroderma pigmentosa	12	1.32
Premature graying of hair	3	0.33
Renal	8	0.88
Renal stone	7	0.77
Unilateral abscent kidney	1	0.11
GIT[c]	2	0.22
Hirschsprung disease	2	0.22
Total	908	100.00

[a] CNS = Central nervous system.
[b] CVS = Cardiovascular system.
[c] GIT = Gastrointestinal tract.

mosaic (45,X/46,XX), 2 patients (0.81%) had long arm isochromosome of X chromosome [46,Xi(Xq)] and another 2 patients (0.81%) had short arm deletion of X chromosome [46,X(Xp-)]. In the 130 studied cases with Klinefelter syndrome phenotype, 109 (83.84%) had 47,XXY, 15 cases (11.53%) were mosaic (46XY/47XXY), 3 cases (2.30%) had variant forms of Klinefelter syndrome (48,XXXY; 48,XXYY; 49,XXXXY) and 3 cases (2.30%) had normal male karyotype (46XY). In our study, out of 75 patients with ovotesticular DSD, 64 patients (85.33%) possessed a 46,XX chromosome complement and presented with ambiguous genitalia, 6 patients (8%) had 46,XY chromosome complement and 5 patients (6.66%) had 46,XX/46XY mosaicism, and presented with obscure external genitalia, hypospadias in males and hypertrophy of the clitoris in females (Table 6).

4. Discussion

The frequency of DSD was 3.15% among our patients registered at the Medical Genetics Center and was 0.14% (1.4/1000) among all patients attending the Pediatrics Hospital during the period of the study. However in other localities in Egypt (Alexandria and Giza), DSD represented 7.3% and

Table 5 Multiple mutant genomic errors defined among relatives of 908 DSD index families.

	MMGE	No.	%
I	Definable errors	220	75.34
	Single gene errors	108	36.98
	Febrile convulsions	10	3.42
	Schizophrenia	8	2.73
	1ry optic atrophy	5	1.71
	Oligodactyly	9	3.08
	Insulin dependent diabetes mellitus	14	4.79
	Albinism	13	4.45
	G6PD deficiency	8	2.73
	Myopia	19	6.50
	Squint	9	3.08
	Polydactyly	13	4.45
	Multifactorial errors	102	43.93
	GMME	5	1.71
	Hypertension	20	6.84
	Coronary heart disease	10	3.42
	Cerebral stroke	2	0.68
	Congenital heart disease	12	4.10
	Bronchial asthma	8	2.73
	NTD	2	0.68
	Cleft lip	7	2.39
	Imperforate anus	4	1.36
	Non-insulin dependent diabetes mellitus	23	7.87
	Obesity	5	1.71
	Atopic dermatitis	1	0.34
	Renal stone	2	0.68
	Oncoplasia (please check the spelling of the term Oncoplasia)	1	0.34
	Chromosomal errors	10	3.42
	Down syndrome	10	3.42
II	Non-definable errors	72	24.65
	Mental deficiency	18	6.16
	Deaf-mutism	12	4.10
	Speech defect	9	3.08
	Deformed nose	6	2.05
	Goiter	7	2.39
	Short stature	11	3.76
	Hydrocephalus	9	3.08
	Sum of traceable errors	292	100.00

Table 6 Chromosomal variations defined among the most common DSD.

Cytogenetic findings	No.	%
Turner syndrome	245	26.98
Mosaic (45,X/46,XX)	159	64.89
Classic Turner (45,X)	82	33.46
46,Xi(Xq)	2	0.81
46,X(Xp-)	2	0.81
Klinefelter syndrome	130	14.31
47,XXY	109	83.84
46XY/47XXY	15	11.53
46XY	3	2.30
48,XXXY	1	0.76
48,XXYY	1	0.76
49,XXXXY	1	0.76
Ovotesticular DSD	75	8.25
46,XX	64	85.33
46,XY	6	8.00
46,XX/46XY	5	6.66

