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Original Article

Association between Thyroid Function indices and Thyroid Autoantibodies with Thyroid Ultrasonography Outcomes in Children and Adolescents

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ABSTRACT

Objectives: Thyroid Ultrasonography (US) is recommended as a valuable tool for evaluating the status and function of the thyroid gland. The objective of this study was to compare and analyze the thyroid ultrasonography outcomes in children and adolescents who have normal thyroid function and thyroid antibodies.

Methods: A cross-sectional study was conducted on a group of 233 selected females aged 9 to 14 years old. Blood samples were obtained from the subjects and analyzed for thyroid hormones and thyroid autoantibodies. Additionally, a thyroid ultrasound was performed to provide an in-depth evaluation.

Results: Of all the participants, 25% displayed hypoechogenicity. Individuals with reduced echogenicity had higher median levels of thyroid-stimulating hormone (TSH), thyroid peroxidase antibody (TPO-Ab), and thyroglobulin antibody (Tg-Ab) compared to those with normal echogenicity. Moreover, those with hypoechogenicity had significantly higher thyroid volume (TVol), iodine status, and thyroglobulin levels than their counterparts with normal echogenicity. Hypoechogenicity was also significantly associated with higher levels of TPO-Ab, Tg-Ab, and TSH. Logistic regression analysis revealed that high TSH and TPO-Ab levels were associated with a higher risk of irregular echo patterns and thyroid autoantibodies.

Conclusion: The results revealed that irregular thyroid patterns in the ultrasonography were useful for assessing thyroid structure and dysfunction. Moreover, elevated TPO-Ab, Tg-Ab, and TSH concentrations in the serum may indicate thyroid malfunction. Ultrasound can help to identify early thyroid dysfunction along with the standard thyroid assessment biomarkers.

Keywords: Thyroid Ultrasonography, Urmia, adolescents, TSH, adolescents

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Introduction

In iodine-replete areas, goitre is the most common feature of thyroid dysfunction among children and adolescents (1). Thyroid enlargement results from inflammation such as autoimmune thyroiditis diseases (AITD), nodules, and infection (2, 3). Thyroid dysfunction in children and adolescents is commonly caused by AITDs. Comprehensive testing is necessary to rule out normal or abnormal thyroid functions, as the presentation of goitre is insufficient to predict thyroid dysfunction.

Thyroid Ultrasonography (US) is highly recommended for evaluating the status and function of the thyroid gland (4-7). Recent studies have reported that ultrasonography accurately detects thyroid gland lesions, inflammation, and destruction of thyroid parenchymal tissue, although other biochemical parameters including thyroid function and thyroid antibodies are useful in clinical assessment (4-7). Previous studies have reported that ultrasound is more sensitive in detecting AITD and that there is a link between altered thyroid echogenicity and thyroid function (7-10). In hypothyroid subjects and healthy individuals with positive thyroid antibodies, thyroid ultrasonography provides a significant predictive value for disease development or treatment outcomes rather than traditional thyroid peroxidase antibody (TPO-Ab) or high thyroid-stimulating hormone (TSH) levels. Nonetheless, reduced thyroid echogenicity on ultrasonography is associated with Hashimoto's thyroiditis (HT), hypothyroidism, and goitre (4, 6-11). For example, Rago et al. reported that thyroid hypoechogenicity was an efficient tool for diagnosing or predicting thyroid dysfunction rather than thyroid autoantibodies or high levels of thyrotropin (8).

Evidence concerning the link between thyroid antibodies (TPO-Ab and thyroglobulin antibody (Tg-Ab) and hypoechogenicity is limited and inconclusive. According to the findings of Park et al. (9), there was no statistically significant correlation between thyroid echogenicity, TPO-Ab, and Tg-Ab. However, another study revealed a significant association between thyroid echogenicity and TPO-Ab (12). No significant association between the levels of Tg-Ab and thyroid echogenicity and heterogeneity was also reported (10). To our knowledge, no research has explored the correlation between thyroid ultrasonography echogenicity, thyroid antibodies, and thyroid function in healthy children and adolescents residing in an iodine replete region. This cross-sectional study analyzed the echogenicity in thyroid ultrasound, thyroid antibodies, and thyroid function in healthy young participants without a history of thyroid dysfunction.

