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ORIGINAL ARTICLE

Thyroid

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Anaemia-based screening for resistance to thyroid hormone alpha in children

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Abstract

Background: The hypothyroid phenotype associated with resistance to thyroid hormone alpha (RTH-α) is associated with a diverse clinical picture. On the other hand, thyroid-stimulating hormone (TSH) levels are normal. Free triiodothyronine (fT3) and free thyroxine (fT4) levels can also be normal; however, normo- or macrocytic anaemia is usually present in reported cases. Diagnosis is challenging and there is limited data regarding screening methods.

Objective: The study aimed to assess the efficiency of a screening strategy for RTH-α.

Subjects and Methods: Out of a total of 6540 children evaluated at the outpatient clinics of paediatric neurology over 2 years and who underwent complete blood count and thyroid function tests, 432 were found to have anaemia. Within this group, we identified 42 children without an underlying specific neurological aetiology who exhibited normo- or macrocytic anaemia, normal TSH levels, fT3 levels in the upper half of the normal range or high, and fT4 levels in the lower half of the normal range or low. We excluded one patient who had already been diagnosed with RTH-α and nine patients could not be reached. Subsequently, clinical evaluation, biochemical assessment, and *THRA* sequencing analysis were conducted on 32 children. The findings were compared with those of the known RTH-α patients in our unit.

Results: The median age of the patients was 5.7 (5.1–7.4) years, and 22 of them were males (69%). The main reasons for assessment in paediatric neurology clinics were autism spectrum disorder ($n = 12$, 38%), epilepsy ($n = 11$, 34%), and delay in developmental stages ($n = 8$, 25%). Constipation was present in five of the cases (16%), while the closure of the anterior fontanelle and tooth eruption were delayed in two cases (6%) and one case (3%), respectively. The median length/height and weight standard deviation (SD) scores were 0.3 [(−0.8)–(1.1)] and −0.1 [(−0.8)–(0.3)], respectively. The median fT3, fT4, and TSH levels were 4.6 (4.2–5.0) pg/mL, 0.9 (0.8–1.0) ng/dL, and 2.2 (1.8–3.1) uIU/mL, respectively. Thirteen of the patients (41%) had high fT3 levels, while none of them had low fT4 levels. The normo- or macrocytic anaemia rate was 47% (normocytic/macrocytic, $n = 8/7$) at the time of reassessment. Serum creatine kinase (CK) was elevated in five patients (16%; one

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had anaemia). None of the subjects had a pathological variant in *THRA*. Known RTH-α patients had significantly lower median height SD score, higher rates of delayed tooth eruption and closure of the anterior fontanelle, lower haemoglobin levels, and higher mean corpuscular volume (MCV) and CK levels as compared to those found without RTH-α.

Conclusions: This approach found one known patient with RTH-α but did not reveal any new cases. Notably, normo- or macrocytic anaemia did not persist in nearly half of the screened patients. A screening strategy that takes clinical findings and prominent laboratory features suggestive of RTH-α into account could lower unnecessary genetic analysis of *THRA* in patients presenting with neurological problems.

KEYWORDS

hypothyroidism, impaired sensitivity to thyroid hormone, neurodevelopmental disorder, thyroid hormone receptor alpha

1 | INTRODUCTION

Thyroid hormones (THs: thyroxine, T4; triiodothyronine, T3) have a wide range of physiological functions, such as the regulation of growth and metabolism as well as the maturation of the central nervous system through processes like neurogenesis, cell migration, differentiation, synaptogenesis, and myelination.[1,2] The active thyroid hormone (T3) exerts its effects through nuclear receptors, namely thyroid receptor α (TRα) and thyroid receptor β (TRβ).[3] These receptors play a crucial role in the regulation of gene expression within target tissues.[2–5]

Resistance to thyroid hormone alpha (RTH-α) is characterised by the presence of dominant-negative loss-of-function mutations in the *THRA* gene, responsible for encoding the alpha receptor. This rare genetic disorder manifests symptoms similar to those of congenital and primary hypothyroidism, such as growth impairment, delayed bone development, varying degrees of neuro-developmental retardation, macrocephaly, macroglossia, dysmorphic facies with a flattened nose, constipation, and bradycardia.[5–7]