7% respectively of the total referred cases which is considered high [14]. In Venezuela Alvarez et al.[15] reported that DSD, represented 5.4% among cases referred to the Medical Genetics Center. However our results were higher than that reported in other governorates in Egypt. Mazen et al. [16] reported an incidence of 1:5000 live births of DSD in Giza, while Temtamy et al. [17] reported an incidence of 1:3000 live births in Giza. Mazen et al. [18] also reported an incidence of 2/10,000 in Great Cairo and Qalyubiyah governorates in newborns and infants up to the age of 6 months. Essawi et al. [19] also reported that disorders of sex development (DSD) constitute a significant entity among the birth defect list in Egypt. Nasir [20] in Saudi Arabia reported that, DSD is not uncommon in their community. In United States Kaefer et al. [21] reported that, DSD vary in frequency depending on their etiology. Leonard [22] reported that, the true prevalence of intersex in which chromosomal sex is inconsistent with phenotypic sex, or in which the phenotype is not classifiable as either "male" or "female" is seen to be about 0.018%. According to the Intersex Society of North America (ISNA) definition [23], 1% of live births exhibit some degree of sexual ambiguity and between 0.1% and 0.2% of live births are ambiguous enough to become the subject of Specialist medical attention, including surgery to disguise their sexual ambiguity [24]. Meanwhile according to Fausto-Sterling's definition of intersex [25], 1.7% of human births are intersex.

Age of presentation of DSD will depend upon the degree of dysfunction caused [26]. The current study showed that, the ages of our cases ranged from 1 month to 28 years old, the commonest age of presentation was adolescence (>15–18 years) (36.56%), followed by patients aged 18 years or more (24.88%), neonatal (1 month) (8.70%), prepubertal (8.37%), early childhood (7.15%), late infancy (6.27%), early infancy (5.72%), and late childhood (5.61%). In agreement to our results Hashem et al. [27] reported that adulthood was the most common presenting age group in patients with genitogonadal differentiation errors among Egyptians. Dessouky et al. [28] studied 314 cases presenting with intersex problems in the Pediatric Surgical Division, Cairo University over a period of 14 years, between 1986 and 2000. They reported that the ages at presentation ranged between 10 days and 20 years with a mean of 5.5 years. This means that diagnosis of patients with DSD was usually delayed in Egypt. On the other hand, Hughes et al. [29] reported that DSD typically are diagnosed at birth in infants with ambiguous genitalia while, disorders associated with phenotypic males and females may be diagnosed much later.

Our data demonstrated that sex chromosome DSD (TS and Klinefelter syndrome) were the commonest disorders among our cases as it represented 41.29% of our patients. TS constituted a significant proportion of DSD cases (26,98%) and Klinefelter syndrome represented 14.31%, followed by Mullerian dysgenesis (15.19%), 46,XX DSD (10.13%), hypospadias (9.69%), ovotesticular DSD (8.25%), gonadal dysgenesis (8.14%), and 46,XY, DSD (7.26%). On the other hand, Hashem et al. [27] reported that, gonadal dysgenesis was the most

common disorder of sexual differentiation and development (54.2%), while Mazen et al. [16] reported that, 46,XY DSD was the commonest (65.9%), followed by 46,XX DSD (28%), complex syndrome with associated malformations (1.44%) and ovotesticular DSD (2.88%). Also, Joshi et al. [30] reported that, disorders of gonadal differentiation were found in 10.1%, 8.25% diagnosed with gonadal dysgenesis and 1.83% with true hermaphroditism and the syndromic form of ambiguous genitalia were detected in 1.83%. Similar to our results, Sybert et al. [31] and Sema et al. [32] reported that, Turner's syndrome is the most common sex chromosome abnormality of human females. However, Ketan et al. [33] found that CAH was the commonest cause of DSD (36.2%), followed by gonadal dysgenesis. Ovotesticular DSD was reported in 5.7%, 4.8% and 14.3% of the cases by Arcari et al. [34], Nieman et al. [35], and Rajendran et al. [36], respectively. A retrospective study [20] conducted in Saudi Arabia showed that, out of 81 children with ambiguous genitalia, congenital adrenal hyperplasia being the commonest cause in 96.5% of the patients. A study in Turkey [32] showed that, out of a total of 95 patients, 27.36% had sex chromosome DSD, 47.36% had 46,XY DSD and 25.26% had 46,XX DSD. Kaefer et al. [21] in USA observed that DSD vary in frequency depending on their etiology.

In our study 32.26% of our female patients presented with primary infertility, 27.86% presented with primary amenorrhea, 16.18% presented with male infertility, 10.35% presented with ambiguous genitalia at birth, 6.60% of our patients were females presented with short stature and delayed 2ry sexual characters, 3.96% were males with microtestes, and 2.75% had hirsutism. On the other hand, Sema et al. [32] mentioned that, the main complaints at presentation among 95 DSD patients were ambiguous genitalia in 24.21%, short stature in 20%, isolated perineal hypospadias in 9.47%, primary amenorrhea in 8.42%, late or incomplete puberty in 8.42%, micropenis in 6.31% and clitoromegaly in 5.26%. Maria and Dolores [37] stated that in at least 30% to 50% of infertility cases a male factor abnormality is involved. Furthermore, Folch et al. [38] have demonstrated that mullerian agenesis, is the second most common cause of primary amenorrhea. While Nieman et al. [35] reported that, the commonest cause of ambiguous genitalia is congenital adrenal hyperplasia (CAH).

