## *Brief Reports*

# 21-Hydroxylase Deficiency: Clinical Features, Laboratory Profile and Pointers to Diagnosis in Indian Children

**Anurag Bajpai, Madhulika Kabra and P.S.N. Menon**

*From the Division of Pediatric Endocrinology, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi, India.*

*Correspondence to: Dr P.S.N. Menon, Professor, Genetic Unit, Department of Pediatrics, Old Operation Theatre Block, All India Institute of Medical Sciences, New Delhi 110 029, India. Email: psnmenon@hotmail.com*

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*We evaluated clinical features, laboratory profile and pointers to diagnosis of 21-hydroxylase deficiency in children presenting to the Pediatric Endocrine Clinic of our hospital from 1990 to 2002. Of the 94 patients included in the study 46 had salt wasting form (SW, 21 girls), 44 simple virilizing form (SV, 34 girls) and 4 non-classical form of the disease (NC, all girls). No difference was observed in the mean (95% confidence interval) age at diagnosis in boys and girls with salt wasting (2.3 mo (0.7-3.9 mo) against 1.3 mo (0.9-1.7 mo), p not significant) despite the presence of genital ambiguity in all girls at birth. Diagnosis of salt wasting was missed at admission in 18 boys (72%) and 3 girls (14.3%) highlighting the need for high index of suspicion for the disorder. Eight patients with 46 XX karyotype (14.5%) had male-like external genitalia with cryptorchidism emphasizing the need for evaluation of boys with cryptorchidism for female pseudohermaphroditism. Our study reiterates the need for early recognition and management of 21-hydroxylase deficiency in children in countries where neonatal screening programs are not feasible.*

**Key words:** *Ambiguous genitalia, Congenital adrenal hyperplasia, 21-hydroxylase deficiency.*

Congenital adrenal hyperplasia (CAH) comprises a group of inherited disorders of enzymes involved in steroid biosynthesis(1). 21-hydroxylase deficiency, the commonest form of the disease accounting for 95% of all cases, is characterized by deficiency of glucocorticoid and mineralocorticoid on one hand and androgen excess on the other(2). Manifestations of the disease depend on the residual enzyme activity and vary from the most severe salt wasting form (SW) to virilizing form (SV) and to the mildest non-classical form (NC)(3). Early identification of the disease is essential for reducing the mortality and morbidity associated with the disease. There is paucity of epidemiological data on 21-hydroxylase deficiency in developing countries(4,5). This study was conducted to evaluate clinical and laboratory features and to identify the pointers to diagnosis of 21-hydroxylase deficiency in Indian children.

## Subjects and Methods

Children with 21-hydroxylase deficiency presenting to the Pediatric Endocrine Clinic of our hospital from 1990 to 2002 were included. 21-hydroxylase deficiency was diagnosed in

appropriate clinical setting with elevated basal and/or ACTH stimulated 17-hydroxyprogesterone (17OHP) levels(6). Diagnostic criteria used in the study are provided in *Table I*(7). Patients were evaluated for age at onset of symptoms and at diagnosis, presenting features, diagnosis at first contact and clinical features. Family history of 21-hydroxylase deficiency, deaths in siblings and consanguinity were noted. Prader staging was done to assess the extent of ambiguity(8).

Investigations included estimation of blood pH, bicarbonate, sodium, potassium, urea, creatinine, dehydroepiandrosterone (DHEA), testosterone and basal 17OHP in all subjects. Adrenocorticotropin (ACTH) stimulation test was performed if baseline 17OHP levels were not sufficient to establish the diagnosis. Additionally, karyotyping and genitogram were done for girls; and testicular ultrasound and GnRH stimulation test for boys, if indicated. Unpaired student's t test was used for comparing quantitative parameters. Values are expressed as mean (95% confidence interval) [range] unless otherwise specified.

## Results

Ninety-four patients of 21-hydroxylase deficiency (46 salt wasting, 44 simple virilizing and 4 non-classical) were enrolled in the Pediatric Endocrinology Clinic of our hospital during the period of study. Salt wasting form was encountered more frequently in boys (25 against 21) while the simple virilizing form was commoner in girls (34 against 10). Family history of 21-hydroxylase deficiency was present in seven families with salt wasting (10 patients) and two with simple virilizing (four patients) while sibling deaths were reported in seven patients with salt wasting. Family history of consanguinity was present in nine patients.

