



Cytomorphological Correlation of Hashimoto's Thyroiditis with Clinical, Biochemical and Immunological Markers

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Abstract

Background and Aim: The present study was performed to study the cytomorphological observations in autoimmune thyroiditis and to correlate with the clinical features, thyroid function tests and antithyroid antibody profile. The cytomorphology in an aspirate is dependent on the phase of the disease.

Materials and Methods: The study was carried out in the department of Pathology, Meenakshi Medical College Hospital and Research Institute from January 2020 to January 2021. FNAC was performed in patients with palpable/visible thyroid swelling. Among them, 75 cases showing the cytomorphology of autoimmune thyroiditis were selected. It is correlated with their clinical features, thyroid function test and antithyroid antibody profile. Out of the 75 cases under study, 10 patients underwent thyroidectomy and sent for histopathological examination. Their results were studied.

Results: Among the 75 cases under study, majority of cases (58.6%) were categorized into cytological grade II. 40% of cases presented with subclinical hypothyroidism and 33.3% of cases presented with overt hypothyroidism. 89.3% of cases showed elevated levels of antithyroid peroxidase antibody.

Conclusion: Even though serology is a useful adjunct in the diagnosis of autoimmune thyroiditis, in cases with negative antibody titers and/or biochemically euthyroid & subclinically hypothyroid cases, FNAC continues to be the 'gold standard' in the diagnosis of Hashimoto's Thyroiditis

Keywords: Hashimoto's Thyroiditis, FNAC, Antithyroid antibody profile

Introduction

The thyroid gland, stimulates oxygen consumption in most of the cells of the body, helps regulate carbohydrate and lipid metabolism and thus it has influence over body mass and mental functions.⁽¹⁾ During fetal and neonatal life, deficiency of thyroxine leads to severe mental retardation and dwarfism.⁽¹⁾ In adults, diseases of thyroid are those associated with its hyper function, hypo function and neoplasm of thyroid gland.⁽²⁾ In our country, during the recent days, thyroid disorders have become increasingly common. Though they are distressing,

most are amenable to treatment. Various epidemiological studies carried out in India have estimated that around 42 million people suffer from thyroid disorders. Laboratory tests for screening and diagnosis of thyroid disorders have become more sensitive and specific over the past decade. Once diagnosed, highly satisfactory treatment modalities are available for most of the thyroid disorders. Endemic goiter due to iodine deficiency are prevented by universal iodination of salt. By keeping a check on excessive iodination, certain autoimmune diseases and its complications can be

prevented.⁽³⁾ In the year 1912, Dr.Hakaru Hashimoto described a new and distinct thyroid disorder based on the histologic changes in the thyroid tissue, which is now referred to as Hashimoto’s thyroiditis (HT) . Today, it is the leading cause of hypothyroidism in iodine sufficient areas of the world.^[2,4] It represents different phases of an organ specific, immune mediated inflammatory disorder characterized by production of autoantibodies that leads to destruction of thyroid follicles and alters thyroid function.^(5, 6, 7, 8)

Hashimoto’s thyroiditis is the most frequent form of thyroiditis, observed clinically.⁽⁹⁾ Although it can occur at any age, it most commonly occurs between 30 and 50 years of age with a marked female predilection with a male:female ratio ranging from 1:4 to 1:20. ^(2,5,9-13) Clinically, it presents as a diffuse, firm thyroid enlargement in most of the cases, although nodular and asymmetric enlargement may also be seen.^(2,14,15) The immune - mediated insult may initially cause diffuse or nodular hyperactivity and eventually causes atrophy of the gland, resulting in hypothyroidism^(15,16,17) although its incidence may vary in different studies.⁽¹³⁾ Fine needle aspiration cytology (FNAC) is a well established method for sampling of thyroid tissue. It aids in the diagnosis of various diseases affecting the gland and helps to alleviate many unnecessary surgeries.⁽¹⁸⁾ There is a well established cytomorphologic criteria for diagnosing Hashimoto’s thyroiditis . Many cases of Hashimoto’s thyroiditis may go undiagnosed by mere serologic examination. The utility of fine needle aspiration in the diagnosis of serologically negative cases has been highlighted. Hence, the importance of a combined clinical, biochemical, immunological and cytomorphologic approach towards the diagnosis of Hashimoto’s thyroiditis is emphasized.^(19,20)

Grading of Thyroiditis:⁽²¹⁾

Grade	Morphological features
GRADE I (Mild)	Few lymphoid cells infiltrating the follicles / increased number of lymphocytes in the background.

