Research Article

Evaluating the role of anti-TPO antibody in detecting Hashimoto's Thyroiditis - a report from a tertiary care hospital of Northeast India.

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ABSTRACT

Background: Hashimoto's thyroiditis (HT) is considered as one of the most common cause of hypothyroidism and it represents a significant public health challenge. Fine needle aspiration cytology (FNAC) alongwith thyroid function tests are two main diagnostic modalities used for its detection. This study evaluate the actual role of anti-thyroid peroxidase (anti-TPO) antibodies in detecting HT. The correlation between thyroid profile parameters, like TSH, fT3, fT4, and anti-TPO were evaluated to establish the efficacy of integrating anti-TPO to enhance diagnostic precision.

Materials and Methods: The study was done in Cytopathology section, Department of Pathology, Gauhati Medical College & Hospital. It is a retrospective cross-sectional study done on patients with confirmed hypothyroidism that were referred for FNAC within a period of 1 year. The patients presenting in the OPD with clinical features that indicate thyroid disorder and underwent hormonal tests like T3, T4, TSH and anti-TPO were subjected to FNA of thyroid and the samples were examined for cytological evaluation.

Results: Out of the total 120 cases, 9 were male and111 were female. Among the total cases, 97 cases were confirmed by FNAC as Hashimoto's thyroiditis. Out of these, 88 cases showed high levels of anti-TPO antibodies and 9 cases showed negative results for anti-TPO antibodies. The anti-TPO shows positive correlation with Hashimoto status ($p \leq 0.05$) which makes sense as anti-TPO is a key marker for Hashimoto's disease.

Conclusion: The anti-TPO antibodies is a non-invasive and valuable method to predict HT, but further confirmatory test like FNAC and histopathological are examination required.

Keywords: : Fine Needle Aspiration Cytology (FNAC), Anti-TPO, antibodies, Hashimoto's thyroiditis, Thyroid profile.

1. INTRODUCTION

Hashimoto's thyroiditis (HT) has been characterized as "struma lymphomatosa" that is an enlarged thyroid gland which has been infiltrated with lymphocytes [1]. In iron-sufficient areas, Hashimoto's thyroiditis is one of the most common cause of primary hypothyroidism (PH) [2, 3]. The incidence of Hashimoto's thyroiditis is around 0.3-1.5 cases per 1000 people, and the female to male predominance is 7-10:1. The Hashimoto's thyroiditis is also known as chronic autoimmune thyroiditis and chronic lymphocytic thyroiditis. It is an autoimmune disease where the thyroid cells are destroyed by the cell and antibody-mediated immune processes. The pathophysiology of Hashimoto thyroiditis is considered to be complex that involves the formation of anti-thyroid antibodies which attack the thyroid tissue and cause progressive fibrosis. The diagnosis of HT is challenging and sometimes it remains undiagnosed untill late in the disease process. The common laboratory reports shows elevated

thyroid-stimulating hormone (TSH) with low thyroxine (T4) levels and there is increase in anti thyroid peroxidase (anti-TPO) antibodies. The females are more affected by HT. The female-to-male ratio is approximately 10:1. Mostly the diagnosis occurs in the fifth decade of life but there are also reports where women are diagnosed in the age group of 30 to 50 years. The etiology of Hashimoto disease is still not completely understood. Generally the patients develop antibodies to numerous thyroid antigens and the most common one is anti-TPO. Also, anti thyroglobulin (anti-Tg) and TSH receptor-blocking antibodies (TBII) also form. All these antibodies together attack the thyroid tissue and these lead to production of thyroid hormone inadequately. A small population of around 10% -15% shows serum antibody negative although they show clinically evident disease. The positive TPO antibodies report is a sign of the clinical syndrome [4, 5]. As per previous studies, the prevalence of Hashimoto‘s thyroiditis varies between 0.55% to 13.4 % [5]. Fine needle aspiration cytology (FNAC) and the presence of anti-TPO are the common diagnostic procedure to detect Hashimoto’s thyroiditis. Several studies has been reported that shows that the anti-TPO method is sensitive, precise and antigen specific method and has the ability to reveal quantitative fluctuations [6]. This study aims to evaluate the combined role of anti-TPO antibodies, FNAC findings and thyroid profile in detecting Hashimoto’s thyroiditis.