Materials & Methods

Studied population

A sampling method was used to recruit 233 female school children and adolescents aged 9 to 14 years old at the endocrinology clinic at Imam Khomeini Teaching Hospital (Urmia, Iran). Those with a history of thyroid dysfunctions, recent anti-thyroid therapy, regular consumption of multivitamin, trace element supplements, and those on a special diet were excluded from the study. The Ethics Committee of Urmia University of Medical Sciences, Urmia, Iran (IR.umsu.rec.1388.32) approved the study, and all participants provided verbal consent before participating.

Blood collection

Five millilitre blood samples were collected by venepuncture and allowed to stand at room temperature for 10 minutes. The samples were then centrifuged at 1000 x g for 15 minutes, and serum aliquots of 250 µL were transferred into Eppendorf tubes. The samples were stored at a temperature of -70°C until further analysis.

Urine collection

Fasting urine samples (10 mL) were collected. Aliquots (1 mL) were transferred into Eppendorf tubes and kept at -70°C until analysis.

Assessment of thyroid volume (TVol) and thyroid echogenicity

The thyroid ultrasound was conducted by the senior radiologist, with subjects examined in a supine position and their necks hyperextended. Real-time sonography was used to estimate the thyroid volume (TVol) using a 7.5 MHz linear transducer (Toshiba Nemio30, Japan). The examiner performed both longitudinal and transverse scans to measure the depth (d), width (w), and length (l) of each lobe. The volume of each lobe was calculated using the formula:

$V(ml)=0.479\times d\times w\times l(cm)$ (13). The total thyroid volume was the sum of both lobes, with the isthmus volume excluded (14).

Thyroid function and antibody tests

The levels of TSH, fT4, and fT3 were measured using ELISA (Pishtaz Teb in Tehran, Iran). The levels of TPO-Ab, Tg-Ab, and thyroglobulin were measured by enzyme immunoassay (EIA; AESKU Inc, Hamburg, Germany). Reference ranges, intra- and inter-assay values are presented in Table 1.

Assessment of urinary iodine concentration (UIC)

To determine urinary iodine excretion, the Sandell-Kolthoff reaction method was used as described elsewhere (15, 16). Initially, the urine samples were thawed at room temperature, vortexed to release any

Table 1: Reference ranges, as well as intra- and inter-assay values of all parameters evaluated

Parameters	Reference range	Intra-assay value (%)	Inter-assay value (%)
TSH (mIU/L)	0.32-5.2	3.9-6	7-8.4
fT3 (pg/mL)	1.9-4.3	4.0-4.8	5.5-7.9
fT4 (ng/dL)	0.7-1.9	3.5-6	3.9-7.1
Tg (ng/mL)	2-50	5-10	8-20
TPO-Ab (IU/mL)	Negative: ≤40 Intermediate: 40-59 Positive: ≥60	3-5	3.5-6.5
Tg-Ab (IU/mL)	Negative: ≤120 Intermediate: 120-179 Positive: ≥180	3.9-5.8	3.9-7.1

