

Original Research Article

Association of eosinophils in Hashimoto's thyroiditis on fine needle aspiration cytology

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Abstract: Hashimoto's thyroiditis (HT) is an autoimmune chronic inflammatory condition characterized by destruction of thyroid gland that may end up in a lifelong thyroxine supplementation. FNAC has a diagnostic accuracy of 90% in diagnosing HT, but has some inherent limitations. Therefore, we should search for some additional cytological findings which can improve the efficacy of FNAC in diagnosing HT. The aim was to study the association of eosinophils in Hashimoto's thyroiditis on fine needle aspiration cytology. This was a case-control study, where 50 diagnosed cases each of HT and colloid goiter were served as cases and controls respectively. FNAC smears of diagnosed HT and colloid goiter cases were reviewed, number of eosinophils and neutrophils per high power field were counted and ratio of eosinophils to neutrophils were calculated and compared using unpaired t-test. In our study we observed higher eosinophil to neutrophil ratio in smears diagnosed as HT than colloid goiter. On applying the unpaired-t test, the P-value was <0.0001 with a 95% confidence interval, which was extremely significant statistically. Thus, there was a higher association of thyroid tissue eosinophilia with HT.

Keywords: Eosinophils, Neutrophils, Hashimoto's thyroiditis, Colloid Goiter, fine-needle aspiration cytology

INTRODUCTION

Hashimoto's thyroiditis (HT) was described in 1912 by Dr. Hakuru Hashimoto and is the most common form of thyroiditis with a prevalence rate of 1-4% and incidence of 3-6/10000 population per year.[1-3] Hashimoto's thyroiditis is about 15-20 times more common in women than in men and frequently involves people between the ages of 30 and 50 years of age and may be associated with hypothyroidism, euthyroidism or occasionally hyperthyroidism.[3] It is important to diagnose HT because patients can become severely hypothyroid and may need lifelong thyroxine supplementation. Also, there is an increased risk of extra nodal marginal B-cell lymphoma in patients with HT and the frequency of carcinoma varies between 0.5 - 23.7%.

FNAC is considered as a superior, cost effective and highly sensitive tool in diagnosing HT, with a diagnostic accuracy of 92% and thus helps in preventing unnecessary surgeries. However there are certain pitfalls of FNAC in diagnosing HT. Diagnosis of HT is likely to be missed in smears showing cytological evidence of hyperplasia or abundant colloid, [4-6] overlap of cytological features of Grave's disease and

follicular cells exhibiting some features of papillary carcinoma, could be observed in a cytology smear of HT, leading to diagnostic pitfalls. [7]. we observed eosinophilic infiltrates in many of the FNAC smears diagnosed as HT. This rare finding inspired us to study the association between eosinophilic infiltrates in thyroid FNAC and Hashimoto's thyroiditis.

MATERIALS AND METHODS

The present study was a case-control study conducted in the department of Pathology, K.V.G. Medical College, Sullia, and Karnataka between December 2014 to December 2015. Smears obtained by FNAC of 50 cases diagnosed as HT served as cases while smears of 50 cases diagnosed as colloid goiter (CG) served as controls. Relevant clinical details like age, sex, thyroid status, Absolute eosinophil count (AEC) and antibody titres were noted.

Under aseptic precautions and following the consent of the patient, FNAC of thyroid was done with a 23G needle and a 10ml disposable plastic syringe. On an average, four slides per case were taken. Out of four, two slides were immediately fixed in 95% alcohol and stained with routine Haematoxylin and Eosin (H & E)

stain. The other two sides were air dried and stained with Leishman stain. The diagnosis of HT was made as per the diagnostic criteria given in the Orell & Sterrett's Fine Needle Aspiration Cytology, 5th edition [8], i.e. by the presence of lymphoid cells impinging on follicular cells, hurthle cell changes, lymphoid and plasma cells in the background. The diagnosis of Colloid Goiter was made by the presence of follicular cells in monolayered sheets or poorly cohesive clusters or singles, many bare nuclei, pigment laden histiocytes and abundant thick or thin colloid.

FNAC smears diagnosed as both HT and Colloid Goiter were studied and following parameters were evaluated in all slides:

- Number of Eosinophils per HPF (high power field) was counted.
- Number of Neutrophils per HPF was counted (to eliminate eosinophilia due to admixture with blood).
- At least 10hpf were checked and average was taken.
- Ratio of Eosinophils to Neutrophils was calculated.

The comparison of observations in both HT (i.e. cases) and Colloid Goiter (i.e. controls) were statistically analyzed.