Since the input of patients presenting with these features are on the rise in our region, this study is undertaken to document the cytomorphological spectrum in the course of Hashimoto’s thyroiditis and its correlation with clinical features, thyroid function tests and antithyroid antibody profile.

Materials and Methods

A prospective cohort study was carried out in the Department of Pathology, Meenakshi Medical College Hospital and Research Institute, Kanchipuram over a period of one year from January 2020 to January 2021, after obtaining approval from the ethical committee of our institute. The study includes 75 cases of Hashimoto’s thyroiditis (HT). Histopathology of 10 thyroidectomy specimens of HT were also studied. Inclusion criteria were patients with Hashimoto’s thyroiditis diagnosed by Fine needle aspiration cytology and or antithyroid antibody titres. Exclusion criteria were other inflammatory lesions, non inflammatory lesions and neoplastic lesions of thyroid. Relevant clinical history was elicited and clinical examination was done and documented.

Sample Collection

After obtaining a written consent from the patients, fine needle aspiration is done under aseptic precautions in patients with visible and/or palpable thyroid gland by aspiration/non-aspiration technique. 2 aspirates were taken from different sites to procure enough material. Smears were made, promptly fixed in 95 % isopropyl alcohol and stained with Haematoxylin and Eosin stain.

Cytomorphology:

Detailed examination of the cytology smears were done. The severity of the disease was graded as per the criteria devised by Bhatia et al.⁽²¹⁾

GRADE II (Moderate)	Moderate lymphocytic infiltration or mild lymphocytic infiltration with Hurthle cell change and anisonucleosis.
GRADE III (Severe)	Florid lymphocytic infiltration with germinal center formation, very few follicular cells left.

Thyroid Function Tests And Antithyroid Antibody Profile:

The total tri-iodothyronine (T₃), thyroxine (T₄) & Thyroid stimulating hormone (TSH) levels were measured using ADVIA centaur chemiluminescent analyzer from SIEMENS by the method of chemiluminescence immunoassay. Titres of antithyroid peroxidase (Anti-TPO) and antithyroglobulin (Anti-Tg) antibodies were measured by Electro-Chemiluminescence immunoassay “ECLIA”.

Statistical Analysis

All the statistical operations were done using SPSS Software for windows evaluation version 16.0 (SPSS-Chicago, Illinois, USA).Chi-square test,

independent sample t -test, ANOVA, and pearson’s correlation (p-value) were used to analyze the data. Independent sample t -test, and ANOVA were used to compare when the categories were 2 or more than 2 respectively. A p-value of < 0.05 is considered to be statistically significant.

Results

Clinical Presentation Of The Thyroid Swelling:

Out of the 75 cases of Hashimoto’s thyroiditis, majority (81.3%) presented with diffuse enlargement of the thyroid gland. 14.7% of the cases presented as multinodular goiter and 4% presented as a solitary nodule. Table.1. shows that the clinical presentation of the thyroid swelling.

Table 1: Clinical Presentation Of Thyroid Swelling

CLINICAL PRESENTATION	NO. OF CASES	OPPERCENTAGE (%)
DIFFUSE THYROMEGALY	61	81.3
MULTINODULAR GOITER	11	14.7
SOLITARY NODULE	3	4
TOTAL	75	100

Clinical Symptoms & Signs Of Patients Under Study:

Among the 75 cases, majority of the cases (69.3%) suffered from increased fatigability, 42.7% cases had weight gain, 40% cases had menstrual irregularities, 21.3 % cases had dryness of skin, 26.7 % cases suffered from constipation, 20% cases had dry skin. Hoarseness of voice was seen in 14.7% of cases and pedal edema was seen in 8% of the cases under study.