*Age at diagnosis*

Patients with salt wasting form of disease were diagnosed at an earlier age (1.8 mo (0.9-2.7 mo) [1 d-18 mo]) than those with simple virilizing form (31.5 mo (20.2- 42.8 mo) [10 d-10 yr 9 mo]), ($p < 0.001$). Boys were diagnosed later than girls in both the forms. Girls with non-classical form of the disease were diagnosed at a mean (SD) age of 8 (1.8) yr.

Most patients with salt wasting form of the disease were diagnosed between second and sixth weeks of life. Significant gap was noted in the age at onset of symptoms and that at

**TABLE I--***Diagnostic Criteria for 21-hydroxylase Deficiency*(7).

| Category | Salt wasting* | Prenatal virilization** | 17OHP*** |
|---|---|---|---|
| Salt wasting (SW) | Yes | Yes | > 20,0000 ng/dL |
| Simple virilizing (SV) | No | Yes | 10000-20000 ng/dL |
| Non-classical (NC) | No | No | 1500-10000 ng/dL |

* Salt wasting defined as shock and serum sodium < 125 mEq/L or potassium > 6 mEq/L or serum sodium < 128 mEq/L and potassium >5 mEq/L.

** Labial fusion considered as a marker for prenatal virilization. Girls with isolated clitoromegaly without salt wasting and labial fusion were categorized as non-classical form. All boys diagnosed as simple virilizers in the absence of salt wasting.

*** Basal/ ACTH stimulated(6).

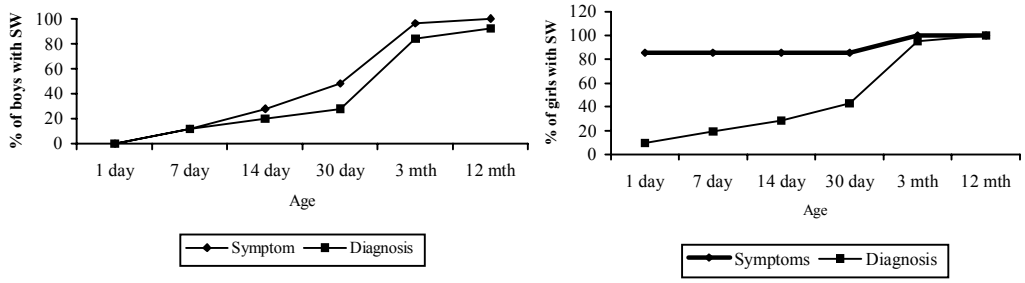
diagnosis in girls; no such difference was observed in boys (*Fig. 1*). Genital ambiguity was noted in 18 girls with salt wasting form of the disease (85.7%) at birth; the diagnosis of 21-hydroxylase deficiency was however established in only eight girls before the advent of clinical features of salt wasting. Diagnosis was delayed in three girls with salt wasting beyond the age of six weeks due to failure to identify genital ambiguity. Two of these had been assigned male sex at birth; third had mild clitoromegaly. Seven boys with salt wasting form of disease were diagnosed before the onset of clinically evident salt wasting due to biochemical features of adrenal insufficiency in five and family history of disease in two.

*Clinical features*

Clinical features of salt wasting form are presented in *Table II*. All boys with simple virilizing form of the disease presented with precocious puberty. Majority of these subjects had prepubertal testicular volume; pubertal testicular volume was observed in three boys. GnRH stimulation test in these subjects was suggestive of central precocious puberty. Testicular ultrasound did not reveal evidence of adrenal rests in these patients. Genital ambiguity was noted in fifty-five girls (*Table III*). Eight had been assigned male sex at birth while no sex assignment had been done in five. Prader stage III and above was observed in a greater proportion of girls with simple virilizing form of disease compared to those

**TABLE II**–*Clinical Features and Diagnosis of Patients with Salt Wasting Form.*

| Clinical features | Boys (n = 25) | Girls (n = 21) |
|---|---|---|
| Salt wasting crisis | 18 (72%) | 13 (61.9%) |
| Failure to thrive | 14 (56%) | 12 (57.1%) |
| Hyperpigmentation | 10 (40 %) | 7 (33.3%) |
| Recurrent vomiting | 7 (28%) | 6 (28.6%) |
| Polyuria | 7 (28%) | 4 (19%) |
| Pubarche | 3 (12%) | 3 (14.3%) |
| Hypoglycemic seizures | 3 (12%) | 1 (4.8%) |
| Renal vein thrombosis and hematuria | 2 (4.3%) | – |
| Ambiguous genitalia | – | 21 (100%) |
| Penile enlargement | 4 (16%) | – |
| **Diagnosis at admission** | | |
| CAH | 7 (28%) | 18 (85.7%) |
| Septicemia | 6 (24%) | 3 (14.3%) |
| Gastroenteritis | 5 (20%) | – |
| Hypertrophic pyloric stenosis | 3 (12%) | – |
| Renal failure | 3 (12%) | – |
| Intracranial hemorrhage | 1 (4%) | – |




