Thyroid function in cord blood

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ABSTRACT

Objective: To determine the biochemical parameters of thyroid function in the cord blood of Saudi infants.

Methods: Cord blood samples sent to the Pathology Department for screening for congenital hypothyroidism were used to determine the reference ranges for thyrotropin, free thyroxine, free triiodothyronine, thyroxine-binding globulin and thyroglobulin. All the measurements were carried out by immunoassay (Elisa, microparticle enzyme immunoassay or chemiluminescence immunoassay). Reference ranges were calculated after exclusion of outliers.

Results: Reference ranges for thyrotropin, free thyroxine and thyroxine-binding globulin were similar to published values, whereas those for free triiodothyronine and thyroglobulin were different.

Conclusion: For correct interpretation of the parameters of thyroid function in cord blood it is essential to have reference ranges based on the laboratory's current methods and derived from the local population.

Keywords: Cord blood, thyroid function, reference ranges.

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Among the many tests listed by Fisher and Vanderschueren-Lodeweyckx[1] for the diagnosis of thyroid disease in children, the main ones are thyrotropin (TSH) and free thyroxine (FT4), with free triiodothyronine (FT3), thyroxine-binding globulin (TBG) and thyroglobulin (TG) less frequently required. These biochemical parameters of thyroid function are known to change with age, particularly in infancy.[2,3] Also, in a survey of infants born in California, Lorey and Cunningham[4] have shown that neonatal plasma total T4 varies with sex and ethnicity. Local reference ranges are therefore essential for correct interpretation of patients' results.

With the volume of cord blood available routinely it is possible to run follow-up tests when the screening results for congenital hypothyroidism are abnormal, and give neonatologists very early on an indication of the possibility of congenital hypothyroidism.

In many laboratories cord blood is routinely available whereas it is more difficult to obtain blood from healthy neonates to determine reference ranges. Even well known reference texts such as Pediatric Clinical Endocrinology[2] have to contend with numerous instances of pediatric reference ranges obtained on very small number of subjects.

Since April 1985 our laboratory has operated a neonatal screening program for congenital hypothyroidism based on measurement of cord blood TSH. Presumptive positives (TSH > 30 mIU/L) are investigated further with measurement of FT4, and if necessary FT3, TBG and TG. Cord blood samples sent for the routine screening were used for determining reference ranges for the biochemical parameters of thyroid function.[5] It was assumed that most of the infants born in the hospital were healthy and had no thyroid abnormality.

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As for any reliable biochemical diagnosis, that of thyroid abnormality in infants requires up-to-date reference ranges based on the local population. The reference ranges for the biochemical parameters of thyroid function in cord blood used in our laboratory are updated regularly as instruments and methodologies change.

Methods. Blood was obtained from the placental side of the cord before delivering the placenta. Five mL of blood was collected into a plain vacutainer tube (Beckton Dickinson) and transported to the laboratory in a cool box. The sample was centrifuged and the serum stored at 2-8°C until assayed. Sera were frozen at -20°C if assay was delayed longer than 24 hours.

Cord blood TSH, FT4, FT3, TBG and TG levels were measured by the methods listed in Table 1. The cord blood samples were chosen at random without any consideration as to the state of health of the infants, gestational age, birth weight or any other neonatal parameters. Data were analyzed for outliers by percentiles.[6] Any values falling outside the check limits of median $\pm$ 3 midspreads were excluded.

The histograms of the raw data for cord blood TSH and free T4 are shown in Figures 1 and 2.

All assays were subject to internal and external quality assurance surveillance. The assay characteristics for TSH and free T4 were determined from routine assays as described by Strike,[6] and are summarized in Table 2. For the other analytes control batches changed too frequently to allow within and between-batch imprecision to be calculated in the same way.

All samples used for TG measurement were also assayed for anti thyroglobulin antibody. TG results from samples containing anti thyroglobulin antibody were excluded from calculations of TG reference range.

All data were checked for gaussian distribution (Kolmogorov-Smirnov test) before analysis:[6] (a) reference limits were set as mean $\pm$ 2 sd for normally distributed data, or after suitable transform (log, exponential or modulus) of non-parametric data. and (b) percentiles with limits set at 2.5 and 97.5 th percentiles. The wider limits are used in practice for reference values in the laboratory.

Results. Reference ranges for cord blood analytes based on mean $\pm$ 2 sd are shown in Table 3. Reference ranges for cord blood analytes based on 2.5 and 97.5 th percentiles are shown in Table 4.

In all cases the wider limits were selected as the reference ranges for use in the laboratory. These reference ranges are compared with published data,[2] as shown in Table 5.

Table 1 - Methods for measurement of the biochemical parameters of thyroid function.

Analyte	Method	Instrument
TSH	Elisa/1 step sandwich assay	Boehringer-Manheim ES 700
FT4	Elisa/competition assay	Boehringer-Manheim ES 700
FT3	MEIA	Abbott AxSYM
TBG	Chemiluminescence immunoassay	DPC Immulite
TG	Chemiluminescence immunoassay	DPC Immulite

MEIA=microparticle enzyme immunoassay

Table 2 - Assay characteristics for TSH and free T4.

Assay	Mean	Standard Deviation	
		Within batch	Between batch
TSH	1.78	0.041	0.090 mIU/L
TSH	8.98	0.243	0.414
Free T4	18.98	0.445	0.530 pmol/L
Free T4	50.21	0.766	2.409

Table 3 - Reference ranges for cord blood; based on mean $\pm$ 2sd.