Patients with RTH-α often present with varying degrees of delayed milestones, including deficits in motor and speech abilities such as slow initiation of motor movements, impaired fine and gross motor coordination, variably reduced IQ, intellectual disability, and delayed speech development.[7–9] Furthermore, there have also been reported cases of autism spectrum disorder (ASD) or childhood epileptic seizures in individuals with RTH-α.[10–12]

RTH-α patients typically have near-normal thyroid function tests (TFTs): free T4 (fT4) levels are low or in the lower half of the normal range and free T3 (fT3) levels are high or in the upper half of the normal range. Thyroid-stimulating hormone (TSH) levels are normal due to the intact functionality of TRβ, which is responsible for the regulation of negative feedback of THs in the hypothalamus–pituitary–thyroid axis (HPT).[7,8,13] Mild, usually normo- or macrocytic anaemia is prevalent in RTH-α patients. The presence of anaemia has been identified in approximately 80% of reported paediatric cases.[8] Additionally, these patients may exhibit elevated levels of serum creatine kinase (CK).[8,9,14]

Diagnosis is essential, as levothyroxine treatment effectively alleviates some clinical symptoms in RTH-α patients.[10,11,13,15] The process of diagnosing RTH-α presents challenges due to the inconsistency observed between TH levels and clinical phenotypes, as well as its potential association with uncommon phenotypes, such as ASD, potentially leading to a delay in referring patients to paediatric endocrinologists. However, there is a lack of knowledge regarding screening methods for RTH-α in the literature.

Based on the observed prevalence of anaemia among most RTH-α patients, the primary objective of this current study was to evaluate the effectiveness of a screening strategy for RTH-α in patients referred to paediatric neurology outpatient clinics.

2 | SUBJECTS AND METHODS

2.1 | Patients

A retrospective single-center study was carried out, involving children who were assessed in paediatric neurology outpatient clinics between 2018 and 2020. Via using the electronic file system of the hospital, we detected 6540 children in whom both complete blood counts and TFTs were performed. Out of this group, 432 children were found to have anaemia. Among them, the number of children without an underlying specific neurological aetiology presenting with normo- or macrocytic anaemia, normal TSH levels, fT3 levels in the upper half of the normal range or high, and fT4 levels in the lower half-normal range levels or low were 42. Nine patients could not be reached for further evaluation. Moreover, we excluded one patient who had already been diagnosed with RTH-α. Consequently, the remaining 32 children within the group underwent a clinical assessment, biochemical evaluation, and *THRA* sequencing analysis (Figure 1).

