
DISORDERS OF ADRENAL GLANDS AND SEX DEVELOPMENT IN CHILDREN: INSIGHTS FROM THE TROPICS

Nishant Raizada MD, DM Endocrinology, Associate Professor and Head, Department of Endocrinology, Center for Diabetes, Endocrinology and Metabolism, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India. drnishantraizada@gmail.com

Phibakordor L. Nonglait MD, Senior Resident, Department of Endocrinology, Center for Diabetes, Endocrinology and Metabolism, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India. phiba27@gmail.com

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ABSTRACT

The adrenal gland is essential for survival and its function is compartmentalized into specific zones. Disorders of the adrenal gland can be classified as those affecting the adrenal cortex or medulla. Pediatric adrenal disorders can have distinct presentations and etiologies in comparison to adults, such as adrenal insufficiency associated with genetic syndromes or Cushing's syndrome associated with adrenocortical tumors and primary pigmented nodular adrenocortical disease. Congenital adrenal hyperplasia (CAH) has been commonly reported from the tropics, and rare variants of CAH have also been recognized in populations where consanguinity is prevalent. Pheochromocytomas and paragangliomas (PPGL) have been reported from tropical countries, some with rare presentations. The frequent rate of heritability and mutations in PPGL highlights the importance of genetic studies among children. The role of functional imaging is evolving for PPGLs as data is emerging from cohort studies. Disorders of Sex Development (DSD) comprise a heterogeneous group of disorders that can present in any age group. DSDs in childhood usually present with ambiguous genitalia and a multidisciplinary approach is required for its management. The diagnosis of adrenal disorders can sometimes pose a challenge in tropical countries due

to resource constraints, lack of awareness, and access to medical care. However, available data from cohort studies and case reports have highlighted differences in etiology and presentation as compared to other parts of the world and the need for further studies.

INTRODUCTION

Adrenal disorders commonly seen in the tropics include adrenal insufficiency, congenital adrenal hyperplasia, adrenal Cushing's syndrome, and pheochromocytoma/paragangliomas

Adrenal Insufficiency

It is characterized by decreased production of cortisol by the adrenals. The identification of adrenal insufficiency in children requires a high index of suspicion. This is important not only to prevent an adrenal crisis but to identify the associated comorbidities. Acute adrenal crisis can present in infancy as a salt-wasting crisis or precipitated in children due to stressors such as illness, trauma, or surgery. They often present as an emergency with abdominal pain, vomiting, hypotension, hypoglycemia with seizures, and hyponatremia which eventually leads to shock and cardiovascular collapse if

undiagnosed. Chronic adrenal insufficiency presents as prolonged neonatal jaundice, failure to thrive, hyperpigmentation, anorexia, fatigue, nausea and vomiting, salt craving, diarrhea, abdominal pain, postural hypotension, and tachycardia. A study from Pakistan characterized the presentation of children with adrenal insufficiency of which 19% presented with an adrenal crisis following an acute illness (1). The chronic symptoms reported were not different from that seen in another cohort from South Africa (2). Rare primary presentations of adrenal insufficiency as infantile cholestasis (3) and gigantism with motor delay have been reported (4).

The causes of adrenal insufficiency in children are different as compared to adults. Etiologically it can be divided into primary and secondary adrenal insufficiency. It can also be seen as an isolated condition or in association with specific syndromes.

Primary adrenal insufficiency may be related to an underlying genetic or metabolic cause. Congenital Adrenal Hyperplasia (CAH) is the most common cause of primary adrenal insufficiency. Autoimmunity, infections, and hemorrhage are also important causes of primary adrenal insufficiency. The largest cohort study from Sudan diagnosed 80 children with adrenal

insufficiency. The etiology ranged from Allgrove syndrome (36%), auto-immune polyendocrinopathy syndrome (11%), adrenoleukodystrophy (9 %), bilateral hemorrhage (1%), to unspecified (42%) (5). Case reports and series also reported similar causes such as Allgrove syndrome (6-8), adrenoleukodystrophy (9), to rare causes such as familial primary glucocorticoid deficiency (3), Steroidogenic acute regulatory protein (StAR) deficiency (10), Nuclear receptor subfamily 0, group B, member 1 (NR0B1) gene or DAX1 gene mutation (11) as well as primary multidrug-resistant adrenal tuberculosis (12).

