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Original Research Article

Immunohistochemical Subtyping of Hashimoto Thyroiditis With Respect to IgG4 Marker

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Abstract

Background: Hashimoto thyroiditis (HT) is the most commonest form of autoimmune thyroiditis with heterogenous clinical presentation. Recently a type of Hashimoto thyroiditis with dense lymphoplasmacytic infiltration and marked fibrotic changes with increased number of IgG4-positive plasma cells and serum IgG4 have been reported in the literature and they have a close relationship to IgG4-related disease (IgG4-RD). On the basis of immunostaining for IgG4, HT was divided into an IgG4 thyroiditis group and a non-IgG4 thyroiditis group and their clinical and histopathological features were studied. *Aims and Objectives:* To categorize Hashimoto thyroiditis with reference to IgG4-positive plasma cell infiltration and to study the histopathological characteristics of each group. *Materials and Methods:* It was a retrospective cross sectional study for a period of one and half year. Cases which were histopathologically diagnosed as Hashimoto thyroiditis were included in the study. Immunohistochemistry marker done for IgG4 and the foci with the highest density of positive cells was evaluated. *Results:* Among the 13 cases of Hashimoto thyroiditis 8 cases were subtyped into IgG4 thyroiditis group and 5 cases into non IgG4 thyroiditis group based on immunohistochemistry. All the cases were females in both the groups and the age of presentation in IgG4 thyroiditis was younger when compared to non IgG4 thyroiditis. Histopathologically, IgG4 thyroiditis showed marked fibrosis and moderate to severe lymphoplasmacytic infiltration with increased IgG4 positive plasma cell infiltration when compared to non IgG4 thyroiditis. *Conclusion:* Immunostaining of IgG4 can help in the subtyping of Hashimoto thyroiditis and are closely related with IgG4 related diseases. IgG4 Hashimoto thyroiditis presents with a distinct histopathological features when compared to non IgG4 thyroiditis.

Keywords: Hashimoto thyroiditis; IgG4; Immunohistochemistry; Fibrosis; Histopathology.

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Introduction

Hashimoto thyroiditis(HT) is the most common form of autoimmune thyroiditis and was first described by Hakaru Hashimoto.[1] It is also known as chronic lymphocytic thyroiditis or struma lymphomatosa. Being an autoimmune disease, characterized by elevated circulating antibodies to thyroglobulin and thyroid peroxidase. Clinically it presents as multinodular goitre, diffuse goitre or a solitary nodule with features of hypothyroidism or hyperthyroidism. Microscopically characterized by infiltration of lymphocytes, plasma cells, germinal center formation with atrophy of the follicles and Hurthle cell changes. Atypically some may exhibit extensive fibrosis with replacement of the thyroid parenchyma and are termed as fibrosing variant of Hashimoto thyroiditis.

Recently from immunohistochemical analysis, a unique subtype of Hashimoto Thyroiditis has been identified as IgG4 thyroiditis showing increased numbers of IgG4-positive plasma cells, and higher levels of serum IgG4 levels. This group was closely related to IgG4-related sclerosing disease (IgG4 -RSD) which was characterized by increased serum IgG4 levels, and the classical histological features are dense lymphoplasmacytic infiltration, storiform type fibrosis, obliterative phlebitis and, clinically alleviation of symptoms after steroid therapy.[2] Both IgG4 thyroiditis and non-IgG4 thyroiditis present with different clinical features but IgG4 thyroiditis was usually associated with rapid progression with subclinical hypothyroidism showing high levels of circulating antibodies, and show diffuse low echogenicity radiologically.[3] Hence the purpose of the study was to categorize Hashimoto thyroiditis into two subgroups with reference to IgG4-positive plasma cell infiltration.

Aim

To categorize Hashimoto thyroiditis with reference to IgG4-positive plasma cell infiltration and to determine the histopathological characteristics of each group.