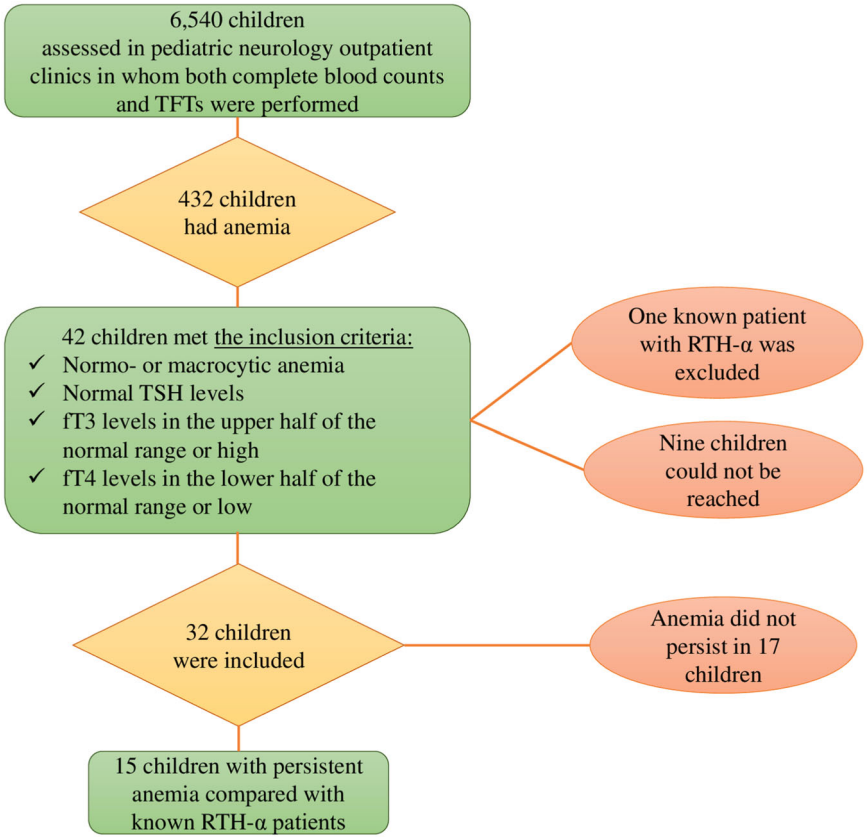


FIGURE 1 The flow diagram including the inclusion criteria of the study. fT3, free triiodothyronine; fT4, free thyroxine; TFTs, thyroid function tests; TSH, thyrotrophin-stimulating hormone.

2.2 | Data collection

Demographic and clinical data, including age, gender, anthropometric measurements, physical examinations, and laboratory findings were obtained. Detailed information was collected concerning the family history of thyroid disease, motor, and mental developmental milestones, frequency and consistency of defecation, school performance, and mental health status. The height of the patients was measured using the Harpenden stadiometer with a precision of 0.1 cm. Additionally, body segments were measured, and body proportions were calculated. The body weight of the subjects was measured using an electronic scale (SECA) with a precision of 0.1 kg while ensuring the subjects were without shoes and wearing light clothing. The body mass index (BMI) was determined by dividing the body weight (kg) by the square of the height (m^2). Additionally, head circumference was measured using tape, beginning from the midpoint of the forehead and extending to the farthest point at the back of the head. Standard deviation (SD) scores for weight, height, BMI, and head circumference were calculated using Child Metrics,[16] utilising the reference data for the Turkish population as provided by Neyzi et al.[17] According to Tanner's standards, patients who had reached a pubertal stage of ≥2 were classified as pubertal.[18] Blood pressure measurements were taken by a single investigator twice on the right arm after a 10-min resting period in a seated position using a calibrated sphygmomanometer. Subsequently, blood pressure percentiles were calculated considering age, gender, and height.[16]

2.3 | Laboratory analyses

Serum fT3, fT4, and TSH levels were measured by chemiluminescent microparticle immunoassay (Beckman Coulter), and reference ranges of the kit were used. The complete blood count, total cholesterol, CK, and insulin-like growth factor-I (IGF-I) were all measured from blood samples taken between 8 and 10 AM after 10–12 h of fasting during the night using standard laboratory procedures. Anaemia was defined as haemoglobin or haematocrit at or below the 2.5th percentile for age and sex, based on reference data from healthy children. It was classified into three types: microcytic (mean corpuscular volume [MCV], ≤2.5th percentile for age and sex), normocytic (MCV, between the 2.5th and 97.5th percentiles for age and sex), and macrocytic (MCV, ≥97.5th percentile for age and sex).[19] IGF-I SD scores were calculated based on age and gender reference values.[20]

2.4 | Genetics

Using the genomic DNA obtained from peripheral blood, the entire exon and exon-intron boundaries of the *THRA* gene were amplified. The resulting product was purified using ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific). Then, NEXTFLEX® Rapid XP V2 DNA-Seq Kit (Perkin Elmer), "Fragmentation, End-repair & Adenylation", "Adapter Ligation", "Post-Ligation PCR", "Bead-Based

Library Normalisation" and "Clean Up" steps were completed. After the library preparation, sequencing of the products was carried out with the Illumina MiSeq NGS System (Illumina, Inc.) using the MiSeq Reagent Nano Kit v2 (Illumina, Inc.). The "FASTQ" formatted data obtained from the NGS run was evaluated with the "SEQ" variant analysis software (Genomize), and the comparison of variants was done using reference sequence NM_199334.5.

2.5 | Ethics

This study was approved by the local Ethics Committee (Approval number: 2023/23-15) and performed in line with the principles of the Declaration of Helsinki.

2.6 | Statistical analysis

Statistical analyses were performed using the SPSS application for Windows version 24.0 (IBM Co.). The data were tested for normality using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Clinical data were presented as a number (%) for categorical variables and medians with the respective 25–75th percentile values for data that were not normally distributed. Comparisons were conducted using the Pearson Chi-Square test and the Fisher Exact test for categorical variables, and the Mann–Whitney *U* test for continuous variables. A two-sided *p*-value of <.05 was considered statistically significant.

