Value of selective screening for congenital adrenal hyperplasia in Hungary

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SUMMARY Measurement of the 17-hydroxyprogesterone concentration in blood spots was used to identify cases of congenital adrenal hyperplasia among patients with inappropriate virilisation, or salt wasting, or both. Between 1978 and 1986 61 were identified among 707 patients (278 neonates, 204 infants, and 225 children).

The incidence of classic congenital adrenal hyperplasia was calculated for a seven year prospective trial period using the blood spot 17-hydroxyprogesterone method in selective screening. There were 38 salt losers and 14 simple virilisers in 968 303 live births, an incidence of congenital adrenal hyperplasia of 1:18 000 in the Hungarian population. Selective screening led to earlier diagnosis of congenital adrenal hyperplasia and a pronounced decrease in mortality. A central laboratory to measure the blood spot 17-hydroxyprogesterone concentrations is valuable for the investigation of patients at risk for congenital adrenal hyperplasia in countries where blood steroid assays are not readily available.

Congenital adrenal hyperplasia is the result of 21-hydroxylase enzyme deficiency in more than 90% of cases.¹ The plasma 17-hydroxyprogesterone concentration is usually raised and its measurement is a rapid and reliable test in patients suspected of having congenital adrenal hyperplasia.^{2–4} Though the assay is routinely available in steroid laboratories in western countries, in other countries such as Hungary the techniques for the provision of rapid results are seldom available.

A method for the measurement of 17-hydroxyprogesterone in blood spotted on to filter paper was first reported in 1977.⁵ The method was originally intended for the screening of neonates for congenital adrenal hyperplasia, but a modified blood spot method was developed for the rapid diagnosis of patients suspected of having congenital adrenal hyperplasia throughout Hungary.⁶ Preliminary experience with the use of a blood spot 17-hydroxyprogesterone assay to detect infants at risk of congenital adrenal hyperplasia has been previously reported.⁷ This paper reports on the use of this test to screen more than 700 patients at risk during an eight year period. The results also provide information on the incidence of congenital adrenal hyperplasia in Hungary.

Patients and methods

Between October 1978 and December 1986, 707 patients considered to be at risk of developing congenital adrenal hyperplasia were studied. The indications for investigation were inappropriate virilisation at any age, or salt wasting during the first month of life. The number of patients investigated in each age group was as follows: 1–3 days, 67; 4–7 days, 73; 8–30 days, 138; 2–3 months, 145; 4–12 months, 59; 2–8 years, 145; 9–18 years, 80.

Capillary blood was spotted on to the filter paper cards that are routinely used for the screening of neonates for phenylketonuria and congenital hypothyroidism. Samples were collected during the morning from patients older than 2 months of age because of the decline in 17-hydroxyprogesterone concentrations that occurs later in the day.⁸ Cards were posted to a central laboratory; the stability of steroids in dried blood spots kept at room temperature for 150 days has been reported previously.⁹ Serial capillary blood samples were also collected from some patients with congenital adrenal hyperplasia before treatment was started.

The assay for the measurement of blood spot 17-hydroxyprogesterone concentration has been

published in detail.^{6 10} The sensitivity of the method was 10 pg/disc, equivalent to 10 nmol/l of serum. Results were available within 24 hours of receiving the sample in the laboratory.

Results

During the eight year study period 61 patients with 21-hydroxylase deficiency were identified using the blood spot 17-hydroxyprogesterone method among 707 patients with suspected congenital adrenal hyperplasia. The distribution of blood spot 17-hydroxyprogesterone values in each age group is shown in figs 1, 2, and 3.

Twenty six infants with salt losing congenital adrenal hyperplasia were identified among 278 patients investigated at age 1–30 days because of ambiguous genitalia, or salt wasting, or both (fig 1). The values of 17-hydroxyprogesterone at age 4–7 days were higher in ill babies born at full term and healthy premature infants compared with healthy babies born at full term (range: 10–140 nmol/l). Using a cut off value of 17-hydroxyprogesterone of

Fig 1 Frequency distribution of blood spot 17-hydroxyprogesterone values in infants at risk of congenital adrenal hyperplasia during the first month of life. Closed bars represent infants with congenital adrenal hyperplasia; there were six of 67 at age 1–3 days, three of 73 at age 4–7 days, and 17 of 138 at age 8–30 days. The arrow denotes the selected cut off value for 17-hydroxyprogesterone.