Materials and Methods

Study setting: The present study was a retrospective cross sectional study carried out in the Department of Pathology, Sri Manakula vinayagar Medical college and hospital for a period of one and half year from December 2017 to May 2019. 13 Cases which were histopathologically diagnosed as Hashimoto thyroiditis were included in the study. The thyroidectomy specimens either partial or total sent for histopathological examination were received in formalin preservative. After grossing, the representative sections were processed, embedded in paraffin and stained by Haematoxylin and Eosin staining.

The morphology of the lesions were studied first in the haematoxylin and eosin stained sections and were evaluated for the intensity of lymphoplasmacytic infiltration, Lymphoid follicle formation, follicular destruction and grading of stromal fibrosis. Since most of the histopathological parameters were subjected to assessment by visual analogue scale, the slides were reviewed by two pathologists to confirm histological findings.

Later the slides were subjected to Immunohistochemical marker IgG4 (Rabbit Anti Human–IgG4 Monoclonal antibody – Clone EP138). For the quantification of IgG4-positive cells, the areas with the highest density of positive cells were evaluated. Five, high-powered fields were analyzed in the section and the average number of positively stained cells per high-power field was calculated. If >20/ hpf IgG4-positive plasma cells were present, it was considered as IgG4 positive and Hashimoto thyroiditis was subtyped into IgG4 thyroiditis or Non IgG4 thyroiditis respectively.[4]

The results of Thyroid function tests including FT3, FT4, and Thyroid Stimulating Hormone (TSH) were obtained from the patients case records.

Results

In the present study, we subtyped 13 cases of Hashimoto thyroiditis into two groups based on immunohistochemistry into IgG4 thyroiditis and Non IgG4 thyroiditis and the criteria used was presence of >20/ hpf IgG4-positive plasma cells.

On immunostaining, 8 cases showed diffuse infiltration of IgG4 positive plasma cells (40 ± 5.41/ hpf) (Fig. 4) and were grouped as IgG4-positive thyroiditis group whereas 5 cases showed low IgG4 positive plasma cells (11 ± 2.42/hpf) and were grouped as Non IgG4 thyroiditis group and the *p*-value was found to be statistically significant ($p < 0.05$).

The clinical and laboratory parameters of these two groups were compared and the results are summarized in (Table 1). All the 8 cases in IgG4 thyroiditis group were found to be females and the mean age of presentation was 30.13 ± 7.2

years whereas the non-IgG4 thyroiditis group also consisted only 5 females with a mean age of 34.6 ± 10.0 years. Additionally the duration of the disease in IgG4 thyroiditis (12.75 ± 10.8 months) was found to be shorter when compared to non IgG4 thyroiditis (23.6 ± 12.1 months). In IgG4 thyroiditis 4 cases showed euthyroidism followed by hypothyroidism (3cases) and hyperthyroidism (1 case) whereas in non IgG4 thyroiditis 3 cases showed euthyroidism and 1 case showed hypothyroidism and none of the case showed hyperthyroidism.

Table 1: Comparison of clinical features between IgG4 thyroiditis and non-IgG4 thyroiditis

Clinical features	IgG4 thyroiditis (*n*-8)	Non-IgG4 thyroiditis (*n*-5)	*p*-value
Age (years)	30.13 ± 7.2	34.6 ± 10.0	0.664
Sex (male/female)	0/7	0/5	–
Disease duration (months)	12.75 ± 10.8	23.6 ± 12.1	–
Thyroid functional status (hypothyroidism/euthyroidism/ Hyperthyroidism)	3/4/1	2/3/0	0.709

The histological features between IgG4 thyroiditis and non-IgG4 thyroiditis were compared and the results are summarized in Table 2.

