

## A Clinical, Biochemical and Cytomorphologic Study on Autoimmune Thyroiditis

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### Abstract:

**Background:** Autoimmune thyroiditis is the second most common thyroid lesion diagnosed on FNAC after colloid goitre. Study was done to undertake clinical and cytological parameters of all thyroid swellings and also to assess the thyroid functional status and to find out the serum anti-TPO antibody levels in patients diagnosed as autoimmune thyroiditis (AT).

**Materials and methods:** The study was carried out in the Department of Pathology and Biochemistry, North Bengal Medical College over a period of one year. After obtaining detailed history and clinical examination, FNAC was performed and the cytological diagnoses were rendered. Estimation of serum free T4, TSH and anti-TPO antibody was carried out in cytologically diagnosed cases of autoimmune thyroiditis.

**Results:** Out of 186 patients, in 173 cases (93%) adequate aspirate was obtained in our study, of which 153 cases (88.4%) were benign non-neoplastic, 14 cases (8.1%) were neoplastic and 6 cases (3.5%) were indeterminate lesion. Among the benign non-neoplastic cases, 103 (59.5%) colloid goitre and 31 (17.9%) AT were recorded. Among 31 cases of AT 17 cases (54.8%) were found in the third decade of life, 26 cases (83.9%) presented with diffuse swelling and all were female. In 31 cases of AT, 16 (51.6%) were hypothyroid, 12 (38.7%) were euthyroid and 3 (9.7%) were hyperthyroid. 28 cases (90.3%) were anti-TPO positive.

**Conclusion:** FNAC is a safe, simple and fairly accurate first line investigation in thyroid swellings. Clinical correlation, serological studies and cytological findings are essential in diagnosis as well as management of autoimmune thyroiditis.

**Key words:** Goitre, FNAC, AT-autoimmune thyroiditis, free T4, TSH, anti-TPO antibody

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### I. Introduction

It is now widely accepted that the thyroid diseases traditionally known as lymphocytic thyroiditis and Hashimoto's thyroiditis represent different phases or manifestations of an organ-specific immune-mediated inflammatory disorder generically designated as autoimmune thyroiditis and characterized functionally by the production of autoantibodies that alter thyroid function.<sup>1,2,3,4</sup> Some authors use the terms lymphocytic and Hashimoto's thyroiditis synonymously.<sup>5</sup> The mechanisms leading to autoimmune thyroiditis are of both humoral and cellular in nature.<sup>6,7,8,9</sup> Lymphocytic thyroiditis is the most common form of thyroiditis observed in clinical practice.<sup>10</sup> In 95% of cases, the patient is female and typically middle aged, although the disease can occur in other age groups including children.<sup>11</sup> The majority of patients progress to hypothyroidism over a period of few years. The mean annual incidence rate of autoimmune hypothyroidism is up to 4 per 1000 women and 1 per 1000 men.<sup>12</sup> The disease is characterized by the presence of anti-thyroid peroxidase (anti-TPO) antibodies.<sup>1</sup> The annual risk of developing clinical hypothyroidism is 4%, when subclinical hypothyroidism is associated with positive thyroid peroxidase (TPO) antibodies.<sup>12</sup> Fine needle aspiration cytology (FNAC) of the thyroid gland is now a well-established, first line diagnostic test for the evaluation of diffuse thyroid lesions as well as of thyroid nodules with the main purpose of confirming benign lesions and thereby, reducing unnecessary surgery.<sup>13</sup> The exact appearances in an aspirate are dependent on the phase of the disease. Early in the disease abundant lymphocytes are present and later Hurthle cell change and fibrosis predominate.<sup>14</sup>

## II. Materials and Methods:

The present study on thyroid lesions with particular reference to autoimmune thyroiditis was carried out in the Department of Pathology and Biochemistry, North Bengal Medical College (NBMC), Darjeeling, West Bengal over a period of one year from July 2014 to June 2015.

