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Primary Blood TSH/Back Up TSH Measurements: An Improved Approach for Neonatal Thyroid Screening

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Objective: Neonatal hypothyroidism is one of the most common endocrine disorders related to mental impairment and growth retardation in newborns. In many countries, the neonatal thyroid screening programs are performed for rapid diagnosis and treatment of hypothyroidism. The major aim of this investigation was to improve the thyroid screening program using primary blood TSH/back up TSH measurements as some patients are missed due to technical and human errors. *Methods*: A total of 9,118 neonates were evaluated on the protocol. On top of that, the quality control procedures were applied to improve the sampling technique and the laboratory results. *Results*: Three missed neonates by current programs using the cutoff point more than 20 mU/l for blood TSH were found by our approach. *Conclusion*: Results showed that the programs based on the primary blood TSH/back up TSH measurements improve the thyroid screening results. J. Clin. Lab. Anal. 25:61–63, 2011.

Key words: hypothyroidism; newborn screening; TSH; T4

INTRODUCTION

Neonatal hypothyroidism is one of the most common endocrine disorders in the world (1). Since rapid diagnosis and treatment of hypothyroidism during the first weeks of life prevent from mental impairment and growth retardation in neonates (2–5) thus, the newborn thyroid screening programs are extensively developed in the world during the past 35 years (6–8). However, most of the current programs are switched on the TSH measurement but even in the more sensitive ones, 5–10% of neonates are missed due to technical and technician errors (9–11).

Three programs known as primary blood TSH/back up T4 measurements, primary blood T4/back up TSH measurements, and simultaneous measurements of T4 and TSH are currently used in newborn screening programs (12–14). The primary blood TSH/back up T4 measurements are used often in Europe, Japan, Mexico, and the United States. In this approach, cases with hypothyroxinemia, central hypothyroidism, and thyroid binding globulin deficiency together with the delayed TSH elevation are missed during the screening. Other approaches, such as the simultaneous measurements of T4 and TSH, however, are more sensitive for the thyroid screening but suffer from some problems such as high expenses. In almost all the programs, the dried blood TSH between 20 and 25 mU/l has been used as a cutoff point to recall neonates (15).

The aim of this investigation was to improve the previous protocol (16) by reducing the recall rate. In this study, we screened 9,118 neonates by the approach of primary blood TSH/back up TSH measurements.

NEONATAL SCREENING PROGRAM

The neonatal thyroid screening program, however, is performed by the government, but the expenses of laboratory tests are paid by families in Iran. At the beginning of the program, the cut off point for the dried blood TSH was 20 mU/l. Since several screened cases were found as hypothyroidism thus, the cutoff point was diminished to 5 mU/l. On this protocol, the neonates

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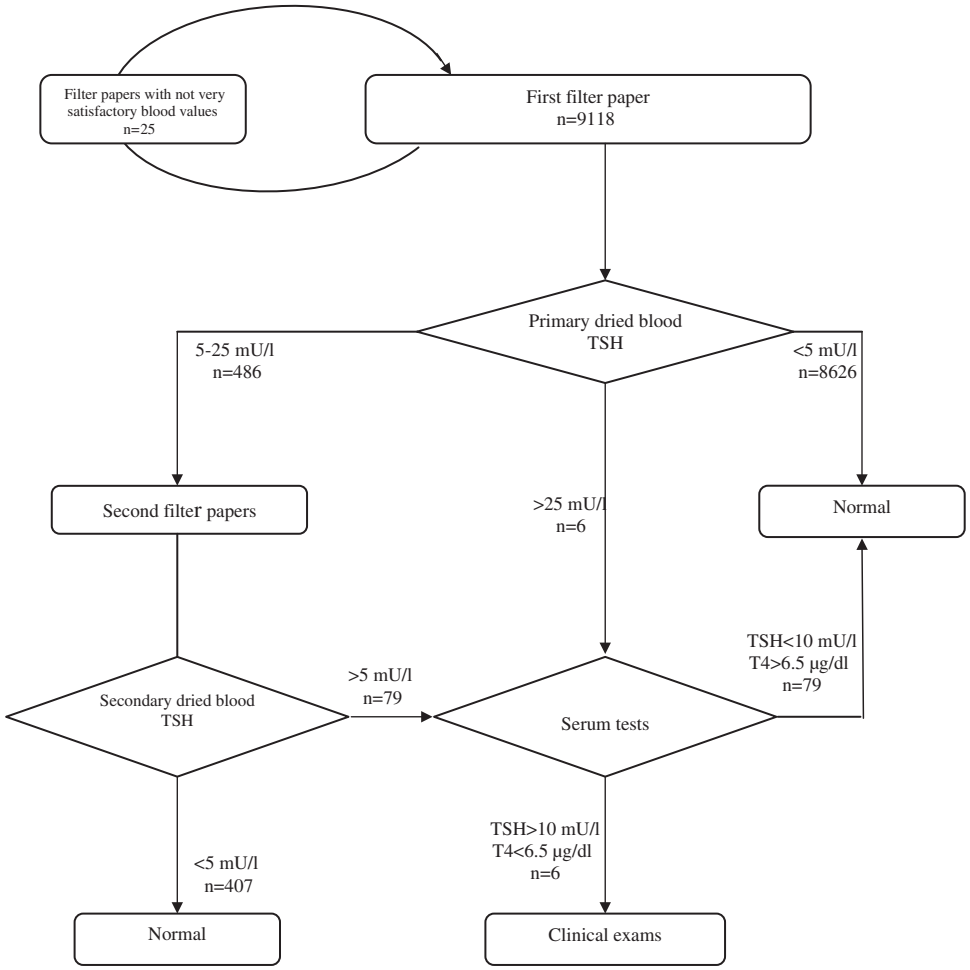