Cytogenetic variants of our studied patients with Turner syndrome phenotype showed that, 64.89% were mosaic (45,X/46,XX), 33.46% were classic Turner (45,X), 0.81% had X isochromosome, 46,Xi(Xq), and 0.81% had deletion 46,X(Xp-). On the other hand, Huang et al. [39] reported, 45,X in 53%; mosaicism 45X/46XX in 15%; X isochromosome, 46,Xi(Xq) in 10%; mosaicism 46,Xi(Xq)/46XX in 8%; deletions 46XXp- or 46XXq- in 6%; other mosaicism in 8%. Another study on Turner syndrome patients in northeastern Malaysia [40] showed that, the incidence of the most frequent karyotype of the Turner syndrome was found to be 45,X (57.1%), followed by 46,Xi(Xq) (21.4%), 45,X/45,X,+mar (7.1%), 45,X/46,Xi(Xq) (7.1%) and 45,X/46,XY (7.1%).

In our study 47,XXY is the most frequent karyotype of the Klinefelter syndrome patients; followed by 46XY/47XXY, 46XY, 48,XXXY, 48,XXYY and 49,XXXXY. Their relative frequencies in the series are 83.84%, 11.53%, 2.30%, 0.76%, 0.76%, and 0.76%, respectively. This is in consistency with the findings of Wikstrom and Dunkel [41] who reported that, the 47,XXY variant was found in 80–90% of patients with Klinefelter syndrome, 10% of patients had mosaicism; (46,XY/47,XXY; 46,XY/48,XXXY; and 47,XXY/48,XXXY), and remaining cases include variants such as the 48,XXYY; 48,XXXY; 49,XXXYY; and 49,XXXXY karyotypes. About 1% of cases are due to a structurally abnormal X in addition to a normal X and Y, such as 47,Xi(Xq)Y and 47,X,del(X)Y.

The karyotype of our cases with ovotesticular DSD was 46,XX in 85.33% of cases, 46,XY in 8% of cases and 46,XX/46XY in 6.66% of cases. However, Ketan et al. [42] Joshi et al. [30] Göllü et al. [43] and Rajendran et al. [36] reported that 46XY was the commonest karyotype in patients with ovotesticular DSD. While Tran et al. [44] reported an uncommon 45,X/46,X,idic(Y)(q11.23) mosaic karyotype in an infant born with ovotesticular disorder of sex development.

Genealogic analysis of pedigrees of index patients with disorders of sex development revealed an apparently high incidence of other genetic anomalies which manifested simultaneously or segregated randomly among many of propand's relatives. The identification of isolated disorders of sex differentiation as well as isolated mental retardation or speech defect among certain index kindreds, could advocate the possibility of linkage of mutant gene loci mediating both types of anomalies [45].

In our study mental retardation was reported in 3.41% of index cases and in 6.16% among the relatives of index cases. However Michela et al. [46] identified duplication of Xp21.2 in patients with isolated gonadal dysgenesis who presented with gonadal dysgenesis as part of a more complex phenotype, including mental retardation and/or malformations. Also Louise et al. [47] reported that, mild mental retardation was present in 14.8% of patients with mixed gonadal dysgenesis (MGD) or pseudohermaphroditism (MPH). Khalifa et al. [48] screened DNA samples from 1205 patients originally referred for fragile X syndrome (FRAX) testing, because of mental retardation of unknown etiology and detected 8 Klinefelter syndrome patients. They reported that Klinefelter syndrome might be the most common cause of mental retardation of unknown etiology among prepubertal males. Maier and Koch [49] also reported a 26-year-old woman who had müllerian dysgenesis associated with a chromosomal anomaly (46XX [13p+]) and mental retardation.

