Serum Thyroglobulin Levels in Preterm Infants with and without the Respiratory Distress Syndrome. I. Cord Blood Study

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ABSTRACT. Cord serum levels of thyroglobulin (Tg) and thyroid stimulating hormone (TSH) in 147 term and preterm infants were related to gestation age, birth weight, respiratory distress syndrome (RDS), and several perinatal factors by means of multiple linear regression analysis. None of the perinatal factors influenced Tg and TSH cord serum levels. However, in infants who developed RDS, Tg and TSH cord serum levels differed significantly from values in infants who did not develop this syndrome. In RDS infants, significantly higher Tg values were found. Tg cord serum levels increased with birth weight in the "average" RDS infant (*i.e.* infants with birth weights according to the 50th percentile of the growth chart for their gestation age), while these levels decreased in the "average" non-RDS infant. In RDS infants TSH cord serum levels increased with increasing birth weight, while these levels did not vary in non-RDS infants. Although Tg and TSH cord serum levels in RDS infants increased during gestation, no correlation between Tg and TSH cord serum levels could be demonstrated. There was no correlation between Tg and TSH cord serum levels in non-RDS infants. Since we found a clear correlation between Tg cord serum levels and gestation age, but no correlation between Tg and TSH cord serum levels, we suggest that other phenomena are responsible for the high Tg levels such as organ immaturity. (*Pediatr Res* 20: 996-1000, 1986)

Abbreviations

Tg, thyroglobulin
TSH, thyroid stimulating hormone
BW, birth weight
GA, gestation age
RDS, respiratory distress syndrome
hTg, human thyroglobulin
RIA, radioimmunoassay

Cord serum Tg levels in term infants are higher than in adults (1). Preterm infants have higher Tg cord serum levels than term infants, and there is a negative relationship of Tg cord serum levels with gestation age (2). In adults Tg synthesis and secretion are under control of TSH (3). TSH cord serum levels show a slight decrease (4) or do not vary (5) with GA, and no correlation between Tg and TSH cord serum levels has been demonstrated

(2, 6). Apparently, in the fetal period, Tg cord serum levels are not under control of TSH. The decrease of Tg cord serum levels with gestation age may indicate that the thyroid gland becomes less permeable (to Tg) with increasing maturation (2).

It is generally accepted that the development of the RDS reflects a delayed maturation of the lungs. Thyroid hormones are known to stimulate lung maturation in animals (7, 8). Thyroid hormones in cord serum increase with GA (9). Lower thyroid hormone levels in cord serum have been demonstrated in infants who develop RDS (10-12) than in those who do not. Since Tg cord serum levels may indicate the degree of maturation of the thyroid gland, we advance the hypothesis that in RDS infants higher Tg cord serum levels will be found. Herein we evaluate this idea. Furthermore we investigate the influence on Tg cord serum levels of several perinatal factors which may influence the maturation. The relationship between TSH and Tg cord serum levels is studied as well.

PATIENTS AND METHODS

Patients. Cord blood samples were obtained from 147 infants (123 preterm and 24 term infants) admitted into the neonatal department because of prematurity or low birth weight for GA. BW varied from 675 to 3640 g; GA varied from 25 to 40 wk of gestation. GA was calculated from the mother's last menstrual period. In case of doubt GA was estimated from the Dubowitz score (13).

Each neonate was evaluated for the idiopathic RDS. There were 117 infants without RDS and 30 infants who developed the syndrome. The diagnosis of RDS was made using the criteria of Usher (14). In addition to the following perinatal factors were recorded for each patient: treatment with tocolytics (non-RDS 36, RDS nine), premature rupture of the membranes (non-RDS 25, RDS two), betamethasone treatment (non-RDS 28, RDS 11), the mode of delivery: caesarean section (non-RDS 20, RDS three) or vaginal delivery (non-RDS 97, RDS 27), sex (85 boys: non-RDS 66, RDS 19; and 62 girls: non-RDS 51, RDS 11), neonatal asphyxia: Apgar score of less than 7 (non-RDS nine, RDS nine), multiple birth (non-RDS 14, RDS 11).

