Etiology of congenital hypothyroidism using thyroglobulin and ultrasound combination

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Abstract. Methods currently employed to establish the etiology of congenital hypothyroidism include thyroid ultrasound and scintigraphic exams. Thyroglobulin is a protein almost exclusively secreted by thyroid tissue and indirectly reflects the amount of follicular cells. Even though thyroglobulin is easy to measure, it has been not frequently used because of discordant results to distinguish mainly athyreosis and ectopy (dysgenesis). Knowing the differences in inheritance and prognosis of thyroid dysgenesis and dyshormonogenesis, it is important to define the etiology of CH, combining tools that are easy, fast and available in most medical centers. Our objective was to evaluate and compare color Doppler ultrasound and serum thyroglobulin with radionuclide scan to define the etiology of congenital hypothyroidism. We evaluated 38 children above 3 years-old off-treatment that performed serum thyroglobulin by immunofluorometric assay, color Doppler ultrasound and radionuclide study. On color Doppler ultrasound, 11 patients had athyreosis, 5 ectopic glands, being 1 associated to hemiagenesis. Twenty one had topic thyroid (3 goiters, 10 normal, 8 hypoplastic). Hemiagenesis and cystic lesion were not revealed by radionuclide scan. We observed substantial agreement between color Doppler ultrasound and radionuclide scan (kappa=0.745, p<0.0001). Serum thyroglobulin in athyreosis ranged from <1.0 to 18.7 µg/L. Patients with ectopic glands showed wider thyroglobulin range (4.5 to 123 µg/L, median 28.4 µg/L). Only one patient showed thyroglobulin deficiency. By using color Doppler ultrasound and serum thyroglobulin levels as valuable combined tools, we established the etiology of congenital hypothyroidism limiting excessive and harmful exams in children, like radionuclide scan.

Key words: Thyroglobulin, Thyroid dysgenesis, Congenital hypothyroidism, Scintigraphy, Ultrasound

CONGENITAL hypothyroidism (CH) is a neonatal disorder characterized by low thyroid hormones at tissue level. The most common causes of primary CH are alterations in thyroid gland development (dysgenesis) and thyroid hormone synthesis defects (dyshormonogenesis) [1-3].

Dysgenesis encompasses thyroid development defects, such as hemiagenesis, ectopy and athyreosis. This is often sporadic and has a genetic origin in only 3% of cases. Mutations in thyroid transcript factors, TSH receptor and PAX8 gene have been described [4, 5]. Dyshormonogenesis comprises defects in any hormonal synthesis steps, including hyporesponsiveness to TSH, defects in thyroidal iodide transport and organification and thyroglobulin synthesis. These are all characterized by a normally located thyroid gland, with normal or enlarged volume [6, 7].

All neonatal patients must be submitted to neonatal screening for CH, using neonatal TSH and/or T4 measurement approaches [8, 9]. When alterations are detected the patient is recruited for clinical evaluation and followed up with repeated measurements [10]. Methods currently employed to establish the etiology include thyroid ultrasound and radionuclide scan, either with 123I or 99mTc Pertechnetate. Perchlorate discharge test is only performed to define organification defects. There is some disagreement to whether a thyroid scan should be performed in all babies because of the unknown risk of radiation exposure, particularly in centers where only 131I is used and relatively large doses of isotope are administered. Unfortunately, in
31 patients with dysgenesis and suggested firstly to be performed in patients with CH and if its level is undetectable, the radionuclide scan could be avoided [22]. This approach, thyroglobulin and radionuclide scan, permits to distinguish between patients with athyreosis or ectopy, but not dysormonogenesis.

In the present study we proposed to evaluate patients with primary congenital hypothyroidism by combining serum thyroglobulin levels and thyroid color Doppler ultrasound (CDUS) findings and comparing with radionuclide scan to define etiology.

**Patients and Methods**

All patients above 3 years-old with primary CH were recruited between 2006 and 2008 from the Association for Parents and Friends of Disabled Individuals (APAE) of a São Caetano outpatient clinic, and referred to the Governmental Neonatal Screening Program Service to be studied at the Hospital das Clinicas – FMUSP. Informed consent was obtained from all parents, and the protocol was approved by the Institution’s Ethics Committee. The initial diagnosis of CH was established in all patients by routine neonatal screening, using neonatal TSH and/or the T4 approach. All patients started levothyroxine treatment after initial diagnosis and were followed-up.

Total T3 and T4, free T4 (FT4), TSH, thyroglobulin (TG) and anti-TG antibody were measured by immunofluorometric assays (Autodelfia®, Wallac Oy, Turku, Finland) in all patients after 4 weeks washout without levothyroxine treatment.