(*a*) (*b*)

*Fig. 1. Age at first symptom and diagnosis in salt wasting form a) boys, b) girls.*

**TABLE III–***Details of Female Patients with Ambiguous Genitalia.*

| Feature | Salt wasting (n = 21) | Simple virilizing (n = 34) | Total (n = 55) |
|---|---|---|---|
| **Prader staging** | | | |
| I | 3 (14.3 %) | 2 (5.9%) | 5 (9.1%) |
| II | 5 (23.8%) | 7 (20.6%) | 12 (21.8%) |
| III | 9 (42.8%) | 20 (58.8%) | 29 (52.7%) |
| IV | 4 (19.1%) | 3 (8.8%) | 7 (12.7%) |
| V | 0 | 2 (5.9%) | 2 (3.7%) |
| **Sex assignment at birth** | | | |
| Female | 16 (76.2%) | 26 (76.5%) | 42 (76.4%) |
| Male | 3 (14.3%) | 5 (14.7%) | 8 (14.5%) |
| Unassigned | 2 (9.5%) | 3 (8.8%) | 5 (9.1%) |

with salt wasting form (73.5% against 61.9%); the difference was however not significant (p >0.05). Uterus could not be identified on ultrasound in seven girls and magnetic resonance imaging was required.

*Diagnosis*

The diagnosis of salt wasting form of the disease had been missed in 21 patients {18 boys (72%) and 3 girls (15.7%)} at admission (*Table II*). The diagnosis of salt wasting form was suspected in these patients only after the investigations were suggestive of adrenal insufficiency. Treatment with hydrocortisone was started as an emergency measure in seven patients before endocrine investigations. Diagnosis was confirmed in these patients later by ACTH stimulation test.

## Discussion

The number of patients with salt wasting form of 21-hydroxylase deficiency was similar to those with simple virilizing form in this study. Previous case survey based studies on the epidemiology of 21-hydroxylase deficiency in the developing countries have shown that simple virilizing form of the disease is more common than the salt wasting form (4,9-14). Salt wasting form accounted for only 36.3% patients in a study involving 219 Turkish children with classical 21-hydroxylase deficiency(11). Similar findings were observed in studies from India and Singapore, which showed a salt wasting to simple virilizing ratio of 1:1.6 and 1:1.8 respectively(4,13). The case survey based studies in the developed countries show that the salt wasting form of 21-hydroxylase deficiency is nearly twice more common than the simple virilizing form (9,10,12). This suggests that a greater proportion of patients with salt wasting form are being missed in developing countries compared to the developed countries.

Girls with salt wasting form have ambiguous genitalia at birth and are therefore more likely to be diagnosed compared to boys who do not have any external abnormality. This can explain female preponderance observed in case survey based studies on 21-hydroxylase deficiency despite the fact that

## Key Messages

- Diagnosis of salt wasting 21-hydroxylase deficiency is missed in a significant proportion of Indian boys.
- Diagnosis of 21-hydroxylase deficiency is delayed in Indian girls despite the presence of ambiguous genitalia.
- Salt wasting form of 21-hydroxylase deficiency should be considered in all neonates with genital ambiguity, cryptorchidism, features of septicemia in the absence of obvious infection, hyperpigmentation, polyuria, recurrent vomiting, unexplained failure to thrive and biochemical features of adrenal insufficiency.

the disease is inherited in an autosomal recessive fashion. However in our study number of boys with salt wasting form of disease was similar to that of girls and no difference was noted in the age at diagnosis between the two sexes. This suggests that the diagnosis of salt wasting form of the disease was missed in girls as well as boys. This may be due to delay in referral of patients with ambiguous genitalia. This emphasizes the need of increasing awareness on the part of primary health care providers about the need of the urgent evaluation of children with ambiguous genitalia.