2. Materials and Methods:

The study has been done in the Cytopathology section, Department of Pathology, Gauhati Medical College & Hospital, which is one of the largest tertiary care hospital in Northeast region of India. It is a retrospective cross-sectional study conducted among patients who have confirmed hypothyroidism and were referred to cytopathology section for fine needle aspiration cytology within a period of 1 year from 2023 to 2024. Consent forms were duly signed by the patients or their guardians during the FNAC procedure and the necessary details were filled in the proforma. The Out Patient Department (OPD) patients which showed clinical features that indicated thyroid disorder underwent hormonal tests fT3, fT4, TSH and anti-TPO. These cases were subjected to Fine Needle Aspiration of the thyroid and the samples were examined for cytological evaluation. The analysis of T3, T4, TSH were performed using Vitros 5600 integrated system and Immunoassay method. Anti-TPO was done using the electrochemiluminescence method. A 23 gauge needle was used to do FNA and MGG and PAP stains were used.

3. Results & Observation:

Out of the total 120 cases, 9 were male and other 111 were female (Figure 1). The commonest age group is 31-50 years. Among the total cases, 97 cases were confirmed as Hashimoto’s thyroiditis by FNAC. Gauge needle was used to do FNA and stained using MGG and PAP stains. Figure 2 shows the MGG stained smears of Hashimoto thyroiditis.

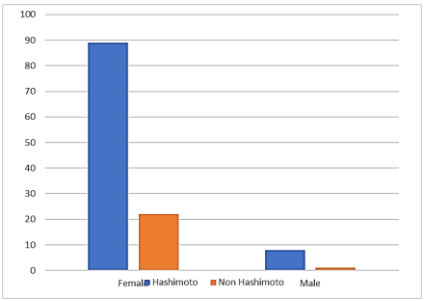
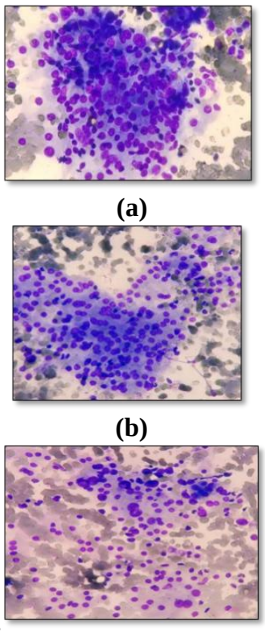


Figure 1: Bar diagram showing Male and Female cases among the Hashimoto and Non-Hashimoto cases.

Out of the 97 confirmed Hashimoto’s thyroiditis cases, 88 were positive and showed high anti-TPO antibodies and in 9 cases the anti-TPO antibodies were lower. Out of total 23 non-Hashimoto thyroid cases, only 3 showed high positive values for anti-TPO antibody.

The results obtained for T3, T4, TSH and anti-TPO antibody assays were collected and tabulated along with the clinical information. Statistical analysis was done using GraphPad Prism: Version 10.4. Among the positive Hashimoto’s thyroiditis cases, significant associations ($p \leq 0.05$) were found for age, sex, T3, T4, suggesting that these factors play a critical role in the disease's occurrence (Table 1, Table 2, Table 3). As recorded, the TSH levels varied and were mostly elevated. Also, Chi-square test shows significant associations of anti-

TPO antibodies and Hashimoto's thyroiditis with p value ≤ 0.05 (Table 4). This shows that elevated level of anti-TPO antibodies can be considered as a key marker for diagnosis of Hashimoto's thyroiditis and also the patients with high anti-TPO antibodies have higher chances of developing this disease.



(a)

(b)

(c)

Figure 2: (a,b,c): Microscopic images of Hashimoto's thyroiditis FNAC. MGG stained smears (40 X) show thyroid follicular cells in clusters, sheets as well as singly dispersed. Lymphocytic impingement seen in few clusters. Few oncocytic cell collections also noted. Background show lympho-histiocytic cell collections, giant cell & scant colloid.