Seizures are the most common pediatric neurologic disorder. Four to ten percent of children suffer at least one seizure in the first 16 years of life. The incidence is highest in children less than 3 years of age, with a decreasing frequency in older children [50]. In this study seizures were reported in 23 cases (2.53%) of our patients [8 cases had Klinefelter's syndrome, 7 cases had CAH, 5 cases had Turner syndrome, 3 cases had mullerian dysgenesis]. Striano et al. [51] reported a Turner patient showing severe neurological impairment, refractory epilepsy and MRI findings of bilateral perisylvian hypoplasia. On the other hand, Tatum et al. [52] described the clinical spectrum of patients with Klinefelter's syndrome and seizures. The authors described 3 American patients and 9 additional patients from two European centers previously reported with Klinefelter's syndrome and seizures. The most common profile of patients with Klinefelter's syndrome and seizures includes mental retardation, behavioral problems, epileptiform electroencephalograms (EEGs), and generalized tonic–clonic seizures. Also Kawawaki et al. [53] reported seizures in 22 children with congenital adrenal hyperplasia (CAH), eight of whom had seizures associated with fever. Their findings implicate unknown

factors in the pathogenesis such as excess secretion of corticotropin releasing factor (CRF) under stress, prolonged elevation of CRF during fetal life and linkage between CAH and febrile seizures on the chromosome 6. Grosso et al. in Italy [54] reported that, few syndromes seem to show peculiar clinical and EEG associations, such as patients with Turner's syndrome and Klinefelter's syndrome.

Congenital heart disease (CHD) is a public health problem among infants and young children and remain the leading cause of death from congenital malformations. We reported CHD in eleven cases (1.21%) of our patients, where 7 of them (0.77%) had Turner's syndrome, 3 patients (0.33%) had CAH, 1 case (0.11%) had Klinefelter syndrome. In agreement to our results, Mazzanti et al. [55] reported that, there is a high prevalence of congenital heart defects in patients with Turner's syndrome, the prevalence of cardiac malformations was 23% [bicuspid aortic valve (12.5%), aortic coarctation (6.9%), and aortic valve disease (3.2%) were the most prevalent malformations]. Also Kusuma et al. [56] reported that ambiguous genitalia are one such rare anomaly that is associated with CHD among other genital abnormalities. The possible causes for this association could be pseudohermaphroditism, which in turn, may be due to congenital adrenal hyperplasia. They reported a very rare case of CHD associated with ambiguous genitalia. Chromosomal analysis of the proband revealed a normal female complement, echocardiographic examinations revealed a ventricular septal defect (VSD) along with atrial septal defect (ASD).

Abnormalities of the genitals and urinary tract are among the most common birth defects. Some of these abnormalities are minor and may cause no symptoms (example: having two ureters leading from one kidney to the bladder). Such minor defects often go undiagnosed unless the child has an X-ray, ultrasound examination or surgery for a related or unrelated problem [57]. Other abnormalities can cause problems such as urinary tract infections, blockages, pain, and kidney damage or failure. Abnormalities of the genitals and urinary tract are affecting as many as 1 in 10 babies. These defects can affect the kidneys, ureters, bladder, urethra, and the male and female genitals. A few genital and urinary tract defects or disorders are inherited from parents who have the disorder or carry the gene for it [58]. Specific causes of most of these conditions are unknown, however, genetic and environmental factors presumably play various roles during development of these organs [59]. In our study, we reported renal affection in 0.88% of our patients [5 cases (0.55%) had Turner syndrome, 2 cases (0.22%) had gonadal dysgenesis and one case (0.11%) had mullerian dysgenesis], where 0.77% of cases suffered from renal stone and 0.11% had unilateral abscent kidney. Saenger [60] reported that renal anomalies are very common in Turner syndrome and a renal ultrasound should be obtained at the time of diagnosis. Mojtaba et al. [61] reviewed the records of 170 patients with unilateral and 18 patients with bilateral Wilms tumor and 6 patients with congenital mesoblastic nephroma for abnormalities of the external genitalia. There were 4 patients with cryptorchism, 1 with hypospadias, 1 with mixed gonadal dysgenesis, and 3 with male pseudohermaphroditism.

Non-insulin dependent diabetes mellitus was reported in 7 cases (0.77%) of our patients, where 4 cases (0.44%) had Klinefelter's syndrome, while the other 3 cases (0.33%) had Turner's syndrome. Also, in our study, non-insulin dependent diabetes mellitus was defined in 23 cases (7.87%) among relatives of index cases. Stephen and Anna [62] reported that, diabetes may be associated with many genetic disorders. The type of diabetes varies in these syndromes. The increased frequency of diabetes in Klinefelter's syndrome, and Turner's syndrome leads to the hypothesis that non-disjunction may, in some way be associated with the predisposition to diabetes. Saenger [60] reported that, obesity, which is present in about 40% of women with Turner syndrome, may increase insulin resistance.