Table 2: Clinical Symptoms & Signs Of Patients With Hashimoto's Thyroiditis

CLINICAL SYMPTOMS & SIGNS	NO. OF PATIENTS	PERCENTAGE (%)
Fatigue	52	69.3
Weight gain	32	42.7
Pedal edema	6	8
Hoarseness of voice	11	14.7
Dry skin	16	21.3
Constipation	20	26.7
Menstrual irregularity	30	40

Functional Status Of Patients Based On Thyroid Function Tests:

Based on the concentrations of triiodothyronine (T_3), thyroxine (T_4) and thyroid stimulating hormone (TSH) in the serum, the patients were biochemically categorized. In the study, among 75 cases, majority were in a state of hypothyroidism. Patients in whom peripheral thyroid hormones T_3 and T_4 were in the normal range, but mildly elevated TSH were referred to as subclinically hypothyroid or mild thyroid failure.

25 cases showed overt hypothyroidism and 30 cases were in a state of subclinical hypothyroidism. 18 cases were euthyroid and only 2 cases were hyperthyroid.

Table 3: Functional Status Of Ht Cases Based On Thyroid Function Tests

FUNCTIONAL STATUS	NO. OF CASES	PERCENTAGE (%)
Overt hypothyroid	25	33.3

Subclinical hypothyroidism	30	40
Euthyroid	18	24
Hyperthyroid	2	2.7

Thyroid Antibody Profile :

The titers of antithyroid peroxidase antibody (ATPO Ab) & antithyroglobulin antibody (Anti-Tg Ab) were measured in the 75 cases under study. Among them, 67 cases showed elevated titers of ATPO Ab and 54 cases showed elevated titers of Anti-Tg Ab. Both the antibodies were elevated in 54 cases. 8 cases showed very low titers for both the antithyroid antibodies under study.

Table 4 : Status Of Antithyroid Peroxidase Antibody & Antithyroglobulin Antibody In The Present Study

ANTIBODY PROFILE	NO. OF CASES	PERCENTAGE (%)
ATPO Ab elevated	67	89.3%
ATg Ab elevated	54	72 %
Both Abs elevated	54	72%
Either ATPO Ab/ ATg Ab Elevated	67	89.3%
Both Abs- very low titers	8	10.6 %

Cytomorphology Observed In Hashimoto’s Thyroiditis:

Fig 1: Photomicrograph of FNAC smear shows thyroid follicular epithelial cells, scattered lymphocytes and a plasma cell, in a background of thin colloid - Grade I thyroiditis. (H &E, 40 X).

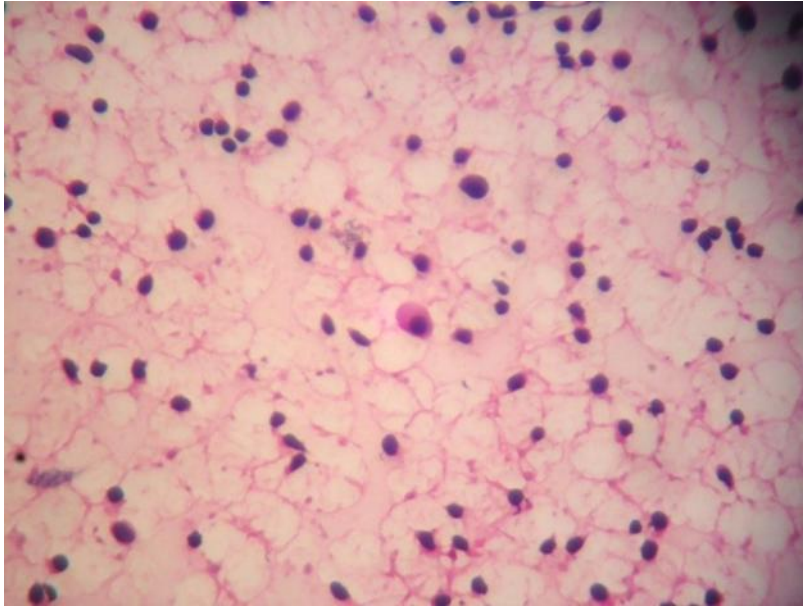


Fig 2: Photomicrograph of FNAC smear, shows a cluster of Hurthle cells with abundant eosinophilic granular cytoplasm and marked anisonucleosis.(H &E, 400 X) - grade II thyroiditis

