J. Clin. Chem. Clin. Biochem.
Vol. 23, 1985, pp. 753–758

Serum Thyroglobulin Concentrations in the First Weeks of Life as Measured with an Immunoluminometric Assay

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(Received May 13/July 24, 1985)

Summary: In addition to the determination of thyrotropin (TSH) on the 5th day of life as a screening parameter for congenital hypothyroidism, serum thyroglobulin concentrations were first measured with a commercially available immunoradiometric assay in those cases presenting with elevated thyrotropin levels. As this thyroglobulin assay required at least 400 μ l serum for a duplicate determination, it was decided to employ a sensitive immunoluminometric assay (detection limit 50 amol/tube) instead, the amount of serum needed being reduced to 100 μ l, the sensitivity to under 3 μ g/l.

We describe the serum thyroglobulin concentrations determined by the immunoluminometric assay in both cord and venous blood and in both full-term and pre-term babies divided into 4 main groups with respect to thyroid function. The criteria for the groups with thyroid dysfunction were determined as a result of

- a) isolated thyrotropin elevation measured in the dry blood spot test (thyrotropin above 4 mU/l),
- b) lowered thyroxine:thyroxine binding globulin ratio together with elevated thyrotropin both prior to and under substitution with *L*-thyroxine.

In full-term babies thyroglobulin levels in serum fell steadily over the first months of life. The same effect was seen in pre-term babies.

The effect of *L*-thyroxine on the suppression of serum thyroglobulin levels appeared to be dose-dependent, as newborns from experimental criterium group a) (defined above) showed no suppression of serum thyroglobulin levels when under partial substitution with *L*-thyroxine (median 84 μ g/l), whereas those in group b), i. e. with full *L*-thyroxine substitution showed a noticeable suppression of serum thyroglobulin (median 27 μ g/l).

Immunoluminometrische Bestimmung von Thyroglobulin im Serum während der ersten Lebenswochen

Zusammenfassung: Zusätzlich zum Thyrotropin-Neugeborenen-Screening am 5. Lebenstag wurde in Fällen mit erhöhter Thyrotropinkonzentration die Thyroglobulinkonzentration immunoradiometrisch bestimmt. Da aber für diese Thyroglobulinbestimmung 400 μ l Serum benötigt werden, wurde ein empfindlicher immunoluminometrischer Assay entwickelt. Es werden für eine Doppelbestimmung lediglich 100 μ l Serum benötigt. Die Empfindlichkeit liegt hier unter 3 μ g/l.

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Die Blutentnahme erfolgte entweder aus der Nabelschnur oder aus einer peripheren Vene. In Abhängigkeit von der Schilddrüsenfunktion wurden die Neugeborenen in 4 Hauptgruppen eingeteilt. In den Gruppen wird die Schilddrüsendysfunktion nach folgenden Kriterien definiert:

- a) Erhöhung der Thyrotropinkonzentration, gemessen im trockenen Blutstropfen (Thyrotropin größer als 4 mE/l), und
- b) Erniedrigung des Thyroxin:Thyroxin-bindendes Globulin-Quotienten vor und unter Substitution mit *L*-Thyroxin.

Sowohl bei reifen als auch bei unreifen Neugeborenen fallen die Thyroglobulinkonzentrationen in den ersten Lebensmonaten kontinuierlich ab. Die Wirkung des Thyroxins zeigt sich dosisabhängig. Bei Neugeborenen der Gruppe a) unter partieller Substitution mit *L*-Thyroxin findet sich keine Erniedrigung des Thyroglobulins, (Medianwert 84 µg/l), während in Gruppe b) die Thyroglobulinkonzentration unter vollständiger Substitution deutlich supprimiert ist (Medianwert 27 µg/l).

Introduction

Thyroglobulin concentrations were previously determined in serum from newborns who showed biochemical evidence of overt hyperthyroidism using a commercial immunoradiometric assay, because thyroglobulin concentrations are, according to the literature (1, 2), related to the morphology of the thyroid gland. As the amounts of serum needed for a duplicate determination were often prohibitive (400 µl), a sensitive immunoluminometric assay was developed in this laboratory which needed 100 µl serum and which had a sensitivity under 3 µg/l. The assay time was reduced at the same time from 48 h to 5 h.