Conductive hearing loss (CHL) was detected in 7 patients (0.77%), in our study, Turner's syndrome was diagnosed in 5 of them and the other 2 cases had congenital adrenal hyperplasia. A retrospective cohort study of 23 individuals with Turner syndrome was undertaken and otologic status was assigned by Monique et al. [63]. The results showed that middle ear disease affected 91% of patients. Otologic disease is a common problem in Turner syndrome patients due to a combination of small dysfunction Eustachian tube, palatal dysfunction and cochlear malformation. So hearing screen should be performed in all patients with Turner's syndrome [64].

The association of sex differentiation errors with chromosomal abnormalities like Down syndrome is considered coincidental. Clinically, 4 of our patients (0.44%) had most of the phenotypic features of Down syndrome as well as variable features characteristic of Turner or Klinefelter syndrome. Also Zaki et al. [65] presented three Egyptian patients with double aneuploidy involving chromosome 21 and sex chromosomes. They all had the classical non-disjunction trisomy 21; that was associated with monosomy X in two of them and double X in the other. Sparagana et al. [66] reported that Trisomy 21 had been observed in association with sex chromosomal anomalies such as in Klinefelter's syndrome, XYY syndrome, triple X syndrome, Turner's syndrome, and mosaic 45,X/47,XY,+21. Musarella and Verma [67] also reported a case of double aneuploidy involving Down and Turner cell lines in a female child with a massive capillary hemangioma of the left orbit and mild clinical features of Down syndrome.

In our work, pedigree analysis revealed that consanguineous marriage was reported among 55.56% of parents of our patients, compared to 35.3% of consanguineous marriage among Egyptian populations in Egypt [68]. High degree of consanguinity in DSD was also reported in other localities in Egypt (61–65%) [16,19,32,37] and it is considered a long-standing social habit among Egyptians.

In the current work, 27.87% of cases had a positive family history of expressing the same DSD. Nevertheless, Mazen et al. [16] reported that, only 10.1% of their patients had a positive family history.

A central clinical issue in neonates with major genital anomalies is assigning the sex-of-rearing. Such assignation determines or dictates myriad postnatal and subsequent social dynamics, including parenting implications, social trajectories, surgical interventions, and often, lifelong sex-hormone administration — not to mention the psychosocial and psychosexual developmental ramifications for the child [69]. Interestingly, we observed that, at the time of presentation, the percentage of patients with intersex problems reared as males was 60.35% while 39.64% were reared as females. In Egyptian society, female infertility precludes marriage, which also affects employment prospects. This drives the family of the patients to rather choose the male sex to create economic independence. This assumption was supported in our study. In agreement

to our results Schweizer et al. [70] reported that adult gender identity outcome of their participants is characterized by high male and low female gender identity. However, Ismail et al. [71] reported that, 80% of their patients were initially assigned as females. In Turkey, Sema et al. also [32] reported that, out of 95 patients met the criteria for DSD, 22 patients were raised as males and 36 as females. Establishing a precise diagnosis in DSD is just as important as in other chronic medical conditions with lifelong consequences [24]. The birth of an intersex child prompts a long term management strategy that involves a myriad of professionals working with the family [72]. The medical management of differences of sex development (DSD)/ intersex in early childhood has been criticized by patients' advocates as well as bioethicists from an ethical point of view. The pattern of surgical practice in DSD is changing with respect to the timing of surgery and the techniques employed. It is essential to evaluate the effects of early versus later surgery in a holistic manner, recognizing the difficulties posed by an ever evolving clinical practice [30].

5. Conclusion

To conclude pediatricians have a key role in relieving psychological distress of families and patients by coordinating the diagnostic evaluation, helping families understand their child's medical condition and maintaining open communication between family and other healthcare team. The availability of pediatric endocrinologists is a key factor in determining whether or not the cause of DSD can be investigated. It is essential to have a sound knowledge about DSD to analyze the attitude of parents and to provide adequate counseling. Uniform classification, good cytogenetic facilities and individualized approach with emphasis on integrated team management form the mainstay of management of DSD. More studies including larger population sizes, using molecular genetic analyses to detect the actual incidence of genital anomalies and DSD in Egypt are required in order to serve those patients and their families.