Each patient was clinically assessed. Detailed history was taken, clinical examinations to note the features of goitre, along with general and systemic examinations were performed to make a provisional diagnosis. Procedure of FNA and smear preparation and fixation was performed as per standard procedure.<sup>15</sup> Leishman-Giemsa stain<sup>16,17</sup> and Haematoxylin & Eosin stain<sup>16,18</sup> were done following standard guidelines. Criteria for adequacy was also followed.<sup>19,20</sup> Biochemical examinations (free T4 & TSH and anti-TPO antibody) were also done. Anti-TPO antibody was estimated only in cytologically diagnosed cases of autoimmune thyroiditis. Serum free T4 was estimated by competitive Enzyme Linked Immunosorbant Assay (ELISA) method (Measuring range: 0.1- 8.0 ng/dl, Biological reference range: 0.8-2.0 ng/dl) and serum TSH by non-competitive ELISA method (Measuring range: 0.01-61µIU/ml, Biological reference range: 0.39-6.16µIU/ml). Estimation of serum anti-TPO antibody was done by competitive Electrochemiluminescence immunoassay (Measuring range: 5-600 IU/ml. Biological reference range: < 35 IU/ml).

The following characteristics were noted while reporting of cytology smears:

- Cellularity: high, moderate, mild, scanty
- Pattern of distribution: clusters, 3-dimensional, papillary, acini, dissociated
- Follicular cells: monomorphic, pleomorphic, infiltration by lymphocytes, degenerative changes, fire flares
- Individual follicular cells-

Nucleus: size/shape nuclei, nuclear margin, chromatin pattern, nuclear grooves and inclusions, nucleolus.

Cytoplasm: amount, staining characteristics, granules.

- Foam cells: few, many
- Colloid: amount, thick/thin, colloid globi
- Metaplasia: Hurthle cells, squamous cells
- Other cells: spindle cells, giant cells, epithelioid granulomas, lymphocytes and other inflammatory cells, etc.

Considering the above mentioned criteria cytological diagnoses were given and the lesions were categorised. Cases considered non-diagnostic where cytological diagnosis could not be given due to material inadequacy after repeated aspiration. The various thyroid lesions obtained by FNAC were divided into three broad categories non-neoplastic, neoplastic and indeterminate lesions. Indeterminate lesion were those, which were suspicious to be neoplastic but definite opinion could not be given in spite of adequate specimen.

Special emphasis was given on cytologically diagnosed cases of autoimmune thyroiditis where cytomorphologic features were reviewed and graded according to lymphocyte infiltrate and other parameters, for example, Hurthle cells, degree of anisonucleosis, giant cells, and so forth as per Bhatia et al (2007).<sup>21</sup>

Grade	Morphological features
1 (Mild)	Few lymphoid cells infiltrating the follicles or increased number of lymphocytes in the background
2 (Moderate)	Moderate lymphocytic infiltration or mild lymphocytic infiltration with Hurthle cell changes/giant cells/anisonucleosis
3 (Severe)	Florid lymphocytic infiltration with germinal centre formation, very few follicles left

## III. Results

Out of 186 patients with thyroid swelling, adequate aspirate was obtained in 173 cases (93%). 151 (87.3%) were females out of 173 cases with adequate aspirates. Most of the cases were found in their third (31.2%) and fourth (23.1%) decades [Table-1].

153 cases (88.4%) were non-neoplastic, 14 cases (8.1%) were neoplastic and 6 cases (3.5%) were indeterminate lesion out of 173 cases with adequate aspirates. Among non-neoplastic cases majority of cases were colloid goitre (59.5%) and autoimmune thyroiditis (17.9%). Major neoplastic lesions were, papillary carcinoma (4%) and of follicular neoplasm (2.6%) among patients with adequate aspirate. [Table-2].

All (31) cases of autoimmune thyroiditis in this study were females. Most of the cases of autoimmune thyroiditis were found in third decades of life (54.8%) [Table-3].

When all goitre cases and autoimmune thyroiditis cases were divided into two age group categories (≤ 30 years and >30 years). It was found that there were significant difference in age wise distribution between two groups (p=0.004) [Table-4].

Diffuse pattern of presentation were seen in 26 cases (83.9%) out of 31 AT cases. Only five cases (16.1%) were presented with nodular thyroid swelling. When autoimmune thyroiditis cases were graded cytologically into mild (grade-1), moderate (grade-2), and severe (grade-3) according to the extent of lymphocytic infiltration and other features like Hurthle cell changes, degree of anisonucleosis, giant cells and

germinal centre formation. 10 (32.3%), 13 (41.9%) and 8 (25.8%) cases of three respective grades were found [Table-5].