The diagnosis of salt wasting form of disease was not considered in as many as 72% boys at the time of admission. This reiterates the need for identification of pointers to the early diagnosis of salt wasting form in boys. Presence of failure to thrive, recurrent vomiting, polyuria, hyperpigmentation, biochemical features of adrenal insufficiency and family history of 21-hydroxylase deficiency or sibling death in an infant strongly favors the diagnosis of salt wasting form of 21-hydroxylase deficiency and warrants steroid replacement after obtaining sample for 17 OHP(15). Treatment should be started even if estimation of levels of 17OHP is not possible. Diagnosis of the disease can be confirmed later by an ACTH stimulation test while on treatment. If ACTH stimulation test is not possible, discontinuing steroids and estimating 17 OHP after four times the half-life of the steroid (2 days for hydrocortisone and 6 days for prednisolone) can help in diagnosis(16).

The extent of ambiguity in girls in our study was not influenced by the form of disease (salt wasting or simple virilizing) or by the age at diagnosis. This suggests that prenatal androgen exposure is the primary determinant of the extent of ambiguity. Postnatal androgen exposure can cause mild clitoral enlargement and virilization. Normal male phenotype with cryptorchidism has been reported in girls with 21-hydroxylase deficiency as observed in eight patients in our series(19). Cryptorchidism in a boy thereby may indicate the diagnosis of female psuedo-hermaphroditism, which should be further evaluated by confirmation of mullerian structures (rectal examination and imaging) followed by estimation of 17 OHP levels. Identification of mullerian structures with ultrasound may be difficult in infantile period as observed in our study. It is however important to emphasize that elevated 17 OHP levels in the presence of normal karyotype (46 XX) is sufficient for the diagnosis of 21-hydroxylase deficiency in a child with ambiguous genitalia. MRI pelvis for

identification of mullerian structures is only of academic significance under these circumstances.

Our study emphasizes the need for early diagnosis and management of children with 21-hydroxylase deficiency. Neonatal screening for 21-hydroxylase deficiency is not feasible in resource poor countries like India making early clinical identification of the condition important. This can be achieved by increasing the awareness about indicators of this life threatening disease, as outlined in our report.

*Contributors:* AB, MK and PSNM were involved in management of patients. AB planned the study, collected data, performed literature review and drafted the manuscript. MK was involved in planning the study and reviewed the manuscript. PSNM designed the study and critically reviewed the manuscript and will act as its guarantor.

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# Long-term Antibody Response and Immunologic Memory in Children Immunized with Hepatitis B Vaccine at Birth

**M.J. Saffar and M.S. Rezai**

*From the Pediatric Infectious Diseases Ward, Department of Pediatrics. Mazandaran Medical Sciences University, Boalisina Hospital, Pasdaran Boulevard, Sari- Iran.*

*Correspondence to Dr. M.J.Saffar, Department of Pediatrics, Mazandaran Medical Sciences University, Boalisina Hospital, Pasdaran Boulevard, Sari- Iran. E-mail: saffar@softhome.net.*

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*Four hundred and fifty three healthy children immunized with a course of hepatitis B vaccine beginning at birth were tested at 10-11 years of age for persistence of anti-hepatitis B-S antigen antibody (anti-HBs); and responses of children without protective antibody to different doses of hepatitis B vaccine booster were evaluated. Although nearly 42% of them were not seroprotected, but most of boosted subjects (87.3%) retained robust immunologic memory and rapidly retained a protective anti-HBs antibody titer of at least 10 IU/L after booster vaccination.*

**Key words:** *Hepatitis B vaccine Booster, Immunologic memory, Long-term immunity.*

Hepatitis B virus (HBV) infection remains a worldwide health problem. There are an estimated number of 350 million hepatitis B carriers globally, who are faced with significant morbidity and mortality(1). Hepatitis B vaccine has been shown to be immunogenic and effective in preventing HBV infection(2). A potential problem of HBV immunization is that vaccine-induced anti-HBs antibody titers decline with time(3,4). Instances of late infection, occasionally resulting in HBV carriage, were documented in some long-term follow-up studies(4-6). The rapid decline of anti-HBs levels in children immunized at < 1 year of age poses some serious concern regarding the duration of immune response in this age group(3,5,7).

There is an increased risk for HBV infection during the sexually active years. Therefore, the duration of protection must last into these years. If the protection induced by HB vaccination at infancy dose not last until the adulthood, booster doses should be administered at the preschool entry ages, or during adolescence(3,4,8-11).