RESULTS

The age of patients studied varied from 15 to 65yrs. Among them HT as well as colloid goiter affected predominantly in the age range of 26 to 35 yrs (i.e.20 cases and 17 controls) (Table.1). All diagnosed cases of HT were of female gender and diagnosed cases of colloid goiter showed a female: male ratio of 15.7: 1 (Table. 2). Increased infiltration of eosinophils in thyroid tissue was observed in many of the FNAC smears that were diagnosed as HT [Figure 1 & 2].

Table 1: Age Distribution of cases

Age Range (in years)	Cases Diagnosed as HT	Cases Diagnosed as CG
15-25	06	05
26-35	20	17
36-45	15	12
46-55	08	13
56-65	01	03

Table 2: Gender distribution among HT and CG diagnosed patients

Gender	Male	Female	TOTAL
HT	0	50	50
CG	3	47	50

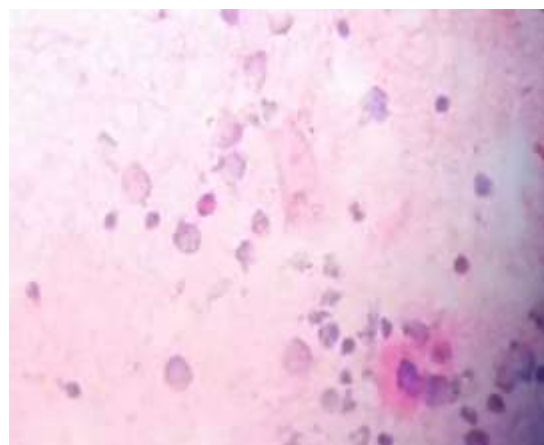


Fig-1: Smear from diagnosed HT cases showing bi-lobed eosinophil along with lymphocytes (Haematoxylin & Eosin stain, High power)

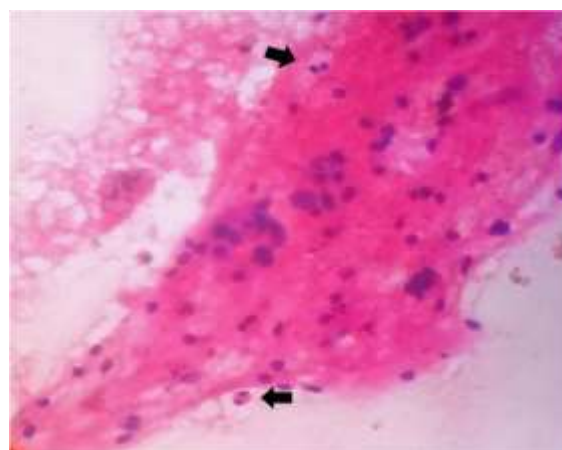


Fig-2: Smear showing eosinophils (black arrows) within and near follicular cells. (Haematoxylin & Eosin stain, High power)

Table 3: Number of Eosinophils per high-power field in smears diagnosed as HT and CG

No. of eosinophils per HPF	No. of cases diagnosed as HT (%)	No. of cases diagnosed as CG (%)
0.0 – 0.5	25 (50)	44 (88)
0.6 – 1.0	17 (34)	06 (12)
1.1 – 1.5	04 (08)	00
1.6 – 2.0	03 (06)	00
>2.0	01 (02)	00

Table 4: Number of Neutrophils per high-power field in smears diagnosed as HT and CG

No. of neutrophils per HPF	No. of cases diagnosed as HT (%)	No. of cases diagnosed as CG (%)
0.0 – 0.5	04 (08)	00
0.6 – 1.0	18 (36)	03 (06)
1.1 – 1.5	09 (18)	02 (04)
1.6 – 2.0	07 (14)	06 (12)
>2.0	12 (24)	39 (78)

The number of eosinophils per high power field in smears diagnosed as HT was found to be higher when compared with smears diagnosed as colloid goiter. Number of eosinophils per high power field were found to be >1 in 25 HT cases (50%), while >88% (44 cases) of colloid goiter showed a value < 0.5 (Table.3). Number of neutrophils per high power field was higher in colloid goiter than in HT cases, and in these cases number of eosinophils was also increased, which prove that high eosinophil to neutrophil ratio in colloid goiter was due to admixture with blood (Table 4, 5). The ratio of eosinophils to neutrophils in the smears diagnosed as HT was found to be significantly greater than in smears diagnosed as colloid goiter (Table .5). The findings were analyzed using unpaired t-test (SPSS software – version 22). On applying the test, the P-value was <0.0001 with a 95% confidence interval, which is extremely significant statistically. Thus, there is a higher association of eosinophilic infiltration of thyroid tissue with HT. Colloid goiter cases with E/N ratio > 0.5 were absolutely absent. Thus the ratio of eosinophils to neutrophils per high power field more than 0.5 should be considered as a cut off limit, above which it is considered pathologically significant to diagnose as thyroid tissue eosinophilia, a diagnostic clue towards Hashimoto’s thyroiditis.

