# THYROID AUTOIMMUNITY IN AN IODINE-REPLETE POPULATION: A RESEARCH ARTICLE

*Peter Manoharan C[1], Himagirish K. Rao[2], Achu Jacob Philip[3], Rahul Raj C. L[4], Gautham P. J[5], Varun[6]*

*[1]Professor, Unit Chief, Department of General Surgery, Pondicherry Institute of Medical Sciences.*
*[2]Assistant Professor, (Endocrine Surgeon), Department of General Surgery, Pondicherry Institute of Medical Sciences.*
*[3]Post Graduate, Department of General Surgery, Pondicherry Institute of Medical Sciences.*
*[4]Post Graduate, Department of General Surgery, Pondicherry Institute of Medical Sciences.*
*[5]House Surgeon, Pondicherry Institute of Medical Sciences.*
*[6]House Surgeon, Pondicherry Institute of Medical Sciences.*

**ABSTRACT**

**INTRODUCTION**

In recent times, the incidence and prevalence of thyroid disorders has been increasing in the Indian population. Autoimmune thyroiditis or Hashimoto's thyroiditis is one of the most common causes of thyroid disease. Antithyroid antibodies rarely develop before 20 years of age, but they may be a prelude to the development of subsequent hypothyroidism. It is universally known that iodine deficiency causes hypothyroidism. However, sustained unnecessary iodine supplementation may be harmful. Goitre, thyroid dysfunction (both hypo- and hyperthyroidism) and thyroid autoimmunity have been reported as a result of sustained supplementation in the iodine-replete state. Data on the impact of iodisation on thyroid function in adults is sparse. A study was conducted with an objective to estimate the problem of thyroid autoimmunity in patients who presented to the OPD.

**PATIENTS AND METHODS**

Patients who presented to the surgical OPD with clinical features of thyroid disease were included in the study after obtaining informed consent. Demographic details and clinical features of thyroid disease were noted. Thyroid status was estimated with the help of serum levels of thyroid stimulating hormone (TSH), free l-thyroxine ($FT_4$), and free tri-iodothyronine ($FT_3$). Auto-antibodies to thyroid peroxidase (Anti-TPO) were estimated. Findings were tabulated and analysed.

**RESULTS AND CONCLUSION**

The prevalence of antibody positivity was 69.7% (209 out of the 300 patients) in this study. Among age-groups, the maximum prevalence was found in the third decade of life (75/99 patients, 75.8%). Among those who were antibody-positive, 69.9% were euthyroid, 26.8% were hypothyroid and 3.3% were thyrotoxic. Hypothyroidism (elevated S. TSH) had a significant positive correlation ($r = 0.324$, $p = 0.003$) with antibody-positivity (elevated S. AMA).

**KEYWORDS**

Anti-TPO, Antibody status, Thyroid autoimmunity, Autoimmune thyroiditis, Hashimoto's thyroiditis, Thyroid status.

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**INTRODUCTION:** In recent times, the incidence and prevalence of thyroid disorders has been increasing in the Indian population. Autoimmune thyroiditis, or Hashimoto's thyroiditis, is one of the most common causes of thyroid disease. The autoimmune basis of Hashimoto's thyroiditis was established in 1956 by Roitt and Doniach.[1] Since then, various thyroid auto-antibodies have been described. Important among these are the antibodies to the microsomal fraction of the thyroid cell (AMA), thyroxine ($T_4$), tri-iodothyronine ($T_3$) and antibodies to the Thyroid Stimulating Hormone (TSH) receptor. It was found that this microsomal fraction of the thyroid cell was none other than Thyroid peroxidase (TPO), the enzyme that is pivotal to the synthesis of thyroid hormones in the thyroid epithelial cells. So, it was found that the anti-microsomal antibodies (AMA) were synonymous anti-TPO antibodies.

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*Corresponding Author:*
*Dr. Peter Manoharan,*
*Pondicherry Institute of Medical Sciences,*
*Ganapathichettikulam, Kalapet, Puducherry-605014, India.*
*E-mail: peternirmala@yahoo.com*
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Hashimoto's thyroiditis is a disorder of immune surveillance, characterised by dysfunction of suppressor T cells. Helper T lymphocytes produce circulatory auto-antibodies to TPO resulting in alterations of the thyroid cell membrane and eventual destruction of thyroid epithelial cells.

