



## RESEARCH ARTICLE

## Serum thyroid autoantibodies in malignant thyroid nodules

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### ABSTRACT

**Background:** Thyroid cancer is one of the most common malignant tumours of the endocrine system. Thyroid autoantibodies were reported to be associated with malignant thyroid nodules. This study aimed to assess the diagnostic accuracy of serum thyroid autoantibody levels for malignant thyroid nodules.

**Methods:** From March 2023 to January 2024, we recruited 104 consecutive patients with thyroid nodules confirmed by ultrasonography in three departments of Bangabandhu Sheikh Mujib Medical University. Out of these, 52 were diagnosed with malignant thyroid nodules using fine needle aspiration cytology, while the remaining 52 had benign thyroid nodules. Serum thyroid autoantibodies were measured using the immunoassay technique.

**Results:** The mean levels of serum thyroid peroxidase antibody (TPOAb) were significantly higher ( $P<0.001$ ) in the malignant group compared to the benign group (36.1 versus 24.0 IU/mL). The mean levels of serum thyroglobulin antibodies (TgAb) were also significantly higher ( $P<0.001$ ) in the malignant group (39.8 IU/mL versus 29.1 IU/mL). Elevated TPOAb ( $>40$  IU/mL) and TgAb ( $>35$  IU/mL) showed reasonable accuracy (60% to 65%) in detecting malignancy of thyroid nodules.

**Conclusions:** Thyroid malignancy is positively associated with TgAb and TPOAb. Therefore, thyroid autoantibodies could be considered for screening malignancy in thyroid nodules.

**Keywords:** thyroid nodules, thyroid cancer, thyroid peroxidase antibody, thyroglobulin antibody

### INTRODUCTION

The American Thyroid Association has defined thyroid nodules as discrete lesions within the thyroid gland, radiologically distinct from surrounding thyroid parenchyma.<sup>1</sup> The prevalence of thyroid nodules in the general population increases from 8% to 76% with high-resolution ultrasound instead of clinical examination.<sup>2</sup> The incidence of thyroid cancer has been increasing rapidly in recent years, with approximately 14.3 cases per 100,000 per year.<sup>3</sup> Its incidence is 43.8 cases per 100,000 person-years among the East Asian people.<sup>4</sup> In India, the incidence of thyroid cancer is 5.8 per 100,000

people.<sup>5</sup> The incidence has increased worldwide in the last three decades.<sup>6</sup>

Thyroglobulin antibody (TgAb), an immunoglobulin G glycoprotein secreted from lymphoid B cells, becomes hyperfunctioning during malignancy.<sup>7</sup> Thyroid peroxidase antibodies (TPOAb) usually indicate autoimmune thyroid disease when they are positive.<sup>8</sup> An early study showed that the prevalence of positive TgAb in patients with thyroid cancer was 2.5 times higher than in the general population.<sup>9</sup> Kim *et al.* first reported that TgAb could be used as an independent predicting factor for thyroid cancer diagnosis regardless

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**HIGHLIGHTS**

1. Limited research exists on thyroid antibodies in malignant thyroid tumours.
2. Thyroid autoantibodies (thyroid peroxidase antibody, thyroglobulin antibody) are associated with thyroid malignancies.
3. These two antibodies can be used to screen for malignancy of thyroid nodules.

of autoimmune thyroid disease, especially in young patients.<sup>10</sup> Measuring thyroid autoantibodies such as TPOAb and TgAb is a cost-effective and reliable test. Thus, this study aimed to assess the diagnostic accuracy of serum thyroid autoantibody levels for malignant thyroid nodules.

**METHODS****Study design**

This cross-sectional study was done from March 2023 to February 2024 in the Departments of Laboratory Medicine, Endocrinology and Metabolism, Otolaryngology-Head and Neck Surgery, Microbiology and Immunology, and Pathology at Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh.

**Subjects**

A non-randomized consecutive sampling method was used to enrol participants in this study. We included men and women aged 18 years or older who had recently been diagnosed with a thyroid nodule or nodular goitre, confirmed by fine needle aspiration cytology (FNAC) reports. Based on the FNAC report, 52 patients with malignant thyroid nodules and 52 with benign thyroid nodules were recruited.

The patients taking medications that might alter thyroid function (e.g. steroids, biotin), levothyroxine or anti-thyroid drugs (e.g. carbimazole, methimazole), pregnancy and lactation, and symptoms of thyroid disorders (Most of the thyroid nodules are asymptomatic)<sup>11</sup> were excluded from the study.

