Neonatal Screening for Congenital Hypothyroidism in West Black Sea Area in Turkey

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The aim of this study was to investigate the incidence of congenital hypothyroidism (CH) in The West Black Sea Area, a mild to moderate iodine deficient area in Turkey. Neonatal screening for CH was performed using blood specimens collected onto filter paper. Thyrotropin (TSH) was measured by radioimmunoassay and a value >20 microunit/ml was considered a cut-off point for re-examining. Venous serum was obtained to measure TSH, thyroxin (T4), free T4 (FT4), and thyroglobulin (TG). To determine the iodine status of the study area, median urinary iodine was measured in 212 randomly selected neonates and their mothers. A total 18606 neonates were screened from three cities (Bolu, Duzce, and Zonguldak) between 2000 and 2002. With a cut-off point of TSH value >20 microunit/ml the recall rate was found 1.6%. Eight cases of CH were diagnosed (incidence 1/2326). There were 3 cases of transient hypothyroidism, with an incidence of 1/6202. Twenty-six percent of the TSH values were greater than 5 microunit/ml. Median urinary iodine concentrations in neonates and their mothers were 85 microgramg/l and 40 microgram/l, respectively. The incidences of CH, transient hypothyroidism, and the recall rate were higher in our study area than many countries in Europe. The study area has been affected by mild to moderate iodine deficiency. Neonatal screening for CH should be introduced in Turkey without delay. A national comprehensive infantile hypothyroidism and iodine prophylaxis policies should be developed.

Key words: **Congenital hypothyroidism, iodine deficiency, screening, Turkey**

Introduction

Congenital hypothyroidism (CH) is one of the commonest treatable causes of mental retardation and occurs in approximately 1 in 4000 infants worldwide (1). Clinical diagnosis occurs in <5% of newborns with CH because of lack specific symptoms and signs in the early neonatal period (2). Without prompt treatment, irreversible mental retardation, growth failure, and a variety of neuropsychological deficits are inevitable (2,3). These features of CH indicate that diagnosis and treatment are essential as early as possible. Since the development of pilot screening for congenital hypothyroidism in Quebec and Pittsburg in 1974 (4), newborn screening for congenital hypothyroidism has become routine in all developed countries of the world and most of Eastern Europe, and is under development in many developing countries.

Elevated serum TSH in the neonate indicates insufficient supply of thyroid hormones to the developing brain, which is either the main consequence of CH, or iodine deficiency (5). For the latter reason the World Health Organization (WHO), United Nations International Children's Emergency Fund (UNICEF), and International Council for Control

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of Iodine Deficiency Disorders (ICCIDD) included neonatal screening TSH as one of the indicators for assessing iodine deficiency disorders (IDD) and their control (6).

Although Turkey has documented mild to severe IDD (7,8), nationwide screening for CH has still not been adopted. As a pilot study this article discusses the use of neonatal screening for CH with primary measurement of TSH in an endemic iodine deficient area and the current status of CH and IDD.

Material and Methods

The study area includes three cities (Bolu, Düzce, and Zonguldak) located in the West Black Sea Region of Turkey whose a total area is 14368 km^2 and population is 1078 337 inhabitants. In two years, 18606 neonates (8931 boys, 9675 girls) were screened. The study was extended more than 2 years only for follow-up of neonates with thyroid gland in-place to differentiate dyshormonogenetic from transient CH babies. Blood specimens were collected onto filter paper (Schleicher & Schuell, No. 2992) by heel-prick, ideally between the 3rd and 7th days of life, but in all cases before newborn nursery discharge. Information was collected regarding the use of topical iodine-containing antiseptics in mothers or in infants.

