CYTOMORPHOLOGICAL AND SEROLOGICAL STUDIES IN HASHIMOTO'S THYROIDITIS

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ABSTRACT

BACKGROUND

Hashimoto's Thyroiditis (HT), a synonym of chronic lymphocytic thyroiditis or autoimmune thyroiditis is characterised by Hurthle cell change and infiltration of mature lymphocytes into the follicles. The autoimmune process is believed to begin with the activation of CD4+ T Cells, which initiate the recruitment of autoreactive B-cell that secrete variety of thyroid antibodies. The important of them are anti-thyroglobulin antibody, thyroid peroxidase antibody/anti-microsomal antibody and thyroid stimulating hormone stimulation blocking antibodies. It is the most common form of thyroiditis diagnosed on Fine Needle Aspiration (FNA). Different autoantibodies against thyroglobulin and thyroid peroxidase antigen are clinically most important for diagnosis, which are present in less than 60% and over 90% respectively in patients with Hashimoto's thyroiditis.

MATERIALS AND METHODS

In view of all the above facts, a combined cytomorphologic and serologic approach in the evaluation of Hashimoto's thyroiditis has been emphasized in our study. The prospective study was conducted in the Department of Pathology, M.K.C.G. Medical College, Berhampur from October 2012 to September 2015.

RESULTS

Total number of 50 cases of HT were evaluated for cytological features and serological markers. Occurrence of HT was found in maximum 20 to 30 (50%) years of age group. Females are more affected than males with male and female ratio of 1 : 11.5; 28 cases (56%) were hypothyroid, 19 cases (38%) were euthyroid and 3 cases (6%) hyperthyroid. Lymphocytic background was seen in 100% cases, whereas Hurthle cell change and high L : E ratio was observed in 76% of cases. Overall antibody positivity was found in 94% of cases, out of which 60% were AMA positive, both AMA and ATG was positive in 30% cases, whereas both antibodies negative was found to be in 6% cases.

CONCLUSION

We conclude that HT is common on 2nd and 3rd decade of life. Serology is a useful adjunct in diagnosis of HT. Serologically, TPO was found to be a better marker than ATG. In euthyroid patients with negative antibody titre, FNAC is the gold standard to establish diagnosis.

KEYWORDS

Hashimoto's Thyroiditis, FNA, Lymphocytic Epithelial Ratio, Antimicrosomal Antibody, Thyroid Peroxidase Antibody, Antithyroglobulin Antibody.

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BACKGROUND

Hashimoto's Thyroiditis (HT) is part of spectrum of Autoimmune Thyroid Diseases (AITDs) and is characterised by various cell and antibody-mediated immune processes.[1] It was first reported by Hakaru Hashimoto, a Japanese Surgeon working in Berlin, Germany in 1912, described as Struma Lymphomatosa and hence bear the name Hashimoto's thyroiditis.[2] Though, worldwide, the most common cause of hypothyroidism is iodine deficiency. HT remains the commonest cause of spontaneous hypothyroidism in areas of adequate iodine intake. The annual incidence of HT worldwide is estimated to be 0.3 - 1.5 cases per 1000 persons.[3,4] The incidence of HT is estimated to be 10 - 15 times higher in females.[1] The most commonly affected age range in HT is 30 - 50 years with peak incidence in men occurring 10 - 15 years later. The overall incidence of hypothyroidism increases with age in men and women.[1] The initiating process of HT is not well understood.[5,6,7,8] The autoimmune process is believed to begin with the activation of CD4+ T cells, which initiates the recruitment of autoreactive B-cells that secrete variety of thyroid antibodies like TMA and ATG.[9,10]

Diagnosis is based on clinical, cytomorphological and serologic parameters. FNA is an established method of

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sampling thyroid tissue for diagnosing a variety of lesion affecting the organ with a diagnostic accuracy of 92%; however, diagnosis is likely to be missed when cytology smears show evidence of hyperplasia as in Grave's disease or abundant colloid.[11] Thyroid peroxidase and thyroglobulin antigens are clinically most important for diagnosis of HT, which are found in > 90% and < 60% respectively.[12]

It is important to diagnose HT because patients subsequently become hypothyroid and require thyroxine supplementation. Thyroid carcinoma and malignant lymphoma can occur in HT, which emphasizes the need for long-term followup.[13]

This study was carried out to review the cytomorphologic spectrum of HT and correlate with clinical findings including thyroid function and antibody profile.

