Assessment of the Degree of Clinical Suspicion of 21-Hydroxylase Deficiency Prior to the Newborn Screening Result

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Key words
neonatal screening, congenital adrenal hyperplasia, 21-hydroxylase deficiency, salt-wasting form, simple virilizing form

received 20.09.2022
accepted after revision 16.06.2023
accepted manuscript online 16.06.2023

Bibliography
Horm Metab Res 2023; 55: 528–535
DOI 10.1055/a-2111-6571
ISSN 0018-5043

Georg Thieme Verlag, Rüdigerstraße 14, 70469 Stuttgart, Germany

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ABSTRACT

The aim of the study was to analyze the clinical suspicion and where patients were when they received the positive result of the neonatal screening for CAH 21OHD. The present data derived from a retrospective analysis of a relatively large group of patients with classical CAH 21OHD patients nosed by newborn screening in Madrid, Spain. During the period from 1990 to 2015 of this study 46 children were diagnosed with classical 21OHD [36 with the salt-wasting (SW) form and 10 with simple virilizing (SV)]. In 38 patients, the disease had not been suspected before the neonatal screening result (30 SW and 8 SV). Thirty patients (79 %) were at home without suspicion of any disease, as healthy children, 3 patients (8 %) were at home pending completion of the study due to clinical suspicion of any disease (ambiguous genitalia, cryptorchidism) and 5 patients (13 %) were admitted to the hospital for reasons unrelated to CAH (sepsis, jaundice, hypoglycemia). It is relevant to note that 69.4 % of patients (25/36) with SW form were at home with potential risk of adrenal crisis. Six females had been incorrectly labeled as male at birth. The most frequent reason for clinical suspicion was genital ambiguity in women followed by family history of the disease. Neonatal screening provided better results than clinical suspicion. In the majority of patients with 21OHD the diagnosis by screening was anticipated to the clinical suspicion of the disease even in female patients with ambiguous genitalia.

Introduction

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder of adrenal steroidogenesis. More than 90 % of CAH cases are caused by deficiency of the enzyme 21-hydroxylase (21-OHD) [1–6]. It is characterized by a reduced ability to synthesize cortisol and aldosterone, coupled with the overproduction of adrenal androgens. Hormone synthesis can be affected to different degrees that result in a wide spectrum of severity and clinical onset during the neonatal period, childhood or adulthood [1–7]. Classics forms include salt-wasting (SW) forms, for which there is a high risk of life-threatening adrenal insufficiency during the first month of life, and simple-virilizing (SV) forms. In both cases, female neonates present with markedly virilized external genitalia. Salt wasting CAH is a rapidly evolving and life-threatening disorder. Babies with untreated, severe CAH typically present in the first month of life with vomiting, weight loss, dehydration, and shock and may die if the diagnosis and treatment are delayed. Nonclassic forms can manifest with hyperandrogenism later in life and do not warrant early recognition through neonatal screening.

Despite the fact that CAH is one of the most common inborn endocrine disorders, some patients are not identified, or may even die, in an acute salt-losing crisis. Neonatal screening program for

21-OHD began in 1977, when Pang et al. developed a specific radioimmunoassay to analyze 17-hydroxyprogesterone (17-OHP) in dried blood spot specimens collected on filter paper cards [8]. The objectives of 21-OHD screening are to detect newborns with the classic salt-wasting form before potential life-threatening shock develops, to prevent or rectify incorrect sex assignment of virilized female neonates, and to anticipate the diagnosis of boys with simple virilizing forms [1, 2, 9, 10].