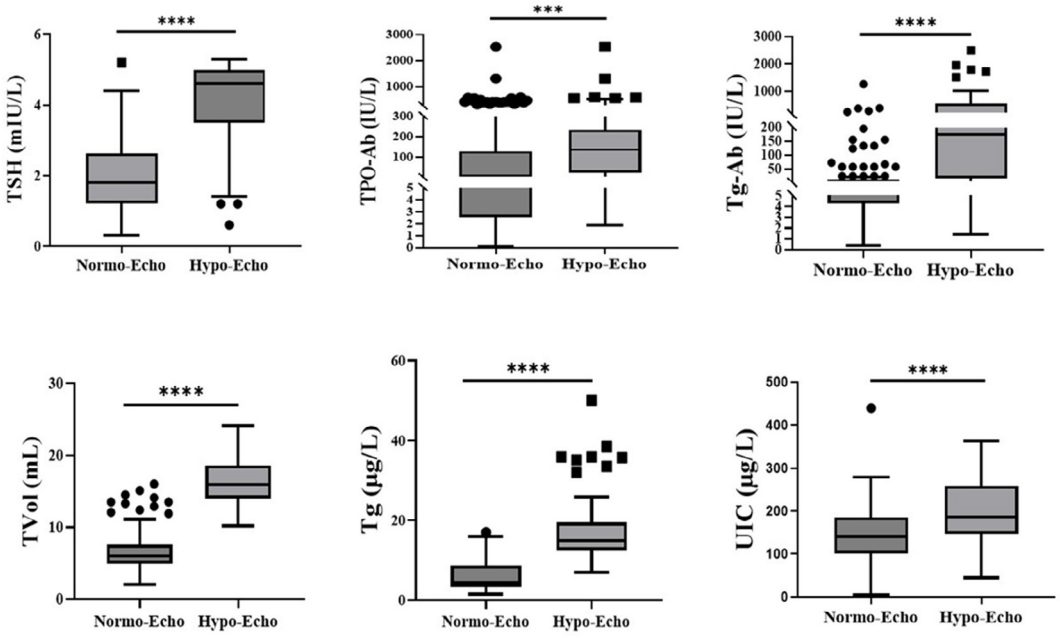

Figure 1: Box and whisker plots showing the levels TSH, TPO-Ab, Tg-Ab, TVol, Tg and UIC in subjects with normal Echo and hypo-echo.

particles, and centrifuged at 15000 rpm for 5 minutes. Then, 250 µL of the urine sample was added to a 10 mL glass tube containing 1 mL of ammonium persulfate solution and after gently shaking the mixture, it was heated at 100 °C for 60 minutes before allowing it to cool for 10 minutes. Finally, 3 µL of cerium (IV) ammonium sulphate (VI) solution was added and the light absorbance was measured at 405 nm using a double-beam UV/Vis Perkin Elmer spectrophotometer at 10-second intervals for 2 minutes.

Statistical analysis

The data analysis was performed using the SPSS software package for Windows version 16.0 (IBM, IL). Depending on the data type, it was presented as Mean ± SD or median value. To assess the relationship between variables, Pearson's correlation coefficient was used. Numeric variables were analysed using Student's t-test or Mann-Whitney U-test (for skewed data), while categorical data was analysed using the $\chi 2$ test. Skewed variables were transformed using the natural logarithm to ensure accurate statistical analysis. The investigation of the relationship between serum TPO-Ab and Tg-Ab with thyroid volume was conducted through simple and multivariate linear regression, with log-transformed thyroid volume as the dependent variable. Furthermore, binary logistical regression was used to examine the relationship between thyroid antibody status and thyroid echogenicity. $P < 0.05$ was considered significant.

Results

According to the results, all subjects were divided into two groups: 1) normal-echogenicity (normal-echo) and 2) hypo-echogenicity (hypo-echo). The percentage of individuals with normal echogenicity was 75% (n=175), and hypo-echogenicity was observed in 25% (n=58). Furthermore, in the whole population, the prevalence of a positive test for either TPO-Ab or Tg-Ab was 33.5% and 14.6%, respectively. The individuals who were positive for both antibodies comprised 11.6%

of the studied population. Furthermore, in hypo-echo subjects, the prevalence of positive TPO-Ab and Tg-Ab were 70.7% and 48.3%, respectively, compared to 21% and 3.4% in normal echo subjects. The means for age and BMI values did not significantly differ between the two groups.

As presented in Figure 1, the mean TVol and Tg were greater in individuals with hypo-echo compared to normal-echo. The concentrations of TSH and fT3 were significantly higher in the hypo-echo group relative to the normal-echo group, whereas higher levels of fT4 were detected in normal subjects compared to the hypo-echo group ($P<0.05$). The levels of Tg, TPO-Ab, and Tg-Ab were greater in the hypo-echo group than those in the normal-echo group (All $P<0.001$). The median UIC in hypo-echo individuals was significantly higher than that of normal-echo subjects (median: 187 µg/L vs. 141 µg/L). TSH was also divided into four quartiles and the level of several parameters in the hypo-echo group was evaluated. Statistical analysis revealed that there were significant differences between TPO-Ab, Tg-Ab, TVol, Tg, UIC, and BMI in quartile 4 with other quartiles (Fig. 2).

Further correlation analysis was performed to measure the relationship between the variables in the whole population. It was found that TVol was positively correlated with TSH, Tg, TPO-Ab, and Tg-Ab, whereas a significant positive correlation was observed between TSH with Tg, TPO-Ab, and Tg-Ab (Fig. 3). Moreover, there was a positive significant correlation between UIC with TPO-Ab and Tg-Ab (r= 0.200, r= 0.243, all P-value <0.05). Pearson correlation analysis in subjects with hypo-echo revealed that there was a negative correlation between fT4 levels with TPO-Ab, Tg-Ab, and TSH (r= -0.617, r= -0.444, r= -0.795, all P-value <0.05). Moreover, a significant association was observed between TVol with TOP-Ab, Tg-Ab, TSH, and Tg, and TPO-Ab and UIC (Fig. 4).