For measurement of capillary blood TSH, one 3/16-inch disk or two 1/8-inch disks per tube were taken. TSH was measured by radioimmunoassay (RIA) using commercially available neonatal TSH kits (DPC, Los Angeles, CA USA) from filter paper blood. The detection limit of the assay is approximately 0.5 μU/ml for both disk sizes. Capillary blood TSH value ≥ 20 μU/ml was used as a cut-off point. TSH values < 20 μU/ml were excluded from further evaluation. In cases in which the screening TSH concentration was ≥ 20 μU/ml but less than 40 μU/ml, another filter paper specimen was obtained before the subsequent screening test. Each child with a filter paper blood TSH result ≥ 40 μU/ml was seen by a paediatric endocrinologist for emergency investigations. Venous serum samples were taken for measurement of serum TSH, thyroxine (T4), free T4 (FT4), and thyroglobulin (TG). Serum TSH, T4, FT4, and TG were determined by using chemiluminescence's enzyme immunoassay on an automatic Immulite Hormone Analyzer (DPC, Los Angeles, CA). The results were interpreted according to the normal levels for age (9). The diagnosis of primary CH was based on the findings of a low or low normal T4 level together with an elevated TSH level. The routine evaluation of CH included a sodium pertechnetate 99m Tc thyroid scan to identify functional thyroid tissue, thyroid ultrasound to identify thyroid tissue, thyroid volume, x-ray image of knee to identify bone age, urinary excretion of iodine, TG measurement to separate the TG synthesis defect from the peroxidase or deiodinase defects, and anti-thyroid peroxidase antibody (anti TPO) measurement to identify autoimmune thyroid disease. Therapy with l-thyroxin (Levotiron tablet) was started as soon as possible after the baseline evaluation, at an initial dose of 10-15 mg/kg per day and L-thyroxin doses were adjusted at each visit to keep the T4 levels in the upper half of the normal range and the TSH levels between 0.1 μU/ml and 5.0 μU/ml. Between the age of 2 and 3 years, TSH, T4 and FT4 were measured after withdrawal of l-thyroxin replacement for 3 weeks. If the T4 was low (<6 μg/dl) and TSH level was elevated (>5 μU/ml), permanent hypothyroidism was confirmed and therapy was reinstituted. If the T4 and TSH concentrations remained in the normal range, euthyroidism was assumed and a diagnosis of transient hypothyroidism recorded.

To asses the effect of iodine status, urinary iodine concentrations in 212 randomly selected newborns and their mothers, and TG in 212 newborns were measured. Colorimetric ceric-arsenious acid method based on Sandell-Kolthoff reaction was used for determination of urinary iodine content (10).

A portable real-time instrument (ALOKA SSD-500, Tokyo, Japan) with a 7.5 MHz transducer was used for estimating of thyroid volume. Thyroid volume was calculated according to the formula 0,479 x with (mm) x length (mm) x thickness (mm) for each lobe (11).

Statistical analysis was carried out using the Statistical Package for Social Science (SPSS Inc., Chicago, IL). Mean values (±SEM) are shown. The confidence intervals for proportion of newborns with a capillary blood TSH > 5 μU/ml were calculated using the exact mid-p method. One-Way Analysis

of Variance (ANOVA) test was applied to compute the significance of differences in mean capillary blood TSH levels, recall rate, the percentage of capillary blood TSH > 5 μU/ml, TG, urinary iodine concentration, and thyroid volume between the three cities. The interrelationship between the mean capillary blood TSH levels, urinary iodine concentration, and TG levels were analyzed by Pearson's correlation test. Descriptive statistical methods were used where appropriate. Statistical significance was established at a P value <0.05.

Results

The number of infants screened from each city was 5210 (28%) from Bolu, 6140 (33%) from Düzce, and 7256 (39%) from Zonguldak. The recall rate, incidence of CH, and transient hypothyroidism are given in Table 1. The mean age at the diagnosis of CH and the beginning of l-thyroxin replacement was 23 ± 14 days. Eleven infants were diagnosed congenital hypothyroidism. Three of the 11 hypothyroid infants were diagnosed the transient CH at about 2.5 years of age and after 3 weeks interruption of therapy. The results of confirmatory investigations in 8 permanent hypothyroid infants are shown in Table 2. Knee x-rays for bone age determining revealed that absence one or both epiphyses or small epiphyses in two cases of thyroid agenesis and one ectopia (all below 36 weeks of gestation), however in the other cases of CH, the bone ages were comparable with the chronological age. Using a dose of 10-15 mg/kg per day l-thyroxin, elevated serum TSH levels at diagnosis were normalized in all hypothyroid infants within six weeks.

The overall capillary blood TSH levels was 4.69 ± 0.08 μU/ml (± SEM, range, 0.03 μU/ml to 603 μU/ml) and the percentage of capillary blood TSH > 5 μU/ml was 26.7%. According to the cities, the mean capillary blood TSH levels were 5.48 ± 0.20 μU/ml in Bolu, 4.91 ± 0.19 μU/ml in Düzce, and 3.93 ± 0.05 μU/ml in Zonguldak. There was a significant difference between the mean capillary blood TSH levels between three cities (p<0.001).

Table 3 shows by city origin of the mean capillary blood TSH levels, recall rates, the percentage of capillary blood TSH > 5 μIU/ml whole blood, median urinary iodine excretion, and TG levels. The recall rate of Zonguldak was found to be significantly different from Düzce (p=0.025) and

Table 1. The number of the screened newborns, recall rate, and incidence of congenital hypothyroidism and transien hypothyroidism.

Newborns screened	18606
Cases of capillary blood TSH > 20 μU/ml	296
Recall rate	1.6 %
Incidence of CH	1/2326
Incidence of transient hypothyroidism	1/6202

CH, congenital hypothyroidism

Table 2. The results of the confirmatory investigations of 8 permanent hypothyroid infants.