However, screening remains controversial, with some main arguments against its routine use. One of them is the proportion of cases for which screening contributes to diagnosis is unclear, as most cases in females are easy to detect clinically and salt wasting can occur before the screening results are obtained. Screening programs do not confirm the diagnosis of 21-OHD and therefore cases must be evaluated individually. Some newborns present elevated 17-OHP levels in consecutive blood determinations that decrease spontaneously within the first months of life and are not diagnosed with CAH. This issue represents a disadvantage in terms of the psychological consequences for the patient's family and the cost to the health system. It is known that in the group of preterm neonates, 17-OHP levels are inversely proportional to gestational age and birth weight [11]. To minimize this limitation, new cutoff levels adjusted for both gestational age and birth weight have been stablished and new methods of analysis are used to decrease cross-reactivity. These anti-screening theses are no longer accepted. Neonatal CAH screening should not be viewed as a cost. Because delays in diagnosis may result in death, especially in 46, XY infants, and may lead to sexual identity preference problems in 46, XX cases.

Neonatal screening for 21-OHD was included in the neonatal screening program of the Madrid region of Spain in 1990, with a 21-OHD incidence of 1/19.211 [12].

The main objective of this study was to analyze the clinical suspicion and where the patients were when they received the result of the neonatal screening for 21-OHD. We retrospectively collected real-life screening data and clinical data for affected neonates to determine whether screening had facilitated case detection before clinical diagnosis.

Patients and Methods

Study population

The present data were derived from a retrospective analysis of a group of patients with classical 21-OHD patients discovered by newborn screening in Madrid, Spain.

In a retrospective study covering the period from 1990 to 2015, we analyzed the clinical suspicion and where the patients were when they received the result of the neonatal screening for 21-OHD and examined the time elapsing before diagnosis of CAH patients with follow-up in our hospital. CAH patients with classical forms were diagnosed according to clinical (SW and SV form) and biochemical data (increased 17-OHP and adrenal androgens, cortisol and aldosterone deficiency) and confirmed by molecular analysis. The patients were cared for in a single institution, over a period of 25 years, ensuring a fairly uniform approach and the continuity of care, thus making the comparisons more reliable.

Variables

The clinical data evaluated were where patients were when they received the results of newborn screening program, birth weight and length, diseases during the neonatal period and family history. The study variables were initial screening levels of 17-OHP, age at diagnosis by newborn screening, values for pH, glucose, electrolytes and serum 17-OHP determined at first evaluation, age of onset of treatment and CYP21A2 analysis.

Screening program

17-OHP was analyzed using dried blood spot specimens (Whatman #903) collected at 48 hours of life. Levels of 17-OHP were determined by fluoroinmmunoassay (AutoDELFIA; PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland).

Values higher than the 99th percentile for gender, born age and weight born are considered pathological. The 17-OHP cutoff limit for a positive test in neonates' weight-term-adequate for gestational age is 15 nmol/l (4.95 ng/ml). Children with 17-OHP > 30 nmol/l (9.9 ng/ml) are recalled immediately to the Pediatric Endocrinology Center for a clinical evaluation. If 17-OHP levels are between 15 and 30 nmol/l, a second analysis is performed. Preterm neonates and those who are small for gestational age have different threshold values, which are adjusted for gestational age and weight, as established by our laboratory.

Parents of newborns with a positive result are contacted by telephone and referred to the Pediatric Endocrinology Department for clinical evaluation. A pediatric endocrinologist then provides confirmatory examination and testing of all at-risk infants to provide the final diagnosis of CAH.

Statistical analyses

Statistical analyses were performed using SPSS 15.5. Qualitative variables are expressed as the frequency and comparisons were performed using the x2-test. Quantitative variables are expressed as the median and mean plus standard deviation (SD). Comparisons were performed using the Mann–Whitney U-test. Statistical significance was set at $p < 0.05$ for all analyses.

Results

Patients

During the study period, a total of 1 594 481 neonates were screened. We analyzed 46 newborns (32 boys and 14 girls) affected by classical forms for 21-OHD for whom the initial evaluation and further monitoring were performed at our hospital. Of these neonates, 36 were affected by SW forms and 10 by SV forms.