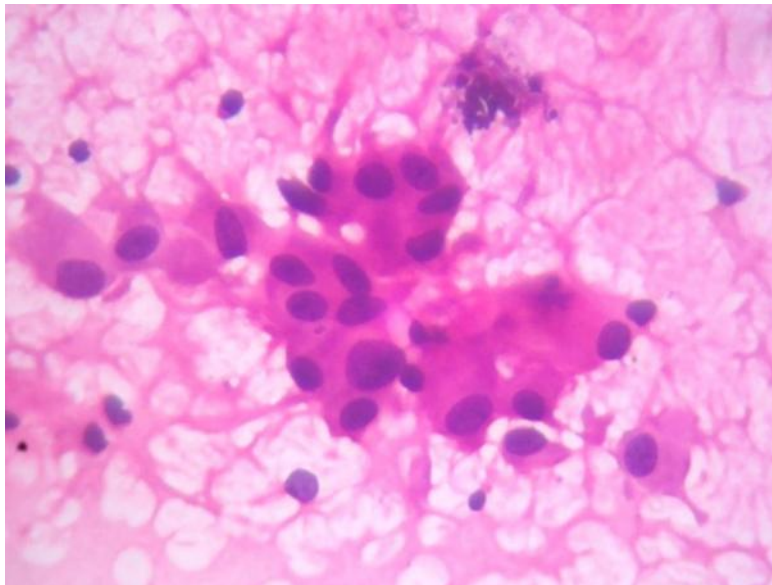
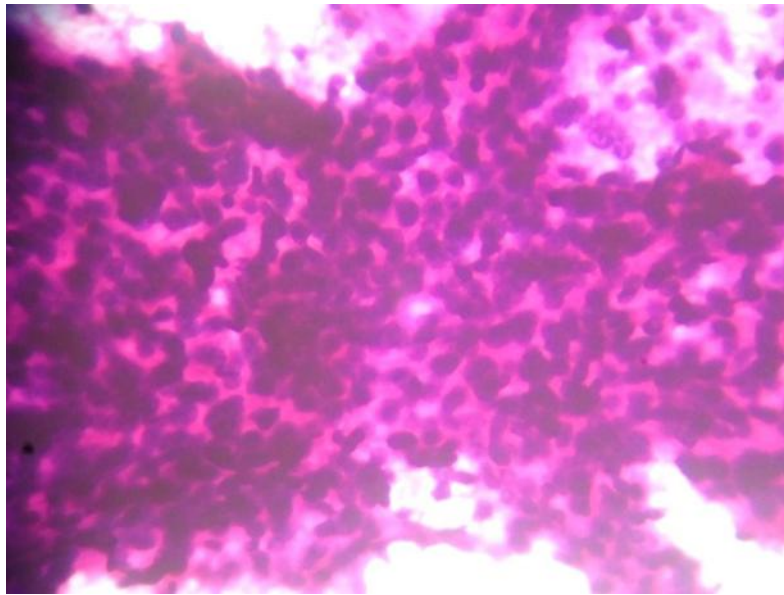


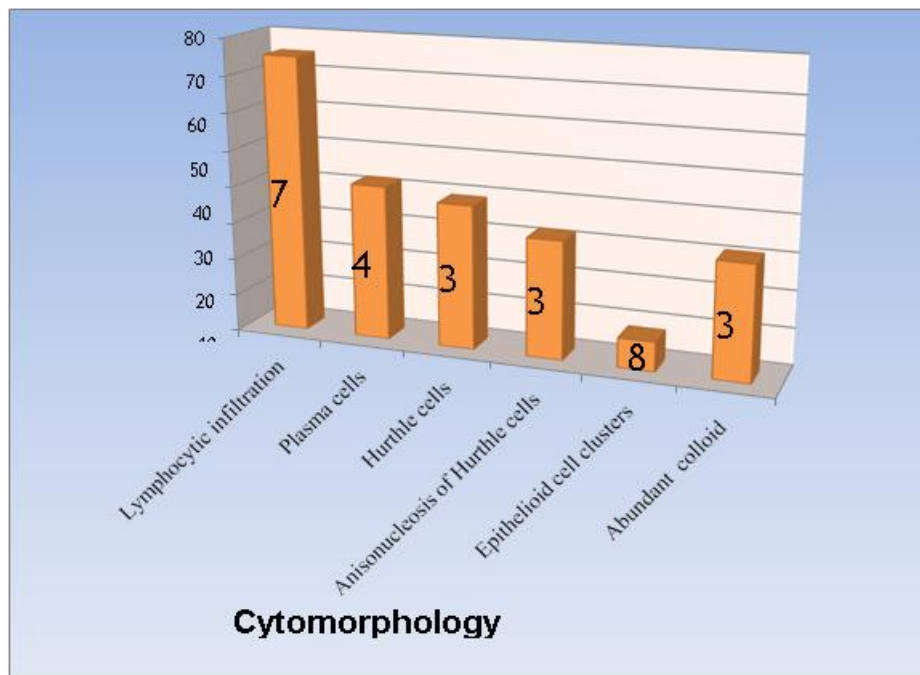
Fig 3: Photomicrograph of FNAC smear shows florid lymphocytic infiltration causing destruction of the thyroid follicular epithelial cells – Grade III thyroiditis, (H & E, 40 X).