The reference range has been established as under 70 µg/l for euthyroid adults, (serum thyrotropin, thyroxine, thyroxine binding globulin and free thyroxine all in reference range and no clinical evidence or history of thyroid disease — age range 15–74 a, median 51 a), but has not been checked for neonates. In establishing the reference ranges for newborns, blood was taken both from the umbilical cord as well as from a peripheral vein, because ranges given in the literature vary widely (2–5) when measured by radioimmunoassay. This discrepancy is also true for published data on premature babies (6).

In adults it has been observed that administration of triiodothyronine (5) or thyroxine (7) in pharmacologically relevant doses suppresses serum thyroglobulin concentrations, in some cases to below the limit detectable with radioimmunoassay. As a result of these findings, newborns treated with *L*-thyroxine were also included in this study.

No detailed evidence exists concerning the relationship between thyrotropin and thyroglobulin (8, 9) and the post-partal courses of both parameters when

elevated in serum appear not to correlate with each other (4). To investigate whether these findings are true under pathological conditions thyroglobulin concentrations were measured in serum of newborns with persisting elevated thyrotropin levels as well as in those with overt hypothyroidism. The fact that all groups have been measured using the same assay methodology allows the removal of any artefacts due to the methodology alone, an important point worth noting.

Materials and Methods

During the past four years, a bank of recall and control sera has been compiled from neonates presenting with hypothyroidism, which has been stored at -20°C . Patient data on 184 serum samples were available, enabling the establishment of three different experimental groups (Groups I–III), according to different clinical and biochemical parameters. A fourth group (Group IV) consisted of 104 blood samples obtained from the placental side of the umbilical cord directly after birth. Serum thyrotropin levels in Groups I, II & IV did not exceed 18 mU/l in any of these samples nor was the dry-spot thyrotropin level elevated on the 5th day after birth or subsequently.

Group I consisted of 95 sera from healthy full-term newborns who had presented with an elevated thyrotropin in the blood spot test on the 5th day postpartum, but which normalised during the subsequent weeks (see below).

Group II was comprised of 35 serum samples from neonates of less than 38 weeks gestational age, but without any thyroid or other abnormalities.

Group III consisted of 54 babies who had an elevated thyrotropin dry blood spot value (above 13 mU/l) as well as persisting elevated serum thyrotropin levels above 4 mU/l. These neonates presented with either latent or manifest hypothyroidism (determined by thyroxine:thyroxine binding globulin ratios above or below 3.0 respectively). There were no athyroid patients in this group.

Group IV has been defined above.

The three experimental groups were divided into separate subgroups depending upon when the sample was taken (Groups I & II) or depending upon the substitution therapy with thyroxine (Group III). Group I aa comprised sera collected within the first 14 days after birth, Group I ab 14–20 days, Group I b 21–41 days, Group I c 42–98 days and Group I d from 5 months to 27 months. Together Groups I aa and I ab made up Group I a. The subdivision of Group I was made in order to demonstrate that the first drop in serum thyroglobulin took place within the third week of life.

Group II a consisted of serum samples taken within the first 20 days of life, Group II b from day 21–118.

Group III a comprised newborns not receiving substitution therapy, Group III b being substituted with *L*-thyroxine.

Serum thyrotropin and thyroxine binding globulin were determined using commercial kits (Henning-Berlin, Berlin West) as was thyroxine (Mallinckrodt Germany, Dietzenbach, D). In each case the kit instructions were followed without modification.

The following reagents were used in the setting-up and performance of the immunoluminometric assay for serum thyroglobulin:

Polystyrene balls (diameter 6.4 mm) for the immobilisation of the antibodies were purchased from SpheroTech Kugeln, Fulda, D.

Rabbit anti-human thyroglobulin antibodies were obtained from Dakopatts, Hamburg, D.

