



## Lympho-epithelial ratio in Hashimoto's Thyroiditis; correlation with Thyroid Antibody and Thyroid Hormone Profile – A Prospective study

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### ABSTRACT

**Background and objectives:** Hashimoto's thyroiditis (HT) is a chronic autoimmune thyroid disease in which genetic and environmental factors play a role in the etiology. In addition, it remains the leading cause of hypothyroidism throughout the world. This study records the lympho-epithelial (LER) ratio in Hashimoto's Thyroiditis and its correlation with the thyroid hormones and antibody.

**Materials and methods:** 100 patients with cytological diagnosis of Hashimoto's thyroiditis (HT) in a period of 1 year were enrolled in the study. We recorded the results of the free triiodothyronine (T3), free thyroxine (T4), Thyroid stimulating hormone (TSH) and anti-thyroid peroxidase (anti-TPO) and also calculated the lympho-epithelial (LER) ratio of each case.

**Results:** Lympho-epithelial ratio showed a statistically significant positive correlation with the thyroid antibody. Furthermore, a negative but statistically significant correlation was determined between the lympho-epithelial ratio and thyroid hormones (T3 and T4).

**Conclusion:** LER can be a valuable and practical parameter for monitoring HT.

**KEYWORDS :** Hashimoto's thyroiditis, cytology, lympho-epithelial ratio, thyroid hormones, anti-thyroid antibodies.

**Introduction:** Hashimoto's thyroiditis (HT) is the most common autoimmune thyroid disease and the most common cause of hypothyroidism. Although autoimmunity plays a role in its pathogenesis, genetic predisposition and environmental factors also are involved. Hashimoto's thyroiditis is characterized by the infiltration of the thyroid gland with autoreactive T and B cells which causes thyroid cell death and the production of antithyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-TG) antibodies. As a result, thyroid-specific T cells also become activated and infiltrate the thyroid. These T cells then induce thyroid cell death, resulting in the gradual destruction of the thyroid gland, hypothyroidism, and goiter. Early studies showed that T cells from HT patients recognize TPO as a specific target molecule. The disease clinically manifests along with hypothyroidism, which leads to the need for thyroid hormone supplementation. Hence, most patients with HT also develop goiter.

This is the first study which analyses the relationship between LER, Thyroid hormones and thyroid antibody in patients with HT. This prospective study was carried out at a tertiary care hospital on 100 patients in a period of one year. We did a cytological analysis on the patients to analyse the lympho-epithelial ratio. Further, we correlated it with the Thyroid antibodies [antithyroid peroxidase (anti-TPO)] and thyroid hormone profile to understand the relationship between these parameters.

**Materials and methods:** This study was conducted in the department of Pathology, KVG Medical College, Sullia, Karnataka, India, on 100 patients with HT. This was a cross-sectional study conducted on patients with cytologic diagnosis of Hashimoto's thyroiditis. Prospective study of patients was done from February 2017 to February 2018. Patients with cytology proven HT with or without associated pathology were included in the study. Patients with cytology of multinodular or colloid goiter were excluded.

Information on clinical symptoms and signs of hypothyroidism and hyperthyroidism was recorded. Investigations noted were thyroid function tests mainly T3 (triiodothyronine) (normal values 0.6-2.02 IU/ml), T4 (thyroxine) (normal values 5.13-14.06 IU/ml), TSH (thyroid stimulating hormone) (normal values 0.27-5.5 IU/ml), FreeT4 (normal values 0.93-1.71 IU/ml). Antithyroid antibody levels mainly anti thyroid peroxidase (anti TPO) (normal values <35 IU/ml) levels were documented.

Cytological study was done in selected 100 cases from fine needle aspiration (FNA) smears. The main parameter studied was lympho-

epithelial ratio.

Statistical analysis was done using SPSS 17.0 (Chicago: SPSS Inc). chi-square test and ANOVA were used and  $p < 0.05$  was considered as significant. Biochemical values of thyroid function, cytological parameters and antithyroid antibody levels were correlated with one another using the Karl Pearson's correlation coefficient.

**Results:** A total of 100 patients with cytology proven HT who were evaluated between February 2017 to February 2018 were included in the study. Of these, 91 patients were female and 9 males, which indicated a significantly higher percentage of females having HT as compared to males. Based on the hormonal profile, there were 67 cases of hypothyroidism, 14 cases of hyperthyroidism and 19 of euthyroidism (Table 1).

Cytology smears were studied. The lympho-epithelial ratio (LER) was observed to be between 2:1 to 10:1 depending upon the stage of the disease (Fig 1). In the florid stage of the disease, the smears were dominated by the presence of lymphoid cells seen in the background of the smear (Fig 2). These are predominantly lymphocytes admixed with a few immunoblasts and plasma cells.

The smear resembled an FNA smear from a reactive lymph node with many microscopic fields in which follicular epithelium is completely absent. We also observed thyroid follicular cells in clusters showing degenerative changes and infiltration by lymphoid cells. In few cases Hurthle cell change was noted which was either focal or diffuse, often showing atypia or infiltration by lymphocytes. The infiltration of follicular and hurthle cells by lymphoid cells showed the characteristic 'ants at a picnic' appearance (Fig 3).