Methods. Tg levels were measured by a double antibody RIA method. The preparation and assessment of purity of isolated Tg from hTg, obtained after autopsy, were conducted as described by Ket *et al.* (2). [¹²⁵I]Tg was obtained after iodination of 10 µg hTg with 1 mCi ¹²⁵I⁻ (15). Rabbit antihuman Tg was used at a dilution of 1:75,000. As second antibody an excess of donkey antirabbit γ-globulin serum (Wellcome, Beckenham, England) was used at a dilution of 1:24. The human sera were diluted 1:10 with goat serum sodium phosphate-salt buffer (1:10) mixture. The composition of the sodium phosphate-salt buffer was 3.5 mM sodium phosphate, 0.15 M NaCl and 0.25 M EDTA,

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pH 7.5. The standard line was obtained by adding Tg to the goat serum sodium phosphate-salt buffer (1:10). The addition of pasturized human plasma containing 5 ng Tg/ml in the ratio 1:10 to the assay tubes did not affect the Tg standard curve. Each sample was determined in triplicate with two controls without added anti-Tg. Nonspecific binding was less than 2% in the presence of approximately 1 µg Tg.

The lower limit of detection was 3 ng Tg/ml diluted serum, the highest value found was 170 ng Tg/ml diluted serum. This means that 30 to 1700 ng Tg/ml serum can be measured accurately. Recovery of hTg added to diluted serum averaged $100 \pm 13\%$ ($n = 9$) in the range of 13 to 90 ng/ml of Tg, with an absolute deviation of about 3 ng/ml. After dilution of two sera (1:10, 1:20, 1:40) containing 510 and 740 ng Tg/ml, respectively, the measured Tg concentrations were within 2.5% of the expected values. Dilution of serum (1:10) containing 55 ng Tg/ml (measured in undiluted serum) gave a value of 6.2 ng/ml diluted serum.

The intraassay coefficient of variation calculated according to Van Herle *et al.* (1) was 4.4% on the basis of values ranging from 3.7 to 13.7 ng/ml in diluted sera ($n = 27$), and 5.3% on the basis of values ranging from 16.8 to 63.9 ng/ml in 15 diluted sera. The interassay coefficient of variation did not exceed 12% for values ranging from 11 to 169 ng/ml obtained on 20 diluted serum samples.

Measurements of anti-Tg autoantibodies were performed with the help of thymune T-kits of Burroughs (Wellcome Ned BV), Weesp, The Netherlands.

TSH determinations were performed by RIA as described by Odell *et al.* (16). The lower limit of detection was 0.5 mU/liter. The intraassay coefficients of variation of values ranging from 2 to 8 mU/liter did not exceed 6%, and those of more than 8 mU/liter did not exceed 13%. The interassay coefficient of variation was approximately 10% for values ranging from 1.6 to 27 mU/liter. All determinations were done in duplicate.

Statistical analysis of the results included analysis of variance and application of the multiple linear regression model (17) after logarithmic transformation (see Appendix). For correlation analysis Pearson's correlation coefficient was used (18).

RESULTS

Tg. Tg was measured in all cord blood samples. One hundred samples were examined for the presence of maternal anti-Tg autoantibodies which can be transferred via the placenta. Autoantibodies were not detected in any of these samples anti-Tg autoantibodies were detectable. This leads to a 95% confidence limit of 3% for the probability of anti-Tg autoantibodies in a single sample.

All Tg values measured were used in the regression analysis. In this analysis it is necessary to test whether the regression of log Tg on BW and GA was parallel for each perinatal factor (see Appendix). The hypothesis could be accepted for all investigated perinatal factors, but not for the respiratory distress syndrome ($F = 14.09_{df2,141}$, $p < 0.01$). Therefore the regression analysis was performed separately in non-RDS ($n = 117$) and RDS ($n = 30$) infants.