3 | RESULTS

Thirty-two children (22 males [69%]; 28 prepubertal [87%]) were enroled in this study, with a median age of 5.7 (5.1–7.4) years. All of them were born with normal birth weights. There was consanguinity among the parents of two (6%) patients, and 11 (34%) patients had a family history of thyroid disease.

The main reasons for presenting to paediatric neurology clinics were ASD (*n* = 12, 38%), epilepsy (*n* = 11, 34%), and delayed developmental milestones (*n* = 8, 25%). Regarding developmental delays, 19 patients (59%) had speech delays, five patients (16%) experienced delayed walking, and three cases (9%) exhibited delayed sitting. The subjects frequently presented with learning disabilities, and 16 (50%) children had special educational needs. Five of the cases (16%) also presented with constipation, while the closure of the anterior fontanelle was delayed in two cases (6%), and tooth eruption was delayed in one case (3%).

The clinical characteristics and laboratory findings of the patients are presented in Table 1. All the subjects had median length/height, upper segment/lower segment ratio, and weight SD scores within the normal range. Except for one patient with a coarse facial appearance, none of them had dysmorphic facial features, macrocephaly, or macroglossia. Bradycardia was not observed in any of the patients, and their systolic and diastolic blood pressures were normal.

TABLE 1 The clinical and laboratory characteristics of the patients (*n* = 32).

Clinical features	
Age, years	5.7 (5.1–7.4)
Weight, SD score	−0.1 [(−0.8)–(0.3)]
Height, SD score	0.3 [(−0.8)–(1.1)]
US/LS ratio	1.02 (0.9–1.1)
US/LS ratio, SD score	−0.03 [(−1.1)–(0.9)]
BMI, SD score	−0.5 [(−1.3)–(0.3)]
Head circumference, SD score	0.6 [(−0.8)–(1.2)]
Heart rate, beats/min	95 (89–105)
Systolic BP, centile	61.5 (39.5–76)
Diastolic BP, centile	65 (48.5–79)
Laboratory	
Free T3, pg/mL	4.6 (4.2–5.0)
Free T4, ng/dL	0.9 (0.8–1.0)
TSH, uIU/mL	2.2 (1.8–3.1)
Haemoglobin, g/dL	11.9 (11.3–12.3)
MCV, fL	79.7 (77.6–82.4)
Creatine kinase, IU/L	107.5 (78.8–156.3)
Total cholesterol, mg/dL	169.5 (144.0–187.5)
IGF- I, SD score	−1.4 [(−2.1)–(−0.4)]

Note: Data are given as median (25p–75p).

Reference values: Free T3, 2.5–3.9 pg/mL; Free T4, 0.5–1.5 ng/dL; TSH, 0.38–5.3 uIU/mL; Haemoglobin, lower limit, 0.5–2 years, 11 g/dL; 2–12 years, 11.5 g/dL; older than 12 years, female, 12 g/dL; male, 13 g/dL; MCV, lower limit, 0.5–2 years, 70 fL; 2–6 years, 75 fL; 6–12 years, 77 fL; 12–18 years, female&male, 78 fL; Creatine kinase, 0–171 IU/L; Total cholesterol, 111–202 mg/dL.

Abbreviations: BMI, body mass index; BP, blood pressure; IGF- I, insulin-like growth factor-1; MCV, mean corpuscular volume; SD score; standard deviation score; T3, triiodothyronine; T4, thyroxine; TSH, thyrotrophin-stimulating hormone; US/LS, upper segment/lower segment.

The median levels of fT3, fT4, and TSH were 4.6 (4.2–5.0) pg/mL, 0.9 (0.8–1.0) ng/dL, and 2.2 (1.8–3.1) uIU/mL, respectively. Out of the patients, 13 (41%) had high levels of fT3, while none showed low levels of fT4. The median hemoglobin and MCV levels were 11.9 (11.3–12.3) g/dL and 79.7 (77.6–82.4) fL, respectively. The normo- or macrocytic anaemia rate was 47%, with eight normocytic anaemia cases and seven macrocytic anaemia cases. The iron and vitamin B12 levels of the patients were within the normal range. The median serum CK level was 107.5 (78.8–156.3) IU/L, and it was elevated in five patients (16%), one of whom had anaemia. IGF-I SD scores were below −2 in eight patients (25%), and total cholesterol levels were over 200 mg/dL in three patients (9%). Molecular genetic analysis of *THRA* was undertaken on all subjects; however, none of them had a pathological variant in *THRA*.