Table 1: Significant correlation of sex in Hashimoto's thyroiditis

	Observed Value			Expected Value		
SEX	Hashimoto (YES)	Hashimoto (NO)	Total	Hashimoto (YES)	Hashimoto (NO)	Total
F	89	22	111	89.73	21.28	111.00
M	8	1	9	1.73	1.73	3.45
Total	97	23	120	91.45	23.00	114.45
p-value: p ≤ 0.05						

Table 2: Significant correlation of T3 in Hashimoto's thyroiditis

	Observed Value			Expected Value		
T3 range	Hashimoto (YES)	Hashimoto (NO)	Total	Hashimoto (YES)	Hashimoto (NO)	Total
Less than 2.6	93	23	116	93.77	22.23	116.00
2.6-4.4	4	0	4	0.77	0.77	1.53
More than 4.5	0	0	0	0.00	0.00	0.00
Total	97	23	120	94.53333333	23	117.5333333
p-value: p ≤ 0.05						

Table 3: Table depicting significant correlation of T4 in Hashimoto's thyroiditis

	Observed Value			Expected Value		
T4 range	Hashimoto (YES)	Hashimoto (NO)	Total	Hashimoto (YES)	Hashimoto (NO)	Total
Less than 1	58	11	69	55.78	13.23	69.00
1-1.6	34	12	46	8.82	8.82	17.63
More than 1.6	5	0	5	4.04	0.96	5.00
Total	97	23	120	68.6333333	23	91.6333333
p-value: p ≤ 0.05						

Table 4: Significant correlation of anti-TPO antibodies in Hashimoto's thyroiditis

	Observed Value			Expected Value		
Anti-TPO range	Hashimoto thyroiditis	Non-Hashimoto thyroid cases	Total	Hashimoto thyroiditis	Non-Hashimoto thyroid cases	Total
Less than 34	9	20	29	23.44	5.56	29.00
More than 34	88	3	91	17.44	17.44	34.88
Total	97	23	120	40.88333333	23	63.8833333
p-value: p ≤ 0.05						

4. Discussion:

Among 120 patients with clinical hypothyroidism, 97 were confirmed as Hashimoto's thyroiditis. Out of it, 88 showed high anti-TPO antibodies. This signifies that anti-TPO antibody can be used as marker in predicting Hashimoto thyroiditis. The prevalence rate of anti-TPO antibodies varies among the hypothyroid cases in India. The anti-TPO positivity was reported to be consistently high in India with five cities recording a prevalence of more than 20% [7]. Another study report from Greece showed the prevalence to be around 30.4% [8]. This hospital based study shows that anti-TPO antibody-positive thyroid disorder is more in this region of the country. In this study, 9(7.5%) were male and 111(92.5%) were female. Among the total 97 Hashimoto's thyroiditis diagnosed cases, 89 (91.75%) were female and 8 (8.24%) male. This is in accordance with the fact that Hashimoto's thyroiditis is more prevalent in females. Studies reveals that the thyroid autoimmunity is significantly greater in women [9]. In this study, the cases of Hashimoto's thyroiditis were in the age range of 30-50 years. This is in accordance with other similar studies which reported that this Hashimoto's thyroiditis was common in the age group of 30-50 years [10]. The root cause of the disease occurring in this age group can be studied in details and explored further. The anti-TPO may be seen before or after a laboratory test reveals a thyroid