References

[1] Ogilvy-Stuart AL, Brain CE. Early assessment of ambiguous genitalia. Arch. Dis. Child. 2004;89:401–7.
[2] Govind BC, Dimitri AP, Kamaldine O, Stephen FM, Paul SB, Foao LPS. Imaging of ambiguous genitalia: classification and diagnostic approach. RadioGraphics 2008;28:1891–904.
[3] Berenbaum SA, Bailey JM. Effects on gender identity of prenatal androgens and genital appearance. Evidence from girls with congenital adrenal hyperplasia. J Clin Endocrinol Metab 2003;88(3):1102–6.
[4] Dacou-Voutetakis C. A multidisciplinary approach to the management of children with complex genital anomalies. Nat Clin Pract Endocrinol Metab 2007;3:668–9.
[5] Maharaj D. Disorders of sex development: a review. Curr Women's Health Rev 2008:223–39.
[6] Teixeira J, Maheswaran S, Donahoe PK. Müllerian inhibiting substance. An instructive developmental hormone with diagnostic and possible therapeutic applications. Endocr Rev 2001;22:657–74.
[7] Diamond DA. Abnormal sexual differentiation. Evaluation and management of the newborn with ambiguous genitalia. In: Wein AJ, editor. Campbell-Walsh Urology. Philadelphia: Saunders Elsevier; 2007, chapter 128.
[8] Smetanova D, Hellerova B. Prenatal diagnosis of abnormal sex differentiation. Ultrasound Obstet Gynecol 2007;30(4):547–653.
[9] MacLaughlin, Donahoe N. Mechanisms of disease: sex determination and differentiation. N Eng J Med 2004;350(4):367–78.
[10] Carillo AA, Berkowitz GD. Disorders of sexual differentiation. In: Lifshitz F, editor. Pediatric Endocrinology. New York: Marcel Dekker; 2003. p. 319–45.
[11] Hideo S, Katsuyuki S, Michiya O, Katuki I, Yoshio O, Hideki Y. Testicular volume measurement: comparison of orchidometry and water displacement. Adult Urol 2007;69(1):152–7.
[12] Theonia KB. Disorders of sexual differentiation. Surg Pathol Clin 2010;3(3):553–601.
[13] Verma R, Babu A. Human chromosomes. Manual of basic techniques. 2nd ed. New York: Mc Graw Hill; 1995.
[14] Mohamed MM, Nahla AN, Hanan SM. Genetic study of abnormal sexual differentiation in Alexandria, Egypt. Egypt J Med Hum Genet 2002;3(1):63–76.
[15] Alvarez-Nava F, Gonzalez-Ferrer S, Soto M. Diagnosis and management of patients with sex differentiation disorders:experience at the Unit of Medical Genetics of the University of Zulia, Maracaibo, Venezuela. Invest Clin 1998;39:237–92.
[16] Mazen I, Hiort O, Bassiouny R, El Gammal M. Differential diagnosis of disorders of sex development in Egypt. Horm Res. 2008;70(2):118–23.
[17] Temtamy SA, Abdel Meguid N, Mazen, Ismail SR, Kassem NS, Bassiouni R. A genetic epidemiological study of malformations at birth in Egypt. East Mediterr Health J 1998;4(2):252–9.
[18] Mazen I, El-Ruby M, Kamal R, El-Nekhely I, El-Ghandour M, Tantawy S, El-Gammal M. Screening of genital anomalies in newborns and infants in two Egyptian governorates. Horm Res Paediatr 2010;73(6):438–42.
[19] Mona E, Hala N, Inas M, Khadiga G, Khalda A, Mona H, Yehia G. Low incidence of androgen receptor mutation among Egyptian children with androgen resistance. Egypt J Med Hum Genet 2008;9(1):111–9.
[20] Nasir AM, Al-Jurayyan.. Ambiguous genitalia: two decades of experience. Ann Saudi Med 2011;31(3):284–8.
[21] Kaefer M, Diamond D, Hendren WH, Vemulapalli S, Bauer SB, Peters CA, et al.. The incidence of intersexuality in children with cryptorchidism and hypospadias: stratification based on gonadal palpability and meatal position. J Urol 1999;162(3 Pt 2):1003–7.
[22] Leonard S. How common is intersex? A response to Anne Fausto-Sterling. J Sex Res 2002;39(3):174–8.
[23] How common is intersex? Intersex Society of North America. Isna.org. http://www.isna.org/faq/frequency. Retrieved 2009-08-21.
[24] Hughes IA, Houk C, Ahmed SF. LWPES Consensus Group; ESPE Consensus Group. Consensus statement on management of intersex disorders. Arch Dis Child 2006;91(7):554–63.
[25] Fausto-Sterling A. Sexing the Body: Gender Politics and the Construction of Sexuality. New York: Basic Books. 2000; ISBN 0465077137.
[26] Brown J, Warne G. Practical management of the intersex infant. J Pediatr Endocrinol Metab 2005;18:3–23.
[27] Hashem NN, Shawky RM, El-Sedfy H, Sherif Y, AbdEl-Aziz. Prevalence and phenotypic expression of various genitogonadal differentiation errors among Egyptians. Egypt J Med Hum Genet 2001;2(1):101–11.
[28] Nabil MD. Gender assignment for children with intersex problems: an Egyptian perspective. Egypt J Surg 2001;20(2):499–515.
[29] Hughes IA. Disorders of sex development: a new definition and classification. Best Pract Res Clin Endocrinol Metab 2008;22:119–34.
[30] Joshi RR, Rao S, Desai M. Etiology and clinical profile of ambiguous genitalia an overview of 10 years experience. Indian Pediatr 2006;43(11):974–9.
[31] Sybert VP, Elizabeth M. Turner's syndrome. New Engl J Med. 2004;351:1227–38.
[32] Sema E, Cengiz K, Ahmet U, Murat A. Etiological classification and clinical assessment of children and adolescents with disorders