We compared the clinical and biochemical data of subjects with persistent normo-macrocytic anaemia ($n = 15$) with pretreatment data of those of six children from three families with RTH-α who were followed up in our clinic (Table 2).[21] The age, SD scores of weight and head circumference, heart rate, and systolic and diastolic blood pressure values were similar between the groups. However, the median height SD score was significantly lower in RTH-α patients compared to the patients without RTH-α ($p = .01$). There were no statistically significant differences observed in clinical characteristics such as delayed psychomotor development (including sitting, walking, and talking) or constipation (Table 3). However, there were significant differences between the two groups in skeletal findings, such as delayed tooth eruption and late closure of the anterior fontanelle ($p = .02$ and $p = .03$, respectively).

Based on the laboratory findings of the subjects (Table 3), no significant difference was observed between the two groups in terms of the median levels of fT3, fT4, TSH, total cholesterol, or IGF- I SD score. However, it is noted that the median hemoglobin level was significantly lower in patients diagnosed with RTH-α ($p = .03$). Additionally, the median levels of MCV, CK, and the ratio of CK to the reference upper limit were significantly higher in patients with RTH-α compared to those without RTH-α ($p = .01$, $p = .01$, and $p = .03$, respectively).

4 | DISCUSSION

Early diagnosis of RTH-α is essential, as the initiation of levothyroxine treatment during early childhood can improve growth and neuropsychological development, alleviate constipation, and increase basal metabolism in patients with RTH-α.[10,13,15,22–25] Therefore, the development of screening strategies holds importance. The gold standard for the diagnosis of RTH-α is molecular studies targeting *THRA* defects or whole exome sequencing.[26,27] However, it is important to note that genetic analyses can be costly and unnecessary when dealing with many undiagnosed patients. Therefore, it is essential to consider clinical findings and laboratory characteristics before initiating any genetic analysis to evaluate patients. The clinical presentation of RTH-α demonstrates quite a bit of variability. In addition to symptoms associated with hypothyroidism, many patients manifest various neurodevelopmental disorders, including delayed speech and, occasionally, mild-to-moderate intellectual disability, ASD, or epilepsy.[6,9,10,12,22,28–30] In light of these data, we undertook a screening process for patients presenting to paediatric neurology clinics over 2 years. Most of the included patients had ASD and epilepsy. Although there are cases of ASD and RTH-α coexisting in the literature, we could not make any comparisons with our patient group due to the lack of clinical and

TABLE 2 Comparison of clinical characteristics between subjects with anaemia and known patients with RTH-α.

		Subjects with anaemia ($n = 15$; F/M, 5/10)	Patients with known RTH-α ($n = 6$; F/M, 3/3)	p
System	Clinical characteristics			
Auxology	Age, years	5.6 (4.8–7.0)	7.4 (1.8–12.9)	.6
	Weight, SD score	−0.2 [(−0.9)–(0.3)]	0.1 [(−0.8)–(0.5)]	.8
	Height, SD score	0.2 [(−1.0)–(1.1)]	−1.2 [(−1.6)–(−0.9)]	**.01**
	BMI, SD score	−0,7 [(−1.8)–(−0.2)]	1.1 [(0.1)–(1.2)]	**.02**
	Head circumference, SD score	0.3 [(−0,6)–(1.1)]	−0.1 [(−0.6)–(1.6)]	.6
Cardiovascular	Heart rate, beats/min	90 (76–106)	91 (81–104)	.6
	Systolic BP, centile	50 (35–68)	47 (27.5–72.5)	.9
	Diastolic BP, centile	65 (47–79)	55 (47–71.5)	.3
Appearance	Coarse face	1 (7%)	2 (33%)	.2
	Macroglossia	0 (0%)	1 (17%)	.3
	Hoarse cry	0 (0%)	1 (17%)	.3
Skeletal	Delayed tooth eruption	0 (0%)	3 (50%)	**.02**
	Late closure of anterior fontanelle	2 (13%)	4 (6%)	**.03**
Neurological	Delayed sitting	2 (13%)	1 (17%)	.7
	Delayed walking	4 (27%)	3 (50%)	.4
	Delayed talking	7 (47%)	3 (50%)	>.99
Gastrointestinal	Constipation	2 (13%)	3 (50%)	.1

Note: Data are given as median (25p–75p).