Antithyroid antibodies rarely develop before the age of 20. If they do appear, the natural history may follow either of the following courses. The classical course of autoimmune thyroiditis is one where the patients first develop thyrotoxicosis, which is self-limiting. This is followed by euthyroidism and later by hypothyroidism. Recovery is characterised by return to the euthyroid state. The most common picture is one of irreversible hypothyroidism which is clinically and pathologically overt. In about 10% of the cases, antibody titres may regress and become undetectable. Hashitoxicosis refers to a state of persistent thyrotoxicosis with a histopathological picture of Hashimoto's thyroiditis.

It is universally known that iodine deficiency causes hypothyroidism. However, sustained unnecessary iodine supplementation may be harmful. Goitre, thyroid dysfunction (both hypo- and hyperthyroidism) and thyroid

autoimmunity have been reported as a result of sustained supplementation in the iodine-replete state.[2,3] Hashimoto's thyroiditis, seen in iodine-replete individuals, can cause clinically overt hypothyroidism.[4] While the aetiology of autoimmune thyroiditis is not clear, it is widely believed that there are genetic predisposing factors. Environmental factors like certain viruses and chronic excess iodine intake can trigger the phenotypic expression of disease.[5] Moreover, radiation exposure and organic pollutants have also been reported to be associated with thyroid autoimmunity.[6]

The Universal Salt Iodisation (USI) programme has been in force in India since 1984. The impact of this programme on thyroid status has been reported by many Indian workers in school age children.[2,7] However, data on the impact of iodisation on thyroid function in adults is sparse. The prevalence of clinically overt hypothyroidism was 3.9% and that of subclinical hypothyroidism was 9.4% in a population-based study from Cochin.[8] More than half (53%) of the subjects with subclinical hypothyroidism were positive for anti-TPO antibodies. When compared with controls, patients with thyroid disorders were found to be more frequently positive for antithyroid antibodies. They also found that antibody-negative status doesn't necessarily exclude thyroid autoimmunity.[9] On the other hand, there are many cases with antibody-negative, yet histo-pathologically proven Hashimoto's thyroiditis.[10]

When patients with endemic goitre were administered iodine supplementation, thyroid auto-antibodies were seen to develop in 8-20% of them. In addition, lymphocytic infiltration of the thyroid was noted.[11,12] However, these findings were transient – antibody titres and lymphocytic infiltration decreased significantly after cessation of iodine supplements. Iodine supplementation may lead to structural and chemical alterations in thyroglobulin structure, resulting in new antigenic determinants and generation of auto-antibodies.[13,14] In addition, excessive iodine is rapidly oxidised by TPO in the hyperplastic thyroid epithelial cells. The oxidative intermediates, excessive iodine intake is also related to the induction of thyrocyte apoptosis and the development of thyroid autoimmunity.[15,16,17]

Thus, it is evident that the problem of thyroid autoimmunity is a significant one. In addition, the increasing incidence and prevalence of autoimmune thyroiditis is an apparent problem of indiscriminate iodine supplementation. On this background, the investigators conducted a study over the past two years with an objective to estimate the problem of thyroid autoimmunity in a population of iodine-replete individuals and to try to understand the correlation between autoimmunity and the clinical features of thyroid disease.

**PATIENTS AND METHODS:** This study was conducted in the Department of Surgery at Dr. SMCSI Medical College, Karakonam over the past two years. Patients who presented to the surgical OPD with clinical features of thyroid disease were included in the study after obtaining informed consent.

Details with regards to age, gender, nutritional history and symptoms of thyroid disease including insomnia, fatigability, anxiety, depression, hair loss, constipation or diarrhoea, weight loss or gain and menstrual irregularities were recorded. The vital signs were noted. Findings on local examination (solitary thyroid nodule, diffuse goitre or multinodular goitre) were noted. Thyroid status was estimated with the help of serum levels of thyroid stimulating hormone (TSH), free l-thyroxine ($FT_4$), and free tri-iodothyronine ($FT_3$). $FT_3$, $FT_4$ and TSH were analysed with the help of electro-chemiluminescence assay (Vitros ECi analyser). Auto-antibodies to thyroid peroxidase (Anti-TPO) were estimated in all patients with the help of the Calbiotech Thyroid Peroxidase (TPO) IgG ELISA Kit.