Case no	Capillary blood TSH (μU/ml)	Serum TSH[a] (μU/ml)	Serum T4[b] (μg/dl)	Serum FT4[c] (ng/dl)	TG (ng/ml)	UI[d] (μg/L)	Thyroid Volume[e] (ml)	Thyroid scan
1	378.20	110.7	1.3	0.30	0.94	91	0	Agenesis
2	275.00	150.0	2.9	0.59	210.6	173	1.40	Goitrous gland
3	564.90	175.7	1.0	0.21	290.5	144	0.75	Eutopic thyroid gland
4	396.50	135.5	3.1	0.36	36.3	124	0	Ectopic thyroid gland
5	433.11	154.4	1.4	0.14	298.7	151	1.71	Goitrous gland
6	575.54	182.5	3.7	0.62	108.1	137	0.91	Eutopic thyroid gland
7	260.15	148.6	2.6	0.47	72.6	15.5	0.45	Eutopic thyroid gland
8	603.36	152.4	0.95	0.12	3.1	15.6	0	Agenesis

TSH, thyroid stimulating hormone; T4, thyroxin; TG, thyroglobulin; UI, urinary iodine

[a], The normal age-range of serum TSH values, 0.6-10 μU/ml

[b], The normal age-range of serum T4 values, 1-2 weeks 9.8-16.6 (μg/dl); 2-4 weeks 7.0-15.0 μg/dl

[c],The normal age-range of serum FT4 values

[d], Non-iodine deficient range of UI, >50 μg/l (12)

[e], The normal range of thyroid volume in mature newborns who have normal thyroid function tests and normal urinary iodine excretion in this study, 0.98 ± 0.14 ml

Bolu ($p<0.001$). H owever, there was an insignificant difference between the recall rates of Bolu and Düzce ($p > 0.05$).

The percentage of capillary blood TSH > 5 μIU/ml was 32.1% in Bolu, 26.0% Düzce, and 23.2% in Zonguldak (Table 3). There was a significant difference between the percentage of capillary blood TSH > 5 μIU/ml of Bolu, Düzce, and Zonguldak ($p<0.001$).

The median urinary iodine concentration of Zonguldak showed a significant difference from Bolu and Düzce ($p<0.001$). There was no significant difference between the median urinary iodine concentration of Bolu and Düzce ($p>0.05$).

The overall percentage of capillary blood TSH > 5 μU/ml, median TG, and median urinary iodine concentration are shown in Table 4. Median urinary iodine in neonates (85 μg/l) was significantly highe than their mothers (40 μg/l; $p<0.001$). The correlation coefficients between the mean capillary blood TSH recall rate, percentage of capillary blood TSH > 5 μU/ml capillary blood, median urinary iodine and TG are given in Table 5.

The questionnaire relating to the use of iodine solution as an antiseptic or disinfectant during the newborn period confirmed that all centres responding used povidone-iodine (Betadine) as a local skin antiseptic in mothers or newborns.

Table 3. City origin of the mean capillary blood TSH levels, recall rate, the percentage of capillary blood TSH >5 μU/ml, thyroglobulin, and urinary iodine concentrations.

	City		
	Bolu	Düzce	Zonguldak
Capillary blood TSH (μU/ml)			
Mean ± SEM	5.48 ± 0.20	4.91 ± 0.19	3.93 ± 0.05
Median	3.62	3.10	3.10
N	5210	6140	7256
Recall rate (%)	2.1	109	2.0
N	122	0.9	65
Capillary blood TSH > 5 μIU/ml (%)	32.1	1672	26.0
N	1596	23.2	1683
TG (ng/ml)			
N	64	72	76
Mean ± SEM	43.1 ± 8.0	48.6 ± 8.2	40.7 ± 11.9
Median	28.1	25.9	21.3
UI (μg/l)			
N	64	72	76
Mean ± SEM	77.8 ± 9.5	80.3 ± 8.2	122.5 ± 10.8
Median	61.2	82.7	154.6
Maternal UI (μg/l)			
N	57	68	65
Mean ± SEM	48.8 ± 8.7	55.4 ± 6.1	71.6 ± 9.8
Median	31.4	44.2	75.2

TSH, thyrotropin; SEM, standard error of mean; TG, thyroglobulin; UI, urinary iodine

Table 4. Comparision the results of percentage of capillary blood TSH > 5 μU/ml, median thyroglobulin, and urinary iodine concentrations with the endemic iodine deficiency criteria (*).