Fig. 1. Newborn thyroid screening program's algorithm.

with the primary dried blood TSH more than 5 mU/l were recalled for measurements of the serum TSH, T4, and T3 and clinical examinations (16). However, the protocol improved the newborn screening program but the recall rate was high (3.6%).

In new approach, the newborns with primary dried blood TSH values between 5 and 25 mU/l were recalled for the secondary blood TSH between 10th and 15th days of age. Then, the ones with the secondary blood TSH above 5 mU/l and those with the primary blood TSH above 25 mU/l were recalled for the clinical examinations and serum tests (Fig. 1).

In addition, the neonates with birth weight more than 4,000 or less than 2,500 g and twins were screened between the third and fifth days and at 2 weeks of age.

DRIED BLOOD TSH ASSAY

The dried blood samples were collected between the third and seventh days of life on a filter paper (Schleicher and Schuell, NO 903) (17). The evaluation of spotted blood on circular areas of filter papers was under strict supervisions and those with not very satisfactory values were recalled for the second filter paper (2.8 by 1,000 filter papers). As the coefficient of within-run variation (CV_{TSH}) of disks was between 11.28 and 25.5% thus, all the discs were prepared from the center of circle and TSH was measured by ELISA technique (Stat-Fax 3100; Awareness Technology, Inc., Palm city, Florida) (18).

Five standard samples (0, 5, 10, 30, and 60 mU/l used for standard graph), two calibrators (low and high values), and three standard replicates equal to the cutoff point (5 mU/l) were applied in each 96-well plate.

The CV value of the three standard replicates was between 10.5 and 13%. The acceptable precision for each run was 5 ± 1 mU/l and acceptable one-way error (AOE) was calculated to be $(CV \times 5)/100$.

Actual cutoff point (ACP) for each run was also calculated by the linear regression equation ($ACP = -0.0052A + 5.847$), as there was a correlation ($R^2 = 0.999$) between the replicates and absorbance (A) of standard sample (TSH = 5 mU/l).

The ACP ± AOE was the criteria for rechecking the filter papers. The AOE and ACP values were calculated to be 0.3–0.8 mU/l and 4.7–5.2 mU/l, respectively, and the repeat rate was 2.8 by 1,000 filter papers.

FINDINGS

On our screening protocol, six neonates with primary dried blood TSH above 25 mU/l were visited by the physician (Fig. 1). Three neonates had the serum TSH more than 50 mU/l and serum T4 less than 6.5 μg/dl. In addition, 486 neonates had the primary dried blood TSH values between 5 and 25 mU/l. The blood TSH values in seventy-nine neonates were confirmed by the secondary dried blood TSH, two neonates were found as cases of hypothyroidism, and a neonate was under the clinical inquiries.

The recall rate for the measurements of serum tests was reduced to 0.85% in comparison with the previous report (3.6%). On the screening policy, however, some cases of hypothalamic–pituitary hypothyroidism with the normal TSH would be missed but the newborns with dyshormonogenesis or the thyroid gland dysgenesis characterized with the low T4 and the delayed TSH elevation can be found during an interval for the measurement of secondary dried blood TSH (19). In addition, we applied the quality control procedures covering almost all sectors in the program to improve false-negative results (20).

In conclusion, the study showed that the thyroid screening approach based on the primary blood TSH/back up TSH measurements and strict supervisions on the sectors of program improve the neonatal screening results.

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