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Cytomorphological study of chronic lymphocytic thyroiditis: A correlation between cytological grade with lymphoid: Epithelial ratio, thyroid status and anti-thyroid antibodies

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Abstract
Chronic lymphocytic thyroiditis /Hashimoto's thyroiditis (HT) is an autoimmune disorder whose diagnosis is based on cytology and raised levels of anti-thyroid antibodies. The aim of the present study was to analyse its varied cytological spectrum along with grading and correlation with lymphoid: epithelial (L:E) ratio and serological parameters. Hundred patients diagnosed as HT on cytology were included in the study. Triiodothyronine (T3), thyroxine (T4), thyroid stimulating hormone(TSH) and antithyroid antibody levels were estimated. There was a female preponderance comprising 92% cases with majority cases presenting in the third decade. There were 63% cases with grade 2, 24% with grade 1 and 13% with grade 3 thyroiditis. Antithyroperoxidase levels were raised in 87% cases and anti-thyroglobulin antibodies were positive in 78% cases. A statistically significant correlation was found between grading and L:E ratio but not with serological parameters. The present study highlights the cytological spectrum seen in HT which is useful for its diagnosis for prompt patient management.

Keywords: anti-thyroid anibodies, cytology, grading, hashimoto's thyroiditis, lymphoid: Epithelial ratio

Introduction
Chronic lymphocytic thyroiditis or Hashimoto's thyroiditis (HT) is the most common form of thyroiditis observed clinically [1, 2]. It has a prevalence rate of 1-4% and an incidence of 30- 60/100000 population per year [3]. It usually presents in middle aged females with a diffuse thyroid swelling. Depending on the stage of presentation patients may be hyperthyroid, euthyroid or hypothytroid. Initially patient may be asymptomatic. However in the later stages of the disease, patient presents with features of hypothyroidism. Diagnosis of HT is based on clinical, radiological, cytomorphological and serologic parameters [4]. Fine needle aspiration cytology (FNAC) is highly sensitive in diagnosing Hashimoto's thyroiditis, with a diagnostic accuracy rate of 92% and is hence considered as a gold standard in its diagnosis [5]. Cytological diagnosis is primarily based on presence of lymphocytes in the background, Hurthle cell change and intraepithelial infiltration by lymphocytes. Depending on the extent of lymphocytic infiltration and other cytomorphological features HT has been divided into three grades [6, 7]. The diagnosis of HT can be missed when it coexists with other conditions like goitre, papillary carcinoma or Hurthle cell neoplasm. Moreover not all cases may show presence of antithyroid antibodies. Hence the present study was undertaken to provide an account of the cytomorphological spectrum of HT along with its grade and correlate these features with L:E ratio, levels of T3,T4,TSH and antithyroid antibodies.

Materials and Methods
A prospective observational study was carried out in the department of pathology of HBT medical college and Dr. R. N Cooper Hospital for a period of 2 years between May 2015 to April 2017. An approval of ethics committee was obtained. Prior written informed consent was taken from all the patients. One hundred cases diagnosed as Chronic lymphocytic thyroiditis on FNAC were analysed. Patients already on treatment were excluded from the study. Detailed clinical presentation, ultrasonography findings, serum T3 ,T4 ,TSH levels

and values of anti-thyroid peroxidise and anti-thyroglobulin antibodies were noted in each case .Any additional lesion observed in association with HT was also recorded. FNAC was done with a standard technique using 10cc syringe and 23-24 gauge needle. Smears were stained with Hematoxylin-eosin stain ,Papanicolaou stain and the May-Grunwald –Giemsa stain. Different cytological features of HT were noted. Lymphoid: epithelial ratio was graded as low to high depending on the relative proportion of lymphoid and epithelial cells and a value of more than 1:1was considered high [8]. Grade 1 included cases showing a few lymphocytes infiltrated follicles with an increase in the background lymphocytes. The presence of a moderate lymphocytic infiltration of follicles or mild infiltration of follicles with hurthle cell change/giantcells/anisonucleosis was grade 2. Florid lymphocytic infiltration with germinal center formation with a very few thyroid follicular cells were features of grade 3 thyroiditis [6]. A correlation was done between the cytologic grade with L:E ratio, thyroid function test and anti thyroid antibodies. Statistical analysis was done using SPSS 16.0 software. The cytological grading and serologic parameters were correlated using the chi square test and a p value of ≤ 0.5 was considered as statistically significant.