Fig 2 Frequency distribution of blood spot 17-hydroxyprogesterone values in children aged 2–12 months at risk of congenital adrenal hyperplasia; (a) indicates an infant with 11 β -hydroxylase deficiency. Closed bars represent infants with congenital adrenal hyperplasia; there were 13 of 145 at age 2–3 months and one of 59 at age 4–12 months. The arrow denotes the selected cut off value for 17-hydroxyprogesterone.

240 nmol/l for the diagnosis of congenital adrenal hyperplasia there were five false positive and no false negative cases. Three of these infants were boys with hyponatraemia secondary to renal tract malformations and infection. The other two infants were premature girls in whom the clitoris was prominent.

Fig 4 shows the results of serial blood spot 17-hydroxyprogesterone measurements in infants with congenital adrenal hyperplasia compared with those from healthy babies born at full term, and very low birthweight premature infants without congenital adrenal hyperplasia. There was some overlap in 17-hydroxyprogesterone values on the first day of life, when three very low birthweight premature infants had concentrations above 500 nmol/l. Their raised values declined rapidly over a few days, however, whereas blood spot 17-hydroxyprogesterone concentrations in infants with congenital adrenal hyperplasia showed pronounced increases.

Fourteen patients with 21-hydroxylase deficiency (two of whom were not salt losers) were identified from 204 infants aged 2–12 months who were investigated for problems such as failure to thrive, dehydration, and enlarged clitoris with labial fusion.

Fig 3 Frequency distribution of blood spot 17-hydroxyprogesterone values in children at risk of congenital adrenal hyperplasia at 2–18 years; (a) and (b) indicate patients with 11 β -hydroxylase deficiency and virilising adrenal carcinoma, respectively. Closed bars represent infants with congenital adrenal hyperplasia; there were 16 of 145 at age 2–8 years and five of 80 at age 9–18 years. The arrow denotes selected cut off value for 17-hydroxyprogesterone.

(fig 2). Blood spot 17-hydroxyprogesterone cut off limits of 180 and 120 nmol/l at 2–3 and 4–12 months, respectively, identified all the patients with congenital adrenal hyperplasia. Of 11 false positive results, one infant had 11 β -hydroxylase deficiency; the other 10 patients had normal blood spot 17-hydroxyprogesterone concentrations when the test was repeated. Among 225 patients aged 2–18 years who had either premature pubarche or hirsutism there were 21 cases of congenital adrenal hyperplasia (including 14 who were not salt losers, and seven who had the late onset form) who were identified using a cut off value of 17-hydroxyprogesterone of 60 nmol/l. Different cut off points for 17-hydroxyprogesterone in each age group were needed to avoid false negative results and ensure as low a number as possible of false positive results. Among the false positive results were a patient with an adrenal carcinoma and two cases of 11 β -

hydroxylase deficiency. The median time from birth to the diagnosis of congenital adrenal hyperplasia in 38 salt losing cases was 17 days (range four to 38). This compares with 35 days (range 7–120) in a comparable number of salt losing cases analysed in a retrospective study.

Table 1 shows the number of patients with congenital adrenal hyperplasia born in Hungary during a 20 year period from 1966 to 1985. The cases were found by examination of hospital medical records and later (from 1979 to 1985) prospectively

Fig 4 Serial blood spot 17-hydroxyprogesterone values in eight infants with congenital adrenal hyperplasia and in 12 infants born at full term and four very low birthweight infants without congenital adrenal hyperplasia during the first 20 days of life. Figures are given as mean (SD).