Regression analysis indicated that thyroid hypo-echogenicity was affected by TSH, TVol, Tg, TPO-Ab, and UIC. Furthermore, the odds ratio for TSH was 5.6, meaning that subjects with a high level of TSH were prone to get hypo-echogenicity 5.6 times higher than individuals with a low level of TSH (Table 2).

Discussion

In this study, the potential correlation between thyroid ultrasound, thyroid volume, thyroid function, and thyroid antibodies in a population of healthy pediatric and adolescent individuals with no history of thyroid dysfunction was investigated. According to sonographic features, the population was divided into two groups: hypo-echogenic and normal-echogenic groups. The mean TVol value was doubled in the hypo-echo group relative to the normal-echo group. In the population as a whole, a significant positive correlation was found between TVol with TSH, TPO-Ab, and Tg-Ab. The prevalence of thyroiditis in the whole population was 31% and 14.6% according to the positive data for TPO-Ab and Tg-Ab, respectively. Binary forward regression was also carried out to evaluate the impacts of the biomarkers on thyroid echogenicity. It was found that TSH and UIC were significant factors that could alter thyroid echogenicity in the whole population.

Thyroid ultrasonography is a useful tool to detect thyroid autoimmune diseases in apparently healthy subjects (4, 5, 10, 17). Vejbjerg et al. (10) reported that

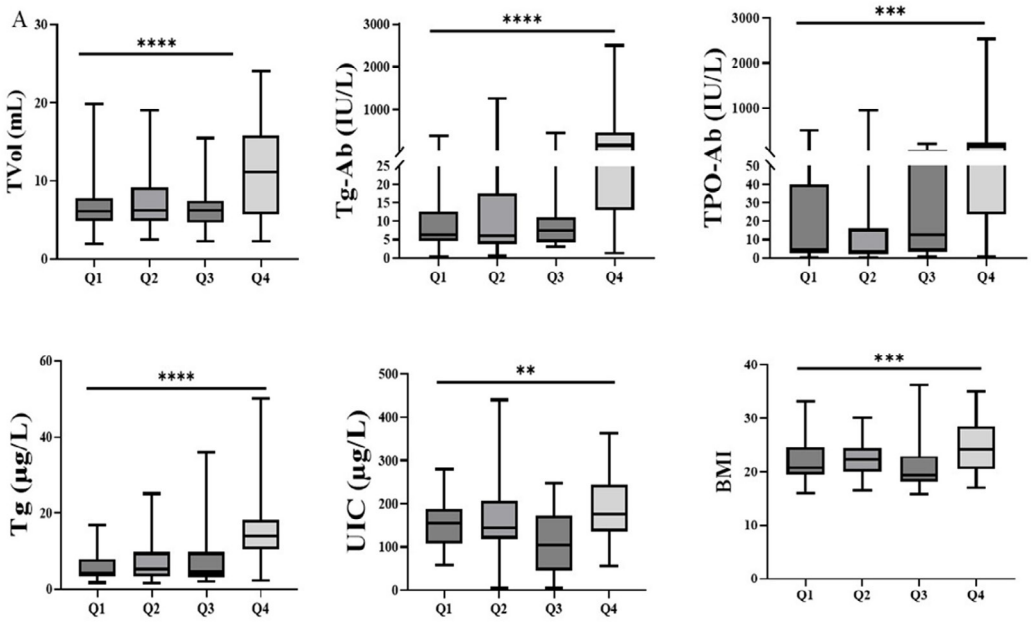

Figure 2: Box plot of TPO-Ab, Tg-Ab, TVol, Tg, UIC and BMI levels in four quatiles of TSH in females of hypo-echo group.