Result

The age of the patients ranged between 11 and 75 years and maximum patients presented in the age group between 21-30 years(28%)followed by 31-40 years (23%).Ninety two patients were females(92%) and remaining eight cases (8%) were males. Clinically 87% of the cases presented with a diffuse enlargement of thyroid, while only 13% of cases presented as a nodular swelling. On ultrasound examination a diagnosis of HT was given in 67% cases. There were 10 cases of HT with coexisting goiter, 3 with follicular hyperplasia and one with papillary carcinoma of thyroid while 86% patients had no associated lesion. On thyroid profile 55% cases were euthyroid, 29% were hypothyroid and 16% were hyperthyroid. Anti-thyroperoxidase (TPO) antibody was positive in 87% cases , anti-thyroglobulin (ATG) titres were raised in 78% cases, while overall antibody positivity was seen in 90% cases. FNAC smears of all 100 cases were examined and showed moderate cellularity in 84% cases , low cellularity in 11% cases and were very cellular in only 5%cases. Table -1 shows all the cyto-morphological features observed along with their frequency.

Table 1: Cytomorphological features of lymphocytic thyroiditis

Findings	Percentage of cases
Background Lymphocyte	100
Lymphocytic infiltration of Thyroid follicles	100
Colloid	29
Hurthle cells change	68
Anisonucleosis	53
Granulomas	12
Giant cells	27
Germinal centre cells	51
Eosinophils	12
Plasma cells	2
Macrophages	37
Fire Flares	3

The lymphoid: epithelial ratio (L:E) was high in 62% cases and low in 38% cases. Out of the 62 cases with high L:E ratio 58 cases(93.5%) were antibody positive while in 38 cases with low L:E ratio only 32 cases(84.2%) had antithyroid antibodies.

On grading of thyroiditis the majority of cases were observed to be of grade 2 (63%), followed by grade 1 (24%) and grade 3 (13%).Correlation between the grades of thyroiditis was done with L:E ratio and antithyroid antibodies. Table- 2 shows the thyroid profile in different grades of thyroiditis and in cases with high L:E ratio

Table 2: shows correlation between cytological grades, L: E ratio and thyroid status.

Grades	Total cases	Hypothyroid	Hyperthyroid	Euthyroid
Grade I	24	8 (33.3%)	4 (16.7%)	12 (50%)
Grade II	63	15 (23.8%)	11 (17.4%)	37 (58.7%)
Grade III	13	6 (46.1%)	1 (7.6%)	6 (46.1%)
High L:E ratio	62	18(29%)	9(14.5%)	35(56.5%)

Table-3 shows the positivity of antithyroperoxidase and antithroglobulin antibodies and high L:E ratio in different grades of thyroiditis

Table 3: Antibody profile and L:E ratio in different grades of thyroiditis

Grades	Total	Both ATPO & ATG+	Only ATPO+	Only ATG +	Both ATPO & ATG negative	Antibodies positive	High L:E ratio
1	24	14(58.4%)	4(16.6%)	1(4.2%)	5(20.8%)	19 (79.19%)	0(0%)
2	63	51(80.9%)	6(9.6%)	1(1.6%)	5(7.9%)	58 (92%)	49(77.8%)
3	13	10(76.9%)	2(15.3%)	1(7.8%)	0(0%)	100%	13(100%)
Total	100	75	12	03	10		62(62%)

ATP- Antithyroperoxidase antibodies. ATG- Antithyroglobulin antibodies
L:E ratio- Lymphoid:Epithelial cell ratio

On statistical analysis the cytological grades were correlated with L:E ratio,thyroid status and antithyroid antibodies (antithyroperoxidase and antithyroglobulin). There was a strong statistically significant correlation between L:E ratio and grade with a p value of <0.00005. No statistically significant correlation was found between cytological grades and thyroid function test (p=0.675), and grading and antithyroid antibodies (p=0.085).No statistically significant correlation was found between L:E ratio and thyroid status (p=0.870), and L:E ratio and antithyroid antibodies (p=0.207).

Discussion

Hashimoto's thyroiditis is the most prevalent autoimmune thyroid disorder, where lymphocytic infiltration of the thyroid gland is often followed by gradual destruction and fibrous replacement of the thyroid parenchymal tissue. Table-4 compares the age, gender, clinical presentation, grading, thyroid status and presence of antibodies in the present study as compared with other studies.

Table 4: Comparison of the clinical presentation, grading, thyroid status and presence of antibodies with other studies.

