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Original Research Article

Antithyroid antibody profile and viral markers in autoimmune thyroiditis in Chennai population

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ABSTRACT

Background: Autoimmune thyroid disease (AITD) is the result of a complex interaction between genetic and environmental factors, which leads to failure of one or more mechanisms responsible for controlling thyroid-reactive T and B cells. The most frequently occurring clinical forms of autoimmune thyroiditis include Hashimoto's thyroiditis and Grave's disease. Viruses have been implicated in the onset of autoimmune disorders. This study aims to investigate the association between *Hepatitis C virus*, *Parvovirus B19* and *Enteroviruses* in autoimmune thyroiditis by assessing virologic and immunologic parameters of cases with clinical indication of AITD.

Materials and Methods: Anti-thyroid peroxidase (TPO) and Anti-thyroglobulin (TG) antibodies were evaluated using ELISA. The presence of Hepatitis C virus (HCV) IgG antibodies and *Parvovirus B19* IgM antibodies were evaluated using ELISA. The presence of *Enterovirus* RNA was investigated by RT-PCR.

Results: A total of sixty study subjects were involved in this study. Sixteen male patients (26.66%) and forty-four female patients (68.33%) were positive for Anti-TPO antibodies. Four male patients (6.66%) and twenty-five female patients (41.66%) were positive for Anti-TG antibodies. Four male patients (6.66%) and twenty-two female patients (36.66%) were positive for both Anti-TPO and Anti-TG antibodies. One female patient tested positive for HCV IgG antibodies. Sixteen patients (26.66%) were positive for *Parvovirus B19* IgM by ELISA of which 3 patients (5%) were male and 13 patients (21.66%) were female. None of the samples were positive for *enterovirus* RNA.

Conclusion: We conclude that viral infection may be involved in triggering autoimmune mechanisms. Further studies with a larger population are necessary to establish an association between HCV, *Parvovirus B19* and *Enteroviruses* in the pathogenesis of autoimmune thyroid disorders.

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1. Introduction

Hyperthyroidism, hypothyroidism, autoimmune thyroiditis, Goitre (diffuse or nodular), and neoplasm are the main conditions affecting the human thyroid gland. The human Auto Immune Thyroid Diseases (AITD) broadly include Graves' disease (GD) and Hashimoto's thyroiditis (HT)

which are the most common causes of dysfunction of thyroid gland. Hashimoto's thyroiditis is characterized by intrathyroidal mononuclear cell infiltration and production of autoantibodies to thyroid peroxidase (TPO) and thyroglobulin (TG), often followed by hypothyroidism due to the destruction of thyroid follicles. The disease is diagnosed based on symptoms of hypothyroidism and the presence of TPO Ab and/or TG Ab.^{1,2}

Thyroglobulin is a glycoprotein with a molecular weight of 660 kDa that consists of two components. Thyroglobulin

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is produced in thyroid cells and released into the colloid through granule exocytosis.^{3,4} The oxidation and interaction of iodide with the released thyroglobulin is mediated by thyroid peroxidase, a membrane-bound enzyme located in the thyrocyte apical membrane.¹ The annual incidence of Hashimoto's thyroiditis worldwide is estimated to be 0.3–1.5 cases per 1000 persons, whereas Graves' disease is estimated at about 5 per 10,000 people.⁵

Association of viral infections are suggested to be a cause of triggering autoimmune mechanisms.^{6–8} Chronic hepatitis C patients have been reported more likely to show signs of autoimmunity, including autoantibodies and autoimmune diseases when compared to healthy controls. It had been hypothesized that hepatitis C virus might share partial sequences in a few amino acid segments with thyroid tissue antigens. Studies of Tran et al (1993), Preziati et al (1995), Matsuda et al 1995, Deutsch et al. 1997, Custro et al.(1997), show a positive relationship between chronic hepatitis C infection and thyroid autoimmunity.^{9–16}

Human pathogenic *parvovirus B19* is known to cause fifth disease (erythema infectiosum). Most infections occur during childhood and adolescence.¹⁷ There is compelling evidence that certain cases of Hashimoto's thyroiditis are caused by acute *parvovirus B19* infections. Kouki Mori et al demonstrated that B19 DNA has been persistently detected in the thyroid in a patient with Hashimoto's thyroiditis.¹⁸

Coxsackie virus, an *Enterovirus* (EV), it is most often associated with subacute thyroiditis. *Enteroviruses* are known to induce low-grade persistent infection. Experimental studies have shown that enteroviruses such as *coxsackievirus B*, can replicate and persist in thyroid cells.^{19–21}

From the above literature, we have evidence linking certain viral infections to autoimmune thyroid disease. This study was carried out to investigate the correlation between *Hepatitis C virus*, *Parvovirus B19* and *Enteroviruses* in patients with autoimmune thyroiditis.