Non-RDS infants. Log Tg in non-RDS infants was found to depend on BW and GA only. Insertion of the other perinatal factors did not improve the regression equation (none of the perinatal factors tested had a p value of less than 0.05).

The regression equation was found to be:

$$\text{Log Tg} = 2.86 + 0.152 \text{ BW} - 0.031 \text{ GA} \pm 0.25 \quad 1)$$

$$(r = 0.31, p < 0.01) \text{ (BW in kg, GA in wk)}$$

As can be seen from the regression coefficients and their associated SEs (Table 1), log Tg increases significantly with increasing BW when GA is constant, and decreases significantly with in-

creasing GA when BW is constant. In Figure 1 we show the relationship between Tg and BW for different gestation ages. This illustrates the differences in the height of the Tg cord serum levels between the lighter and the heavier infants with the same GA. Since BW increases during normal development of the fetus, we calculated the estimated Tg values in "average" infants, *i.e.* infants with BW according to the 50th percentile of the Kloostermans' growth chart (19) for several different GA. The broken line, constructed from the estimated Tg values, represents Tg in the "average" infant in the last trimester of gestation.

RDS infants. For RDS infants the regression equation was found to be:

$$\text{log Tg} = -1.06 - 0.836 \text{ BW} + 0.155 \text{ GA} \pm 0.30 \quad 2)$$

$$(r = 0.61, p < 0.01) \text{ (BW in kg, GA in wk)}$$

Insertion of the perinatal factors did not improve the regression equation. The SEs of the regression coefficients are given in Table 1. It can be seen that in RDS infants log Tg decreases significantly with increasing BW when GA is constant, and increases significantly with increasing GA when BW is constant. Thus the dependence of log Tg on GA and BW in RDS infants is the opposite of that for non-RDS infants. Figure 2 shows the results.

Table 2 summarizes the relevant mean data of Tg values in the infants subdivided according to their gestation ages; an extra column is added showing the estimated log Tg values (*i.e.* values computed from the regression equation).

TSH. As with Tg, TSH cord serum levels in RDS infants showed a different dependence on BW and GA compared with non-RDS infants ($F = 3.30_{df2,141}$, $p < 0.05$). Therefore the regression analysis was performed separately for RDS and non-RDS infants. Neither in the non-RDS nor in the RDS infants did any of the perinatal factors improve the regression equations. The following regression equation was found for non-RDS infants:

$$\text{log TSH} = 0.49 - 0.077 \text{ BW} + 0.016 \text{ GA} \pm 0.27 \quad 3)$$

$$(r = 0.15, p = 0.20) \text{ (BW in kg, GA in wk)}$$

As can be seen from the regression coefficients and their associated SEs (Table 1), the contributions of BW and GA are not significant and can be ignored. This implies that in non-RDS infants no dependence on either BW or GA is demonstrated.

The equation for RDS infants was:

$$\text{log TSH} = -0.43 + 0.29 \text{ BW} + 0.027 \text{ GA} \pm 0.33 \quad 4)$$

$$(r = 0.41, p = 0.10) \text{ (BW in kg, GA in wk)}$$

As can be seen from the regression coefficients and their associated SEs (Table 1), either of the two factors (GA or BW) can be omitted from the model. However, after omitting GA a specific

Table 1. Results of the regression equations of multiple linear regression analysis for log Tg and log TSH in non-RDS and RDS infants*

	BW			GA		
	b ₁	SE	p	b ₂	SE	p
Log Tg						
non-RDS	0.152	0.055	<0.01	-0.031	0.009	<0.01
RDS	-0.836	0.238	<0.01	0.155	0.040	<0.001
Log TSH						
non-RDS	-0.077	0.059	NS	0.016	0.010	NS
RDS	0.290	0.262	NS	0.027	0.044	NS

* The regression coefficients (b₁ and b₂) and their associated SEs are given with their p values (BW in kg, GA in wk).