Color Doppler ultrasound was performed using a Phillips scanner with a 7.5-12 MHZ transducer focused on the thyroid gland and cervical region, from mandible bone to manubrium. Total thyroid volume was calculated as described elsewhere [23] and compared according to height [24], sex and age, body surface and sex [23, 25]. Two investigators analyzed the images of ultrasonography independently and the results were compared. They were blinded to radionuclide scan findings by design.

Radionuclide scan was performed after 4 weeks without levothyroxine and 2 weeks with a low iodine diet. Uptake measurement was made at 2 and 24 hours after oral administration of $^{131}$I (5 µCi). Uptake measurements were taken again on the following day after intravenous injection of $^{99m}$Tc Pertechnetate. Two investigators analyzed the images independently and
All patients presented TSH increment after discontinuing levothyroxine treatment, thus confirming primary CH. Three patients showed positive anti-TG, and consequently serum TG was rejected from analysis.

CDUS results

On CDUS, 11 patients had athyreosis and 5 ectopic glands, all located in the submentum region. One patient presented simultaneously hemiagenesis and an ectopic thyroid tissue, due to visualization of a hypoplastic right thyroid lobe (2.0 x 1.6 x 1.5 cm) and hypoechoic tissue with vascularization flow at sublingual region (1.5 x 1.2 x 0.8 cm) (Fig. 1). Twenty one patients had topic thyroid, comprising 3 goiters, 10 normal volumes and 8 hypoplastic glands. All patients had homogeneous parenchyma, except one patient showed a hypoplastic with one cyst (0.4 cm) that occupies almost the entire right lobe of the thyroid gland (0.9 x 0.4 x 0.4 cm)(Fig. 2).

Radionuclide scan revealed 7 patients with athyreosis, 11 with ectopic thyroids in the submentum region, and 10 topics with normal iodine uptake. Ten patients deficient. All patients presented TSH increment after discontinuing levothyroxine treatment, thus confirming primary CH.

Three patients showed positive anti-TG, and consequently serum TG was rejected from analysis.

Statistical analysis

The agreement between ultrasonographic and scintigraphic evaluations was measured by Kappa. The level of significance was 5%.

Results

We evaluated 38 patients, 26 females and 12 males, aged from 3 to 13 years old (median 4.6). Only one patient had short stature and was diagnosed as SHOX deficient. All patients presented TSH increment after discontinuing levothyroxine treatment, thus confirming primary CH.
had high iodine uptake at 2 hours, including 3 goiters. These patients were submitted to the PCIV test, 6 of which showed a positive early iodine discharge test with normal hearing, thereby defining a thyroperoxidase deficiency.

**Comparison between US and radionuclide scan**

We found discrepancies in 5 patients diagnosed as having athyreosis on CDUS yet presented ectopic glands on scintigraphy. The hemiagenesis was not revealed by radionuclide scan, but CDUS clearly demonstrated thyroid tissue (Fig. 1). One patient presented a cystic lesion on CDUS which was not diagnosed on scintigraphy. The agreement between all ultrasound and radionuclide scan results kappa coefficient = 0.745 (p<0.0001).

**Comparison between thyroglobulin and radionuclide scan**

Thyroglobulin levels in athyreosis detected on radionuclide scan ranged from <1.0 to 18.7 µg/L (median 4.7 µg/L). The highest thyroglobulin level was detected in the patient with cystic thyroid lesion. Ectopic glands showed a wider range, from 12.2 to 123 µg/L for thyroglobulin (median 28.4 µg/L). Only one patient with high iodine uptake showed low thyroglobulin level (2.1 µg/L) denoting the thyroglobulin deficiency. The remaining patients with high iodine uptake had thyroglobulin levels which ranged from 39.8 to 287 µg/L (median 70.2 µg/L). We did not consider patients with positive anti-TG antibody.

**Combining thyroglobulin, CDUS, radionuclide scan, PCIV and absence of deafness**

At the end of all evaluations, we reached the following final diagnoses: 6 athyreosis, 10 ectopia, 1 ectopia and hemiagenesis, 8 hypoplasia, 7 thyroperoxidase deficiencies, and 3 thyroglobulin deficiencies. We were unable to determine the etiology of CH in three patients. Besides slightly increased TSH, these patients demonstrated variable serum TG along with normal thyroid on CDUS and radionuclide scan. We sequenced all exons and exon-intron boundaries of the TSH receptor gene, but no mutation was found [26].

After ruling out the presence of thyroglobulin antibodies, using only serum thyroglobulin and CDUS in first approach we would consider athyreosis those patients with low to undetectable serum thyroglobulin and no visible gland on CDUS. If serum thyroglobulin was detectable or high and no thyroid tissue was detected on CDUS, the patient could have an ectopic gland with such a small tissue that could not be seen in CDUS. Patient with low thyroglobulin level and normal or enlarged thyroid on CDUS would be diagnosed as thyroglobulin deficient patient. In the presence of high thyroglobulin level, a normal or enlarged gland detected on CDUS, organification defect is the most probable diagnosis. In the absence of deafness, thyroperoxidase deficiency is the most probably etiology (Fig. 2).