**Sample size determination**

Considering the formula<sup>12</sup>

$$n = \frac{\{u\sqrt{[\pi_1(1-\pi_1)+\pi_0(1-\pi_0)]}+v\sqrt{[2\bar{\pi}(1-\bar{\pi})]}\}^2}{(\pi_0-\pi_1)^2}$$

Where n (number of cases); u=1.96 for 1% level of significance; v=0.52 (from z table) at 70% power of the test;  $\pi_0$  (mean in group I)=44;  $\pi_1$  (mean in group II) = 27;  $\bar{\pi} = (\pi_0 + \pi_1)/2=35$ . Therefore, the sample size for each group was 52, and the total sample size was 104.

**Specimen collection**

Venous blood (3 mL) samples were collected in a biochemistry tube from the antecubital vein. After an incubation period of 30 minutes, the biochemistry tube was centrifuged at 3,000 revolutions per minute for 5 minutes. The separated serum specimen was kept in an Eppendorf tube and stored at -20° C until analysis was performed. Laboratory investigations were done at the Department of Microbiology and Immunology, BSMMU. All samples were tested on four successive occasions within one month of sample collection.

With all aseptic precautions, a 10 ml syringe was attached to a syringe holder. Two fingers of the free hand firmly grasped the nodule while the other hand held a pistol grip syringe holder. The needle was then inserted into the nodule through the skin. Once the needle tip was in the nodule, gentle suction was applied. The needle was moved in and out vertically within the nodule. This allowed the cellular material to dislodge and easily aspirate into the needle. Suction was maintained, and as soon as aspirate appeared in the half of the needle, the suction was released, and the needle was withdrawn. The needle was reattached to the syringe with the beveled end facing down, and one drop of aspirate material was ejected onto several glass slides. All the slides were labelled.

**Measurement of thyroid autoantibodies**

TgAb and TPOAb were measured using the immunoassay technique (ADVIA centaur XPT, Siemens) in the Department of Microbiology and Immunology, BSMMU.

**Statistical analysis**

Data were entered, cleaned, and analysed using SPSS version 26. Quantitative data were described using mean and standard deviation, and compared using the t

test. The results of TgAb and TPOAb were considered elevated if these were  $>40$  IU/mL<sup>13</sup> and  $>35$  IU/mL<sup>14</sup>, respectively. Qualitative data were described using frequency and percent and association was measured using chi-square test. Logistic regression analysis was done to examine the associated factors for malignant forwards. Sensitivity, specificity, predictive values and diagnostic accuracy<sup>15</sup> were calculated.  $P < 0.05$  was considered as significant.

### Ethical issues

Following the ethical approval, we have explained the potential risk of the FNAC procedure to the participants. Informed written consent was taken from the participants. Confidentiality of the personal information was maintained.

## RESULTS

The mean age of the patients with malignant (43.9 years) and benign (42.4 years) nodules were similar. The mean serum TgAb levels are higher in the malignant group (39.8 IU/mL) compared to the benign group (29.1 IU/mL). TPOAb levels exhibited higher in the malignant group (36.1 IU/mL) compared to the benign group (24.0 IU/mL) (TABLE 1). These differences persisted even after adjustment of the potential confounders using a multivariate logistic regression analysis (data not shown). Elevated levels of TgAb were significantly associated with thyroid malignancy (OR 2.6; 95% CI 1.1, 5.9). This was true for TPOAb (OR 4.0; 95% CI 1.7, 9.5) also (TABLE 2).

The diagnostic performance of TgAb and TPOAb in distinguishing malignant tumours from benign tumours was moderately high. Sensitivity, specificity, positive predictive value and negative predictive value for TgAb were 46.2%, 75.0%, 64.9% and 58.2%, respectively.

**TABLE 1** Mean (standard deviation) of age and antibodies in malignant and benign thyroid nodule groups (n=104)

Demographic variables	Malignant (n=52)	Benign (n=52)	P
Age in years	43.9 (15.8)	42.4 (13.9)	0.61
Serum TgAb (IU/mL) <sup>a</sup>	39.8 (5.9)	29.1 (9.5)	<0.001
Serum TPO Ab (IU/mL) <sup>b</sup>	36.1 (5.6)	24.0 (8.5)	<0.001

<sup>a</sup>Thyroglobulin antibody, <sup>b</sup>Thyroid peroxidase antibody

These were 51.9%, 78.9%, 71.1%, and 62.1%, respectively, for the TPOAb (FIGURE 1). They had modest diagnostic accuracy: TgAb, 60.6%, and TPOAb, 65.4%.