We recorded the patients thyroid hormone profile and anti-TPO antibody levels and correlated them with the Lymph-epithelial ratio (Table 2). We found that the patients who had a high LER (5:1 to 10:1) (n=74), also showed an increase in the anti-TPO levels (n=71) and TSH (n=67) with a decrease in the T3 and T4 hormone levels (n=67). Similarly patients with low LER (2:1 to 4:1) (n=14) showed a decrease in the antibody levels (n=9) and TSH (n=14) and an increase in T3 and T4 levels (n=14) respectively.

So we established that there is a statistically significant positive relationship of LER with anti-TPO and TSH levels and a negative but statistically significant relationship with the T3 and T4 hormone levels.

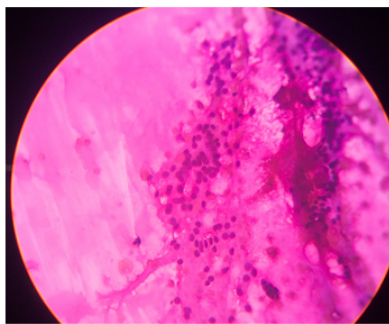
**Table 1 : Statistical analysis**

LE ratio		5:1 to 10:1 (high)	2:1 and 4:1 (low)
T3	Normal ( 19 )	Increased ( 14 )	Decreased ( 67 )
T4	Normal ( 19 )	Increased ( 14 )	Decreased ( 67 )
TSH	Normal (14 )	Increased ( 67 )	Decreased ( 14 )
Anti-TPO	Normal	Positive ( 71 )	Negative ( 29 )

**Table 2: Comparison of LER with Thyroid hormones and anti-thyroid antibody**

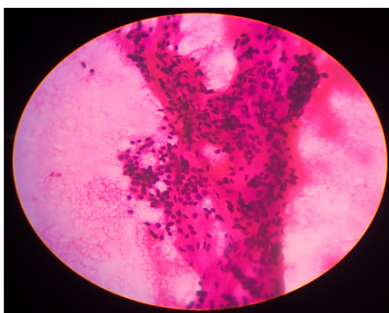
		T3	T4	TSH	Anti-TPO
	Pearson's Correlation (r)	-0.031	-0.031	0.054	0.065
LER	P	0.380	0.380	0.570	0.640

**Figure 1:** The lympho-epithelial ratio (LER) was observed to be between 2:1 to 10:1 depending upon the stage of the disease.



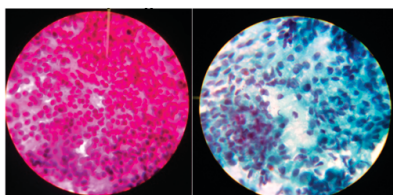
Haematoxylin and eosin stain

**Figure 2:** Florid stage of the disease, the smears were dominated by the presence of lymphoid cells



Haematoxylin and eosin stain

**Figure 3:** The infiltration of follicular and hurthle cells by lymphoid cells showed the characteristic 'ants at a picnic' appearance



Haematoxylin and eosin stain

Papanicolaou stain

**Discussion:**

The first report of chronic thyroiditis (struma lymphomatosa) was described by Haku Hashimoto in 1912, and the disease now bears his name.

Patients suffering from this disease usually present with a diffuse enlargement of the thyroid gland or, less frequently, with one or two prominent nodules. Recently, the incidence of HT has been increasing,

and this has been linked to excess iodine intake, particularly in coastal areas.

Thyroid diseases are always reported to be higher in the female population. Our study also shows the same comprising of 91% females.

Most of our patients were hypothyroid (subclinical and overt), and of these only 38 per cent showed overt hypothyroidism. These results were similar to previous studies from India and abroad. In our study, 14 per cent patients had hyperthyroidism, not seen in other studies.

We found 71 per cent of our patients were anti TPO positive . There is a well-recognized discrepancy between antibody levels and cytology in Hashimoto's thyroiditis in children and young adults. This could be because that in early stage of disease, antibody production is confined to intrathyroidal lymphocytes. On the other hand, patients with significant titres may not have cytology proven Hashimoto's thyroiditis. This can be explained by the fact that focal HT which is an early lesion may be missed by cytology.

In fine needle aspiration cytology of thyroid, L:E ratio (lymphoid:epithelial ratio) in Hashimoto's thyroiditis ranges from 2:1 to 10:1. An increase in number of epithelial cells raises suspicion of epithelial proliferative process associated with HT like malignancy. Plasma cells were seen in many of our cases. Plasma cells are in diagnosing early Hashimoto's thyroiditis where lymphocytic infiltration of follicles is insignificant.

Our study showed a statistically significant positive correlation of LER with Thyroid antibodies ( anti-TPO) and a negative correlation with thyroid hormones (T3 & T4).

Our study had several limitations. The lack of complete data for all variables in all patients has reduced the sample size in the analysis of the various parameters. Antithyroid antibody levels were considered mainly for anti-TPO and not anti-TG, cost constraints being the main limiting factor.

In conclusion, lympho-epithelial ratio (LER) can be an indicator to know the progress of Hashimoto's thyroiditis. LER has a statistically significant positive correlation with anti-thyroid antibody (anti-TPO) and TSH. Furthermore, it also has a statistically significant negative correlation with the thyroid hormones ( T3 & T4). Since the lympho-epithelial ratio (LER) correlates well with the anti-thyroid antibody and thyroid hormonal profile, a good assessment of LER ratio in the FNAC is enough to prognosticate the disease.

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