2. Materials and Methods

2.1. Study design

The present study was conducted in the Immunodiagnostic Laboratory and Department of Virology, King Institute of Preventive Medicine and Research from April 2022 until August 2022. Proposal had gone through Institute Ethical Committee review by the approval number 014/KIPMR/2022/ Date: 12.05.2022. Informed consent was obtained from the patients involved in the study. Study subjects who were not willing to participate in the study and patients under immunocompromised category were excluded from the study.

The blood samples were collected from the participants under aseptic conditions by venipuncture, serum separation was completed, aliquoted into properly labelled 2 ml

Eppendorf tubes and stored at -20°C until further use. The clinical specimens were collected from 60 patients attending the tertiary care centres in Chennai.

Quantitative measurement of IgG class autoantibodies against thyroid peroxidase (TPO) and thyroglobulin (TG) in human serum were completed using commercial kit by Orgentec Diagnostika. Qualitative detection of IgG antibodies against HCV in human serum or plasma using Hepalisa (J. Mitra) kit. Qualitative determination of IgM Parvovirus antibodies B19 in human serum or plasma using Novalisa kit. In vitro nucleic acid amplification for the detection of *Enterovirus* specific RNA was performed using the HELINI *Enterovirus* Real-time PCR kit. Reagents and primers were prepared and used according to the kit instructions.

3. Results

A total of 60 samples were subjected to anti Thyroid peroxidase ELISA and 95% positivity was found among the study population. The presence of anti-thyroglobulin antibodies was observed in 48.3%. This study investigated *Hepatitis C Virus*, *Parvovirus B19* and *Enterovirus* viral markers among 60 patients with Hashimoto's thyroiditis.

The target population was selected based on the presence of Anti-TPO and/or Anti-TG antibodies in hypothyroid patients. Forty-four patients (73.32%) were female and sixteen patients (26.64%) were male. The majority of the population were between the age group of 20–40 years (46.66%) and predominantly female (35%). The female to male sex ratio was 2.75. Out of 60 patients, 16 male (26.66%) and 41 females (68.33%) were positive for anti-TPO antibodies. Anti-TG antibodies were positive in 4 male patients (6.66%) and 25 female patients (41.66%). In this study, 4 male patients (6.66%) and 22 female patients (36.66%) were positive for both Anti-TPO and Anti-TG antibodies (Table 1).

Table 1: Gender wise distribution of TPO, TG, TPO/TG and Viral markers

	Male (n=16) 26.7%	Female (n=44) 73.3%
TPO	16 (26.7%)	41 (68.3%)
TG	4 (6.7%)	25 (41.7%)
TPO&TG	4 (6.7%)	22 (36.7%)
HCV	-	1 (1.7%)
Parvovirus	3 (5%)	13 (21.7%)
EBV	1 (1.7%)	4 (6.7%)

3.1. Viral markers

One female patient who was Anti-TPO positive was also positive for HCV Ab by ELISA. Fifty-nine (98.33%) of the patients positive for Anti-TPO and/or Anti-TG were negative for HCV antibodies.

In this study sixteen patients (26.66%) were positive for *Parvovirus B19* IgM by ELISA of which 3 patients (5%) were male and 13 patients (21.66%) were female. The prevalence of *Parvovirus B19* IgM was more in patients with TPO antibodies. None of the 60 hypothyroid patients were positive for *Enteroviruses* RNA panel.

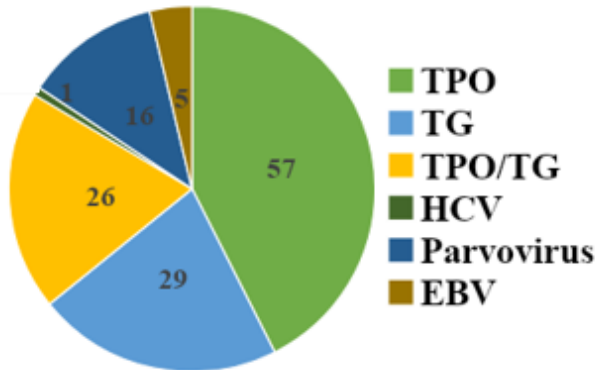


Figure 1: Analysis of Anti-Thyroid peroxidase (TPO), Anti-Thyroglobulin (TG), TPO/TG and Viral markers in Autoimmune Thyroiditis