Table 1 Number of patients with congenital adrenal hyperplasia born in Hungary, 1966–1985

Enzyme defect	Girls	Boys	Total No
21-hydroxylase:			
Salt losers	37	42	79
Simple virilisers	25	18	43
Late onset	11	2	13
11 β -hydroxylase	1	3	4

Table 2 Incidence of classic congenital adrenal hyperplasia in Hungary

Method of analysis	Period of survey	No of live births	No of cases			Estimated incidence
			Salt losers	Simple virilisers	Total No	
Retrospective case survey	1966-1978	2 119 727	41	29	70	1:30 282
Prospective selective screening	1979-1985	968 303	38	14	52	1:18 621

by measurement of blood spot 17-hydroxyprogesterone values in patients at risk. There was an equal incidence in boys and girls and two thirds were salt losers. The incidence of congenital adrenal hyperplasia among live births during the 20 year period was 1:25 312. The incidence for the last seven years of this period is shown in table 2. The estimated incidence was higher during the period of selective screening compared with that obtained by retrospective analysis. The presumed incidence of congenital adrenal hyperplasia in Hungary between 1979 and 1985 was 1:18 600 with 95% confidence limits of 1:14 642 to 1:25 572. One country area had 11 salt losers during this period (1966-1985), an incidence five times greater than that in the whole country.

Discussion

This study shows the value of 17-hydroxyprogesterone measurements in identifying congenital adrenal hyperplasia among patients within a wide age range with inappropriate virilisation or salt wasting. The restriction of screening to a population at risk rather than mass screening at birth is an option recently introduced in Italy.¹¹ The present study reports the largest series of infants with congenital adrenal hyperplasia identified during the neonatal period by the blood spot 17-hydroxyprogesterone method. The results confirm previous reports of the accuracy of this method for screening for congenital adrenal hyperplasia in the newborn.¹²⁻¹⁴ We found a higher false positive rate because those patients at risk were more likely to have, for example, salt wasting than the population identified by mass screening. Acute illness in infants, particularly if they are premature, can lead to the secretion of large amounts of adrenocortical hormones.¹⁵⁻¹⁶ Steroid concentrations normally fall rapidly once the stressful period is over. Diagnosis when there are only moderate rises of 17-hydroxyprogesterone concentrations above the cut off value for age, as in 11 β -hydroxylase deficiency, requires detailed plasma steroid analysis and specific gas chromatographic determination of urinary steroid excretion.

In a recent workshop on screening for congenital adrenal hyperplasia¹⁷ it was emphasised that the increased awareness of cases at risk (listed in table 3) and the ready availability of a rapid 17-hydroxyprogesterone assay⁴ should result in a low incidence of undetected classical cases of congenital adrenal hyperplasia. A retrospective survey of 117 patients with congenital adrenal hyperplasia in Birmingham during a 30 year period showed an improvement in early diagnosis in children born after 1970.¹⁸ The change was attributed to the factors previously mentioned, and the authors concluded that a neonatal screening programme for congenital adrenal hyperplasia in the UK was not necessary. The need is clearly different in Hungary where routine 17-hydroxyprogesterone measurements are not yet available. A central laboratory that is able to provide rapid results of 17-hydroxyprogesterone concentrations in blood spots collected from a restricted group at risk is a satisfactory alternative. The procedure has led to earlier diagnosis and treatment, and a reduction in mortality from 27% in 1966-78 to 8% in 1979-85.

Data are now available from a national register of all cases of congenital adrenal hyperplasia in Hungary. The ratio of salt losers to simple virilisers in classic congenital adrenal hyperplasia and the incidence of 1:18 000 are similar to those in other European countries.¹⁹⁻²¹ One region of Hungary seems to have an increased incidence of salt losing congenital adrenal hyperplasia, although it is still lower than those reported from Alaska²² and La Reunion.¹⁴ Selective screening for congenital adrenal hyperplasia is a practical option that can yield valuable clinical information and more accurate estimate of incidence.

Table 3 Cases of congenital adrenal hyperplasia readily identifiable at birth

- All affected girls (theoretically)
- Salt losers
- Apparent cryptorchid boys with hypospadias
- Those with a previous family history of congenital adrenal hyperplasia
- Those with a positive prenatal diagnosis

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