Cytomorphological Findings In 75 Cases Of Hashimoto’s Thyroiditis :

The FNAC smears showed polymorphous population of lymphoid cells in the background and / or impinging on the thyroid follicular epithelial cells in all the 75 cases. Plasma cells were seen in 42 cases (56%). In 39 cases (52%), follicular epithelium showed Hurthle cell change with abundant granular eosinophilic cytoplasm and anisonucleosis. Moderate to marked anisonucleosis of Hurthle cells was seen in 32 cases (42.6%). Abundant colloid material was seen in the background of 31 cases (41.3%) . In 8 cases (10.6%), plump epithelioid – like cell clusters were seen.

Figure 4. Cytomorphological Findings In 75 Cases Of Hashimoto’s Thyroiditis



Cytological Grading Of Hashimoto’s Thyroiditis:

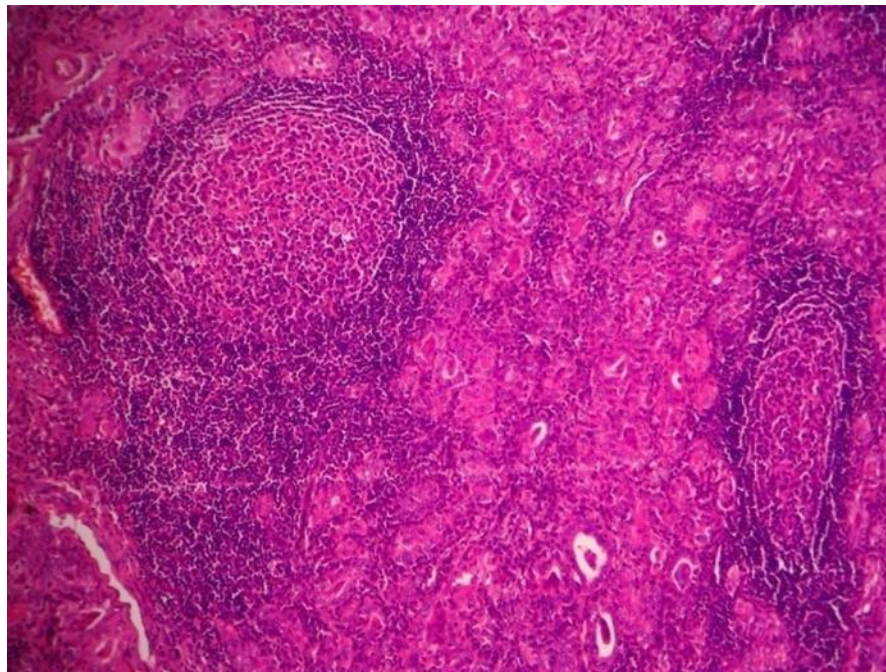
The criteria devised by Bhatia et al(21) was followed to subclassify Hashimoto’s thyroiditis based on cytomorphology. Out of the 75 cases, 21 cases showed few lymphoid cells infiltrating the follicles or increased number of lymphocytes in the background(grade I thyroiditis). 44 cases showed mild lymphocytic infiltration along with Hurthle cell changes and varying degrees of anisokaryosis (grade II thyroiditis). 10 cases showed a pattern of florid lymphocytic infiltration with very few follicular epithelial cells (grade III thyroiditis).