The diagnosis of adrenal insufficiency is made by a peak cortisol value less than 18 mcg/dl on ACTH (Synacthen) stimulation test. A raised plasma ACTH level confirms primary adrenal insufficiency. The dose of Synacthen recommended for children less than 2 years is 15 µg/kg body weight and for children more than 2 years 250 µg im. However, Synacthen is not easily available in many countries. Acton Prolongatum, a long-acting synthetic ACTH preparation of the 39-amino acid native porcine sequence in a carboxymethylcellulose base has been studied and validated in India for the diagnosis of adrenal insufficiency in children > 5 years (13).

| Table 1. Acton Prolongatum (ACTH Stimulation) Test | |
|---|---|
| Indication | To diagnose adrenal insufficiency * |
| Preparation | Injection Acton Prolongatum® Ferring pharmaceuticals (Saint Prex, Switzerland) is available as a 5-mL vial with a concentration of 60 IU/mL. To prepare 25 IU** of Acton Prolongatum, 0.4 ml of Acton Prolongatum is taken in 1 ml syringe and diluted with 0.5 ml NS |
| Performing the test | After overnight fast, basal sample for cortisol is taken at 8 AM and 25 IU of Acton Prolongatum is injected intramuscularly over the deltoid. One hour (9 AM) post stimulation, a second cortisol sample is taken |
| Interpretation | Peak cortisol (at 60 minutes) <18 mcg/dl: suggestive of adrenal insufficiency (94% specific and 57% sensitive) Peak cortisol (at 60 minutes) >22 mcg/dl : rules out adrenal insufficiency |

NB: * Test is validated for children above 5 years (13). ** Studies in adults have also been done with 30 IU of Acton Prolongatum (0.5 ml) (95) (96).

Congenital Adrenal Hyperplasia

CAH is a group of autosomal recessive disorders characterized by enzymatic defects in adrenal steroidogenesis and diminished cortisol synthesis. The accumulation of precursors proximal to the blocked pathway and hypocortisolemia are responsible for the clinical features of these disorders. The presentation is varied and includes early classic presentation of salt-wasting (SW) and simple virilizing (SV) disorder to the non-classical presentation. Rare presentations of CAH as adrenal insufficiency (14), genital ambiguity (15) (16), hypoglycemia (17, 18), and precocious puberty (19) due to enzyme defects other than 21 hydroxylase deficiency have also been reported.

Newborn screen (NBS) for CAH from India revealed a prevalence of 1 in 576 (20). However retrospective data in the absence of NBS revealed the presentation of adrenal crisis in >80% of subjects with 70% presenting as SW-CAH and a delayed diagnosis in boys as compared to girls highlighting the importance of NBS (21).

CAH due to 21 hydroxylase deficiency (21OHD) accounts for 90-95% of the cases followed by 11 β -hydroxylase deficiency (11 β OHD), and 3 β -hydroxysteroid dehydrogenase 2 (3 β HSD2). Regional

differences in the prevalence of enzyme deficiencies confirmed by genetic tests have been described from Cameroon (n=24) which found that 11 β OHD was more common (66.6%) followed by 21OHD and as well from Algeria (n=273) which showed that 3 β HSD2 (5%) was the second most common form after 21OHD. These differences may be attributed to the founder mutations (22, 23).

Diagnosis is made by screening for 17 OH Progesterone (OHP) which is elevated, followed by 17 OHP and other steroid responses to synacthen test. However, confirmation of specific enzyme deficiency requires genetic testing. The spectrum of genetic mutations has been described in various cohorts for CYP21A2, which was able to diagnose mutations in 80-96% of the subjects, and genotype-phenotype correlations have been established for various forms of CAH (24-27). Additionally, allele-specific PCR for screening common CYP21A2 mutations has been suggested as a cost-effective tool, especially in resource-constraint settings (28). The diagnosis of other enzyme deficiencies is often challenging due to a lack of genetic tests and steroid precursor assays. However, studies are emerging for other CAH variants such as 11 β OHD from India (29) and 3 β -hydroxysteroid dehydrogenase 2 (3 β HSD2) deficiency from Algeria (23) with the discovery of novel mutations

indicating genetic heterogeneity. Combined genetic mutations have also been reported (30).

A child diagnosed with CAH requires lifelong treatment and monitoring. A longitudinal data from Egypt indicated that CAH subjects with older age, poor hormonal control, and frequent hospitalizations have relatively poorer health-related quality of life. The challenges faced in the management of CAH include late diagnosis, poor follow-up (31), and the development of adrenal rest tumors (23, 29).

Cushing Syndrome

Cushing syndrome is suspected in a child who presents with weight gain and growth failure. The characteristic cushingoid features described in adults are usually not seen and they often present with generalized obesity. Endogenous Cushing syndrome varies with the age of diagnosis with adrenal tumors predominating in children < 7 years and Cushing disease after 7 years. However, it is important to note that the most common cause of Cushing's syndrome is exogenous and even topical routes of administration have been implicated in children (32-34).