Fig. 1. Cord serum Tg values of non-RDS infants. The Tg values are indicated by *symbols* according to the gestation age ($\square$, 25–26–27 wk; $\bullet$, 28–29–30 wk; $\circ$ 31–32–33 wk; $\blacksquare$, 34–35–36 wk; $\triangle$, 37–38–39–40 wk). Regression lines (*solid lines*) of Tg cord serum values on BW for different GA are given according to: $\log Tg = 2.86 + 0.152 BW - 0.031 GA \pm 0.25$, $r = 0.31$, $p < 0.01$. The *broken line* indicates log Tg of the “average” non-RDS infant (see text).

Fig. 2. Cord serum Tg values of RDS infants. The Tg values are indicated by *symbols* according to the gestation age ($\square$, 25–26–27 wk; $\bullet$, 28–29–30 wk; $\circ$ 31–32–33–34 wk). Regression lines (*solid lines*) of Tg cord serum values on BW for different GA are given according to: $\log Tg = -1.06 - 0.836 BW + 0.155 GA \pm 0.30$, $r = 0.61$, $p < 0.01$. The *broken line* indicates log Tg of the “average” RDS infant (see text).

effect of BW is found, according to:

$$\log TSH = 0.21 + 0.407 BW \pm 0.33$$

$$(r = 0.39, p < 0.05) \text{ (BW in kg)}$$

4a)

No specific effect for GA alone was demonstrated ($r = 0.36$, $0.05 < p < 0.10$).

Figure 3 shows the TSH values plotted against BW in both RDS and non-RDS infants. There is a significant increase in TSH values with birthweight in RDS infants ($p < 0.05$). In non-

Table 2. Mean BW, GA, and log Tg, and log TSH in cord serum in non-RDS and RDS infants*

Gestational age	n	BW		GA		Log Tg		Geometric mean Tg	Estimated log Tg	Log TSH		Geometric mean TSH
		Mean	SD	Mean	SD	Mean	SD			Mean	SD	
Non-RDS infants												
25-26-27	4	829 ± 167		25.8 ± 1.0		2.01 ± 0.23		102	2.19	0.85 ± 0.15		7.1
28-29-30	19	1392 ± 256		29.0 ± 0.7		2.18 ± 0.34		153	2.17	0.86 ± 0.25		7.2
31-32-33	23	1636 ± 363		32.0 ± 0.8		2.16 ± 0.29		143	2.12	0.89 ± 0.36		7.7
34-35-36	47	2251 ± 439		35.0 ± 0.9		2.11 ± 0.22		129	2.12	0.88 ± 0.29		7.6
37-38-39-40	24	2094 ± 405		37.4 ± 0.9		1.97 ± 0.22		93	2.02	0.94 ± 0.29		8.7
RDS infants												
25-26-27	4	1040 ± 225		26.3 ± 0.9		1.95 ± 0.20		89	2.15	0.55 ± 0.44		3.6
28-29-30	19	1284 ± 169		28.8 ± 0.7		2.43 ± 0.34		269	2.33	0.70 ± 0.32		5.0
31-32-33-34	7	1654 ± 508		32.1 ± 1.1		2.41 ± 0.41		257	2.53	1.01 ± 0.27		10.2

* Infants were subdivided into groups of 3 or more wk of gestation. Estimated log Tg represents the Tg values computed from the regression equations 1) and 2) for mean BW and mean GA.

Fig. 3. Cord serum TSH values of non-RDS infants (●) and RDS infants (□). The slope of the regression line of RDS infants is significantly different from the slope of non-RDS infants (■): coinciding points of RDS and non-RDS infants.

RDS infants TSH values are not significantly dependent on BW. However, the slope of the regression line of RDS infants is significantly different from that of non-RDS infants ($p < 0.05$).

No correlation could be demonstrated between log TSH and log Tg cord serum values either in non-RDS or in RDS infants ($r = -0.04$ and 0.007 , respectively).

DISCUSSION

We have demonstrated that in RDS and non-RDS infants Tg cord serum levels are differently related to GA and BW. We found the Tg pattern of the "average" non-RDS infant (Fig. 1) to be comparable to the results reported by Ket *et al.* (2). This result is also in accordance with the decrease of Tg with chronological age in childhood (20).