Findings were tabulated and analysed. The antibody status was correlated with age, gender, goitre grade and thyroid status.

Data was analysed using Statistical Package for the Social Sciences (SPSS), version 17.0. Continuous variables were correlated using Pearson's correlation coefficient. Chi-square test was used to test the significance of statistical association. If the p-value was less than 0.05, then such statistical associations were considered to be significant.

**Definitions:** S. $FT_4$ stands for the level of free l-thyroxine in serum. S. $FT_3$ stands for serum level of free tri-iodothyronine. S. TSH stands for serum level of thyroid-stimulating hormone (TSH). The normal range for S. $FT_4$, S. $FT_3$ and S. TSH were 0.8 - 2.0 ng/dL, 1.4 - 4.2 pg/mL and 0.4-4.2 mIU/L respectively. Patients with normal $FT_4$ level (0.8-2 ng/dL) and normal TSH level (0.4-4.2 mIU/L) were considered to be euthyroid. If S. $FT_4$ was less than 0.8 ng/dL or if S. TSH was more than 4.2 mIU/L, such patients were considered hypothyroid. Subjects with S. $FT_4$ > 2 ng/dL or TSH < 0.4 mIU/L were considered to be hyperthyroid.

**Subclinical Thyroid Disease:** Some patients were included in the study S. TSH was more than 4.2 mIU/L or less than 0.4 mIU/L, although they had no symptoms of thyroid disease. Such patients were considered to have subclinical hypothyroidism or hyperthyroidism.

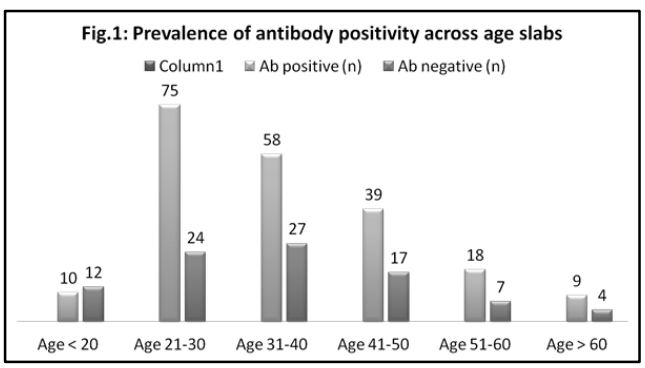
**Anti-TPO Antibody Status:** If the level of Anti-TPO Ab was 50 IU/L or more, such patients were considered to be Antibody-positive. If it was less than 50 IU/L, then the status was Antibody-negative.

**Goitre Grade:** While a solitary nodule of the thyroid is not a goitre by definition, it was included as 'goitre' for the purpose of this study. If no goitre was made out either on inspection of the neck or by palpation, such patients were considered to have grade 0 goitre. If the patients had thyroid nodules or goitre which was palpable but not evident on inspection, then such patients were deemed to have grade 1 goitre. Those who had goitres or nodules on inspection as well as palpation were deemed to have grade 2 goitre.

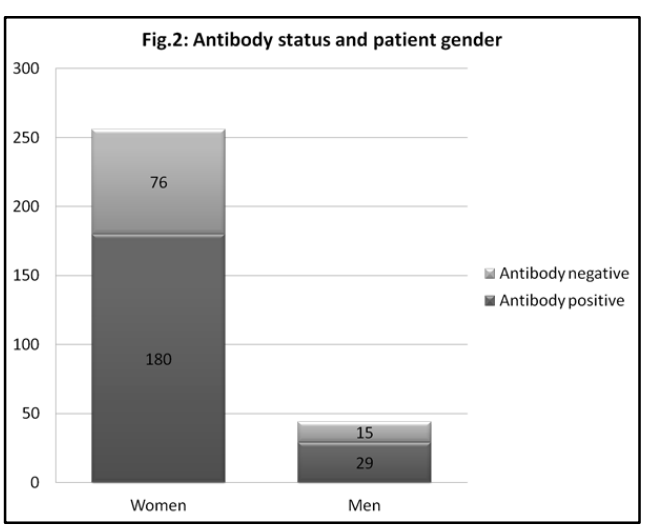
**RESULTS:** A total of 339 patients presented to the OPD with the clinical features mentioned above. Out of these, 39 patients did not consent for inclusion in the study. 300 patients were included in the final analysis in this study.