Variables	Mild IDD	Moderate IDD	Severe IDD	Presented study
Median UI i n mother (μg/l)	50 - 99	20 - 49	< 20	40
Median UI in infants (μg/l)	35 - 50	15 - 34	< 15	85
Capillary blood TSH > 5 μU/ml (%)	3.0 - 19.9	20.0 - 39.9	≥ 40	26.7
Median TG in newborns (ng/ml)	10.0 - 19.9	20.0 - 39.9	> 40	26

IDD, iodine deficiency disorders; UI, urinary iodine; TSH, thyrotropin; TG, thyroglobulin

* Adopted from Delange (5,12) with permission

Table 5. The correlation coefficient between the mean capillary blood TSH level, recall rate, the percentage of capillary blood TSH >5 μU/ml, median urinary iodine concentrations, and median thyroglobulin.

Variable	Median TSH r/p[a]	Recall rate r/p[a]	TSH>5 μU/ml % r/p[a]	Median UI r/p[a]	Median TG r/p[a]
Median TSH	-	0.61**/<0.001	0.41**/<0.001	0.19/0.09	0.32**/<0.001
Recall rate	-	-	0.71**/<0.001	0.11/0.07	0.21*/0.02
TSH>5 μU/ml %	-	-	-	-0.14*/0.03	0.51**/<0.001
Median UI	-	-	-	-	-0.34*/0.01

TSH, thyrotropin; UI, urinary iodine; TG, thyroglobulin

[a], Pearson correlation test

*, Correlation is significant at the 0.05 level

**, Correlation is significant at the 0.01 level

Discussion

The incidence of CH in European countries was reported as high as 1:2634 in Finland (13), as low as 1:5632 in Hungary (14), it is about 1:3000-1:4000 in Europe (15). The incidence of CH in central part of Turkey was reported 1:2736 in 30097 screened newborns (16). Our pilot study was limited to three cities in Turkey with 1078 337 inhabitants and yearly live-newborn birth rate about 14290. Among screened 18606 newborns, which represented about 65% of all newborns during two years study period, eight neonates were diagnosed with permanent CH (incidence 1:2326).

In this study, with a cut-off point of capillary blood TSH value ≥ 20 μU/ml the overall recall rate was 1.6 %, which is in the higher range of recall rates reported by European countries. With cut-off points of capillary blood TSH values 25 μU/ml or 30 μU/ml, most of the European countries reported their recall rate usually between 0.16 and 2.7% (5) and (17). In one pilot study, recall rate with a cut-off point of capillary blood TSH value >20 μU/ml 2.3% in Turkey (16). The wide variability of recall rates under the same methodology and similar incidence of permanent CH in different parts of the same country may reflect the degree of iodine deficiency in each region. Bolu, Düzce, and Zonguldak are affected by mild to moderate iodine deficiency (8). It is well known that low maternal iodine intake disturbs the thyroid function of neonates transiently or permanently and is expressed by higher TSH levels in neonates (12,18).

The median urinary iodine concentration in newborns was reported lower than median urinary iodine concentration of the school-age children or adults (12). In contrast, in this study, median urinary iodine concentration in newborns was found higher than in their mothers. Although there were limited studies that include simultaneously newborn screening for CH, and median urinary iodine excretion in newborns and their mothers, some studies confirm our findings. Urinary iodine excretion in breast-feeding neonates was reported significantly higher than in their mothers in Ireland (an area of moderate dietary iodine intake (19). A similar level of median urinary iodine excretion in neonates and mothers who had not taken artificial iodine supplementation during pregnancy period was reported in a mild endemic iodine deficiency area (20).

Many studies have shown that topical iodinated antiseptics increase not only the recall rate, but also the median urinary iodine excretion in neonates (21,22). Our questionnaire showed that topical iodine-containing antiseptics (Betadine) were extensively used in our study area. This means that median urinary iodine in neonates may be equal to or even higher than adult levels, if iodine containing antiseptics are used extensively in newborns. TSH levels show a great variability in the perinatal period (23). Accepting capillary blood specimens for CH within the first 24 hours of life resulted in more than the usual number of "false-positive" results or high rate of recall rate (24). Potential problems are the possibility of missing infants with a slowly rising TSH or failure to obtain a specimen before discharge might result in a delayed or missed diagnosis. Many experienced centres suggest accepting all capillary blood specimens from neonates before discharge from neonatal surgery (1,24). We accepted all capillary blood specimens from neonates before discharge from neonatal nursery. These two factors

(extensively using iodine containing antiseptics and collecting capillary blood specimens in the first or second day of life) may explain the reason of our high re-call rate. The high recall rate may be decreased using cut-off point of TSH level according to day of obtained screening blood specimens or increased the level of cut-off point of TSH level from 20 μU/ml to 30 μU/ml.

In conclusion; the incidence of congenital hypothyroidism in our study area is higher than many of European countries. Iodine deficiency and using of iodinated antiseptics in the neonatal period have an important effect on the data of neonatal screening for CH. Neonatal screening for CH using primary measurement of TSH should be the preferred method in Turkey, since it detects not only permanent CH, but also transient congenital hypothyroidism and iodine status of the study area.

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