MATERIALS AND METHODS

The study is a prospective study conducted in the Department of Pathology, M.K.C.G. Medical College, Berhampur over a period of extending from October 2012 to September 2015. The cases referred for FNAC of palpable thyroid swelling to the cytology section of Department of Pathology were taken as the study group. The patients of either sex irrespective of age group ranging from 10 to 70 years were subjected to FNAC. After diagnosis of HT on the basis of FNAC, the patients were subjected for evaluation of thyroid function tests as well as anti-thyroid antibodies estimations.

FNAC was done by a 23-G needle with suction being provided by a 10 mL syringe. The characteristic of the aspirate was noted. The smears were stained with Diff-Quik, May-Grunwald-Giemsa and Papanicolau's strains. Diagnosis of HTs were made on the conventional cytological feature and graded according to criteria devised by Bhatia et al[14] into mild (Grade - 1), Moderate (Grade - 2) and Severe (Grade - 3).

Grade - 1 : Few lymphocytes infiltrated into follicles.
Grade - 2 : Moderate lymphocytes infiltrated into follicles with Hurthle Cell changes.
Grade - 3 : Florid lymphocytic infiltration with germinal centre.

Following cytological interpretation, 5 mL of blood was collected for evaluating Thyroid Function Tests (TFTs) and anti-thyroid antibody profile. The total triiodothyronine (T3) and Thyroxine (T4) levels were measured by the competitive Chemiluminescent Immunoassay (CLIA) method.

The thyroid stimulating hormone (Thyrotropin, TSH) level were determined by ultrasensitive sandwich Chemiluminescent Immunoassay. The anti-thyroid Peroxidase (TPO)/Anti-microsomal Antibody (AMA) were measured by Enzyme-Linked Immunosorbent Assay (ELISA) method.

Finally, the cytomorphology and serologic parameters (thyroid hormone status and anti-thyroid antibodies) were correlated using X^2 ($P < 0.05$) was considered statistically significant.

RESULTS

A total number of 645 cases with thyroid enlargement were subjected for FNAC during period of 3 years. The cytological diagnosis of HT was suggested in 154 cases. Among these diagnosed cases of HT, 50 cases were turned up for both thyroid function tests and antibodies estimation constituted the study population. Maximum number 25 (50%) cases were within 21 - 30 years of age group; 46 (92%) were females and only 4 (8%) cases were males. Out of 50 cases studied 28 (56%) patients had hypothyroidism, 3 (6%) patients had hyperthyroidism and 19 (38%) were euthyroid.

Both TPO and APG were positive in 15 (30%) cases, TPO positive in 30 (60%) and ATG positive in 2 (4%) cases. Both antibodies were negative in 3 (6%) cases.

Cytologic Features	No. of Cases	Percentage
Lymphocytic infiltration	50	100
Colloid	22	44
Lymphocytic background	50	100
Hurthle Cells	38	76
Reactive lymphoid cells	31	62
Fire flare	3	6
Macrophages	21	42
Giant cells	21	42
Epithelioid like cells	20	40
High L:E Ratio	38	76
Plasma cells	22	44

Table 1. Cytomorphologic Features of Hashimoto's Thyroiditis (n = 50)

Table - 1 highlights various cytomorphological features of HT. Lymphoid background and lymphocytic infiltration was observed in all 50 (100%) cases. Hurthle cell change and high lymphoid and epithelial cell ratio (L:E) was observed in 38 (76%) cases, reactive lymphoid cells were seen in 31 (62%) cases, plasma cell in 22 (44%) cases and colloid in 22 (44%) cases, whereas macrophages and giant cells were seen in 21 (42%) cases.