Out of them, 256 (85.3%) were women and 44 (14.7%) were men. The patients were aged between 18 yrs. and 81 yrs. with a median age of 41. When they were categorised on the basis of age, the most number of patients were in the third decade (99 patients, 33%) and the fourth decade (85 patients, 28.3%) of life.

With regards to antibody status, 209 out of the total 300 patients (69.7%) were found to be antibody-positive with significant titres of Anti-TPO antibodies in blood. The other 91 (30.3%) were found to be antibody-negative. When analysed with regards to decades of life (the 2nd decade, 3rd decade, 4th decade and so on), the prevalence of antibody-positivity was maximum in the 3rd decade of life (75/99 patients, 75.8%; Fig. 1).




With regards to the gender of the patients, it was found that the prevalence of antibody-positivity among women was 70.3% (180 out of 256 women were antibody-positive). Among the men, the prevalence of antibody-positivity was 65.9% (29 out of 44 patients). The difference was not found to be significant (Fig. 2).




When antibody status was correlated with thyroid status, it was found that out of the 209 patients who were positive for the Anti-TPO antibody, 146 (69.9%) were euthyroid, while 56 patients (26.8%) were hypothyroid and 7 patients (3.3%) had hyperthyroidism. Out of the 210 patients who were euthyroid, 146 (69.5%) were AMA positive. Prevalence of AMA positivity among the hypothyroid patients (56/70, 70%) and hyperthyroid patients (7/10, 70%) was identical (Table 1).

| Thyroid status | Antibody positive | Antibody negative | Total |
|---|---|---|---|
| Euthyroid | 146 | 64 | 210 |
| Hypothyroid | 56 | 24 | 80 |
| Thyrotoxic | 7 | 3 | 10 |
| **Total** | **209** | **91** | **300** |

***Table 1: Correlation of Antibody Status with Thyroid Status***

With regards to grade of goitre, 219 out of the 300 patients (73.0%) had no visible or palpable goitre. Grade I goitre was present in 49 patients (16.3%) and grade II goitre was seen in 32 patients (10.7%). When this was correlated with antibody status, it was found that the majority of antibody-positive patients had no clinically evident goitre (149 out of 209 patients, 68.0%). A further 34 patients (16.3%) had grade 1 goitre, while the other 26 patients (12.4%) had grade 2 goitre (Table 2).

| Goitre grade | Antibody positive | Antibody negative | Total |
|---|---|---|---|
| Grade 0 | 149 | 70 | 219 |
| Grade 1 | 34 | 15 | 49 |
| Grade 2 | 26 | 6 | 32 |
| **Total** | **209** | **91** | **300** |

***Table 2: Correlation of Goitre Grade with Antibody Status***

The prevalence of antibody positivity was 68.0% among those without clinically evident goitre (149 patients out of 219), 69.4% among patients with grade I goitre (34/49) and 81.3% among patients with grade II goitre (26 out of 32 patients). However, this difference was not found to be significant.

**DISCUSSION:** Elevated titres of Anti-TPO antibodies and anti-thyroglobulin antibodies (ATG) are the diagnostic hallmarks of Hashimoto's thyroiditis. The prevalence of these auto-antibodies in various countries has been reported to be 7-20%. Significant titres of AMA and ATG are seen in 95-100% of autoimmune hypothyroidism and 80-90% of Graves' hyperthyroidism. In addition, about 10-15% of patients with multinodular goitre and 30-40% of patients with thyroid cancer are positive for these antibodies. When they are present without thyroid dysfunction, the positive predictive value of future thyroid dysfunction is 40-60%.[18,19]

The status of anti-TPO antibodies is indicative of disease activity in autoimmune thyroid disease. ATG, however, is not associated with disease activity either in clinical terms or in pathological terms. It has also been seen that among the general, apparently healthy adults, 6 to 8% are auto-antibody positive.

