Neonatal screening for congenital adrenal hyperplasia

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SUMMARY Capillary blood samples from 42 930 infants born in the Italian region of Emilia-Romagna were collected for 17-hydroxyprogesterone radioimmunoassays on days 2, 3, 4, 5, 6, and 7, or between days 7 and 15 of life. A microfilter paper method modified from that of Pang *et al.*¹ was used for this assay. Pathologic values of 17-hydroxyprogesterone were found in 5 infants giving an incidence in this homogeneous Caucasian population of 1:8586. We also investigated 17-hydroxyprogesterone values in relation to the day of sampling and the possible correlation between 17-hydroxyprogesterone values and birthweight and gestational age. We concluded that neonatal screening for congenital adrenal hyperplasia caused by 21-hydroxylase deficiency was possible by this method and that the infants' maturity and the particular day of collection of the samples affect the values but not the validity of the screening.

The considerable variation in the reported incidence of congenital adrenal hyperplasia (CAH)²⁻⁸ may be explained partially by the lack of a valid screening method. We have reported on CAH screening in 22 233 neonates⁹ in the region of Emilia-Romagna in Italy (a homogeneous Caucasian population) and in this study we enlarge on and update that information. We screened 42 930 infants for CAH caused by 21-hydroxylase deficiency by assaying 17-hydroxyprogesterone (17-OHP) values. In addition we investigated 17-OHP values in relation to the sampling day and a possible correlation between 17-OHP values and gestational age and birthweight.

Materials and methods

Capillary blood samples taken from 42 930 neonates and collected on filter paper (Sleicher and Schuell) were used to determine 17-OHP concentrations. A microfilter paper (1/8 inch diameter paper disc) method modified from that of Pang *et al.*¹ that has been reported previously¹⁰ was used. Blood samples were taken on day 2 of life from 134 infants (0.2%), on day 3 from 801 (2.1%), on day 4 from 6945 (15.3%), on day 5 from 19 271 (46%), on day 6 from 9063 (2.8%), and on day 7 from 1660 (3.4%). The remaining 5056 samples (12.2%) were collected between days 7 and 15 of life.

For the first 1634 infants screened a 17-OHP concentration as high as 9 pg/disc was regarded as a

threshold value calling for a repeat test. We found that infants with 17-OHP concentrations up to 20 pg/disc had no clinical or biochemical evidence of CAH, however, and a new threshold was set at 20 pg/disc for the remaining infants. Where values were higher than this they were confirmed by a second assay and the serum 17-OHP concentration was determined.

Day of sampling, gestational age, and birthweight were all considered in the analysis of the results. With regard to gestational age, infants were divided into 2 groups; either 'term'—those delivered between 37 weeks and 42 weeks of gestation, or 'preterm'—delivered before 37 weeks' gestation. The data reported here include only those infants who were screened using a threshold value of 20 pg/disc.

Statistical analysis was performed using Student's *t* test and χ^2 test and the correlation coefficient 'r' was calculated. The 95th percentile confidence limits of estimated incidence rate were calculated according to the confidence limits for the expectation of the Poisson variable.¹¹ The gene frequency and the carrier frequency were calculated according to the Hardy-Weinberg law¹² which states that:

Gene frequency = square root of the incidence of the homozygous state.

Heterozygote frequency = 2 pq; where p = frequency of normal gene and q = frequency of CAH gene.

Table 1 Clinical and hormonal features of the 5 infants with congenital adrenal hyperplasia

Case No	Age (Days)	Gestation	Birthweight (g)	17-OHP value		External genitalia
				1st spot (pg/disc)	Serum (ng/dl)	
1 (M)	4 24	Term	4000	>250	>5000	Mild macrogenitosomia
2 (F)	5 150	Term	3300	28*	5000	Ambiguous
3 (F)	5 30	Term	3870	47	1400	Normal
4 (F)	4 14	Term	3000	>250	>5000	Ambiguous
5 (F)	3 7	Term	3200	>250	>5000	Ambiguous

*This patient's blood, collected on filter paper, was stored in unfit conditions, which may explain her relatively low 17-OHP value.