Out of 31 autoimmune thyroiditis cases, 16 (51.6%) were hypothyroid, 12 (38.7%) were euthyroid and only 3 (9.7%) were hyperthyroid [Table-6].

28 (88.5%) AT cases were anti-TPO positive and only three cases (9.7%) were anti-TPO negative [Table-7].

Autoimmune thyroiditis cases were divided into three categories according to the serum level of anti-TPO antibodies. The categories were <201 IU/ml, 201-400 IU/ml and >400 IU/ml. It was found that most of the cases (41.9%) have anti-TPO antibody level in the range of 201-400 IU/ml [Table-8].

A comparison was made between different grades of autoimmune thyroiditis and thyroid profile. It was found that as the grade increased the patients significantly become hypothyroid ( $p=0.04$ ) [Table-9]. Another comparison was made between different grades of autoimmune thyroiditis and different level of anti-TPO antibody. It was also found that as grade increased serum anti-TPO level become significantly positive at higher serum level ( $p=0.007$ ) [Table-10].

#### IV. Discussion

In this study of 173 goitre cases with adequate aspirates, maximum patients were within the age group of 21-30 years (31.2%) followed by 31-40 years (23.1%). Singh et al<sup>22</sup> (2013) also found maximum number of cases in 3<sup>rd</sup> and 4<sup>th</sup> decades as in our study. Most of our reported patient were females (87.3%) with female to male ratio 6.86:1 was comparable to the ratio of 6.35:1 and 6.7:1, in studies of Guhamallick et al<sup>23</sup> and Singh et al<sup>22</sup>

Out of 173 cases in this study, 153 (88.4%) were benign, 14 (8.1%) were neoplastic and 6 (3.5%) were indeterminate lesions. It was compared to the study of Swamy et al<sup>24</sup> (2011), where among 120 cases, 100 (83.66%) were benign and 20 (16.66%) were malignant lesion. Whereas, Singh et al<sup>22</sup> (2013), in a study of 122 cases, found 48 (39.3%) non-neoplastic, 70 (57.3%) neoplastic and 4 (3.4%) indeterminate lesions.

Among 173 cases diagnosed cytologically, the non-neoplastic lesions were, 103 (59.5%) colloid goitre, 31 (17.9%) autoimmune thyroiditis, 7 (4%) adenomatoid nodule, 5 (2.9%) hyperplastic nodule, 3 (1.7%) thyroglossal cyst, 2 (1.2%) subacute thyroiditis, and 2 (1.2%) acute suppurative lesion.

India has the world's biggest goitre belt in the sub-Himalayan region. In these iodine-deficient areas, the incidence of colloid goitre among thyroid nodules is much higher as shown in the study by Patel et al (2013), where they got 111 colloid goitre cases (57.22%) out of 194 cases which were comparable to the this study.<sup>25</sup> Handa et al (2008)<sup>26</sup> also found 57.60% colloid goitre among thyroid nodules in these iodine-deficient areas were also comparable to this study.

In this study, 31 cases (17.9%) of autoimmune thyroiditis were found. Handa et al (2008) reported an incidence of 27.4% of HT in their study of 434 thyroid lesions.<sup>26</sup> A high incidence of HT, 22.8% and 31.4% were also reported by Chabchoub et al (2010)<sup>27</sup> and Sood et al (2014)<sup>28</sup>.

In this study series 17 (54.8%) cases of autoimmune thyroiditis were found in the third decade of life. Sood et al (2014)<sup>28</sup> and Fatima et al (2014)<sup>29</sup> also reported 21-30 years as the commonest age group of lymphocytic thyroiditis. It was also found that there were significant difference ( $p=0.004$ ) in age wise distribution of goitre patients between autoimmune thyroiditis patients and all other goitre patients.