Study	Number of patients	Commonest age group	M:F ratio	Clinical presentation	Cytological Grading	Thyroid status	Anti TPO antibodies	ATG antibodies
Present Study	100	21-30 yrs	1:12	Diffuse-87% Nodular-13%	I-24% II-63% III-13%	Euthyroid-55% Hypothyroid-29% Hyperthyroid-16%	87%	78%
Sood *et al*. [9]	55	21-30 yrs	1:10	Diffuse-NA Nodular-NA	I-21.82% II-30.91% III-47.27%	Euthyroid-NA Hypothyroid-NA Hyperthyroid-NA	94.5%	NA
Chandanwale *et al*. [10]	52	21-30 yrs	1:16	Diffuse-98.08% Nodular-1.92%	I-38.46% II-46.15% III-15.38%	Euthyroid-15.38% Hypothyroid-61.53% Hyperthyroid-23.1%	61.5%	NA
Anila *et al*. [11]	60	31-40 yrs	1:11	Diffuse-77% Nodular-23%	I-45% II-36.67% III-18.33	Euthyroid-65% Hypothyroid-30% Hyperthyroid5%	95%	66.6%
Bhatia *et al*. [6]	76	31-40 yrs	1:11	Diffuse-97.37% Nodular-2.63%	I-38.67% II-44% III-17.3%	Euthyroid-25% Hypothyroid-73.68% Hyperthyroid-1.32%	65.7%	NA
Singh *et al*. [8]	150	21-30 yrs	1:14	Diffuse-95.3% Nodular-4.7%	I-29.3% II-48% III-22.7%	Euthyroid-37.3% Hypothyroid-57.3% Hyperthyroid-5.3%	79.3%	67.3%
Jayram G *et al*. [12]	40	NA	0:40	Diffuse-NA Nodular-NA	I-13.51% II-62.16% III-24.32%	Euthyroid-48.5% Hypothyroid-39.7% Hyperthyroid-11.7%	93%	83%
IHA *et al*. [13]	31	<40	1:30	Diffuse-87.09% Nodular-12.90%	I-9.7% II-67.7% III-22.6%	Euthyroid-16.12% Hypothyroid-54.80% Hyperthyroid-29%	83.8%	NA

M:F ratio- Male:Female ratio. Anti TPO- Antithyroperoxidase. ATG- Antithyroglobulin

The age of our patients ranged from 11 to 75 years with the majority of patients (28%) presenting between 21-30 years followed by 31- 40 years (23%).Similar results were seen in study by Chandanwal *et al*. [10] and Singh *et al*. [8] while Anila *et al*. [11] and Bhatia *et al*. [6] and observed the commonest age group to be the 3rd to 4th decade. Male female ratio in our study was 1:11.5 with marked female predominance which has also been reported in other studies. Diffuse enlargement was seen in 87% of our cases while nodular presentation was seen in 13% patients which is higher than many other studies. On ultrasonography 67% of our cases were diagnosed as Hashimoto's thyroiditis which is similar to other studies.[10] There were only 29% cases in our study with hypothyroidism which is lesser compared to other studies. This variation in thyroid profile can also be explained because in different studies patients presented at different stages of disease. In initial stages of HT there is transient hyperthyroidism followed by a euthyroid state. While in later stages of the disease, the patient presents with hypothyroidism. Also, different iodine deficient zones and local factors contribute to the variation in thyroid status of the patient. The younger age of our study population more proportion of nodular presentation and euthyroid status can be explained with the early presentation of the disease in our patients who belongs to urban population with adequate iodine intake due to fortified salts. A higher incidence of Hashimotos thyroiditis has been reported among younger girls in post iodinistion phase in India in study done by Marwaha RK *et al*. [14].

In our study, presence of 87% cases with ATPO positivity and 78% of the cases with ATG positivity is in concordance with the fact that TPO antibodies are considered to be more specific than TG antibodies. Jayaram *et al*. [12] and Marwaha RK *et al*. [14] also concluded that TPO antibodies are more specific than TG antibodies in HT. According to the experience of Guarda *et al*. [15] not all the cases of HT have elevated titres of thyroid antibodies. In their study only 67.9% of tested patients had positive antibodies in their serum. They reported that antibody positive and negative cases are morphologically indistinguishable. Negative serology in cases of HT causes a considerable diagnostic dilemma, however it is now well documented that localized intrathyroidal immune destruction occurs much earlier than serologic evidence of the disease. Hence, the antibody titres might change with time but cytomorphologic features persist during the course of HT [15].