Analyte	n	Lower limit	Upper limit	Unit
TSH	1000	2.38	19.06	mIU/L
Free T4	260	11.698	19.64	pmol/L
Free T3	71	<1.7	2.23	pmol/L
TBG	86	17.2	36.40	mg/L
TG	78*	12.2	113.50	ug/L

*After excluding results from samples (12/90) containing anti thyroglobulin antibody from calculation of reference ranges.

Table 4 - Reference ranges for cord blood; based on 2.5 and 97.5 percentiles.

Analyte	n	Lower limit	Upper limit	Unit
TSH	1000	2.4	20.60	mIU/L
Free T4	260	12.0	19.40	pmol/L
Free T3	71	<1.7	2.29	pmol/L
TBG	86	18.4	37.70	mg/L
TG	78	12.1	109.00	ug/L

Table 5 - Riyadh Al Kharj Hospital programme (RKH) reference ranges for cord blood and adapted published data (2). The mean values are given between brackets).

Analyte	RKH reference ranges	Published ranges (2)	Units
TSH	2.4-20.6 (6.7)	1-20 (10)	mIU/L
Free T4	12.0-19.6 (14.8)	13.3-22.3 (17.8)	pmol/L
Free T3	<2.29	1.2-4.6 (2.9)	pmol/L
TBG	18.4-37.7 (26.8)	8-52 (30)	mg/L
TG	12.2-113.5 (49.7)	2-54 (24)	ug/L

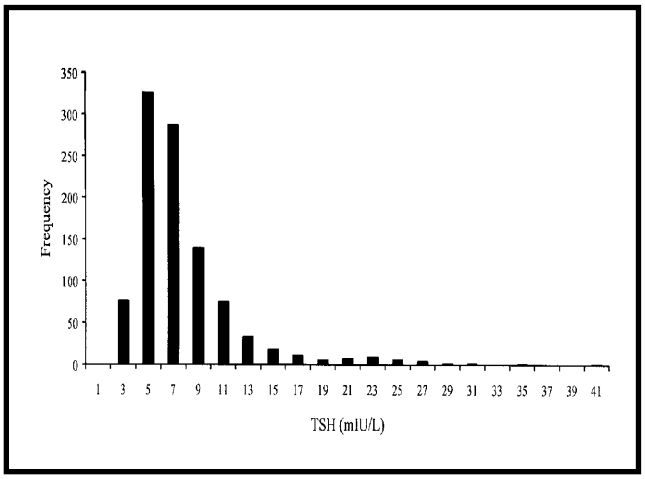

Figure 1 - Histogram of raw data for cord blood TSH.

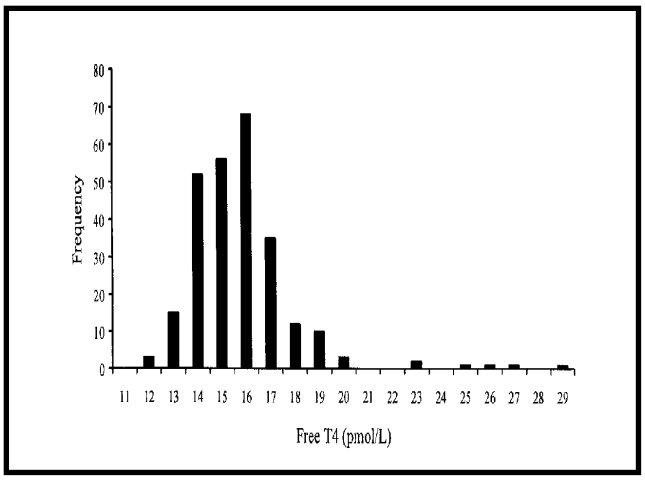

Figure 2 - Histogram of raw data for cord blood FT4.

Discussion. The confirmation of a presumptive positive diagnosis of congenital hypothyroidism is a multidisciplinary task with neonatologists relying on input from clinical chemistry, radiography and nuclear imaging.

The crucial role of the clinical chemist depends on reliable data for interpretation of the infants' thyroid function test results at the screening stage and during further investigation of any presumptive positive results.

Our own reference ranges for cord blood TSH, FT4 and TBG were similar to published data[2] but different with respect to FT3 and TG. As the published data were not sufficiently informative it was not possible to determine if the differences between the respective ranges in the laboratory and reference groups were statistically significant. The discrepancies may be due to methodological differences (equilibrium dialysis as opposed to immunoassay for free T3) or ethnicity. Whatever the reasons, they underline the imperative for local reference ranges.

Cord blood FT3 does not play any part in the diagnosis congenital hypothyroidism but it may be valuable in the diagnosis of the rare cases of neonatal hyperthyroidism.

Laboratories measuring cord blood total T4 for their screening may need TBG measurement to further investigate samples with low T4, in case the abnormal results are due to low levels or absence of TBG.

Some studies[7] have indicated that cord serum TG measurement may be useful in differentiating infants with normal thyroid from those with athyreosis or ectopia. Complementary evidence of thyroid abnormality should be provided by radioisotope scan using ^{123}I or ^{99m}Tc. However, radioisotope scanning is not always possible. In our own limited experience cord blood TG measurement has correlated quite well with radioisotope scan, low levels being found in athyreosis and raised levels in ectopia.

As anti thyroglobulin antibody is known to cross the placenta, all samples for TG measurement were also assayed for the antibody as its presence renders the TG immunoassay invalid. Twelve of 90 cord blood samples (13.3%) had anti thyroglobulin antibody, and were excluded from the calculations of reference ranges. This proportion of infants with anti thyroglobulin antibody is a close reflection of the proportion of adults with the antibody (our own unpublished data).

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