Results

Eighty eight (0.2%) of the 42 930 infants tested showed a 17-OHP value beyond the threshold of 20 pg/disc and were retested (Table 2). Retest blood samples were taken from 2 of the children (0.25%) initially tested on day 3 of life, from 2 (0.03%) initially tested on day 4, from 25 (0.13%) on day 5, from 20 (0.22%) on day 6, from 10 (0.6%) on day 7, and from 29 (0.57%) after day 7. In 5 of the infants who were retested (5.7%) the pathologic values were confirmed giving an incidence of 1:8586 neonates. These infants had a 17-OHP value >20 pg/disc and were born at term; their clinical and hormonal details are summarised in Table 1.

Table 2 shows the concentrations of 17-OHP in relation to the day of sampling. No statistically significant differences were found between the various days. The number and percentage of second tests according to gestational age (term and preterm) are set out in Table 3. The percentage was slightly higher for preterm infants. Retest percentages and 17-OHP values according to gestational age and birthweight are shown in Figs. 1 and 2.

Table 2 Relation between 17-hydroxyprogesterone values and day of sampling

Day	Cases	Value pg/disc (mean (SD))	Retest cases	Retest (%)
2	134	5.73 (1.03)		0.00
3	801	6.18 (2.19)	2	0.25
4	6,945	6.82 (2.83)	2	0.03
5	19,271	6.73 (2.99)	25	0.13
6	9,063	7.74 (3.25)	20	0.22
7	1,660	9.15 (9.55)	10	0.60
>7	5,056	8.96 (8.93)	29	0.57
Total	42 930		88	0.2

Table 3 Number and percentage of retests according to gestational age (term and preterm)

	Patients tested (%)	Retested (%)
Term infants	37,364 (90.47)	27 (0.07)*
Preterm infants	3,935 (9.53)	61 (1.55)*

* $\chi^2 = 298$ ($P < 0.000001$).

Fig. 1 Retest percentage according to gestational age (term and preterm) and birthweight.

$P < 0.001$ between group with birthweight <2500 g and all other groups (as regards the children born at term);
 $P < 0.001$ between groups with birthweight 800–2000 g and the groups with birthweight >2000 g (as regards the preterm children);
 $P < 0.05$ between term and preterm children in the group with birthweight 2500–3000 g.

Fig. 2 17-hydroxyprogesterone values (pg/disc) SD according to gestational age (term and preterm) and birthweight. (In brackets number of cases.)

$P < 0.05$ between group with birthweight < 2500 g and the groups with birthweight > 3500 g (as regards the children born at term); $P < 0.001$ between group with birthweight 800–1500 g and the group with birthweight > 2000 g (as regards the preterm children); $P < 0.05$ between term and preterm children in the group with birthweight 2500–3000 g.

Among term infants, the group with birthweight < 2500 g had a higher retest percentage than all other groups ($P < 0.001$), and the 17-OHP concentrations were higher than those of infants with birthweight 3500 g ($P < 0.05$). Among preterm infants the retest percentage in the groups of infants with birthweight between 800 and 2000 g was similar, and was higher ($P < 0.001$) than for those groups with a birthweight > 2000 g. The 17-OHP value was greater ($P < 0.001$) in infants weighing 800–1500 g than in those > 2000 g. In infants of 1500–2000 g the 17-OHP concentration was greater than in infants weighing > 2500 g.

The difference in retest percentages was significant ($P < 0.05$) between term and preterm infants in the group with birthweight 2500–3000 g. The 17-OHP value was greater in preterm than in term infants ($P < 0.05$). A negative correlation existed ($P < 0.05$) between birthweight and 17-OHP values in retested infants.