Family and perinatal history

Median gestational age was 39.7 weeks (range 38–40.2). Five patients presented preterm delivery. Three of them were born at 36 weeks, one at 34 weeks, and one girl at 31 weeks (950 g), who had received prenatal treatment with dexamethasone for a previous affected sibling. No differences were found in gestational age according to sex and clinical form. The Z-score of weight (3210 g; 3000–3550) and length (50 cm; 48.5–51) at birth in our patients did not differ from that found in the general population.

Six patients (12.2 %) were found to have a history of consanguinity in the parents. Two families (4.3 %) had a child previously die of unknown cause in the first 3 months of life. In both families, the child included in the study had a form of salt loss.

Four (8.16 %) patients had a previous affected sibling who had been the index case in the family. Of the 4 families that after having a first affected child had a second child also affected by the disease, 3 families requested treatment and prenatal diagnosis and one of them refused treatment and prenatal diagnosis. Three pregnant women received prenatal treatment with dexamethasone. In one of them, treatment was suspended after the male fetal sex was known and in the remaining two families dexamethasone was maintained until the end of gestation due to the affected female fetal sex.

Clinical suspicion before the results of neonatal screening

In 38 (82.6 %) patients (30 SW and 8 SV), the disease had not been suspected before the result of newborn screening but only in 8 (17.4 %) patients, the disease had been suspected before the result of newborn screening (▶ **Table 1**). Of these 30 patients with SW form, 24 were boys and 6 were girls. Six of the 8 patients affected by SV form without clinical suspicion of the disease were boys and 2 were girls. In almost all (30 of 32) of the boys, the disease had not been clinically suspected. In 6 of 14 girls, sex assignments were incorrect until the screening result was known.

The 8 patients with clinical suspicion of the disease, 6 of them were affected by SW form. The disease had been suspected by previous relative affected in 4 patients (2 girls with SW form, 1 boy with SW form and 1 boy with SV form). In 4 females the suspicion was made because of ambiguous genitalia at birth (3 were affected with SW form and 1 with SV form) (▶ **Fig. 1**). The disease was clinical suspected in 6 of 14 girls and 2 of 32 boys, that is, the disease is suspected in females but not in males ($p < 0.001$).

Clinical situation on recall

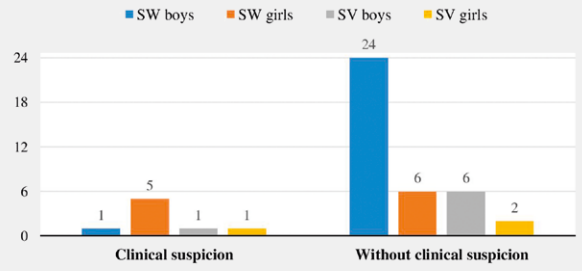
The clinical situation when the result of neonatal screening was obtained in 38 patients without clinical suspicion was 30 patients (78.9 %) were at home without suspicion of illness, 3 (7.9 %) were at home but with hospital follow-up due to clinical suspicion of illness and 5 (13.2 %) newborns were admitted to the hospital for different reasons before the screening results were available (▶ **Fig. 2**).

- Patients at home without clinical suspicion of disease (n = 30): 23 patients affected by SW form (22 boys and 1 girl in with incorrect sex assignment at birth) and 7 by SV form (6 boys and 1 girl with Prader II).
- Patients at home in follow-up for suspected illness (n = 3): 2 females with SW form with incorrect sex assignment at birth like males with cryptorchidism at birth and 1 female with SV form under study by ambiguous genitalia classified as Prader III.
- There were 25 patients with SW (22 boys and 3 girls) in their homes with morbidity and mortality risk like healthy neonates.
- Hospitalized patients (n = 5): all of them affected by SW form, 3 of them were admitted with suspicion of sepsis (2 girls and 1 boy), 1 boy with jaundice and 1 girl with hypoglycemia.

▶ **Table 1** Patients with/without clinical suspicion for 21-hydroxylase deficiency.