Cytological Grading Of Ht Cases

CYTOLOGICAL GRADE	NO. OF CASES	PERCENTAGE (%)
I (MILD)	21	28%
II (MODERATE)	44	58.67%
III (FLORID)	10	13.33%

Histopathology Of Cases Of Hashimoto’s Thyroiditis

Fig 5 : Photomicrograph of a section from thyroidectomy specimen with Hashimoto’s thyroiditis shows lymphoid follicles with germinal centre formation and Hurthle cell change in the follicular epithelium.(H & E, 40 X)



Comparison of Antithyroid Antibody Levels in Patients With Subclinical Hypothyroidism And Euthyroidism .

	FUNCTIONAL STATUS	n	MEAN	S.D	p value (2 tailed)
ATPO Ab	Subclinical	30	106.82	121.48	0.003
	Hypothyroidism				
	Euthyroidism	18	32.24	35.15	
ATg Ab	Subclinical	30	75.75	68.26	0.000
	Hypothyroidism				
	Euthyroidism	18	13.76	20.52	

Table.5.. The standard deviation of anti-thyroid peroxidase antibody & anti thyroglobulin antibody levels are statistically different in patients with subclinical hypothyroidism and euthyroidism & p values were 0.003, 0.000 respectively.(p value < 0.05 is considered as statistically significant)

Discussion

Hashimoto’s thyroiditis (HT) is an autoimmune condition characterized by destruction of thyroid follicles. It is essential to diagnose Hashimoto’s thyroiditis as patients subsequently become hypothyroid and require lifelong thyroxine supplementation. There is also an increased risk of extranodal marginal B cell lymphoma and thyroid carcinoma in patients with HT, which emphasizes the need for long term follow up. It is also important not to over-diagnose this entity as neoplasms, so that unnecessary surgery can be avoided.⁽²²⁾

On the basis of thyroid function tests, most of the patients in the present study were in a state of subclinical hypothyroidism (40%), followed by overt hypothyroidism (33.3%). 24% of the patients were

euthyroid and only 2.7% were hyperthyroid. This was somewhat similar to the observations made by Singh et al⁽¹⁹⁾ in which 58% were hypothyroid and 19% were subclinically hypothyroid, but majority (63.3%) were in a euthyroid state. On the other hand, Bhatia et al⁽²¹⁾ found 98.68% of their patients in a state of subclinical hypothyroidism.

Normal T3, T4 levels in the presence of elevated TSH indicates subclinical hypothyroidism (SCH). The incidence of SCH was higher in the present study compared to Western population . Bagchi et al⁽²³⁾, who reviewed prevalence of thyroid dysfunction in US population, found subclinical disease in 8 -17% of their subjects. Whereas, studies in the Indian population showed a higher incidence of SCH as in the present study.

Comparison Of Incidence Of Subclinical Hypothyroidism In Various Studies

Study	% of subclinical hypothyroidism
Bagchi et al ⁽¹³³⁾	8.17%
Bhatia et al ⁽²¹⁾	98.68%
Singh et al ⁽¹⁹⁾	19.3%
Kini et al ⁽²⁴⁾	42.3%
Present study	40%

Cytomorphologic Analysis Of Hashimoto's Thyroiditis:

The diagnostic accuracy of FNAC was high and multiple aspirations were very helpful in many cases. Hamburger et al⁽²⁵⁾ stressed on the usefulness of increased number of aspirations and found that as the number of aspirations increased, the rate of false negativity decreased. It has been pointed out that in equivocal cases, testing for antibody levels is very helpful, but if it is within normal limits, a repeat FNAC⁽²⁶⁾ becomes the preferred choice.

The FNA yield in case of HT depends on several factors. The most important is the technique of aspiration. The clinical presentation of thyroid enlargement is also said to determine the cell yield. Friedman et al,⁽¹⁵⁾ noted that FNA from a nodular form of HT provided higher yield of diagnostic material. It should be noted that HT and colloid goiter with follicular hyperplasia can co-exist and influence the cellularity of FNA smear.

Changes in the follicular epithelium :

Jayaram G et al⁽¹⁸⁾, from Maulana Azad medical college, studied the cytomorphology of HT in 51 cases and described that it was characterized by Hurthle cell changes and a significant lymphoid population consisting of mature and transformed lymphocytes, often impinging on follicular cells. 'Hurthle cell change' has been described by many authors in HT in a wide range from 48 to 98%. They have emphasized on moderate to marked anisokaryosis of Hurthle cells as a diagnostic feature of HT and this feature is very much helpful in distinguishing HT from Hurthle cell neoplasm, which paradoxically tends to show a lesser degree of nuclear variability.