Cytologic Features	No. of Cases	Euthyroid	Hypothyroid	Hyperthyroid
Hurthle cells	38	8 (21.05%)	27 (71.05%)	3 (7.89%)
Reactive lymphoid cells	31	7 (22.58%)	23 (74.19)	1 (3.22%)
Plasma cells	22	8 (36%)	14 (64%)	0
Fire flares	3	0	1 (33.33%)	2 (66.66%)
Macrophages	22	9 (42.85%)	12 (57.14%)	0
High L:E ratio	38	9 (23.68%)	28 (73.68%)	1 (2.63%)

Table 2. Correlation of Cytologic Features of Hashimoto's Thyroiditis with Thyroid Functional Status (n = 50)

Table 2 shows the correlation of thyroid function tests with different cytologic features of HT. Out of 50 cases studied, 38 patients showing Hurthle cell change were hypothyroid state in 27 (71.05%) cases, in 8 (21.05%) cases euthyroid and only 7.89% cases were hyperthyroid. Out of 31 patients having reactive lymphoid cells 35 (74.19%) were in hypothyroid state, 7 (22.58%) were euthyroid and 1 (3.22%) hypothyroid. In 22 patients having plasma cells, 14 (64%) cases were hypothyroid and euthyroid in 8 (36%) cases. Out of 3 patients with fire flare change, 2 (66.66%) were in hyperthyroid state. 38 patients having high L:E ratio were mostly 28 (73.68%) of hypothyroid state and only 9 (23.68%) cases were euthyroid state. No statistical significant association was found between high L:E ratio and hypothyroid state (p = 0.24). No significant association was found between cytological features and hormonal status (p = 0.24).

Cytologic Features	No. of Cases	AMA		ATG	
		+ ve	- ve	+ ve	- ve
Hurthle cells	38	28 (73.68%)	10 (26.31%)	15 (39.47%)	23 (60.52%)
Lymphocytic infiltration	50	41 (82%)	9 (18%)	30 (60%)	20 (40%)
Plasma cells	22	4 (18.18%)	18 (81.81%)	2 (9.09%)	20 (90.90%)
High L:E ratio	38	35 (92.10%)	3 (7.89%)	22 (57.89%)	16 (42.10%)

Table 3. Correlation of Cytomorphologic Features of Hashimoto's Thyroiditis with Antibodies (n = 50)

Table 3 shows correlation of varied cytologic features of HT with their antibody titres. Out of 50 cases of study group, 38 cases showing Hurthle cell change with AMA positivity in 28 (73.68%) cases, whereas only 15 (39.47%) cases had ATG positivity. Almost all cases of lymphocytic infiltration showed AMA positivity in 41 (82%) cases and 30 (60%) cases showed ATG positivity. Plasma cells were associated with AMA positivity in 4 (18.18%) cases only. ATG positivity was seen in 2 (9.09%) cases. Patients having high L:E ratio showed 92.10% AMA positivity and only 57.89% cases had ATG positivity. Statistical significant correlation between high L:E ratio and AMA titres were observed (p = 0.003).

Grades of Lymphocytic Infiltration	Case (No.)	Hormonal Status			Antibody Status	
		Euthyroid	Hypo	Hyper	+ ve	- ve
Grade 1	8	3 (37.5%)	5 (62.5%)	0	7 (87.5%)	1 (12.5%)
Grade 2	29	5 (17.24%)	22 (75.86%)	2 (6.89%)	28 (96.55%)	1 (3.44%)
Grade 3	13	1 (7.69%)	11 (84.61%)	1 (7.69%)	12 (92.30%)	1 (7.69%)

Table 4. Correlation of Lymphocytic Infiltration with Hormonal and Antibody Status (n = 50)

Table 4 shows grading of lymphocytic infiltration and correlation with functional status and antibody titre. Out of 50 cases, 29 cases were under Grade 2 lymphocytic infiltration followed by Grade 3 in 13 cases and Grade 1 in only 8 cases; 8 cases with Grade 1 lymphocytic infiltration showed hypothyroidism in 5 (62.5%) cases and euthyroidism in 3 (37.5%) cases; 29 cases with Grade 2 lymphocytic infiltration showed hypothyroidism in 22 (75.86%) cases and euthyroid states in 5 (17.24%) cases. Out of 13 cases with Grade 3 lymphocytic infiltration, 11 (84.61%) were hypothyroid and 1 (7.69%) case euthyroid. In Grade 2 thyroiditis, overall antibody positivity was observed in 28 (96.5%) cases and negative in 1 (3.44%) case. In Grade 3 thyroiditis, 12 (92.30%) cases showed overall antibody positivity. Association between antibody titres with Grade 2 and Grade 3 lymphocytic infiltration was statistically significant (p - 0.003), whereas no statistical significant association was found between hormonal status and lymphocytic infiltration (p = 0.25).