	Patients	SW		SV	
Clinical suspicion	8 (17.4 %)	6		2	
		♂ 1	♀ 5	♂ 1	♀ 1
Without clinical suspicion	38 (82.6 %)	30		8	
		♂ 24	♀ 6	♂ 6	♀ 2

SW: Salt-wasting form; SV: Simple-virilizing form.



▶ **Fig. 1** Patients with/without clinical suspicion before the neonatal screening result for 21-hydroxylase deficiency.

Age at diagnosis by newborn screening for 21-OHD

The median age at diagnosis by Neonatal screening Program was 8.5 days (range, 6–10.5 days). The median age of the boys and girls at detection was 9 days (range, 8–12 days) and 7 days (range, 3–9 days), respectively, and was not significantly different. The median age at detection in patients with SW form was earlier (8 days; range, 6–9 days) than in those with SV form (18 days; range, 14.5–31.5), being this statistically significant difference ($p < 0.001$) as detailed in ▶ **Fig. 3**.

As shown in ▶ **Table 2**, 39 % patients affected by SW were diagnosed in the first week of life and 58 % in the second week of life. Thirty percent patients affected by SV form were diagnosed in the first 15 days of life (20 % in the first week and 10 % in the second). Forty percent patient affected by SV form were diagnosed between 16 and 30 days of life and 30 % beyond the month of life. The diagnosis is later in SV forms because it requires several determinations of 17-OHP to arrive at the confirmation of the diagnosis. Neonatal Screening allowed the diagnosis of the SV form in boys in the first month of life (18 days; range, 14.5–31.5 days).

Values of 17-OHP in dried blood spot specimens from neonatal screening

17-OHP initial concentrations detected using dried blood spot specimens was 422.5 nmol/l (range, 294.0–622.0 nmol/l) in SW forms and 58 nmol/l (range, 38.5–93 nmol/l) in SV forms (▶ **Fig. 4**). Neonates with SW forms had higher 17-OHP concentrations than SV forms ($p < 0.001$). The highest values of 17-OHP in absorbent paper were obtained at 2 days (2–6) in SW forms at 18 days of life

in SV forms, this difference being statistically significant ($p < 0.001$). All patients affected by SW forms were identified when performing the first screening test, however, most of the children affected by the SV forms required several repetitions of the screening test (2 to 4 repetitions) because slightly elevated values in some patients.

Physical examination

All of patients included were diagnosed by neonatal screening program of classic forms of 21-OHD. The most common symptom and sign of neonates with 21-OHD was hyperpigmentation (71.7 %). The most frequently affected hyperpigmentation areas were the genitals in 26 (78.8 %), nipples in 2 (6.1 %) and both areas (genitals and nipples) in 5 (15.1 %) patients. Poor feeding was noted in 15 patients affected by SW form.

All girls with 21-OHD, regardless of type, had atypical external genitalia, clitoromegaly and variable degrees of posterior labial fusion at the time of diagnosis except 2 of the 14 girls born with normal female genitals because they had been treated prenatally with dexamethasone. An incorrect assignment of sex at birth had been performed in 6 of the 14 girls (42.8 %).

Biochemical findings

CAH patients tended to present hyponatremia and hyperkalemia, although no relevant differences were observed in pH values. ▶ **Table 3** shows biochemical and neonatal anthropometry data according to clinical form. The median baseline 17-OHP level was 311 ng/ml (range, 69.3–479.5 ng/ml) in SW form and 234 ng/ml (range, 160–276 ng/ml) in SV form, which was not statistically significantly different.