Standards of human thyroglobulin in serum and quality-control sera were obtained from Sorin, Saluggia, I.

6-(N-(4-aminobutyl-N-ethyl)-isoluminol) hemisuccinamide (ABEI-H) for labelling was purchased from LKB-Wallac, Turku, SF.

Table 1 shows the assay scheme for the immunoluminometric assay as set up in this study.

Tab. 1. Thyroglobulin immunoluminometric assay flow scheme.

50 µl serum/standard
150 µl assay buffer*
1 rabbit anti-human thyroglobulin coated polystyrene ball.
Incubate 120 min on horizontal rotator (170–190 min ⁻¹) at ambient temperature
Wash with 2 × 5 ml 0.15 mol/l NaCl / 0.3 ml/l Tween 20
200 µl ABEI-H labelled anti-thyroglobulin
Incubate for 120 min on horizontal rotator and wash as above
Transfer solid-phase to clean measuring cuvette, add 300 µl microperoxidase/NaCl and measure light integral for 12 s in luminometer**

Key:

* Assay buffer — 0.025 mol/l Tris-HCl, 0.08 mol/l NaCl, 0.025 mol/l phosphate, 150 µl/l Tween 20, 2.5 g/l bovine serum albumin, pH 8.0.

** For full details of luminescence measurement and preparation see references 12 & 13. The luminometer used in this study was an LB-950T 300 sample semiautomatic luminometer — Laboratorium Prof. Dr. Berthold, Wildbad, D.

Table 2 shows basic quality control parameters as well as the established reference range for healthy euthyroid blood donors, the latter being based upon serum thyrotropin (basal), total serum thyroxine and the thyroxine:thyroxine binding globulin ratio as well as normal serum aminotransferases and blood sedimentation rate.

Tab. 2. Reference range and basic quality control parameters for the thyroglobulin immunoluminometric assay.

a. Reference range — under 70 µg/l

number of clinically euthyroid subjects = 156
range of values — 4–68 µg/l
16th percentile — 12 µg/l
50th percentile — 31 µg/l
84th percentile — 60 µg/l
mean — 35 µg/l
mean/median — 1.13

b. Quality control

Interassay coefficient of variation from 36 assays.

Serum	Mean (µg/l)	Standard deviation	Coefficient of variation (%)
1	15.3	2.36	15.4
2	89.6	8.95	10.0
3	315	25.8	8.21

Cumulative precision profile from 55 assays — derived from duplicates

Concentration range (µg/l)	No. of samples	Mean coefficient of variation (%)
0–100	644	9.88
100–200	289	5.84
200–300	104	6.12
300–400	51	5.68
400–500	27	5.65
500–600	17	2.99
600–700	14	6.12
700–800	14	6.90
800–900	9	8.68
900–1000	10	5.53

Data from 1179 patients lying within the standard curve (0–1000 µg/l)

Results

Full-term neonates

Thyroglobulin concentrations were highest in cord blood with values scattered over a wide range (15–above 1000 µg/l). The first signs of a decrease in serum thyroglobulin levels was seen between the 14th and 21st days postpartum (tab. 3 b, Group I ab) where median levels fell by more than 50 µg/l. The fall in thyroglobulin concentrations continued until the 14th week (Groups I b & I c, tab. 3 a). Between the 5th and 27th months after birth, (Group I d, tab. 3 a) the median levels had still not reached those for euthyroid adults (tab. 2).

Tab. 3a. Serum thyroglobulin levels in neonates at various ages.

	Group I	Group Ia 0–20 d	Group Ib 21–24 d	Group Ic 43–98 d	Group Id 5–27 mo	Group IV
Number of samples	95	44	32	8	11	104
Range of values (µg/l)	15–937	45–937	38–187	15–141	30–90	16–1000
Concentrations (µg/l)						
Mean	121	173	84	67	56	213
16th percentile	51	58	52	15	33	92
50th percentile	88	114	79	64	50	155
84th percentile	153	243	121	141	89	296
Mean/median	1.38	1.52	1.06	1.05	1.06	1.37

Group I values are derived from peripheral venous blood, Group IV values from cord blood from the placental side of the circulation.