A female to male ratio of 29:1 and 43:1 among patients of HT was reported by Handa et al.<sup>26</sup> and Fatima et al.<sup>29</sup> All the 31 patients of autoimmune thyroiditis were females in our study. Among 31 autoimmune thyroiditis cases, 26 (83.9%) were presented with diffuse swelling. Similar clinical presentation was noted by Kapila et al.<sup>30</sup>

Among autoimmune thyroiditis cases in this study, 16 (51.6%) were hypothyroid, 12 (38.7%) were euthyroid and 3 (9.7%) were hyperthyroid. Similar results were obtained by Fatima et al (2014)<sup>29</sup> in their study on 44 cases of autoimmune thyroiditis, with 21 hypothyroid cases, followed by 20 euthyroid cases and 3 cases with increased thyroxine levels. Similarly hypothyroid state was common association with chronic lymphocytic thyroiditis in a study by Khalil et al.<sup>31</sup> But Kapila et al reported majority of cases as euthyroid at presentation.<sup>30</sup> These findings explain that active phase of disease is transient with clinical manifestations of thyrotoxicosis while the evolution phase and destructive phase manifest with subclinical or overt hypothyroidism.<sup>21</sup>

This study showed anti-TPO positivity in 90.3% (28) cases of autoimmune thyroiditis. Comparable results have been found by Demirbilek et al (2009)<sup>32</sup> and Jayaram et al (2007).<sup>33</sup> Dayan et al (1996)<sup>34</sup> stated that testing of thyroid autoantibodies and measurement of serum thyroglobulin levels confirm the diagnosis of chronic autoimmune thyroiditis. Mariotti et al (1990)<sup>35</sup> stated that a positive test for anti-thyroid peroxidase antibodies is a more sensitive indicator of chronic autoimmune thyroiditis than a positive test for antithyroid microsomal antibodies.

In this study a comparison was made between different grades of autoimmune thyroiditis and thyroid profile. It was found that as the grade increased the patients significantly become hypothyroid ( $p=0.04$ ) [Table-

9]. Another comparison was made between different grades of autoimmune thyroiditis and different level of anti-TPO antibody. It was also found that as grade increased serum anti-TPO level become significantly positive at higher serum level ( $p=0.007$ ) [Table-10].

As per the study of Soodet al<sup>28</sup> the higher the grade of lymphoid infiltrate, the higher the percentage of patients with increased anti-TPO antibodies. They also said that patient with hypothyroidism secondary to autoimmune thyroiditis would have an elevated TSH, a low FT4, and positive anti-TPO antibodies; however, in early stages of the disease, TSH might be normal and anti-TPO antibodies might be positive with or without goitre.

Singh et al<sup>36</sup> said that high lymphoid to epithelial ratio was strongly correlated with thyroid peroxidase positivity and thyroid peroxidase positivity is statistically strongly associated with HT as compared to HT coexisting with follicular hyperplasia/Hashitoxicosis/neoplasm.

## V. Conclusion

So from the above study we can conclude that FNAC is safe, simple, cost- effective, reliable, accurate and first line of investigation in thyroid swellings. Majority of thyroid swellings were due to colloid goitre and thyroiditis which were more prevalent in young females of reproductive age group. Further we have seen a significant correlation between autoantibody levels, thyroid function test and cytological findings on FNAC in autoimmune thyroiditis patients. So clinical correlation, serological study and cytological findings can be used as useful tool in diagnosis as well as management of autoimmune thyroiditis.

## VI. Tables

**Table 1: Age distribution of goitre cases (n=173)**

Age	Number of cases (%)
11-20yrs	23 (13.3)
21-30yrs	54 (31.2)
31-40yrs	40 (23.1)
41-50yrs	24 (13.9)
51-60yrs	18 (10.4)
61-70yrs	14 (8.1)
Total	173

**Table 2: Categorization on goitre cases (n=173)**

Cyodiagnosis	Number of cases (%)
Colloid goitre	103 (59.5)
Adenomatoid nodule	7 (4)
Hyperplastic nodule	5 (2.9)
<b>Autoimmune thyroiditis</b>	<b>31 (17.9)</b>
Subacute thyroiditis	2 (1.2)
Acute suppurative lesion	2 (1.2)
Thyroglossal cyst	3 (1.7)
Papillary carcinoma	7 (4)
Follicular neoplasm	4 (2.3)
SCC thyroid	1 (0.6)
Mets adenocarcinoma	1 (0.6)
Mets squamous cell carcinoma	1 (0.6)
Indeterminate lesion	6 (3.5)
Total	173 (100)