Inflammatory changes:

In autoimmune thyroiditis, the inflammatory component is composed of mature and reactive lymphoid cells and plasma cells. They infiltrate into the thyroid follicular epithelial cell clusters.^(21,23,26)

The identification of plasma cells is very helpful in the diagnosis of HT in its early stages since lymphocytic infiltration of the follicular epithelium is very minimal or insignificant during this stage. Jayaram et al,⁽¹⁴⁾ have highlighted the importance of careful inspection of smears in early stages of HT,

where an occasional plasma cell especially at the periphery of the smear, would provide a clue to the diagnosis of HT. In the present study, plasma cells were seen in 56% of the cases.

Cytological Grading Of Hashimoto's Thyroiditis & Its Correlation With Serologic Parameters:

In the past, grading of thyroiditis was done on resected histological specimens based upon the number of foci of lymphocytes per standard representative section.⁽²⁷⁾ On the other hand, grading of thyroiditis was done on cytology smears only by few authors.

Kumar et al,⁽²⁶⁾ did semiquantitative grading of lymphocytic infiltration in autoimmune thyroiditis. They used a 2-tier grading system of (1) Minimal and (2) Moderate – heavy lymphocytic infiltration. In their study of 55 HT cases, 61.9% of cases showed minimal & 38.1% showed moderate to heavy lymphocytic infiltration. They found a statistically significant correlation existing between lymphocytic infiltration and hormonal status of the patient ($p=0.02$).

In 2007, Bhatia et al⁽²¹⁾ devised a simple, practical & easily applicable criteria for cytomorphological grading of chronic thyroiditis into 3 categories. Accordingly, in the present study, 28%, 58.67% and 13.33% of cases were grouped into grade 1, 2 & 3 respectively. Although these cases were initially diagnosed as HT, following the grading of thyroiditis, it was found that most of the cases of grade 1 (mild) thyroiditis actually represented chronic lymphocytic thyroiditis (CLT), while grade 3 (severe) thyroiditis fell into the category of florid lymphocytic thyroiditis. The intermediate grade 2 (moderate) thyroiditis represented typical Hashimoto's thyroiditis with Hurthle cells, plasma cells and epithelioid cell aggregates and granulomas.

Antibody Profile:

Various studies have shown positivity for anti-TPO Ab in the range of 62.5 to 93% and Anti-Tg Ab in the range of 37.5 to 83%.^(9,10,15,18,26,28,29,30) According to experience of Guarda & Baskin,⁽³¹⁾ not all cases of HT have elevated titres of thyroid antibodies. In their study only 67.9% of tested patients had positive antibodies in their serum. In a study of 150 cases of HT, by Singh N et al⁽¹⁹⁾ the overall antibody positivity was 88.6%. The present

study showed similar observations, in which overall thyroid antibody positivity was seen in 89.3% of cases. Anti-TPO Ab was positive in 89.3 % cases & Anti- Tg in 72% of the cases. 8 cases (10.67% of cases) showed very low titers for both antibodies.

Since most clinicians are in a habit of screening patients based only upon serological findings, and also by considering the expenditure spent on antithyroid antibody tests, this study throws light on FNAC procedure, as a simple, inexpensive and yet an effective tool in diagnosing HT.

When the antibody titers are very low in cases of HT, it causes considerable diagnostic dilemma. However, it is well documented that localized intra-thyroidal immune destruction occurs much earlier than serologic evidence of the disease. Therefore, in such cases, the diagnosis of HT can still be confirmed by FNAC. In the present study, a detailed analysis of cytomorphic features of HT, its serological parameters and its correlation has been carried out.

Conclusion

The present study highlights the importance of FNAC as a simple, effective, reliable and out-patient based investigation in the diagnosis of Hashimoto's thyroiditis, which may often be missed. Even though serology is an useful adjunct in the diagnosis of HT, in cases with negative antibody titers and/or biochemically euthyroid & subclinical hypothyroid cases, FNAC continues to be the 'gold standard' in the diagnosis of HT.

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