Histopathologically, in IgG4 thyroiditis moderate fibrosis was seen in 75% of cases and severe fibrosis seen in 25% cases with predominant cases (75%) showing interfollicular pattern of fibrosis followed by interlobular fibrosis in 25% cases (Figs. 1a and 1b). It was observed that most of the cases in IgG4 thyroiditis group showed moderate (4 cases) to severe (3 cases) lymphoplasmacytic infiltration (Figs. 2a and 2b) and follicular destruction (Fig. 3) and the residual thyroid follicle were found to be atrophic with scanty colloid. Whereas in Non IgG4 thyroidtis, histological features like fibrosis were mild in 2 cases and moderate in 3 cases and none of the cases in this group showed severe fibrosis. Lymphoplasmacytic infiltration were moderate in 4 cases and mild in 1 cases. Lymphoid follicle formation was seen in both the groups.

Table 2: Comparison of common histologic features between IgG4 thyroiditis and non-IgG4 thyroiditis

Common histologic features	IgG4 thyroiditis (*n*-8)	Non-IgG4 thyroiditis (*n*-5)	*p*-value
Stromal fibrosis (Mild/ moderate/ severe)	0/6/2	2/3/0	0.103
Fibrosis pattern (interfollicular/ interlobular/scar)	6/2/0	2/3/0	0.207
Lymphoplasmacytic infiltration (Mild/ moderate/ severe)	1/4/3	1/4/0	0.296
Lymphoid follicle formation (3+, 2+, 1+, -)	0/5/3/0	0/2/3/0	0.928
Follicular destruction (3+,2+,1+, -)	1/5/2/0	0/2/2/1	0.434
Obliterative phlebitis (+, -)	0/8	0/5	–
No. IgG4 + plasma cells per HPF	40 ±5.41/hpf	11 ± 2.42/hpf	0.053

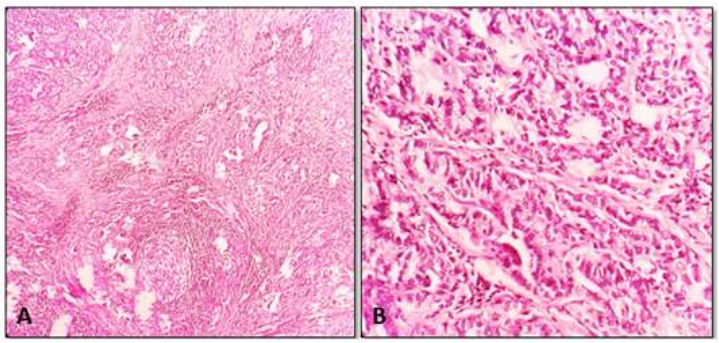

Fig. 1: Histopathology of the study cases showing (A) -Interlobular fibrosis - fibrous tissue is surrounded and extended between the individual lobules (H&E 10X). (B) Interfollicular fibrosis: thyroid follicles are separated by fibrous tissue with lymphoplasmacytic infiltration in the interfollicular area (H&E 40X).

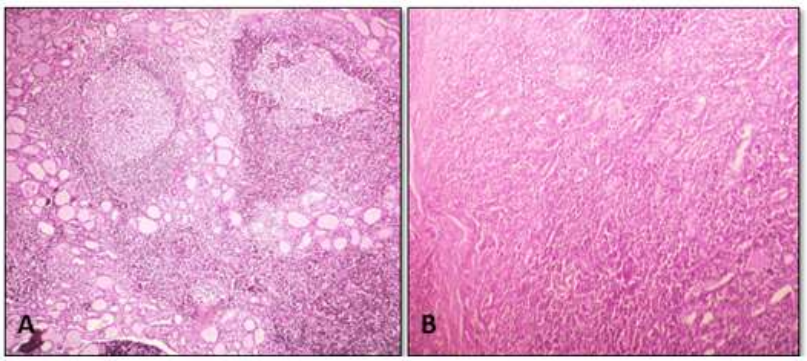

Fig. 2: Histopathology of the study cases showing (A) Moderate inflammatory infiltration, (B) Severe inflammatory infiltration in Hashimoto thyroiditis (H&E 10X).