The direct cost for each screening test was US \$1.63 (£1.05) and this included wages for laboratory staff and all expenses for reagents and supplies.

Discussion

This is the first study to evaluate the true prevalence

of CAH in a homogeneous Caucasian population using a reliable method. The reported incidence of CAH varies internationally from 1:490 among Alaskan Eskimos to 1:67 000 in the United States.^{2–8} In these reports the lack of accurate screening procedures may have made it difficult to assess the incidence. Some infants may have eluded detection because of neonatal death and some boys with delayed virilisation may have been diagnosed as cases of precocious puberty.

Our investigation shows that the incidence of CAH with or without salt loss in the Emilia-Romagna region is 1:8586 live births. This corresponds with a heterozygote incidence of 1:47 for CAH is transmitted as an autosomal recessive trait. The gene frequency is 0.011. The lower and upper 95th percentile confidence limits of these estimated incidence rates indicate that the incidence of CAH may be as high as 1:3678 live births and as low as 1:26 500 live births for homozygous affected cases and as high as 1:30.8 and as low as 1:81.8 for heterozygous cases. The 95th percentile confidence limits of the gene frequency are 0.016–0.006.

The 5 infants with raised 17-OHP concentrations (4 girls and 1 boy) included 3 with salt loss (Table 1, cases 1, 4, and 5), 1 infant had a classic case of CAH without salt loss (Table 1, case 2), and in 1 patient (Table 1, case 3) a diagnosis of late onset^{13–15} or cryptic form¹⁶ of CAH, based on a high 17-OHP value with no clinical signs, was made. The patients in cases 1, 2, 3, and 4 have been described.⁹ Screening made it possible to establish a precise and timely diagnosis and to decide on treatment in the patients in cases 1 and 4.

With a 17-OHP threshold value of 20 pg/disc the number of repeat tests was not appreciably affected by the day on which the samples had been collected. Children examined on day 2 of life showed lower mean values of 17-OHP than those examined later, but this was not statistically significant. This finding may be ascribed to the fact that sampling was usually done before leaving the obstetric ward and it is possible that the infants examined and considered fit to be moved on days 2 and 3 of life were the most mature and therefore showed lower 17-OHP values. In any case, it is evident that by day 2 of life the 17-OHP values were no longer affected by placental production. Those neonates examined from day 7 onward were mostly recovering from premature birth or low birthweight for dates. The stage of adrenal maturity of these infants varied considerably and this may explain the range of values and the higher retest percentage (Tables 2 and 3).

Our study showed that although the number of

repeat tests in preterm infants was high, the 17-OHP value quickly became normal when prematurity was not complicated by other factors. This is confirmed by the first follow up, usually made within the third week of life.

The analysis of retest percentages and 17-OHP values in relation to birthweight and gestational age (Figs. 1 and 2) indicated that the latter had greater influence on adrenal enzymatic maturity in infants of birthweight >2500 g, whereas birthweight seemed to be more important in infants <2500 g. In terms of both retest percentages and 17-OHP values an appreciable difference existed between term and preterm infants weighing 2500–3000 g but this difference was not found in the group of infants with birthweight 2000–2500 g.

The number of false positives (0.2%) was acceptable, but we believe that this could be reduced further. Preterm infants and those of birthweight <2500 g were recalled most frequently and in future we would increase the threshold value to 30 pg/disc for these infants. We were not aware of any false negative values.

We conclude that: (1) neonatal screening for CAH is possible using micropaper discs impregnated with capillary blood and that it is convenient to carry it out in the same laboratory that screens for phenylketonuria and hypothyroidism; (2) immaturity of the neonates and the particular day of sampling affect the value of 17-OHP but not the validity of the screening; (3) although these data alone do not justify wide scale or even national screening for CAH, greater experience is needed before a definite conclusion may be drawn.

We thank Doctor M I New for her comments and advice during the preparation of this paper.

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Received 10 May 1983