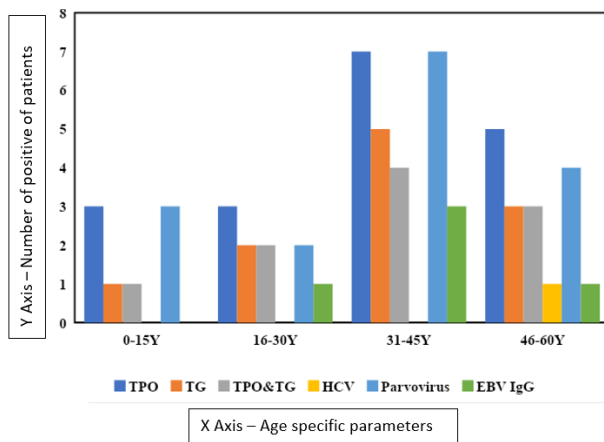


Figure 2: Viral markers in patients with Hashimoto's thyroiditis.

Represented below is the thyroid autoantibody profile of all hypothyroid patients on thyroxine treatment as well as the viral markers tested.

4. Discussion

Autoimmune diseases are more prevalent in recent times. The prevalence of autoimmune thyroid antibodies is on a constant rise in the present era. The current diagnosis of Hashimoto's thyroiditis is based on clinical symptoms correlating with laboratory results of elevated TSH with normal to low thyroxine levels as well as the presence of either Anti-TPO and/or Anti-TG antibodies.^{22–24}

Review studies of Usha Menon suggested that about 16.7% of adult subjects had anti thyroid peroxidase (TPO) antibodies and about 12.1% have anti-thyroglobulin (TG) antibodies. In the same study which involved 971 subjects, when subjects with abnormal thyroid function were excluded, the prevalence of anti-TPO and anti-TG antibodies was 9.5% and 8.5%.

In our study, 95% positivity of anti thyroid peroxidase antibodies and presence of 48.3% of anti thyroglobulin antibodies was observed among the suspected individuals.

Out of the 60 known hypothyroid patients, Anti-TPO and Anti-TG antibody positivity suggests that our study population presents with Hashimoto's thyroiditis.

Duclos Vallee JC, et al., (1994), found a higher prevalence of serum hepatitis C virus antibodies in patients with Hashimoto's thyroiditis than in those with any other thyroid disease. The high prevalence of such antibodies in patients with Hashimoto's thyroiditis compared with other groups of thyroid disease and with the normal population suggests that hepatitis C virus may be responsible for triggering Hashimoto's thyroiditis which was dissimilar to our study where the incidence was too low and found to be 1.66%.²⁵

Acute *parvovirus B19* infections may be involved in the pathogenesis of Hashimoto's thyroiditis, in some cases IgM antibodies to *parvovirus B19* are present for few months after onset of infection and in the present study there is an increased positivity of 26.66% indicating a link between prior Parvovirus infection and autoimmune disease.²⁶

Enterovirus real time PCR using serum samples from hypothyroid patients did not detect RNA. *Enteroviruses* are known to persist and replicate in thyroid cells, indicating the need to collect appropriate sample. Viruses triggering autoimmunity may only be present transiently in the early stages of the disease since they are cleared by the immune system.

5. Conclusion

This study evaluated immunological parameters and viral markers in patients with suspected autoimmune thyroiditis. Presence of HCV antibodies indicating Hepatitis C infection was detected by ELISA. Studies have shown that chronic Hepatitis C infection is involved in the pathogenesis of Hashimoto's thyroiditis. Acute *parvovirus B19* infection was validated by the presence of *B19* specific IgM antibodies. Studies have shown an association between *parvovirus B19* infection and Hashimoto's thyroiditis. The prevalence of autoimmune thyroiditis in India is 7.5% and since the seroprevalence of *parvovirus B19* specific antibodies is much higher (IgM- 7.53%, IgG- 27.96%),^{27–29} it indicates that only a minor proportion of *parvovirus B19* infections might induce thyroiditis. In this study *enteroviruses* did not exhibit an association with Autoimmune thyroid disease.^{30–32} Further studies with a

larger population are necessary to establish an association between HCV, Parvovirus B19 and Enteroviruses in the pathogenesis of autoimmune thyroid disorders.

6. Ethical Approval and Consent to Participate

Ethical clearance was obtained from the Institutional Ethics Committee at King Institute of Preventive Medicine and Research, Guindy.

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7. Conflict of Interest

None.

8. Source of Funding

None.

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