hormone test abnormality [11]. In the study, clinical and laboratory confirmed hypothyroid cases were taken. Although, in some cases, person with Hashimoto's thyroiditis has thyroid hormone values within limit, but many of them eventually develop hypothyroidism [12]. Therefore, a combined test of anti-TPO and thyroid function can be helpful in identifying and detecting individuals who are at potential risk of developing this autoimmune disease. This simple laboratory testing can be life-saving and would prevent long-term morbidity as the anti-TPO antibody is associated with future over hypothyroidism. So, patients with positive anti-TPO should do regular follow ups with serial thyroid function tests and see if there are changes in the thyroid level. This will help in early diagnosis of thyroid disorders. Hashimoto's thyroiditis is an autoimmune disorder and there is inadequate production of the thyroid hormone. The biochemical test always show high thyroid-stimulating hormone (TSH) and low free T4. A low vale of total T4 or free T4 level in the presence of increased TSH levels predicts primary hypothyroidism. The presence of parameters like anti-thyroid peroxidase alongwith anti-thyroglobulin antibodies indicates Hashimoto's thyroiditis. The detection of Hashimoto's thyroiditis is mostly done by FNAC with histopathology. However, FNAC is considered as a gold standard as it has a specificity of 94.2 % and sensitivity of 92.8 % [13]. As per studies, anti-TPO antibody has a specificity of 88.37 % [14]. Anti-TPO antibody estimation is very much essential in finding the etiological diagnosis of autoimmune thyroid diseases [15]. Anti-TPO antibodies must always be done to confirm Hashimoto's thyroiditis diagnosis as only doing T3, T4, TSH is not sufficient [16]. Many studies have shown that there is a positive corelation between antibody positivity and hypothyroidism [15- 21]. It has been reported that there is a significant correlation between TSH or T4 concentration and elevated anti-TPO antibody [22]. However in the present study group, around 9.27% patients showed antibody negative. It has been observed in previous studies also that in some cases of Hashimoto's thyroiditis, the anti-TPO antibody may be negative despite the presence of the autoimmune disease. This is because of the fact that in some less destructive form of Hashimoto's thyroiditis, the thyroid gland may function adequately, leading to lack lack of over hypothyroidism and lower TPO level. The autoimmune process in Hashimoto's thyroiditis can be cyclical with sometimes high antibody levels and in times it may show lower levels or even levels which are undetectable [23]. Although the anti-TPO antibodies are generally high in Hashimoto's thyroiditis, but they are not the only thyroid antibodies that are involved. In certain cases, the role of anti-thyroglobulin antibodies (anti-Tg) may also be there [24]. It has been observed that, clinicians often face issues in choosing the next test to rule in or to rule out Hashimoto's thyroiditis. So, the present study aimed at correlating the anti-TPO and FNAC in predicting Hashimoto's thyroiditis. As per the evaluated data, it has been observed that there is a significant corelation between anti-TPO antibody and Hashimoto's thyroiditis with p value ≤ 0.05. This shows that elevated level of anti-TPO antibodies can be considered as a key marker for diagnosis of Hashimoto's thyroiditis and also it shows that the patients with high anti-TPO antibodies have higher chances of developing this disease.

5. Conclusion:

The anti-TPO antibodies detection is non-invasive and is a valuable method to predict Hashimoto's thyroiditis, but further confirmatory test like FNAC and histopathological examination are required. Hashimoto's thyroiditis has no cure and stays life- long. The only thing that can be done manage the disease with proper medication alongwith routine check-ups and laboratory test. Thus, it is best managed by an inter-professional team of endocrinologist and primary healthcare provider. In case of a resource limited set-up, where the inter-professional team of endocrinologist is not there, management of Hashimoto's thyroiditis primarily involves thyroid hormone replacement therapy with Levothyroxine. Dose adjustment is based on individual need of the patient. It needs lifelong medication and regular monitoring, so

patient education and compliance to treatment is necessary. The best way to handle the disease is to follow up on the levels of thyroid hormone at regular intervals as per the suggestions given by the physician. Sometimes, lymphoma may develop in the patients. So, a regular examination of the neck area is thus required and is recommended in such cases. Primary thyroid lymphoma (PTL) is mostly accompanied by Hashimoto's thyroiditis which is the only identified risk factor. Although Hashimoto's thyroiditis is detected in 80% of primary thyroid lymphomas, only 0.6% of patients with Hashimoto's thyroiditis develop PTL [25]. Trucut needle biopsy can be used for diagnosis of PTL and to avoid unnecessary surgery if FNA results are insufficient. Staging is required for further treatment planning in patients with PTL [26]. Early and non-invasive diagnosis are always preferable and most widely chosen. So, anti-TPO antibodies can be considered as a good non-invasive diagnostic parameter in detecting Hashimoto's thyroiditis.

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