Abbreviations: BP, blood pressure, F, female; M, male; RTH-α, resistance to thyroid hormone alpha; SD score, standard deviation score.

TABLE 3 Comparison of biochemical characteristics between subjects with anaemia and known patients with RTH-α.

	Subjects with anaemia (*n* = 15; F/M, 5/10)	Patients with known RTH-α (*n* = 6; F/M, 3/3)	*p*
Free T3, pg/mL	4.5 (4.1–4.8)	4.5 (4.2–5.9)	.2
Free T4, ng/dL	0.9 (0.8–1.0)	0.8 (0.7–1.1)	.4
TSH, uIU/mL	2.3 (1.7–2.9)	2.0 (1.6–2.3)	.2
Free T3/T4 ratio*	0.6 (0.5–0.7)	0.8 (0.5–1.4)	.2
Hemoglobin, g/dL	11.3 (11.2–11.5)	10.8 (9.5–11.3)	**.03**
MCV, fL	78.9 (76.2–81.3)	86.4 (82.0–88.6)	**.01**
Creatine kinase, IU/L	91 (75–129)	236 (166.5–254)[b]	**.01**
Creatine kinase, relative to the reference range	0.5 (0.4–0.8)	1.4 (0.9–1.5)[b]	**.03**
Total cholesterol, mg/dL	164 (138–186)	154 (142.5–178.5)	.9
IGF- I, SD score	−1.6 [(−2.4)–(−1.2)]	−1.6 ([−2.9]–[1.7])[b]	.6

Note: Data are given as median (25p–75p).

Reference values: Free T3, 2.5–3.9 pg/mL; Free T4, 0.5–1.5 ng/dL; TSH, 0.38–5.3 uIU/mL; Hemoglobin, lower limit, 0.5–2 years, 11 g/dL; 2–12 years, 11.5 g/dL; older than 12 years, female, 12 g/dL; male, 13 g/dL; MCV, lower limit, 0.5–2 years, 70 fL; 2–6 years, 75 fL; 6–12 years, 77 fL; 12–18 years, female&male, 78 fL; Creatine kinase, 0–171 IU/L; Total cholesterol, 111–202 mg/dL.

Abbreviations: F, female; IGF- I, insulin-like growth factor-1; M, male; MCV, mean corpuscular volume; RTH-α, resistance to thyroid hormone alpha; SD score, standard deviation score; T3, triiodothyronine; T4, thyroxine; TSH, thyrotrophin-stimulating hormone.

[a]The data (x) was expressed relative to the relevant reference range with the following formula: (x − lower limit of normal range)/(upper limit of normal range − lower limit of normal range).

[b]*n* = 5

*The free T3 and free T4 values were converted to pmol/L before calculation.

biochemical data on reported children.[12,22] Previous reports have indicated that three patients with RTH-α had experienced epileptic seizures during their childhood.[10,11,28]

Anaemia is a commonly observed clinical feature in patients with RTH-α, typically presenting as normocytic and normochromic and, less frequently, as macrocytic. However, the precise mechanisms underlying this condition remain unclear.[8,9,14,31] Studies conducted on animal models lacking TRα showed diserythropoiesis, pointing to the significant role of TH and its TRα in erythropoiesis.[32,33] To further investigate this, Van Gucht et al. carried out a study focusing on progenitor cells obtained from RTH-α patients. Their findings revealed that THs and TRα are involved in the later stages of human erythropoiesis, and inactivating mutations in TRα resulted in the impaired proliferation and differentiation of human erythroid progenitor cells.[31] Based on previously reported cases, the hemoglobin levels in children with RTH-α ranged between 8.6 and 10.9 g/dL, while levels in adults ranged between 9.6 and 12.9 g/dL.[6,8,10,21,23,29,34,35] In addition, individuals diagnosed with RTH-α typically experience anaemia that is unresponsive to levothyroxine treatment.[15,29,36] Upon re-evaluation of the patients enroled in our study, we found that nearly half of them had persistent anaemia, eight of whom had normocytic anaemia and seven had macrocytic anaemia, with a median hemoglobin level of 11.3 g/dL and MCV level of 79.7 fL. According to our strategy, we conducted molecular analysis of *THRA* in all of the 32 patients we identified. However, we did not observe any pathological variant in any of them.