of sex development. J Clin Res Pediatr Endocrinol 2011;3(2):77–83.
[33] Ketan PK, Inusha P, Reena D, Surinder K, Ram KM. Pediatric disorders of sex development. Indian J Pediatr 2009;76(9):956–8.
[34] Arcari AR, Bergadá I, Rey RA, Gottlieb S. Predictive value of anatomical findings and karyotype analysis in the diagnosis of patients with disorders of sexual development. Sexual Dev 2007;1:222–9.
[35] Nieman LK, Orth DN, Kirkland JL. Treatment of congenital adrenal hyperplasia due to CYP21A2. (21-hydroxylase) deficiency in infants and children. 2004, Uptodate, online 12.1 (www.uptodate.com).
[36] Rajendran R, Hariharan S. Profile of intersex children in South India. Indian Pediatr 1995;32:666–71.
[37] Maria RM, Dolors JL. Investigative urology: review article. understanding new genetics of male infertility. J Urol 2002;168(5):2197–205.
[38] Folch M, Pigem I, Konje JC. Müllerian agenesis: etiology, diagnosis, and management. Obstet Gynecol Surv 2000;55(10):644–9.
[39] Huang B, Thangavelu M, Bhatt S, Sandlin JC, Wang S. Prenatal diagnosis of 45, X and 45, X mosaicism: the need for thorough cytogenetic and clinical evaluations. Prenat Diagn 2002;22:105–10.
[40] Kannan TP, Azman BZ, Ahmad Tarmizi AB, Suhaida MA, Siti Mariam I, Ravindran A, Zilfalil BA. Syndrome diagnosed in northeastern Malaysia. Singapore Med J 2008;49(5):400.
[41] Wikstrom AM, Dunkel L. Testicular function in Klinefelter syndrome. Horm Res 2008;69(6):317–26.
[42] Ketan PK, Inusha P, Reena D, Surinder K, Ram KM. Pediatric Disorders of Sex Development. Indian J Pediatr 2009;76:956–8.
[43] Göllü G, Yýldýza RV, Bingol-Kologlu M, et al.. Ambiguous. Genitalia: an overview of 17 years' experience. J Ped Surg 2007;42:840–4.
[44] Tran CN, Semins MJ, Epstein JI, Gearhart JP. Ovotesticular disorder of sex development with mosaic 45, X/46, X, idic(Y)(q11.23) karyotype and streak gonad. Urology 2011;78(5):1178–81.
[45] American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision. Washington, DC: APA Press; 2000:41–9.
[46] Mikael B, Jacqueline S, Johan S, Sten AI, Anna W. Isolated 46, XY gonadal dysgenesis in two sisters caused by a Xp21.2 interstitial duplication containing the DAX1 gene. J Clin Endocrinol Metab 2007;92(8):3305–13.
[47] Louise T, Aziza l, Olivia Del Pino, Jacques PB, Jean LC. Experience and reason 45,X/46,XY Mosaicism: Report of 27 Cases. Pediatrics 1999;104(2):304–8.
[48] Khalifa MM, Struthers JL. Klinefelter syndrome is a common cause for mental retardation of unknown etiology among prepubertal males. Clin Genet 2002;61(1):49–53.
[49] Maier D, Koch E. Müllerian dysgenesis associated with a chromosomal anomaly and mental retardation. A case report. J Reprod Med 1990;35(3):301–2.
[50] Friedman MJ, Sharieff GQ. Seizures in children. Pediatr Clin North Am 2006;53(2):257–77.
[51] Striano S, Striano P, Tortora F, Elefante A. Intractable epilepsy in Turner syndrome associated with bilateral perisylvian hypoplasia: one case report. Clin Neurol Neurosurg 2005;108(1):56–9.
[52] Tatum 4th WO, Passaro EA, Elia M, Guerrini R, Gieron M, Genton P. Seizures in Klinefelter's syndrome. Pediatr Neurol 1998;19(4):275–8.