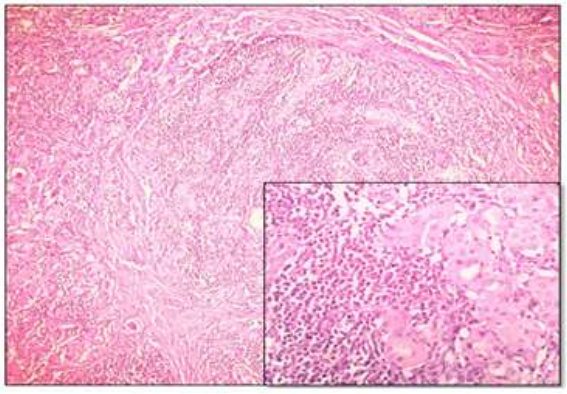

Fig. 3: Dense lymphoplasmacytic infiltration in IgG4 thyroiditis. (H&E 10X) and Inset showing lymphocytes and plasma cells destructing the thyroid follicles and (H&E 40X).

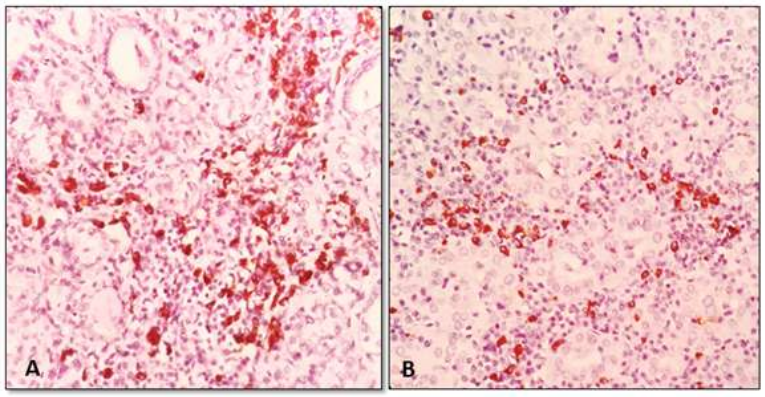

Fig. 4: (A) Immunohistochemistry of IgG4 (B) Shows numerous IgG4-positive plasma cells in thyroid parenchyma seen in IgG4 thyroiditis (40x).

Moreover the IgG4-positive group also shared many histological features which was similar to IgG4-related sclerosing disease in other organs, except obliterative phlebitis. Thus, IgG4 thyroiditis group exhibited a histomorphological features which is distinct from the non-IgG4 thyroiditis group and all these changes were found to be limited by the thyroid capsule.

Discussion

In 1912, Hakaru Hashimoto discovered and described a new autoimmune thyroid disease called Hashimoto thyroiditis.[5] It was an autoimmune disorder which affect the thyroid, presenting with variable spectrum of thyroiditis ranging from self-limiting forms such as focal, silent and juvenile

thyroiditis to atrophic thyroiditis manifesting as hypothyroidism without goiter. It was found to be associated with other autoimmune diseases like Graves disease, Sjogrens syndrome, Systemic Lupus Erythematosus, Rheumatoid arthritis, Auto immune Lymphocytic adrenalitis (Schmidt's syndrome) Pernicious anaemia, Myasthenia gravis, etc.[6] Histopathologically it was characterized by diffuse lymphoplasmacytic infiltration with prominent germinal center, Hurthle cell change of follicular cells and degeneration of thyroid follicle.[7]

In 2001, Hamano et al.[8] first proposed a new disease which was related to autoimmune pancreatitis (AIP) called IgG4-related disease (IgG4-RD). This IgG4-related disease were seen in other organs like pancreas, biliary tract, gallbladder, liver, salivary gland, retroperitoneum, lung, kidney, prostate, orbit, lymph node, lacrimal gland and adventitia of the aorta and share a common histopathological features which includes