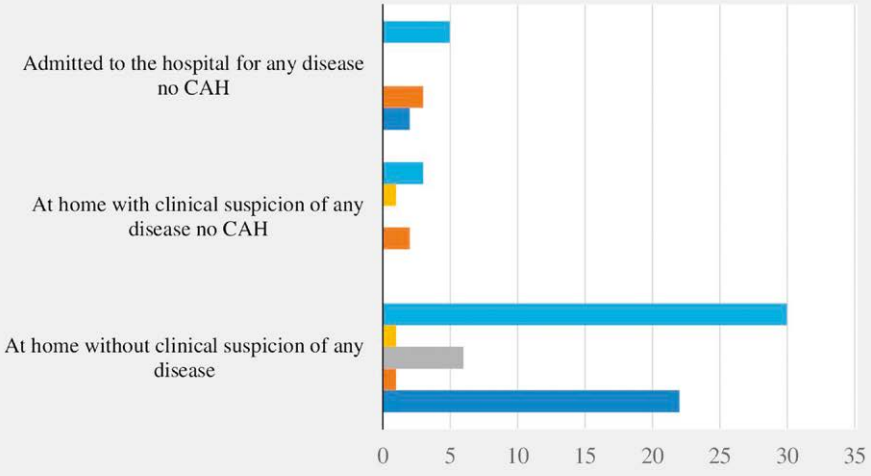
Molecular analysis of the 21-OH gene

Molecular analysis of the 21-OH gene was performed in all patients included. The most frequent type of genetic alteration detected was deletions/conversions found 26/92 of alleles (28.26 %). The most frequent point mutation detected was c.293–13 C > G in intron 2, found 21/92 of alleles (22.82 %).

Four patients with SW presented a homozygous genotype, in one of them there was no history of consanguinity. Homozygosis could be explained because both parents had a mutation considered frequent in the general population (c.293–13 C > G).

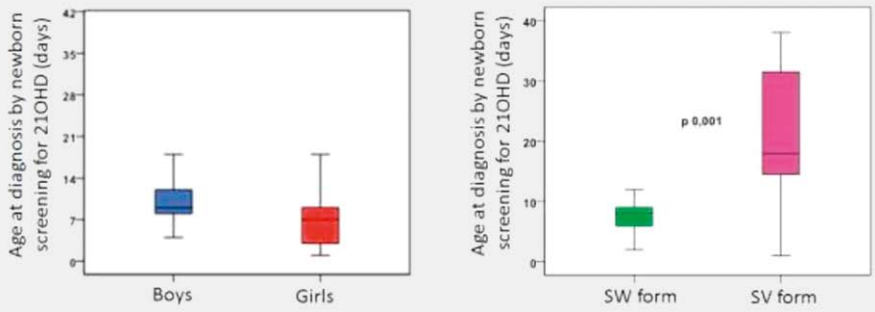
In 6 of 46 patients there were consanguinity history, 3 presented a homozygous genotype and another 3 were compound heterozygotes.

In our cohort there is a good genotype phenotype correlation (94 %) except in 3 patients (▶ **Table 4**), in which the expected phenotype based on the genotype (severe mutation in one allele and slight mutation in the other) would correspond to a non-classical form but the clinical behavior is in a way SV. This phenotype is based on visible signs of virilization from the neonatal stage (macrogenitalism), hyperpigmentation of nipples and genitals, elevation of 17-OHP greater than 100 nmol/l in neonatal screening detection and confirmed in venous blood and even elevation of plasma renin activity in the pubertal stage in one of them that needed to add to the treatment fludrocortisone.



	At home without clinical suspicion of any disease	At home with clinical suspicion of any disease no CAH	Admitted to the hospital for any disease no CAH
Total	30	3	5
SV girls	1	1	0
SV boys	6	0	0
SW girls	1	2	3
SW boys	22	0	2

▶ **Fig. 2** Where were patients when they received the neonatal screening result for 21-hydroxylase deficiency?



▶ **Fig. 3** Age at diagnosis by neonatal screening for 21-hydroxylase deficiency according to sex and clinical form.

▶ **Table 2** Number of patients diagnosed with 21-hydroxylase deficiency according to age.