[53] Kawawaki H, Kusuda S, Kurimasa H, Tomiwa K, Murata R. [Seizures associated with fever in children of congenital adrenal hyperplasia]. No to hattatsu. Brain Dev 2001;33(1):27–30.
[54] Grosso S, Farnetani MA, Di Bartolo RM, Berardi R, Pucci L, Mostardini R, Anichini C, Bartalini G, Galimberti D, Morgese G, Balestri P. Electroencephalographic and epileptic patterns in X chromosome anomalies. J Clin Neurophysiol 2004;21(4):249–53.
[55] Mazzanti L, Cacciari E. Congenital heart disease in patients with Turner's syndrome. Italian Study Group for Turner Syndrome (ISGTS). J Pediatr 1998;133(5):688–92.
[56] Kusuma L, Bharath AP, Savitha RM, Balasundaram K, Nallur BR. A rare case of congenital heart disease with ambiguous genitalia. Indian J Hum Genet 2010;16(3):166–8.
[57] Norwood VF, Chevalier RL. Renal Developmental Disorders of the Fetus and Newborn. In: Rudolph CD, Rudolph AM, editors. Rudolph's Pediatrics. New York, NY: McGraw-Hill; 2003. p. 1638–42.
[58] Bernstein J. The kidneys and the urinary tract. In: Rudolph AM et al., editors. Rudolph's Pediatrics. Stamford, CT: Appleton & Lange; 1996. p. 1347–405.
[59] National Institute of Diabetes & Digestive & Kidney Diseases, National Institute of Health web site. Available online at http://www.niddk.nih.govhealth/kidney/kidney.htm. through http://www.niddk.nih.govhealth.
[60] Saenger P. Turner's syndrome. N Engl J Med 1996;335(23):1749–54.
[61] Mojtaba B, Kent Mancer JF, Brian EH, Bernard MC, John DB. External genital abnormalities associated with Wilms tumor. Urology 1984;24:113–30.
[62] Stephen R, Anna K. 9 Diabetes secondary to genetic disorders. Baillière's Clin Endocrinol Metab 2005;6(4):867–98.
[63] Monique P, Paul W. Hearing loss in Turner syndrome. Int J Pediatr Otorhinolaryngol 2009;73(2):243–7.
[64] Ahmadreza O, Mahin H, Alireza M, Mehdi S, Masoumeh R, Saied HO. Otologic problems in Turner syndrome. Audiology 2011;19(1):57–62.
[65] Zaki MS, Kamel AA, EL-Ruby M. Double aneuploidy in three Egyptian patients: Down-Turner and Down-Klinefelter syndromes. Genetic Counseling 2005;16(4):393–402.
[66] Sparagana M, Wong PWK, Dorsch TR, Casten C, Rauer M, Szego K. J Med Genet 1980;17:319–21.
[67] Musarella MA, Verma RS. An infant with Turner-Down aneuploidy and massive capillary hemangioma of the orbit: a case report with review. Annales de Génétique 2001;44(2):67–70.
[68] Rabah MS, El-Awady Mohamed Y, Elsayed Solaf M, Hamadan Ghada E. Consanguineous matings among Egyptian population. Egypt J Med Hum Genet 2011;12(2):157–63.
[69] William GR. Gender identity and sex-of-rearing in children with disorders of sexual differentiation. J Pediatr Endocrinol Metab 2005;18(6):549–53.
[70] Schweizer K, Brunner F, Schützmann K, Schönbucher V, Richter-Appelt H. J Counseling Psychol 2009;56(1):189–201.
[71] Ismail SI, Mazen IA. A study of gender outcome of Egyptian patients with 46, XY disorder of sex development. Sex Dev 2010;4(4–5):285–91.
[72] Lee PA, Houk CP. Disorders of sexual differentiation in the adolescent. Ann N Y Acad Sci 2008;1135:67–75.