**TABLE 2** Logistic regression analysis for predicting factors thyroid malignancy

Factors	Malignant tumours	Benign tumours	Odds ratio (95% CI) <sup>a</sup>
Age in years (continuous)			1.01 (0.98-1.03)
Sex			
Male	13 (25.0)	7 (13.5)	2.1 (0.8-3.7)
Female	39 (75.0)	45 (86.5)	
Thyroglobulin antibody			
Normal ( $\leq 40$ IU/mL)	28 (53.8)	39 (75.0)	
Elevated ( $>40$ IU/mL)	24 (46.2)	13 (25.0)	2.6 (1.1-5.9)
Serum thyroid peroxidase antibody			
Normal ( $\leq 35$ IU/mL)	25 (48.1)	41 (78.8)	
Elevated ( $>35$ IU/mL)	27 (51.9)	11 (21.2)	4.0 (1.7-9.5)

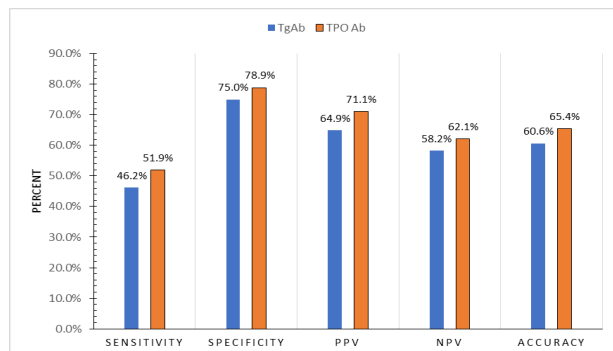
<sup>a</sup>Confidence interval

## DISCUSSION

There is a dearth of information on the usefulness of thyroid autoantibody levels in diagnosing malignant thyroid nodules. We have examined this relationship for the first time in Bangladeshi patients. Although our study had low power (70%), we report here that the TgAb and TPOAb tests have a modest level of diagnostic accuracy.

FNAC is the investigation of choice for diagnosing malignant thyroid nodules. The measurement of thyroid autoantibodies would contribute to a better diagnosis of carcinoma differentiation in thyroid nodules than the FNAC.<sup>16</sup> Although small, there is a chance of spreading the underlying cancer by the FNAC procedure. Scientists have already claimed that serum thyroid autoantibody levels as biochemical markers would provide an opportunity for better management planning.<sup>16</sup>

Boi *et al.* mentioned a higher prevalence of malignancy (18.8%) in individuals with elevated TPOAb levels compared to those (9.2%) with normal TPOAb levels.<sup>17</sup> Krátký *et al.* described that 44% of individuals with malignant nodules had positive TPOAb levels, compared to 27% in benign thyroid nodules. A possible



**FIGURE 1** Diagnostic accuracy of serum thyroglobulin antibody (TgAb) and thyroid peroxidase antibody (TPOAb) for malignant thyroid nodules (n=104)

Serum TgAb: elevated if, >40 IU/mL; thyroid peroxidase antibody (TPOAb): elevated if >35 IU/mL

\*Accuracy= (true positives+true negatives)/n

explanation might be the local inflammatory reaction of tumour cells with the production of TPOAb.<sup>18</sup> Our findings were similar to the findings of the above reports.

In the current study, TgAb levels significantly differed between malignant and benign thyroid nodules. Kim *et al.* observed a similar difference.<sup>10</sup> The sensitivity, specificity, positive predictive value, and negative predictive value for TgAb were 46.2%, 75.0%, 64.9%, and 58.2%, respectively. In our study, the malignant cases of TPOAb were 51.9%, 78.9%, 71.1%, and 62.1%, respectively. However, Wong *et al.* reported higher sensitivity and specificity of thyroid autoantibodies: 74.4% and 70.4%, respectively.<sup>19</sup> This might be due to the differences in study design and participant background.

Human thyroglobulins have four to six epitopes that are recognised by B cells.<sup>20</sup> Lupoli *et al.* recently determined the prognostic relevance of TgAb epitope specificities in thyroid cancer.<sup>21</sup> Therefore, it is possible that occult thyroid cancer stimulates chronic immunologic responses and produces new or more TgAb. This could explain the mechanism of association between positive TgAb and malignancy.

### Conclusion

Although the study findings are based on a small sample, our findings indicate that thyroid cancer is associated with high TgAb and TPOAb levels. They have a modest accuracy in detecting malignant thyroid

nodules. Therefore, elevated levels of TgAb and TPOAb may be considered candidates for screening for nodular malignancy in the thyroid.

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### Author contributions

*Conception and design:* TRA, DP, SS. *Acquisition, analysis, and interpretation of data:* MSY. *Manuscript drafting and revising it critically:* TRA, SIN, MSY, DP, SS, NI. *Approval of the final version of the manuscript:* TRA, DP, MSY, SS. *Guarantor of accuracy and integrity of the work:* DP.

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### Conflict of interest

We do not have any conflict of interest.

### Ethical approval

The study followed the Declaration of Helsinki as the cornerstone of human research ethics. Before commencing the study, this project was approved by the Institutional Review Board of BSMMU (Memo No. BSMMU/2023/10205), dated 31 July 2023.

### Data availability statement

The authors confirm that the data supporting this manuscript's findings will be shared upon reasonable request.

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