- diffuse lymphoplasmacytic infiltration,
- progressive fibrosis,
- occasional eosinophilic infiltration and
- obliterative phlebitis.
- Presence of IgG4-positive plasma cells and increased level of serum IgG4.[9]

IgG4 is the rarest of the IgG subclass and accounts for 3–6% of all the IgG in normal serum.[10] Recent research discovered that serum levels and immunohistochemistry staining with IgG4 antibody were considered to be the diagnostic marker for IgG4 related sclerosing diseases.[11]

But Mayo Clinic experience on diagnosis of autoimmune pancreatitis found a poor correlation between serum IgG4 levels and immunohistochemical findings.[12] Serum IgG4 Levels were low even with high grade of tissue infiltration of IgG4 positive cells. By extrapolating the above observation in the present study the immunohistochemistry of IgG4 positive cells in the thyroid specimens were done to sub-type the Hashimoto thyroiditis into IgG4 (plasma cell rich) thyroiditis and Non IgG4(plasma cell poor) thyroiditis.

Comparison of IgG4 thyroiditis with other studies

In the present study all the cases were females in both groups which was in coherence by Li et al.[4] Also our study showed that IgG4 thyroiditis were younger than those in the non IgG4 thyroiditis which was similar to the findings of Li et al.[4]

Deshpande et al.[2] and Li et al.[4] found that patients with IgG4-positive HT had a significantly higher grade of stromal fibrosis. In this study 6 cases showed moderate fibrosis and 2 showed severe fibrosis in IgG4 Thyroiditis, whereas in non IgG4 thyroiditis 2 cases showed mild fibrosis and 3 cases showed moderate fibrosis but it was not statistically significant. Our study also revealed that marked fibrosis of thyroid parenchyma and IgG4 positive plasma cells were found to be clinically significant than in the Non-IgG4 Thyroiditis. However p value significance could not be obtained due to small sample size.

In this study, IgG4-positive thyroiditis was characterized by moderate to severe lymphoplasmacytic infiltration with moderate to severe stromal fibrosis and an increase in the IgG4-positive plasma cells and these findings met atleast one of the three major histopathological features of IgG4 related disease.

In the current study or in the previous studies none of the cases showed, the typical storiform-type fibrosis and obliterative phlebitis in IgG4-positive HT.

Our study confirms that HT can be divided into two different immunophenotypes i.e. IgG4 thyroiditis and non IgG4 thyroiditis but we could not able to conclude an evidence to relate IgG4 thyroiditis with IgG4-Related Disease in our study. Previous studies found that IgG4 thyroiditis was closely related with IgG4-Related Sclerosing disease, so IgG4 thyroiditis also show better response to glucocorticoid therapy for relieving from local symptoms and improving clinical outcomes and if symptoms persists surgical treatment is needed.[13]

The increased serum IgG4 levels indicates the histological changes that occured in the thyroid gland and help the clinicians to make a differential diagnosis from suspected malignancy and thereby avoiding unnecessary surgeries. So, measurement of serum IgG4 levels as a routine test of HT in the near future may predict the link between HT and IgG4-RSD and the prognosis.[14] Finally, awareness of this disease may help the clinician to guide treatment of patients with HT.

Limitation of the Study

- Sample size is small.
- Special investigations like thyroid antibodies and serum IgG4 levels could not be obtained.
- As it was a retrospective study follow-up of the patient could not done to rule out autoimmune disorders in various organs.

Conclusion

The immunostaining of IgG4 can help in the subtyping of Hashimoto thyroiditis into IgG4 thyroiditis and Non IgG4 thyroiditis. Histopathologically, IgG4 thyroiditis presented with severe fibrosis and lymphoplasmacytic infiltration of thyroid gland which is closely related with IgG4 related diseases (IgG4-RD). Hence we recommend to measure serum IgG4 levels as a routine test of HT, which may help for the prompt treatment for HT and to rule out other systemic autoimmune diseases in the same patients and may aid in predicting the clinical course of these patients.

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