Anti-TPO antibodies cause damage to the thyroid epithelial cells by means of complement activation and antibody-mediated cytotoxicity.[20] High titres are often found in the active phase of the disease. Various other factors like age, gender, stress, infections, trauma and smoking influence disease activity.[21-25] The preponderance of autoimmunity among women indicates hormonal influence.[26] In recent times, there have been many reports of the role of iodine as a key influence on the pathology and clinical manifestation of autoimmune thyroiditis.[27]

In the present study, it was found that the prevalence of antibody-positivity was slightly higher among women, although the difference was not significant. When the patients were categorised on the basis of age, the prevalence of anti-TPO was found to be significantly more in the third and fourth decades of life, when the hormonal cycle is most active. In recent times, there has been a steady rise in the levels of stress in the lives of people across the world as well as among the population that was studied. An objective evaluation of stress and correlation of antibody status with this aspect will be worthwhile.

In the present study, patients with AMA titres higher than 50 IU/L were considered as antibody-positive. Among them, more than three-fourths of them had titres more than twice the upper limit of normal. While it has been widely reported that higher antibody levels correspond to active disease, it was not found so in the present study. The majority of antibody-positive patients were clinically euthyroid.

Vaseghani, et al. have concluded that anti-TPO titres correspond to TSH titres.[28] Zois, et al. correlated hypothyroidism and antibody status in a population of school children from northern Greece. Iodine deficiency has been eliminated in this population. They found in their study that the prevalence of antibody-positivity among patients with sub-clinical hypothyroidism was 100%.[29] A significant correlation between S. TSH or S. $FT_4$ concentration and elevated levels of S. AMA was demonstrated by Ghoraishian, et al.[30] Zimmermann, et al. observed that iodine supplementation in the deficient state was not significant in the induction of auto-immunity.[31]

With regards to correlation of antibody status with goitre grade, it was found in the present study that the prevalence of antibody positivity was 68.0% among those without clinically evident goitre, 69.4% among patients with grade I goitre and 81.3% among patients with grade II goitre. Among the antibody-positive patients, 73.0% had no visible or palpable goitre, 16.3% had grade I goitre and 16.3% had grade II goitre. Clinical assessment alone was employed in the evaluation of goitre. Ultrasound of the neck would reveal the presence of clinically occult nodules or thyroid enlargement. Association of autoimmune thyroiditis with thyroid malignancy, including papillary thyroid carcinoma and thyroid lymphoma, has been reported. So ultrasound evaluation of clinically occult nodules and goitre is significant in the long-term follow-up of patients.

In the present study, it was observed that hypothyroidism (elevated S. TSH) had a significant positive correlation ($r = 0.324$, $p = 0.003$) with antibody-positivity (elevated S. AMA). Even among those who were biochemically euthyroid, the majority were antibody-positive.

The various symptoms of thyroid disease were also documented, tabulated and analysed. The focus of this study was on the estimation of thyroid autoimmunity as a problem. Correlation of antibody status with clinical features alone, without regard to the biochemical thyroid status, is not relevant for this study. However, the significance of autoimmunity in the clinical syndrome of hypothyroidism assumes relevance when one realises that autoimmunity is a significant precursor of hypothyroidism. In that respect, follow-up of this subset of the population will be helpful. Patients who are positive for antibodies at present while being clinically euthyroid develop clinically overt hypothyroidism later. Time will tell as to how many of these patients who are euthyroid but antibody-positive will develop pathologically and/or clinically significant autoimmune thyroiditis.

Correlation of iodine supplementation with autoimmune thyroid disease will be reliable if supplemented and non-supplemented populations can be compared in this regard. This is not possible in this country at present, since marketing, distribution and consumption of uniodised salt is illegal.

**CONCLUSION:** Autoimmunity to thyroid, evident as elevated titres of anti-TPO antibody, is very common in the population studied. While the overall prevalence of anti-TPO positivity was 69.7% (209 patients out of a total of 300), it was maximum in the third decade of life, between ages 21 and 30 (75.9%, 75 of 99 patients). Among those who were antibody-positive, the majority of the subjects were euthyroid (146 out of 209, 69.9%). 56 patients had clinically and/or biochemically evident hypothyroidism (56 out of 209, 26.8%) and a further 7 patients (3.3%) were hyperthyroid. All patients are at present on regular follow-up. Long-term follow-up will reveal the extent to which antibody-positivity will result in pathologically and/or clinically significant thyroid disease.

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