The mean data of the non-RDS infants less than 37 wk of gestation appear to follow the line representing the "average" infant (see Table 2 and Fig. 1). These infants all have mean BW in accordance with the 50th percentile for their gestation age. The non-RDS infants older than 37 wk of gestation in this study, however, appeared on the average to be small for date; therefore their mean Tg values fall below the broken line of Figure 1.

These values can be predicted almost exactly from the regression equation 1) (see also Table 2, estimated Tg). This reflects the accurate description of the data by this model.

With the exception of the early gestation ages, Tg levels found in RDS infants are significantly higher than in non-RDS infants. In non-RDS infants we found an increase of Tg cord serum levels with increasing BW when GA is constant. This appears to be in contrast with the data of Penny *et al.* (21). In their analysis groups were used composed of infants whose gestation age differed by three or more weeks. Therefore their findings reflect the combined effect of GA and BW on Tg cord serum levels. However, our statistical method analyzes the individual effects of BW and GA and gives a more accurate description. On the other hand, their results are consistent with the decrease of Tg in the "average" non-RDS infant found in our study.

Tg cord serum levels reported herein are in a similar range as reported by others (6, 22, 23). On the other hand Black *et al.* (24) reported lower values, ranging from 30–40 ng/ml in both term and preterm infants. Moreover these authors found no difference between ill and healthy preterm infants.

A new finding is that RDS infants show higher Tg cord serum levels than non-RDS infants. Although in RDS infants both TSH

and Tg levels appear to increase with increasing BW (Fig. 2 and 3), no correlation between Tg and TSH levels could be demonstrated. In non-RDS infants TSH shows no dependence on either GA or BW, as was also found by others (5). In the non-RDS infants we could not demonstrate a correlation between Tg and TSH either. These findings are in agreement with other studies (2, 6), but are in contrast with those of Penny *et al.* (21) in newborns. Moreover Roti *et al.* (25) could not demonstrate an increase of Tg cord serum levels in term infants after endogenous TSH stimulation caused by maternal administration of thyrotropin-releasing hormone.

None of the investigated perinatal factors which may stimulate or suppress maturation could be shown to influence Tg and TSH cord serum levels significantly.

We demonstrated a difference in Tg cord serum levels between RDS and non-RDS infants. We hypothesize that in addition to a given level of TSH stimulation, organ immaturity may be responsible for the high Tg cord serum values, especially in RDS infants; an enhanced permeability of the thyroid gland, *e.g.* caused by narrow tight junctions (26), and/or a delayed degradation of Tg by the liver may be involved.

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APPENDIX

The multiple linear regression model is expressed in the following equation:

$$y = a + b_1 x_1 + b_2 x_2 + b_3 x_3 + \dots + b_n x_n$$

where $y = \log \text{ Tg or } \log \text{ TSH}$, x_1 is BW in kg, x_2 is GA in wk, $x_3 \dots x_n$ are the variables to be investigated (RDS and the perinatal factors), a is the intercept and $b_1 \dots b_n$ are the regression coefficients of $x_1 \dots x_n$. Inference from the multiple linear regression model requires the population to be normally distributed; therefore the analysis was performed after logarithmic transformation of the Tg and TSH data. We entered BW and GA as permanent variables in the regression analysis to interpret the specific effect of the other variables. This was necessary because the occurrence of these other variables is dependent on GA and/or BW (*e.g.* tocolysis and betamethasone treatment were given only if pregnancy was less than 33 wk and if no signs of intrauterine growth retardation were present). Thus in each regression analysis the effects of BW and GA were eliminated first. Then the other variables were inserted stepwise in the regression procedure to test their specific influence on Tg or TSH cord serum concentrations. The order of insertion was determined by using the F test to measure the relative importance of variables not yet in the equation. A variable was entered into the regression only at a significance level of $p < 0.05$. After establishing the effects of other variables, the specific effect of BW and/or GA was tested.