	≤ 7 days of life	8–15 days of live	16–30 days of live	≥31 days of life	n
SW form	14 (38.9%)	21 (58.3%)	1 (2.7%)	0	36 (100%)
SV form	2 (20%)	1 (10%)	4 (40%)	3 (30%)	10 (100%)
Total	16 (34.8%)	22 (47.8%)	5 (10.8%)	3 (6.5%)	46 (100%)

SW: Salt-wasting form; SV: Simple-virilizing form.

▶ **Table 3** Biochemical data and neonatal anthropometry for 21-hydroxylase deficiency patients according to clinical form.

	SW	SV	p-Value
Age at diagnosis, days	8.00; 6.00–9.00	18.00; 14.50–3150	<0.05
Screening 17-OHP, nmol/l (Median, p25-p75)	422.5 (294.0–622.0)	58.0 (38.5–93.0)	<0.05
Sodium, mmol/l	128.3 ± 7.5	132.5 ± 1.0	<0.05
Potassium, mmol/l	6.7 ± 1.3	5.3 ± 0.4	<0.05
pH	7.35 ± 0.08	7.35 ± 0.06	
Glycemia, mg/dl	90.7 ± 28.4	81.8 ± 5.6	
Serum 17-OHP, ng/ml (Median, p25–p75)	311.0 (69.3–479.5)	234.0 (160–276)	
Birth weight, SD	0.14 ± 1.20	0.10 ± 0.88	
Birth length, SD	0.12 ± 1.41	0.05 ± 1.53	

SW: Salt-wasting form; SV: Simple-virilizing form; 17-OHP: 17-Hydroxyprogesterone.

Two new mutations not previously described in the literature have been found in two patients. The intronic variant c.292 + 5 G > A which in cis with the slight mutation p.Val282Leu constitutes a serious allele with PS1 and the mutation c.518 T > A (p.Ile173Asn) associated with SV form.

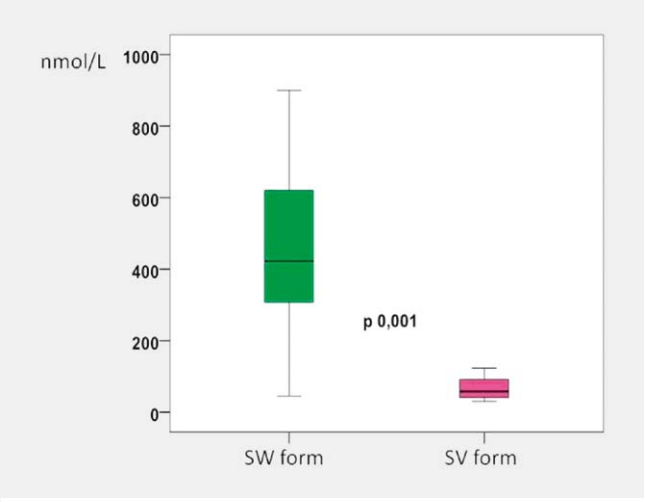
Treatment

The age of onset of hydrocortisone treatment coincides with the age at diagnosis in the SW forms at 8 days (range, 6–9 days), since the treatment begins on the day the diagnosis is made. In the SV forms, the treatment is delayed up to 26 days (range, 22–64 days), since the diagnosis in this case may not be as obvious and the differential diagnosis between simple virilizing form and transient elevations of 17-OHP has to be made.

Of the 10 patients with SV form, 3 required treatments with mineralocorticoid from the beginning due to a sustained elevated PRA. In these patients the treatment with fludrocortisone began at 16.5 days (range, 9–20 days).