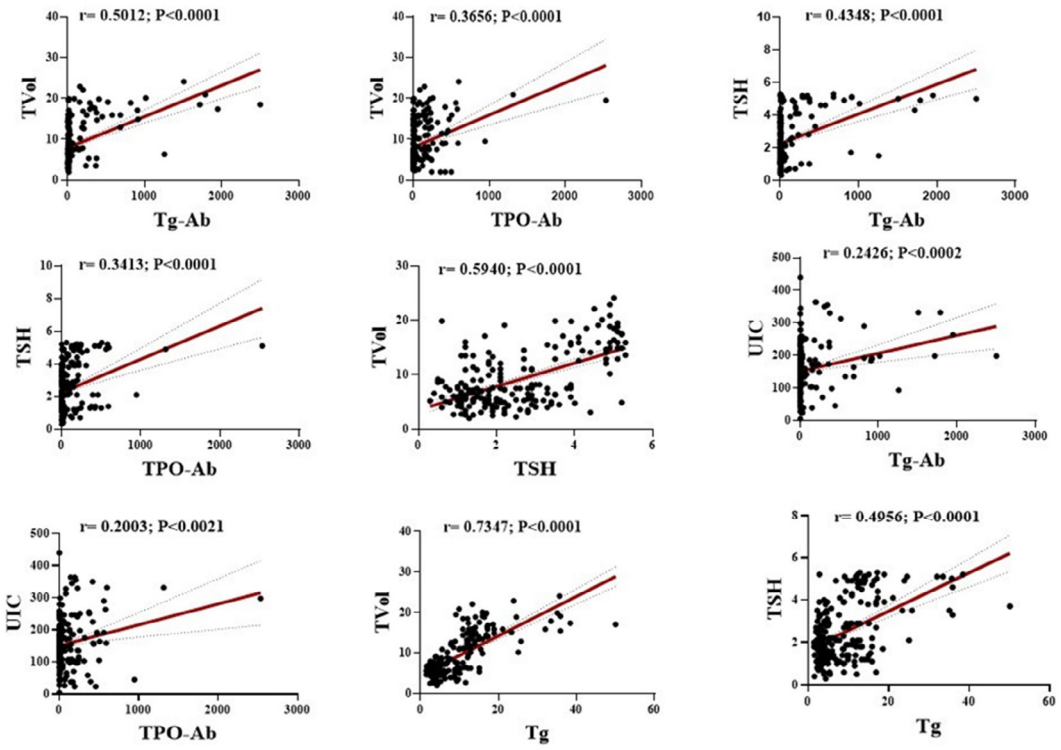

Figure 3: The correlation between the variables in the whole population.

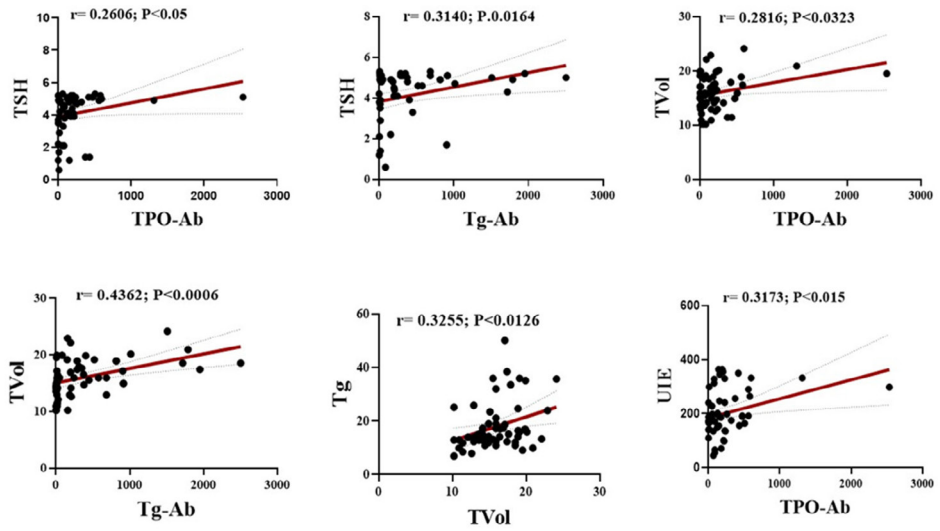

Figure 4: The correlation between the variables in the hypo-echo group.

in normal subjects with markedly hypo-echogenicity, the levels of TSH, TPO-Ab, and Tg-Ab were significantly increased relative to the normal echogenicity group. In a similar study, Rostami et al. reported that in Hashimoto's thyroiditis, high values of TSH and TPO-Ab were associated with thyroid hypo-echogenicity (11, 16). There are also findings that indicate a link between thyroid echogenicity and function, particularly in school-aged children. Additionally, thyroid ultrasound grading has been shown to be connected to TSH levels (6, 9). According to a study conducted by Jeong et al., a significant association was found between the thyroid echogenicity and thyroid function in children and adolescents with Hashimoto's thyroiditis, including those with overt hypothyroidism, subclinical hypothyroidism, and euthyroidism. Notably, a strong correlation was observed between reduced echogenicity and increased TSH levels in the pediatric and adolescent population

