

**DIAGNOSTIC ACCURACY OF ANTI-THYROID ANTIBODIES IN HASHIMOTO'S THYROIDITIS**

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**ABSTRACT****BACKGROUND**

Hashimoto's thyroiditis is the most common cause of hypothyroidism in Iodine sufficient areas. Diagnosis depends on histopathological or cytological features. The accuracy of autoantibodies among hypothyroid patients for diagnosis of Hashimoto's thyroiditis was explored in this study.

**MATERIALS AND METHODS**

A cross sectional study was conducted on persons with laboratory confirmed hypothyroidism at a tertiary care hospital. All subjects underwent determination of anti-thyroid peroxidase antibodies (anti-TPO) and Fine Needle Aspiration Cytology (FNAC) of the thyroid. The sensitivity and specificity of anti-TPO antibodies was determined using FNAC as gold standard.

**RESULTS**

Fatigue was the most common symptom among the hypothyroid patients. The diagnostic sensitivity and specificity of anti-TPO antibodies was 88.37% and 50% respectively for Hashimoto's thyroiditis. Using this sensitivity and specificity, the calculated positive likelihood ratio (LR) is 1.80 and negative likelihood ratio is 0.23.

**CONCLUSION**

Anti-TPO appears to be a relatively sensitive test for diagnosis of Hashimoto's thyroiditis among patients with hypothyroidism; however, its specificity is low. The low negative likelihood ratio transforms it into an effective test to rule out Hashimoto's thyroiditis in populations with low pre-test probability of Hashimoto's thyroiditis.

**KEYWORDS**

Hashimoto's Thyroiditis, Hypothyroidism, Sensitivity, Specificity, Likelihood Ratio.

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**BACKGROUND**

Primary hypothyroidism is a common disorder worldwide and results from a variety of underlying causes. Autoimmune disorders constitute a significant proportion of the aetiology of primary hypothyroidism, Hashimoto's thyroiditis being one form. In the United States it continues to be the most common aetiology for hypothyroidism.<sup>1</sup> The prevalence of Hashimoto's thyroiditis ranges from 0.55% to 13.4% in various studies.<sup>2,3</sup> A definitive diagnosis of Hashimoto's thyroiditis is established by histopathology or more often fine needle aspiration cytology. However, several studies have documented the presence of antibodies to thyroid peroxidase (anti-TPO) in persons with Hashimoto's thyroiditis.

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Whether they can be used as reliable diagnostic markers of Hashimoto's thyroiditis in hypothyroid patients remains unclear. We aimed to determine the diagnostic value of anti-TPO in Hashimoto's thyroiditis among patients with primary hypothyroidism as compared to cytology.

**MATERIALS AND METHODS**

In this cross-sectional study, adult patients aged 18 years or more attending the outpatient services of the hospital between 2011 and 2013, and for whom a report of serum levels of thyroid stimulating hormone (TSH) and free thyroxine (FT4) was available were eligible to be included. From these patients, we enrolled 50 consecutive patients with primary hypothyroidism as evidenced by elevated thyroid stimulating hormone (TSH) above 10 mU/L and a low free thyroxine (FT4) less than 0.8 ng/dl. Patients with secondary hypothyroidism, those on drugs like Amiodarone, Lithium, Sulfonamides and pregnant women were excluded from the study. All patients were interviewed for history of symptoms of hypothyroidism, and examined for signs as well as documentation of any goitre. They were subjected to fine needle aspiration (FNA) of the thyroid following which the sample was examined for cytological evaluation.

The FNA was performed using 23-25-gauge needle and stained using Pap and Giemsa stains. The same experienced pathologist performed and interpreted the cytology specimens of all subjects. Blood was also drawn for serum anti-TPO antibodies. Informed consent was obtained prior to the procedures and for enrolment into the study. Of the 50 patients, only 45 consented for FNAC and 49 consented for serum anti-TPO estimation. Hence 45 patients with results of both cytology and anti-TPO antibodies were included in final analysis.

FT3, FT4 and TSH were analysed by electrochemiluminescence (ECLIA) immunoassay by COBAS e 411 analysers. Normal reference range for FT3 was taken as 1.08-3.14 nmol/L; for FT4 from 0.8-2.3ng/dl and TSH the normal range was 0.4-4.0 mU/L. Anti-TPO was also analysed by ELCIA by COBAS e 411 with a normal range of 7- 50 IU/mL.

The Institute ethics committee approved the study. Written informed consent was obtained from all subjects and confidentiality was strictly maintained.

Statistical Analysis

Baseline characteristics and clinical features were represented using descriptive statistics. To determine diagnostic accuracy of anti-TPO antibodies, sensitivity and specificity were calculated using a 2x2 table with cytology findings as gold standard for diagnosis of Hashimoto’s thyroiditis.

	Hashimoto Thyroiditis (Cytology)	Non-Hashimoto Thyroid Disease (Cytology)	Total
Anti-TPO positive	38	1	39
Anti-TPO negative	5	1	6
	43	2	45

Table 2

Table 2. Two by two table depicting the sensitivity and specificity of anti-TPO as compared to cytology for diagnosis of Hashimoto’s thyroiditis among patients with primary hypothyroidism.