TABLE 5: Ratio of eosinophils to neutrophils per HPF in smears diagnosed as HT and CG

Ratio of eosinophils to neutrophils per HPF	No. of cases diagnosed as HT (%)	No. of cases diagnosed as CG (%)
0.00 – 0.10	02 (04)	34 (68)
0.11 – 0.20	06 (12)	06 (12)
0.21 – 0.30	04 (08)	02 (04)
0.31 – 0.40	07 (14)	06 (12)
0.41 – 0.50	05 (10)	02 (04)
0.51 – 0.60	06 (12)	00
0.61 – 0.70	06 (12)	00
0.71 – 0.80	04 (08)	00
0.81 – 0.90	06 (12)	00
0.91 – 1.00	00	00
1.10 – 2.00	04 (08)	00
TOTAL	50 (100)	50 (100)

Note: On applying unpaired t-test, P-value calculated was <0.0001 with 95% confidence interval, which is extremely significant statistically.

DISCUSSION

HT is an autoimmune chronic inflammatory condition characterized by destruction of the thyroid gland. The incidence of HT seems to be increasing in

the recent times. It is important to diagnose HT as patients subsequently become hypothyroid and require lifelong thyroxine supplementation. Literature reports various neoplastic and nonneoplastic lesions in association with HT like colloid goiter, cellular adenoma, follicular neoplasm, Hurthle cell neoplasm, papillary carcinoma, Non-Hodgkins lymphoma (NHL) and follicular carcinoma, which emphasizes the need for long-term follow up. It is also important not to over-diagnose this entity as neoplasms, so that unnecessary surgeries can be avoided.[9-11]. Many recent studies on cytomorphological aspects of Hashimoto’s Thyroiditis emphasized the presence of thyroid tissue eosinophilia in diagnosed cases of HT. Infiltration of abundant eosinophils was also seen in association with Sclerosing mucoepidermoid carcinoma with eosinophilia (SMECE) , a rare tumor entity of thyroid gland , commonly seen in females, occurs in a background of HT and was considered to be the most distinctive cytological feature to distinguish it from Squamous cell carcinoma [12-16].

In the present study, all diagnosed HT cases were females, showing that HT is predominantly seen in female population. Rathi *et al.*; [17] also reported a female: male ratio of 6.14: 1 among cases of HT. In our study the age group of patients who were diagnosed with HT varied from 15 to 56years and most common age group affected was 23 – 49years.

Most of the cases in the present study were hypothyroid (72%), this is in concordance with other studies [18]. Percentage of HTcases with hypothyroid status in studies conducted by Ekambaram *et al.*; [19] and Rathi *et al* [17] were 84 and 56 respectively. In current study, antibody profile was available in 30 cases and 4 controls, out of which 22cases and one control had elevated level of antibody titres. Due to this false positive and false negative results with antibody titres, many HT cases can be missed or over diagnosed if antibody titre estimation is used alone as the screening indicator. Thus FNAC is superior to antibody screening in the diagnosis of HT. However, FNAC has certain pitfalls in diagnosing HT. Diagnosis of HT is likely to be missed in the presence of hyperplastic follicular cells, abundant colloid, presence of lymphocytic infiltration in Grave’s disease and if there is variation in the proportion of follicular cells and hurthle cells. There is sometimes an overlap in the cytomorphological features of HT and other conditions like subacute lymphocytic thyroiditis, nuclear features of papillary carcinoma, Hurthle cell neoplasms. The summary of the findings in the current study were compared with other studies in the Table.6.

Table 6: Comparison of findings in HT cases of present study with other literature

Findings	Present study	Ekambaram <i>et al.</i> :[19]	Rathi <i>et al.</i> :[17]	Jayaram <i>et al.</i> :[20]
No. of cases studied	50	50	50	88
Common age group involved	26 – 45 yrs	Not recorded	23 – 49 yrs	Not recorded
F : M ratio	All females	Not recorded	6.14 : 1	Not recorded
Thyroid status	Available in 50 cases	Available in 50 cases	Available in 41 cases	Available in 68 cases
Hypothyroid	36 (72%)	42 (84%)	23 (56.09%)	27 (39.7%)
Hyperthyroid	07 (14%)	03 (03%)	03 (7.31%)	08 (11.7%)
Euthyroid	07 (14%)	05 (10%)	15 (36.58 %)	33 (48.5%)
Elevated Ab titres	22 out of 30	26 out of 40	9 out of 11	27 out of 29
Lymphocyte infiltrate impinging on follicular cells	50 (100%)	50 (100%)	36 (72%)	61 (69%)
Hurthle cell change	40 (80%)	Not recorded	37 (74%)	49 (56%)
Increased eosinophilic infiltration	25 (50%)*	22 (44%)*	24 (48%)	15 (17%)
Neutrophilic infiltration	+	+	+	+
E/N Ratio > 0.5	26 (52%)	16 (32%)	Not recorded	Not recorded
Location of eosinophils	In & near follicular cells and within lymphocytic aggregates	Within lymphocytic aggregates	Infiltration of follicular epithelial cells	Infiltration of follicular epithelial cells