**Table 3: Age wise distribution of cases of autoimmune thyroiditis (N=31)**

Age	Number of cases of autoimmune thyroiditis(%)
11-20yrs	4 (12.9)
21-30yrs	17(54.8)
31-40yrs	8(25.8)
41-50yrs	2(6.5)
Total	31(100)

**Table 4: Distribution of cases of autoimmune thyroiditis and other goitre cases with age group (n=173)**

Age	Number of autoimmune thyroiditis cases (%)	Number of other goitre cases (%)	Statistical test
11-20yrs	4 (12.9)	19 (13.4)	$X^2= 8.25$ $df= 1$ $p= 0.004$
21-30yrs	17 (54.8)	37 (26.1)	
31-40yrs	8 (25.8)	32 (22.5)	

41-70yrs	2 (6.5)	54 (38)	Significant
Total	31 (100)	142	173

**Table 5:** Cytological grading of autoimmune thyroiditis

Grade	Lymphocytic infiltrate & others	Number of cases (%)
1	Mild	10 (32.3)
2	Moderate	13 (41.9)
3	Severe	8 (25.8)
Total		31 (100)

**Table 6:** Thyroid function status in AT patients

Thyroid function status	Free T4 level (ng/dl)	TSH level (uIU/ml)	Number of cases (%)
Hypothyroid	<0.8	>6.16	16 (51.6)
Euthyroid	0.8-2.0	0.39-6.16	12 (38.7)
Hyperthyroid	>2.0	<0.39	3 (9.7)
Total			31 (100)

**Table 7:** Anti-TPO positivity in autoimmune thyroiditis cases (n=31)

Anti-TPO status	Number of cases (%)
Anti-TPO positive	28 (90.3)
Anti-TPO negative	3 (9.7)
Total	31 (100)

**Table 8:** Grading of AT cases on the basis of serum anti-TPO antibody levels

Anti-TPO level (IU/ml)	Number of cases (%)
<201	11 (35.5)
201-400	13 (41.9)
>400	7 (22.6)
Total	31 (100)

**Table 9:** Comparison between different grades of autoimmune thyroiditis with thyroid function test

Grading of lymphocytic infiltrate	Number of cases	Thyroid profile		
		Hypo-thyroid (%)	Eu-thyroid (%)	Hyper-thyroid (%)
Grade-1	10	3 (30)	5 (50)	2 (20)
Grade-2	13	6 (46.2)	6 (46.2)	1 (7.6)
Grade-3	8	7 (87.5)	1 (12.5)	0 (0)
Total	31	X <sup>2</sup> =6.15, df=2, p=0.04, Significant		

**Table 10:** Comparison between different grades of autoimmune thyroiditis and different level of anti-TPO antibody

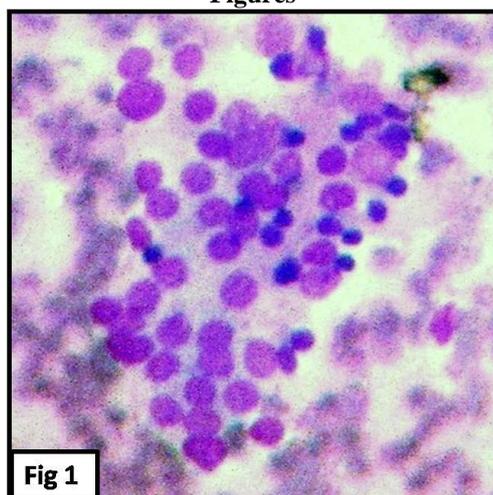
Grading of lymphocytic infiltrate	Number of cases	Serum anti-TPO ((IU/ml)		
		<201 (%)	201-400 (%)	>400 (%)
Grade-1	10	7 (70)	3 (30)	0 (0)
Grade-2	13	4 (30.8)	7 (53.8)	2 (15.4)
Grade-3	8	0 (0)	3 (37.5)	5 (62.5)
Total	31	X <sup>2</sup> =9.73, df=2, p=0.007, Significant		