Subsequently, we compared the clinical and laboratory findings of the subjects with persistent anaemia with those of patients with RTH-α who were followed up in the clinic to find reliable indicators. RTH-α often exhibits similarities to untreated hypothyroidism, including short stature, impaired bone growth, macroglossia, macrocephaly, coarse facial features, chronic constipation, and delayed milestones for motor and speech abilities.[8,9,14] It has been observed that a considerable proportion, nearly 60%, of patients with RTH-α experience short stature. Additionally, constipation and delay in developmental milestones are common clinical findings found in around 85% of cases. According to available data, almost 30% of patients with RTH-α may exhibit coarse facial features, while nearly 70% have macrocephaly. Various skeletal deformities, such as delayed tooth eruption and late closure of fontanelles, were also reported in individuals with RTH-α.[8] In our study, comparison of known patients with RTH-α with screened subjects without RTH-α revealed that RTH-α patients were shorter and had more frequent developmental skeletal abnormalities, such as delayed fontanelle fusion, and delayed dental eruption; but the rates of coarse facies, delay in neurological milestones, and constipation were similar among the groups.

In RTH-α, near-normal TFTs, encompassing normal TSH levels, either high or high-normal fT3 levels, as well as either low or low-normal fT4 levels are encountered.[3,5,8,13,37] For those diagnosed with RTH-α, it has been reported that 61% of children and 78% of adults have normal levels of fT4 and TSH. It has been observed that 33% of

children and 83% of adults have normal levels of fT3, fT4, and TSH.[8] Besides, further biochemical markers indicative of thyroid hormone activity in peripheral tissues, including CK, cholesterol, and IGF-I levels, can also be abnormal in RTH-α. Similar to hypothyroidism, serum CK and total cholesterol levels may be elevated, while circulating IGF-I levels can be low.[6,9,14,21,25,30,34,35] It has been reported that approximately 75% of children with available data have elevated CK levels.[8] In the present study, there were no significant differences found in the median levels of fT3, fT4, and TSH between patients with screened subjects without RTH-α and those with RTH-α. Besides, the median hemoglobin and MCV levels of RTH-α patients who underwent follow-up in our clinic were 10.8 g/dL and 86.4 fL, showcasing more severe anaemia and higher MCV levels compared to those without RTH-α. It is important to note that patients with RTH-α had higher median CK levels and a higher ratio of CK to its upper reference limit compared to those without RTH-α. However, there were no significant differences in total cholesterol and IGF-I levels between the two groups. Therefore, in patients with clinical manifestations of hypothyroidism and near-normal TFTs, moderate (rather than mild) normocytic-macrocytic anaemia or elevated serum CK levels may be deemed reliable indicators that should raise suspicion regarding the presence of RTH-α.

To our knowledge, this study is the first to assess the efficacy of normo-macrocytic anaemia as a screening method for identifying RTH-α in children with neurological problems. This approach identified one known patient with RTH-α, but no new cases were discovered. Moreover, nearly half of the patients did not demonstrate persistent normocytic or macrocytic anaemia. However, the present study also showed that patients diagnosed with RTH-α were shorter, had more severe anaemia, and had higher levels of MCV and serum CK in comparison to those without RTH-α. For this reason, it is important to assess the presence of other clinical or biochemical features that suggest RTH-α before conducting a genetic analysis of *THRA*.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ETHICS STATEMENT

This research complies with the guidelines for human studies and is conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Institutional approval was granted by the Ethics Committee of Dokuz Eylül University Faculty of Medicine (Ethics approval number: 2023/23-15). Both patients and parents were required to sign the informed consent form to participate in the study.

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