Of the ACTH-independent Cushing syndrome, primary pigmented nodular adrenocortical disease (PPNAD) has been the most frequently described from the tropics in case series and reports some of which have been found in association with Carney's complex (35-39). The other important cause reported is in association with Adrenocortical tumors as described below.

Adrenocortical Tumors

These tumors account for 0.2% of all pediatric tumors. The largest case series from India with 17 cases reported that 82% presented with endocrine dysfunction, of which the most common was Cushing syndrome with or without virilization seen in 53% of the subjects (40). Another cohort of 7 children from Sri Lanka also reported peripheral precocious puberty in all the subjects and one boy had the phenotypic

features of Beckwith–Wiedemann syndrome (41). Case reports have also reported similar presentations some of which are the rare variants of adrenocortical oncocytoma (42-49). Large non-functioning adrenal cortical carcinoma can present with mass effects without any features (40, 50). The prognosis depends on the diagnosis with adenomas having complete remission. However, the prognosis of subjects with carcinoma was poor (40) (41).

Pheochromocytomas and Paragangliomas

Pheochromocytoma (PCC) refers to the catecholamine-producing tumor of the adrenal medulla whereas paragangliomas (PGL) are extra-adrenal tumors of sympathetic and parasympathetic ganglia. Of the PPGLs, 10-20% occur in the pediatric age group. There is a high rate of germline mutations and heritability in pediatric PPGLs. A cohort of 30 children from India with PPGL showed that 26.7% of the subjects had syndromic or familial association, of which Von Hippel-Lindau was the most common. Fourteen (46.7%) children had germline mutations (VHL 10 (33.3%), SDHB 2 (6.6%), and SDHD 2 (6.6%). Bilateral pheochromocytomas and symptomatic presentation was more frequent in children as compared to adult PPGL. Children with VHL mutation had more frequent bilateral PCC, coexisting PGL and recurrence (51).

PPGLs often mimic other diseases and rare presentations such as myocarditis (52), diabetes insipidus (53), hypertensive encephalopathy (54) (55), Cushing syndrome (56), pseudo renal artery stenosis (57), and papilledema (58) have been described.

After biochemical confirmation, imaging studies are advised for anatomical localization. Functional imaging is recommended for larger tumors, suspected multifocal or extra adrenal tumors, succinate dehydrogenase subunit B (SDHB) or alpha-thalassemia/mental retardation syndrome X-linked mutations (ATRX) and dopamine secreting PPGLs. A cohort study from India revealed that 68Ga-DOTATATE PET/CT (95%) had a higher sensitivity than 18F-FDG-PET/CT (80%) and 131I-MIBG (65%)

for overall lesions. 68Ga-DOTATATE PET/CT was more sensitive than 131I-MIBG (93 vs. 42%) for detecting metastases (59). The definitive management of PPGL is surgical resection. Pre-operative preparation with experienced anesthetic (60) and surgical team (61) is important for successful outcomes following surgery. The management of metastatic PPGL is challenging especially in countries with limited resources. Fractionated low dose 131 I-metaiodobenzylguanidine (MIBG) therapy has been used in the treatment of metastatic paraganglioma (62). Lifelong surveillance is recommended in children to detect early recurrence (63).

DISORDERS OF SEX DEVELOPMENT (DSD)

DSD is a condition in which chromosomal, gonadal, or anatomical sex is atypical (64). Observational studies from Egypt and Cameroon reported that these constitute 2-9.4% of the subjects presenting to endocrine clinics (65, 66).

Epidemiology

DSDs can be broadly classified into sex chromosomes, 46 XX and 46 XY DSDs. Cohort studies have revealed a prevalence of 5-15 % for sex chromosomal DSDs, 33.7-71% for 46 XY DSD, and 24-51% for 46 XX DSD (65-67). Regional differences were observed in the prevalence of these disorders attributed to consanguinity and endogamous marriages (66).

Table 2. Classification of DSDs

| SEX CHROMOSOME DSD | 46 XY DSD | 46 XX DSD |
|---|--|--|
| Turner's syndrome (and 45X variants) | Disorders of testis development Complete testicular dysgenesis (Swyer syndrome) Partial gonadal dysgenesis Testicular regression | Disorders of ovarian development Gonadal dysgenesis Ovotesticular DSD RSPO gene mutation NR5A1 gene mutation Testicular DSD SRY+ SOX9/SOX3 duplication WNT 4 mutation |
| Klinefelter's syndrome (and 47XXY variants) | Disorders of androgen synthesis STAR mutation CAH 3 β -hydroxysteroid dehydrogenase 2 17 α -hydroxylase/17,20-lyase P450 oxidoreductase Isolated testosterone deficiency 17 β -hydroxysteroid dehydrogenase | Androgen excess CAH 21-hydroxylase 3 β -hydroxysteroid dehydrogenase 2 P450 oxidoreductase 11 β -hydroxylase Glucocorticoid receptor mutations Maternal |