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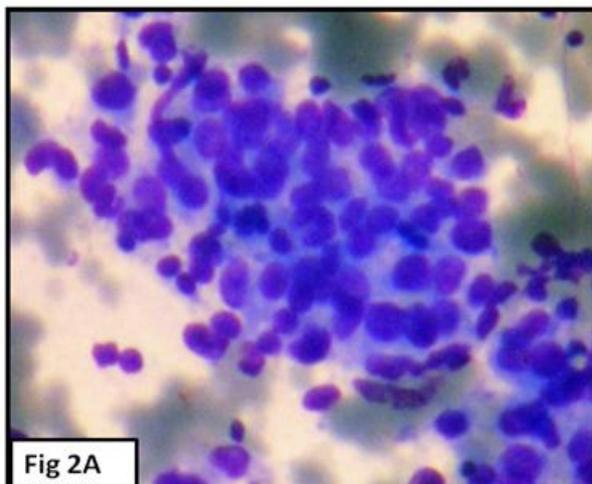
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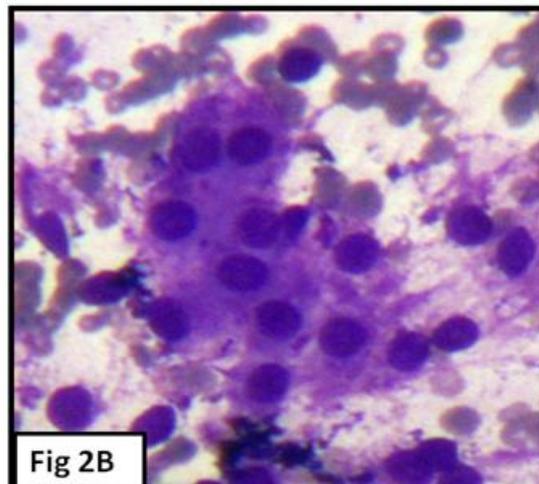
### Figures



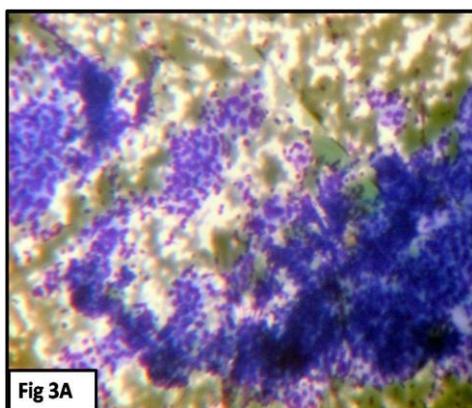
**Figure 1:** Mild lymphocytic infiltration in Grade-1 Autoimmune Thyroiditis (400X magnification), Leishman Giemsa stain



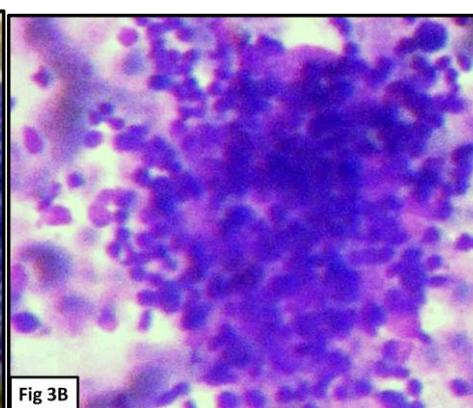
**Figure 2A :** Moderate lymphocytic infiltrate in a case of Grade-2 Autoimmune Thyroiditis (400X magnification), Leishman Giemsa stain



**Figure 2B:** Hurthle Cell changes in a case of Grade-2 Autoimmune Thyroiditis (400X magnification), Leishman Giemsa stain



**Fig 3A:** Severe lymphocytic infiltrate within thyroid follicular cells in a case of grade-3 Autoimmune thyroiditis (100X magnification), Leishman Giemsa stain



**Fig 3B:** Destruction of follicles by polymorphous lymphoid cell population in the same case (400X magnification), Leishman Giemsa stain

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