**Discussion**

The determination of CH etiology is almost pivotal to ascertaining not only parental counseling, but also helping to titrate levothyroxine dose. According to disease severity, some authors suggest higher dose in athyreosis than ectopy and dysshormonogenesis [27, 28]. We suggest combining color Doppler ultrasonographic examination and serum thyroglobulin measurement to help definition of CH etiology. Thyroglobulin measurement is not usually performed during the first assessment of CH patients. Thyroglobulin is the best method of revealing the presence of thyroid tissue, as it is a thyroid-specific protein. In our study, we performed thyroglobulin and TSH measurements following levothyroxine withdrawal. We diagnosed six cases of athyreosis using CDUS with thyroglobulin levels < 10 µg/L. The misdiagnosis of ectopia on CDUS proved on scintigraphy showed thyroglobulin levels ranging from normal to extremely high levels. Therefore, those patients with no thyroid tissue on CDUS and detectable thyroglobulin, we must consider a false-negative ectopia diagnosis. We noted that in ectopic glands with normal thyroglobulin level, little thyroid tissue was seen on scintigraphy. This reflected a low tissue volume to secrete the thyroglobulin protein, and therefore scant thyroid tissue to be detected on CDUS. Anyway is thyroid dysgenesis, ectopia or athyreosis and consequently a permanent CH.

Thyroid ultrasound is a rapid and harmless exam. Moreover, it can be performed at any time without preparation or interruption of treatment. CDUS proved accurate for diagnosing thyroid hypoplasia in our study, using height reference for normal volume in the Brazilian pre-school population [23, 25]. We did not find the best reference for normal thyroid volume in childhood. Ueda et al studied a Japanese popu-
Table 1 Comparison between color Doppler ultrasound and radionuclide scan findings in 38 patients with congenital hypothyroidism.

<table>
<thead>
<tr>
<th>Color Doppler ultrasound (CDUS)</th>
<th>Athyreosis</th>
<th>Ectopy</th>
<th>Eutopic</th>
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<td>7</td>
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<tr>
<td>Ectopy</td>
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<td>5</td>
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<tr>
<td>Total</td>
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<td>5</td>
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Kappa=0.745, p<0.0001

![Fig. 3] Flowchart of congenital hypothyroidism diagnosis.

lution, which certainly had a lower mean height than the population worldwide [24]. We know that thyroid volume can vary not only with height, but also with iodine ingestion, sex, body surface and puberty. But we need a swift and easy reference to use as a normative pattern. Unfortunately, normal thyroid references for populations under 6 years and above 3 years of age are not available [13, 29]. We noticed that serum thyroglobulin levels in a patient with hypoplastic gland is lower than in a patient with a normal gland or goiter due to TPO/THOX2 defect (mean 42.6 versus 122.6 μg/L). Therefore thyroglobulin level can indicate if total thyroid volume measured at CDUS is hypoplastic or goiter.

Patients with normal or enlarged thyroid gland and low or undetectable thyroglobulin levels have an etiology of thyroglobulin deficiency [30-32]. In such cases, scintigraphy and PCIV are redundant. By contrast, patients with normal or enlarged thyroid glands and normal or high thyroglobulin levels must undergo scintigraphy to establish NIS or organogenesis defects [7, 33, 34]. With no iodine uptake, the diagnosis is NIS defect. PCIV need only be performed in patients that have high iodine uptake, a normal audiometric exam, and questionable thyroglobulin levels.

It is important to define the cause of CH, since there are known differences in inheritance and prognosis [4, 5]. Dysgenesis are mostly sporadic events and very few mutations are found, whereas inborn errors of thyroid hormone metabolism are inherited, usually autosomal recessive, important differences to parental gen-
netic counseling. Also for prognosis, many studies in children with CH have shown that those with functioning thyroid tissue (ectopic, goitrous or hypoplastic glands) have better neuropsychological development than those with none [35, 36]. Nevertheless, some authors are favorable to indicate higher levothyroxine dosage in early treatment in athyreosis patients [27, 28, 37].

We propose a flowchart for CH investigation (Fig. 3). After neonatal diagnosis, CDUS and thyroglobulin measurement should be first performed. Depending on the results, it may not necessary to check patients at around 3 years of age. Thus, children would not be submitted to so many exams and the final diagnosis could be made earlier in the majority of cases.

We conclude that by using CDUS and thyroglobulin levels as valuable tools to establish the etiology of CH, excessive exams which are hard to perform in children can be limited while the radionuclide scan can be replaced or rarely indicated.

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### References