| | | |
|---|--|---|
| | 5 α -reductase 2 | Virilising tumors Exogenous androgens |
| Mixed gonadal dysgenesis Ovotesticular DSD | Disorders of androgen action Androgen insensitivity syndrome Luteinizing hormone receptor defects | Others Mullerian agenesis (MRKH syndrome) Uterine abnormalities Syndromic associations (cloacal exostrophy) |
| | Others Persistent mullerian duct syndrome Complex syndromic disorders Isolated hypospadias | |

Clinical Features

DSDs have a varied presentation which includes ambiguous genitalia of varying severity, primary amenorrhea, and virilization at puberty to infertility in adulthood. The recognition of DSDs has critical implications due to their syndromic associations such as Wilm's tumor and renal failure with Denys-Drash syndrome, adrenal insufficiency with CAH, and future risk of gonadoblastoma. In addition, there are long-term social and psychological impacts such as gender of rearing and fertility prospects.

46 XY DSD

46 XY DSD can be classified as disorders of testis development, androgen synthesis, or androgen action.

The most common DSD reported among these are disorders of androgen synthesis of which 5 α reductase deficiency is the most commonly reported with a prevalence of 10%- 33% with a presentation as ambiguous genitalia (65-68). The higher rates reported in recent literature are attributed to the

genetic confirmation some of which are novel and founder mutations, as opposed to the earlier diagnosis based on biochemical ratios of Testosterone: Dihydrotestosterone (69-71). Rare variants of CAH with presentation as infertility, hypertension, or virilization been reported (15, 72-74).

Androgen insensitivity syndrome (AIS); partial (PAIS) or complete (CAIS) is the next most commonly reported 46 XY DSD from various countries with a prevalence of 5-28% (65-67, 75, 76). However, cohort studies with genetic confirmation reported a prevalence of 10-38% (77, 78). A point to be noted was that only 31% of patients with a provisional diagnosis of PAIS had pathogenic variants in the AR gene (78). Patients with CAIS are reared as females and have a later presentation with primary amenorrhea. The presentation of PAIS may be earlier with atypical genitalia or gynecomastia.

The third most commonly reported cause is gonadal dysgenesis which can be partial or complete with a prevalence of 4-10% (65-67, 79). Case reports of gonadal development disorders with dysgenesis are also emerging which include WT-1 mutation (80-82),

Desert hedgehog (DHH) gene (83), and Mitogen-activated protein 3 kinase 1 (*MAP3K1*) gene (84).

Other rare causes such as persistent Mullerian duct syndrome (85, 86) and Leydig cell hypoplasia (87) have been reported from the Middle-eastern countries.

Syndromic causes of 46 XY DSD accounts for 1-1.8% of the cohort studies cited earlier.

46 XX DSD

In contrast to the 46 XY DSDs which can have variable presentation and etiology, the most common cause of 46 XX DSD is CAH of which 21 hydroxylase deficiency is the most common cause. However, Sap et al from Cameroon reported 11 hydroxylase was the most common cause of CAH in their population (66). The prevalence of 46 XX DSD ranges from 20%-55% (65-67).

The other important causes of 46 XX DSD are ovotesticular DSD (16.2%) and vaginal atresia (2%). Rare case reports of aromatase deficiency (88) and isodicentric Y chromosome in 45 X individuals have been reported (89).

Management

The diagnosis and management of DSDs are challenging, especially in countries with low

resources. The most important step in the initial evaluation of ambiguous genitalia is the presence of gonads which gives us a clue in narrowing the cause and guiding further workup. Karyotyping, imaging by pelvic USG or MRI, followed by biochemical evaluation helps in establishing a diagnosis. The emergence of genetic tests has further simplified the evaluation of such patients and will prove to be a valuable tool in the future.

Diagnosis of DSD and gender assignment has lifelong implications for the patients. There have been reports of gender change and gender identity confusion especially in 46 XY DSDs (90-92). However, patients with AIS have less prevalence of gender dysphoria (77, 92).

For 46 XX DSDs with virilization, feminizing genitoplasty is an important concern especially the timing of surgery. An observational study from Malaysia of 59 females with CAH who had undergone feminizing genitoplasty (FG) reported that infancy and early childhood as the best timing for first FG, most preferring single-stage over 2-stage surgery (93).

Data regarding the risk of gonadoblastoma and prophylactic gonadectomy is scarce. A case series of 5 subjects of 46 XY DSD reared as females revealed malignancy in only one patient with CAIS (94).

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