Sensitivity =  $38/43 \times 100 = 88.37\%$   
Specificity =  $1/2 = 50\%$

DISCUSSION

This study on diagnostic value of autoantibodies in Hashimoto’s thyroiditis found that anti-TPO antibodies have a high sensitivity but low specificity. This means that the use of anti-TPO antibodies is likely to miss 50% cases of Hashimoto’s thyroiditis in those with primary hypothyroidism. However, a negative anti-TPO result can effectively rule out Hashimoto’s thyroiditis in patients with primary hypothyroidism. Likelihood ratios would give better indication of the utility of these tests. With the sensitivity and specificity found in this study, the positive likelihood ratio (LR) is 1.80 and negative likelihood ratio is 0.23. With an estimated prevalence (pre-test probability) of 5% in general population, a positive anti-TPO test in such

RESULTS

Of the 45 patients finally included in analysis, one was male and the remaining were females. The mean age of the patients was  $38.3 \pm 10.9$  years. The median serum TSH level was 28.96 mU/L (5-191). The most common symptom was fatigue (87.8%). Other common symptoms are depicted in table 1.

Among the 45 patients who underwent FNA and anti-TPO antibody estimation, 43 were confirmed as Hashimoto’s thyroiditis by FNA cytology. Of these 43 confirmed Hashimoto’s thyroiditis, 38 were positive for anti-TPO antibodies. Using cytology as gold standard, anti-TPO antibody had a sensitivity of 88.37% and specificity of 50% (table 2).

Table 1. Frequency of distribution of symptoms among patients with primary hypothyroidism

Symptom	Frequency (%)
Fatigue	43 (95.5)
Hair loss	36 (80.0)
Psychosis /depression	26 (57.7)
Cold intolerance	22 (48.8)
Puffiness of face	20 (44.4)
Weight gain	18 (40.0)
Irregular menstrual cycle	19 (42.2)
Decreased appetite	18 (40.0)
Constipation	15 (33.3)
Infertility	14 (31.1)

individual is likely to improve the post-test probability to a modest 9%. On the other hand, a negative result would lower the probability of disease to as much as 1%.

In a hypothyroid population where the probability of Hashimoto’s thyroiditis is quite high, the utility may be slightly different. Assuming a pre-test probability of 50% for Hashimoto’s thyroiditis in this subpopulation, a positive test would push up the post-test probability to 65%; a negative result would reduce the post-test probability to 18%. Thus, a negative test has very important role in effectively reducing the unnecessary testing and further evaluation for Hashimoto’s thyroiditis in hypothyroid persons.

Although autoimmune thyroid disease encompasses several disorders, Hashimoto’s thyroiditis and Grave’s disease comprise the two major entities. They share many cytological and serological features.<sup>4</sup> Hashimoto’s thyroiditis has a prevalence of 5%.<sup>5</sup> Among serological markers, anti-TPO is the most important since they fix complement leading to direct damage.<sup>6</sup> Previous studies have also shown correlation between antibody titres and activity in autoimmune thyroiditis.<sup>7</sup> Although many persons with Hashimoto’s thyroiditis are euthyroid, significant proportion

of them develop hypothyroidism eventually.<sup>8</sup> The prevalence of Hashimoto's thyroiditis in elderly patients is estimated to be 40%.<sup>9</sup> However, diagnosis relies mostly on cytology and/or histology. In a study comparing Fine needle aspiration with histopathology, the former demonstrated a very high sensitivity and specificity of 92.8% and 94.2% respectively.<sup>10</sup> Since FNAC is quite simple to perform with minimal expertise, it has become an alternative gold standard for diagnosis of many autoimmune thyroid disorders including Hashimoto's thyroiditis. Hence, we used it as the gold standard in this study.

About 70% of patients with Grave's disease may have positive anti-TPO antibodies.<sup>11</sup> Moreover, thyroid stimulating hormone receptor antibodies that are pathogenic of Grave's disease may be seen in Hashimoto's thyroiditis as well.<sup>11</sup> Further, persons with elevated TSH and positive anti-TPO are likely to develop overt hypothyroidism at a rate of 4.5% per year.<sup>12</sup> Hypothyroidism is a common diagnosis in outpatient departments. Clinicians are often faced with the dilemma of choosing the next test to rule in or rule out Hashimoto's thyroiditis since this is a very prevalent cause of hypothyroidism. So, we aimed at determining the utility of anti-TPO in diagnosis of Hashimoto's disease among people with hypothyroidism. With this study we have found that though anti-TPO has a low specificity, and low positive LR, its ability to rule out Hashimoto's thyroiditis when the test is negative seems very good. In other words, a negative anti-TPO in patients with hypothyroidism effectively reduces post-test probability below testing threshold obviating the need for further unnecessary evaluation of the patient for Hashimoto's thyroiditis. On the other hand, a positive anti-TPO may still need to be supported by further testing like FNAC to confirm the diagnosis.

Our study had a few limitations. First, we had a relatively small number of patients. However, all of them were selected rigorously following standard criteria for hypothyroidism. Second, our gold standard was FNAC, which could have been improved upon by using histopathology. Again, this would not have impacted results too much as several studies have shown very good sensitivity and specificity for FNAC compared to histopathology.

## CONCLUSION

In patients with hypothyroidism, anti-TPO antibodies provide a valuable non-invasive method to effectively rule out Hashimoto's thyroiditis due to their high sensitivity and excellent negative likelihood ratio. However positive anti-TPO tests in such individuals may warrant further invasive testing to confirm Hashimoto's thyroiditis. A negative test saves unnecessary further testing for Hashimoto's thyroiditis.

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