DISCUSSION

HT is the most common form of thyroiditis observed clinically.[15,16] The disease, which was first described in 1912 by Hakaru Hashimoto, is a leading cause of goitre and hypothyroidism in areas in the world where iodine levels are sufficient.[17,18] In iodine deficient areas, it ranks next to endemic goitre as an important cause of hypothyroidism.

In this study, 50 cases diagnosed as HT on FNA were subjected to serological evaluation to provide an account of the correlation between the cytomorphology of the HT with the thyroid function tests and thyroid antibody profile.

HT can occur at any age. In our study, the age group range from 7 - 65 years with a mean of 20.78. Significantly, 50% cases documented were in a population somewhat younger than 21 to 30 years of age at par with Kumar et al (2002)[19] and N. Singh et al (2009).[20] The disease exhibits an obvious female predilection with a female : male ratio of 11.5 : 1. It correlates with the study of the above-mentioned authors.

On hormonal assay 56% of the patients were found to be hypothyroid and 38% cases were euthyroid, while hyperthyroid patients account for only 06% of cases. Our findings were similar to and corroborate with N. Singh et al (2009) and Kumar et al (2002). However, Kusum Kapila et al (1995) reported hypothyroidism in 10.10%, euthyroid in 79.30% and 6.90% were hyperthyroid patients.

Various authors have described Hurthle Cell change in wide range, 48 - 98% of HT cases and also highlighted moderate-to-marked anisonucleosis of Hurthle cells as a diagnostic feature of HT.[16,21,22,23] In the present study we encountered Hurthle cell change in 76% of cases, which corroborates with findings of N. Singh et al.[20]

In the present study, reactive lymphoid cells (62%) and macrophage (42%) were also found. Out of 50 cases, 29 (58%) cases were grouped under Grade 2 lymphocytic thyroiditis, which corroborates with the findings of Poropatich et al (1994).[24] Plasma cells found in 44% of cases in this study did not find significant correlation between the levels of antibody titre and plasma cells, which is similar to findings of N. Singh et al (2009).[20]

L:E ratio is high in HT, ranging from 2:1 to 10:1. In the present study, we found L:E ratio in 76% of cases with Grade 2 or 3 thyroiditis. Our findings were in concordant with Friedman et al (1981).[15] Kini et al (1981)[25] Jayram et al (2007) and N. Singh et al (2000).[20] TPO positive showed a strong statistically significant correlation with high L:E ratio (P = 0.003), seen in 92% of the cases similar to N. Singh et al (2009).

Absent or scanty colloid is a useful feature of HT,[26,27,28] as it is associated with destruction of follicle in long run. In the present study, 44% of the cases had scanty-to-moderate amount of colloid. These findings were similar to that of Kumar et al (2002).[20] But other authors reported N. Singh et al (2009), 59.3% of their cases. Hence, colloid should not be taken as a factor against HT, rather one should carefully search for cytologic features of HT in such cases.

The present study showed an overall thyroid antibody positivity of 94%; the TPO positivity correlates strongly with cytologic diagnosis of HT (P = 0.002). Of the TPO positive cases in our study, 96.55% had the characteristic features of HT on smears. Most studies indicate the higher specificity of TPO/AMA in diagnosing HT, which is a more reliable marker.

However, according to Guarda and Baskin encountered 67.9% of higher antibody titre and found that morphologically the antibody positive cases are indistinguishable from seronegative cases. However, it is well documented that localised intrathyroidal immune destruction occurs much earlier than serologic evidence of disease. So antibody titre might change in time, but cytomorphologic features persists during course of HT.

Therefore, to conclude, the present study highlights the importance of FNAC as a simple and reliable modality in the diagnosis of HT, which often may be missed on more serologic examination. Anti-TPO antibody is a reliable adjunct to diagnosis.

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