*Significant eosinophilic infiltration .i.e. No. of Eosinophils / hpf > 0.6 is included here.

The current study focused mainly on quantitatively assessing the importance of the presence of eosinophils in the diagnosed HT FNAC smears, which is a finding rarely been studied. If the association of eosinophilic infiltration and HT is significantly proved, it can serve as a distinctive cytological feature in the diagnosis of HT, so that false positive and false negative results can be avoided in the future.

Our study has shown a significant increase in the number of eosinophils per high power field (> 0.6) as well as the ratio of eosinophils to neutrophils per high power field in HT cases when compared with the control i.e. colloid goiter cases. 50%(25) of HT cases had significant eosinophilic infiltration, whereas study done by Ekambaram *et al.*[19]; Rathi *et al* [17] and Jayaram [20] *et al.*; had 44%, 48% and 17% respectively .Most of the elevated values of eosinophil per hpf as well as E/N ratio among colloid goiter cases were associated with an increase in number of neutrophils per hpf; thus confirming that elevated levels are due to admixture with blood. Significant E/N ratio i.e. > 0.5 was observed in 52% (26) of HT cases. This is consistent with the study done by Ekambaram *et al.*[19]; where 32% (16) cases showed >0.5 E/N ratio. The eosinophilic infiltration was observed in and near the follicular cells as well as within lymphocyte aggregates.

The relation between age, thyroid status and antibody titres with high levels of eosinophils are yet to be explored. In our study, high E/N ratio is seen among the age group 36 to 45 years, but low among the age groups <26 and >52years. High E/N ratio (>0.5) was seen among hypothyroid cases (23 cases) and hyperthyroid cases (3 cases), but absent in Euthyroid cases. Another observation made was the high levels of antibody titres with high E/N ratio (>1). Most of the patients with high E/N had normal levels of AEC (absolute eosinophil count), thus ruling out the possibility of thyroid tissue eosinophilia in association with peripheral blood eosinophilia.

There are several hypotheses as well as studies which support our proposal of high association of eosinophilic infiltration of thyroid gland in HT. Few animal studies, which report a similar finding, were also found. In a study conducted by Cohen *et al.*; [21], guinea pigs were immunized with thyroid extracts in complete Freund's adjuvant to induce thyroiditis, eosinophilic infiltration were noticed and mainly located in close proximity to small blood vessels, and in large numbers in the fibro-fatty tissue surrounding the gland [were found only in the thyroids of those animals, which have, both thyroid lesions and antibody activity]. The local accumulation of eosinophils appears to be related to the presence of both lymphocytes in the thyroid and circulating antibody, suggesting that the

lymphocyte-derived eosinophil chemotactic factor might be involved in this accumulation [22, 23].

In another study conducted by Sharp *et al.*; [24], guinea pigs that received a passive transfer of rabbit antiserum to guinea pig thyroglobulin developed an eosinophilic infiltrate of the thyroid. In 1969 B. D. Jankovic *et al.*; [25] conducted a study on passive transfer of experimental allergic thyroiditis, delayed hypersensitivity to thyroglobulin and anti-thyroglobulin antibody formation in inbred lewis rats, also showed eosinophilic and neutrophilic polymorphonuclear leucocytes dispersed in thyroid parenchyma. The study proposed that thyroglobulin is also present in the interstitial areas either normally or as a result of the antiserum injection and that the eosinophils appeared in response to the presence of thyroglobulin–antithyroglobulin complexes in these areas.

CONCLUSION

FNAC continues to be a tool of significance in diagnosing HT. Due to the false positive and false negative results with antibody titres, many HT cases can be missed or over diagnosed. So emphasis should be placed on the cytological features of HT in cases of doubt. In our study, we found significant association of eosinophils in FNAC smears of diagnosed HT cases within a small population. This cytological finding can improve the efficacy of FNAC in diagnosing Hashimoto's thyroiditis, if confirmed by further larger, independent and molecular level studies.

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