Group I babies had an isolated thyrotropin elevation in the dry blood spot test on the 5th day of life.

Tab. 3b. Serum thyroglobulin levels (peripheral venous blood) within Group Ia neonates.

Parameter	Group Ia 0–21 d	Group Iaa 0–14 d	Group Iab 15–20 d
No. of samples	44	26	18
Range of values (µg/l)	45–937	45–937	44–187
Mean (µg/l)	173	230	91
16th percentile (µg/l)	58	61	55
50th percentile (µg/l)	114	157	91
84th percentile (µg/l)	243	354	116
Mean/median	1.52	1.47	1.00

Groups defined in the text.

Premature neonates

From table 4 it can be seen that the serum thyroglobulin patterns follow those for the full-term neonates described above. In contrast to the full term babies, the distribution of values in this group was more homogeneous, as seen in the mean/median ratios. Whether this is of any significance cannot be answered as the number of data is too small.

Tab. 4. Serum thyroglobulin levels in premature babies.

Parameter	Group II 0–118 d	Group Ila 0–20 d	Group IIb 21–118 d
No. of samples	35	21	14
Range of values (µg/l)	23–267	28–267	23–175
Mean (µg/l)	96	114	72
16th percentile (µg/l)	34	40	30
50th percentile (µg/l)	78	110	68
84th percentile (µg/l)	162	169	95
Mean/median	1.23	1.04	1.05

In this group the thyrotropin levels and thyroxine:thyroxine binding globulin ratios in serum were normal – thyrotropin under 4 mU/l, thyroxine:thyroxine binding globulin ratio above 3.

Non-substituted and thyroxine-substituted hypothyroid newborns

In view of the results from full-term and premature babies with regard to serum thyroglobulin concentrations and trends, this group was not subdivided according to gestational age.

In neonates presenting with hypothyroidism, but who were not receiving thyroxine-substitution, serum thyroglobulin levels were comparable with healthy euthyroid babies of the same age (21–42 days old). The distribution of results was very wide as can be seen in table 5a, Group IIIa.

The serum thyroglobulin levels in babies receiving *L*-thyroxine substitution were much lower (tab. 5a, Group IIIb) but still widely scattered, as reflected in the mean/median ratios. In a further division of Group III as shown in table 5b, the behaviour of serum thyroglobulin levels with and without *L*-thyroxine substitution becomes more obvious in both latent and overt hypothyroid cases.

Table 6 summarises the serum thyroglobulin levels in 3 patients who were under *L*-thyroxine substitution therapy for part of the observation period.

The following facts became evident:

1. Thyroglobulin levels of newborns with latent hypothyroidism were almost identical with euthyroid neonates of corresponding age groups, independent of *L*-thyroxine substitution.
2. Non-substituted neonates with manifest hypothyroidism had higher serum thyroglobulin levels than those found in the cord blood of euthyroid neonates.
3. In the babies with manifest hypothyroidism under full *L*-thyroxine substitution, serum thyroglobulin levels were much lower than in partially or non-substituted babies.

Tab. 5a. Serum thyroglobulin levels in neonates with and without *L*-thyroxine substitution.

Parameter	Group III	Group IIIa - thyroxine	Group IIIb + thyroxine
a) Age in days			
Median	30	20	74
Mean	74	35	154
Mean/median	2.46	1.75	2.10
b) Concentrations in µg/l			
No. of samples	54	37	17
Range of values	4-1000	17-1000	4-285
Mean	148	191	70
16th percentile	17	38	5
50th percentile	68	84	27
84th percentile	285	473	176
Mean/median	2.18	2.27	2.56

In this group, thyrotropin levels were elevated in both the dry blood spot test and in the subsequent serum control sample. Group IIIb was substituted with *L*-thyroxine hence the heading "+ thyroxine". In Group IIIb the thyroxine:thyroxine binding globulin quotients remained low or normalised under therapy.