Discussion

21-OHD Neonatal Screening Programs have allowed the early diagnosis of the classic form with saline loss (before its clinical expression) favoring that the age of initiation of treatment is increasingly early, so that the adrenal crisis can be prevented and therefore the mortality and morbidity derived from the pathology itself as well as shortening the time of incorrect assignment of sex at birth. Early diagnosis and treatment are crucial to prevent these life-threatening crises and their irreversible consequences such as intellectual disability due to brain damage secondary to hyponatremia. The European Society of Pediatric Endocrinology and the American Society of Pediatric Endocrinology (Lawson–Wilkins) establish that 21-OHD neonatal screening is recommended with a grade of recommendation ++ and level of evidence I [13, 14]. The probability of death due to an adrenal crisis in the neonatal period in



▶ **Fig. 4** 17-Hydroxyprogesterone initial concentrations in dried blood spot specimens.

▶ **Table 4** Allele mutation in congenital adrenal hyperplasia patients without good genotype phenotype correlation.

Clinical form	Mother's allele mutation	Father's allele mutation
SV	c.293–13 C>G	c.844 G>T (p.Val282Leu)
SV	c.1069 C>T (p.Arg357Trp)	c.844 G>T (p.Val282Leu)
SV	c.1360 C>T (p.Pro454Ser)	c.92 C>T (p.Pro31Leu)

SW: Salt-wasting form. SV: Simple-virilizing form.

the absence of screening is controversial but most studies establish that between 0–4 % of children with saline loss due to 21-OHD would die despite being born in highly qualified health centers [15].

In this study, 36 of 46 patients (78 %) had SW 21-OHD, which is similar to other studies reported in Europe and the United States [16, 17].

A key aspect of our study is the possibility of having the results of the screening test in time to prevent the occurrence of the salt loss crisis, which usually occurs between the 2nd and 3rd week of life. The age at diagnosis of patients diagnosed by screening our sample (8.5 days; 6.0–10.5) allows early diagnosis and thus the establishment of treatment in time to avoid the salt loss crisis, adjusting to the fundamental objective of a neonatal screening program on its ability to reduce morbidity/ mortality.

In the studies published in the literature there is a great variability in the age of children at the time of definitive diagnosis of the classic form of the disease (note that some authors present the data in means and others in medians). The age of diagnosis ranges from 6 days to about a month. The study by Chu et al. (Taiwan) [18] establishes an average age at the time of diagnosis by screening of 11.6 days (4–20) and average age at the time of definitive diagnosis of 14.8 days (5–31). Steigert et al. (Switzerland) [19], describe a mean age at onset of treatment of 6.7 days (1–22) in children diagnosed by screening. Gruñeiro-Papendieck et al. (Argentina) [20] places the average age at diagnosis at 6 days, Van der Kamp et al. (Netherlands) [21] reports an average age of treatment initiation in areas with 21-OHD screening at 7 days of life (1–31) and in areas without 21-OHD screening at 14 days of life (0–115). Brosnan et al. (Arkansas, Oklahoma and Texas, USA) [16] describe a mean age at diagnosis by screening in men with SW of 12 days in screening areas and 26 days in areas without screening. The age at diagnosis by screening in the study by Cartigny-Maciejewski et al. (Lille, France) [22] was 18 days on average (5–90) and 9 days on average. The median age at the time of diagnosis by screening for children with SW form reported by Therrell et al. in Texas [9] was 11 days (0–40) and for children with SV form 34 days (0–111). In Sweden, Thilén et al. [23] establish a median age at diagnosis of 9 days in areas with screening and 21 days in areas without screening and in Emilia-Romagna (Italy), Balsamo et al. [24], report a median age at diagnosis by screening of 20 days (12–150) in the period between 1980 and 1983 and 11 days (5–35) in the period between 1991 and 1995. In Israel, Sack et al. [25] reported an age of initiation of treatment before the month of life in areas with screening and in Wisconsin, Allen et al. [26], reveal that the results of the screening were obtained between the 5th and 9th day of life. In Spain, the average age of diagnosis reported by the 5 Detection Centers that perform 21-OHD screening is 10.9 days (5 percentile: 7.3 percentile 99: 15.9) [27], higher than that found in our sample (8.5 days; 6.0–10.5).