Table 2: Stepwise multilinear regression to estimate parameters affecting thyroid echogenicity

	Thyroid hypo-echogenicity		
	Odd Ratio	**CI**	**P-Value**
TSH	5.6	2.052 – 19.646	0.001
TVol	1.87	1.218 – 2.888	0.004
Tg	1.53	1.006 – 2.332	0.047
TPO-Ab	1.013	1.003 – 1.022	0.01
UIE	1.029	1.007 – 1.051	0.008

(6, 9). In the present study, it was also found that TSH, Tg, TPO-Ab, and Tg-Ab were increased in hypo-echo subjects relative to normo-echo groups.

It has been suggested that thyroid autoantibodies are linked to thyroid hypo-echogenicity. Vejbjerg et al. reported that in normal subjects with markedly hypo-echogenicity, the levels of TSH, TPO-Ab, and Tg-Ab were significantly increased relative to the normal echogenicity group (10). Park and colleagues also demonstrated that hypo-echogenicity was significantly associated with TPO-Ab and Tg-Ab. In the hypo-echo group, a significant correlation was found between TPO-Ab and Tg-Ab with TSH and TVol (9). Yan et al. (18) reported that the prevalence of thyroid hypo-echogenicity increased with growing concentrations of TPO-Ab regardless of Tg-Ab status. They also suggested that an elevated TSH concentration was significantly linked with an increased risk of having positive TPO-Ab. In the present study, according to multivariate logistic regression analysis, the risk of elevated TPO-Ab and Tg-Ab levels increased with enhanced TSH levels. In line with previous studies, the results revealed that elevated TSH levels and a history of thyroid dysfunction are required to screen thyroid antibodies in women and susceptible people (18, 19).

In this study, the Urinary Iodine Concentration (UIC) in the hypo-echogenic group was greater than the normal echo group. Furthermore, a positive significant correlation was found between TPO-Ab and UIC in the whole population and hypo-echo subjects, respectively ($r= 0.200$ and $r= 0.317$, $P<0.05$). Therefore, it can be suggested that an increase in the incidence of thyroid autoimmunity and thyroid hypo-echogenicity is followed by a transition from iodine deficiency to sufficient or excessive iodine intake (20-22). In line with these data, Zois et al. (23) indicated that the levels of TSH, TPO-Ab, and Tg-Ab were gradually increased after 5 years of iodine supplementation. Furthermore, Tg was significantly higher in the hypo-echo group than in subjects with normal echo. A significant association was also detected between the levels of Tg and TSH, TPO-Ab, Tg-Ab, and TVol.

This study had several limitations. Firstly, it did not measure the TVol using Doppler sonography. Secondly, since this was a cross-sectional study, it is not possible to establish a causal relationship between the presence of thyroid antibodies and the levels of TVol and thyroid echogenicity (24). Additionally, since the majority of participants belonged to the northwest region of Iran, where iodine intake is insufficient, it may have biased the results due to ethnicity and location.

In summary, the prevalence of hypoechogenicity and positive TPO-Ab and Tg-Ab were high in Urmia County, an iodine-replete area. The research indicated that Autoimmune Thyroid Diseases (AITDs) are linked to reduced echogenicity, changed echo patterns, and high TSH values, even in individuals with normal thyroid function. Furthermore, the results suggest that ultrasonography findings can predict the association of AITDs in young people with enlarged thyroid with no history of thyroid dysfunction. Moreover, ultrasonography can serve as a valuable supplement to biochemical tests in the initial assessment of thyroid status. However, further investigations with follow-up studies are necessary to assess the effectiveness of US in predicting the advancement of abnormal thyroid function.

Conflict of Interest

The author have nothing to declare.

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Authorship contribution statement

Jaffar Nourooz-Zadeh and Rahim Rostami: Conceptualization, Writing – original draft. **Asghar Beiranvand**: Data curation; Formal analysis. **Rahim Rostami, Asghar Beiranvand, Afshin Mohammadi**: Methodology. **Rahim Rostami**: Writing – original draft. **Sarmad Nourooz-Zadeh and Massoumeh Rostami**: Visualization. **Rahim Rostami and Jaffar Nourooz-Zadeh**: Conceptualization, Writing – review & editing. **Rahim Rostami and Jaffar Nourooz-Zadeh**, Supervision. **Jaffar Nourooz-Zadeh**

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