Discussion

Serum thyroglobulin levels decrease significantly during the first two years of life, a fact observed by *Ket et al.* (2). In the present study, as seen in table 3 b, this decrease is first observed during the third week of life. Even after 27 months, the median value for serum thyroglobulin levels have not reached adult levels.

The thyroglobulin levels in cord blood and in serum from newborns less than 14 days old show a wide variation as reflected by the mean/median ratios. This fact has also been reported previously (2, 4). The range of values becomes narrower, the older the children become.

Euthyroid premature and full-term babies behave similarly as far as the concentration and time-dependent reduction in serum thyroglobulin levels is con-

Tab. 5b. Serum thyroglobulin levels in hypothyroid newborns with and without substitution with *L*-thyroxine.

Sub-group (see key)	Latent hypothyroidism			Manifest hypothyroidism		
		no sub- stitution	sub- stitution		no sub- stitution	sub- stitution
a) Age in days						
No. of samples	36	30	6	16	5	11
Range of samples	7—377	7—133	27—377	7—579	7—196	14—579
Median	28	24	90	59	10	73
Mean	51	33	141	124	47	160
Mean/median	1.82	1.37	1.58	2.10	4.74	2.19
b) Concentrations in µg/l						
No. of samples	38	32	6	16	5	11
Range of values	13—703	24—703	13—285	4—1000	17—1000	4—209
Mean	141	151	86	164	444	38
16th percentile	37	38	13	5	20	4
50th percentile	73	80	60	18	473	13
84th percentile	285	305	73	473	1000	92
Mean/median	1.93	1.89	1.44	8.86	0.94	2.87

Key: Latent hypothyroidism (thyrotropin above 4 mU/l, thyroxine:thyroxine binding globulin ratio above 3.0)

Manifest hypothyroidism (thyrotropin above 4 mU/l, thyroxine:thyroxine binding globulin ratio below 3.0)

The median serum thyrotropin level in the group with latent hypothyroidism, not substituted was 8.3 mU/l (range 4.3-27.7 mU/l) and in the group with manifest hypothyroidism, not substituted 300 mU/l (range 25-577 mU/l).

In the groups of latent hypothyroidism and latent hypothyroidism, not substituted the date of sampling was not known in 2 cases, the results being omitted from the age data.

Tab. 6. Serum thyroglobulin levels in three selected patients under *L*-thyroxine substitution.

Patient	N. B.	M. K.	F. H.
Date of birth	1980.04.17	1980.06.06	1982.08.05
Dry spot thyro- tropin (mU/l)	38	32	344
Diagnosis	latent hypothyroid	transient manifest hypothyroid	manifest hypothyroid
Day/thyro- globulin (µg/l)	41/71s 145/90 (s = under substitution)	8/656 72/ 6s 196/ 51	8/577 14/209s 72/ 5s

cerned (2, 6). Babies presenting with manifest hypothyroidism in terms of elevated thyrotropin (above 13 mU/l in the dry spot test) and low thyroxine:thyroxine binding globulin quotient (under 3), have median serum thyroglobulin levels before *L*-thyroxine substitution in excess of those found in cord blood of euthyroid newborns. This may reflect the overstimulation of the foetal thyroid gland, since the median thyrotropin levels in this group were above 300 mU/l in serum, compared with the latent hypothyroid group, which had median serum thyrotropin levels of 8 mU/l.

The thyroglobulin suppression as a result of *L*-thyroxine substitution appears to be dose-dependent (5, 11) and is independent of the age of the infants tested. These facts were supported here in Group III in which partially and fully substituted babies were studied. Logically, it may be argued that exogenous thyroxine suppresses thyroid activity and thyroglobulin production and turnover. If not only thyroxine, but also thyroglobulin is released into the circulation in the perinatal period, it is only to be expected that the

suppression of immunoreactive thyroglobulin levels reflects the reduction of endogenous thyroid gland activity.

Acknowledgement

The authors would like to thank Professor *P. Ball* and Professor *P. C. Scriba* for advice and constructive criticism in preparing the text. The help of Frau *Jutta Jäger* and Frau *Sabine Kurras* in carrying out the assays described here is gratefully acknowledged.

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