There was no clinical suspicion of 21-OHD in 82.6 % (n = 38) patients diagnosed by neonatal screening, this figure exceeds 47 % described by Thilén [23] and 73 % of the Therrell study [9]. Of the patients without clinical suspicion of any disease, 30 patients (79 %) were SW forms with potential risk of adrenal crisis. Among the 30 SW forms without suspicion of disease, 24 were boys and 6 were girls. The remaining 8 (21 %) patients without clinical suspicion of 21-OHD affected by SV form (6 boys and 2 girls) the benefit obtained from the screening has been early diagnosis, shortening the study time of the girl with virilization of the genitals (diagnosis at 18 days of life) and prevention of early pseudopuberty.

The disease was suspected in 17.4 % (n = 8) of the patients. The reason for suspicion was the ambiguous genitalia in women (3 with SW 1 with SV form) followed by family history in 4 cases (in 2 of them with prenatal diagnosis). Therefore, as Therrell et al. describes, the cases detected before knowing the results of the screening were due to the involvement of a sibling or to certain signs of virilization in girls [9].

Taking these results into account, we can say that of the 46 patients affected by 21-OHD, 30 (65 %) affected by saline loss with risk of mortality from adrenal crisis, would have obtained an absolute benefit of the screening. In the retrospective study carried out by Steigert et al. [19], he found that of the 31 patients with confirmed 21-OHD, 15 of them (48.4 %) would be those really benefited from the screening, since in them, the diagnosis had not been suspected by the clinic. They would also have obtained relative benefits, the 6 women with incorrect sex assignment at birth,

the 8 patients affected by SV form without clinical suspicion of disease because thanks to the screening, the time was shortened until the definitive diagnosis was reached.

Newborn girls with 21-OHD are recognized earlier due to their genital ambiguity. The clinical examination makes it possible to suspect, prior to the result of the screening, the classic forms of the disease in some women with ambiguous genitals, consequently the joint use of the clinical examination and the screening test is a very useful strategy to improve the early diagnosis of the illness. Men with SW forms would be the most benefited by this screening because they do not have clinical signs that warn of the disease before salt-wasting. In the absence of screening, there is a percentage of cases with SW form that would die without ever being diagnosed [28, 29].

In contrast to those diagnosed in the prescreening era, most patients detected by neonatal screening were asymptomatic or had mild symptoms upon diagnosis, especially among males. The data here confirm those previous studies [23, 30, 31].

There are no studies in the literature that analyze where patients were when they received the result of neonatal screening. In this study, 79 % (n = 30) were at home without suspicion of any disease and 7.91 % (n = 3) were at home pending study for clinical suspicion of any disease (ambiguous genitalia, cryptorchidism) and 13.2 % (n = 5) were admitted to the hospital for reasons unrelated to CAH (sepsis, jaundice, hypoglycemia).

It is important to highlight that 69.4 % of the patients (n = 25/36) with SW form identified by neonatal screening were in their homes with potential risk of death and that 6 women had been incorrectly labeled as men at birth (3 were in their homes as healthy men without any clinical suspicion, 2 were in their homes pending study for hypospadias, and 1 of them was hospitalized for ambiguous genitals).

In conclusion, this study allows us to detect that in the majority of patients with 21-OHD, the diagnosis by screening was anticipated to the clinical suspicion of the disease even in female patients with ambiguous genitalia. The Neonatal Screening Program allows the identification of affected by CAH newborns and avoids the establishment of severe dehydration and shock conditions; reduce the time of incorrect sex assignment at birth in virilized girls and detect boys with simple virilizing form early.

Acknowledgement

We thank Unidad de Investigación Materno Infantil Fundación Familia Alonso (UDIMIFFA) for its valuable support.

Funding

The study was partially supported by Unidad de Investigación Materno Infantil Fundación Familia Alonso (UDIMIFFA).

Conflict of Interest

The authors declare that they have no conflict of interest.

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