Table-5 compares the cytomorphological features found in the present study as compared to other studies.

Table 5: Comparison of frequency of cytological features with other studies

Study	Hurthle cell change	Fire flares	Granuloma	Giant cells	Anisonucleosis	Colloid	Eosinophils	Plasma cells	Macrophages	Background lymphocytes	Lymphocytes infiltrating follicles
Present study	68%	3%	12%	27%	53%	29%	12%	2%	37%	100%	100%
Chandanwale *et al.* [10]	69.2%	0%	2%	10%	17.3%	5.76%	1.9%	3.8%	3.8%	100%	76.9%
S Chandanwale *et al.* [16]	64%	3%	6%	18%	19%	8%	1%	5%	12%	100%	79%
Bhatia *et al.* [6]	44%	NA	13%	33%	26.3%	NA	NA	NA	NA	98.6%	98.6%
Singh *et al.* [8]	70%	6.7%	56%	38%	47%	59.3%	NA	55.3%	58%	100%	NA
Jayram G *et al.* [17]	56%	24%	45%	45%	NA	NA	17%	23%	39%	NA	77.5%
IHA *et al.* [13]	83.8%	38.07%	16%	38.07%	NA	NA	NA	29%	NA	100%	100%

The presence of lymphocytes and follicular infiltration by lymphocytes was a consistent feature in all studies. Hurthle cell change was observed in 68% of our cases. Singh *et al.* [8] encountered Hurthle cells in 70% of the cases but they did not find a statistically significant correlation between TFTs (Thyroid Function Tests) and Hurthle cell population (p=0.26).

Colloid was found in 29% of the cases in our study. Absent or scanty colloid is a usual feature of HT as it is associated with the destruction of follicles in the long run. However various authors have emphasised the presence of colloid in HT on FNAC [8, 15]. They highlighted the fact that this combination of nodular goiter and HT has not been given much attention in the literature although such combinations are commonly found in resected specimens of the thyroid. As coexistence of HT and colloid goiter is not unusual particularly in the post iodization era, the presence of colloid in the smears will not necessarily exclude HT.

Anisonucleosis was observed in 53% of the cases which is useful in distinguishing HT from a hurthle cell neoplasm (HCN), which paradoxically tends to exhibit a lesser degree of nuclear variability. Neutrophils and eosinophils may be seen adhering to or infiltrating follicular cells in early stages. Occurrence of multinucleate giant cells has been frequently described in HT. Various studies have reported them in 27.3% to 58% of cases [11 ,15, 16, 17]. Epithelioid cells in the form of granulomatous aggregates are documented in 10% of HT cases [17, 18].

L:E ratio is high in HT[11] ranging from 2:1 to 10:1with smears resembling a reactive lymph node. In the present study a high L:E ratio was seen in 62% cases and all these patients had grade 3 or grade 4 thyroiditis which is similar to findings reported in other studies [8]. Though 94% patients with high L:E ratio had APO/ATG antibodies we did not find statistically significant correlation between L:E ratio with thyroid profile or antibody positivity. In present study, majority of the cases of all the three grades were euthyroid and there was no significant correlation between grading and thyroid status of the patient. (p=0.675) which can be due to early presentation. Maximum cases in our study were of grade II which is similar to other studies. No significant correlation was found between grading and antibody positivity on serological findings (p=0.085). Similar results were reported by Bhatia *et al.* [6] and Singh *et al.* [8] while Kumar *et al.* [18] found a significant correlation. Studies have reported various coexisting lesions with HT on FNAC which include goiter, follicular neoplasms, Hurthle cell neoplasms papillary carcinoma and lymphoma. In our study, 10% cases had goiter, 3% cases had follicular hyperplasia and there was one case of papillary carcinoma.

Conclusion

This study highlights the importance of fine needle aspiration cytology as a simple and reliable toolfor diagnosis of Hashimoto's thyroiditis which often may be missed on serology alone. A knowledge of the varied cytomorphological spectrum is necessary for its diagnosis especially in cases where it is has coexisting conditions. Though serology is useful adjunct in the diagnosis of Hashimoto's thyroiditis in biochemically euthyroid patients and in antibody negative patients, fine needle aspiration cytology remains the "gold standard" for diagnosing Hashimoto's thyroiditis. Though cytological grading was found to have a significant correlation with L:E ratio it does not correlate with the the thyroid function tests and antithyroid antibodies.

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