

**Chapter 3. Autoimmunity and
Hypothyroidism: Anti-TPO
antibodies in Hypothyroid
Patients in Gujarat
Population**

I. INTRODUCTION

Hypothyroidism is an endocrine disorder characterized by decreased activity of thyroid gland leading to insufficient production of thyroid hormones. Subclinical or asymptomatic hypothyroidism is characterized by elevated thyrotropin level and normal serum thyroid hormones level. Whereas, there remains elevated thyrotropin but decreased thyroid hormones serum levels in case of overt or clinical hypothyroidism (1) (2).

In India, hypothyroidism used to usually be categorized under the iodine deficient disorders and represented based on total goiter rate. Government of India has adopted the universal salt iodization program and since then there has been a decline in goiter prevalence in various parts of the country (3-7). As per World health organization (WHO) assessment report India has undergone transition from iodine deficient state to iodine sufficient state (8-10). A large, cross-sectional, comprehensive study recently carried out in adult population across the country, indicates about 10.9% prevalence of hypothyroidism (11); whereas, the prevalence of hypothyroidism in the developed countries is about 4-5% (12, 13). This indicates even though most of the regions of India have been made iodine sufficient there is still high prevalence of hypothyroidism. Hence, underlying pathogenesis may involve a complex interplay of genetic, environmental and endogenous factors and not only iodine deficiency. Clinical investigation of patients in India does not include evaluation of thyroid autoantibodies and hence iodine deficiency is believed to be the sole candidate for hypothyroidism pathogenesis which may not be the case.

Autoimmune hypothyroidism is characterized by gradual destruction of the thyroid gland due to loss of thyroid cells, leading to thyroid hormone deficiency. The immunological features of this disorder include the presence of anti-thyroidperoxidase (anti-TPO) antibodies and, less commonly, anti-thyroglobulin (anti-TG) antibodies, abnormalities in the circulating T cell population and a goiter with lymphocytic infiltration (14, 15). Thyroid peroxidase (TPO) is a heme containing microsomal membrane glycoprotein (molecular weight- 105KDa) that catalyzes iodination and coupling of tyrosine residues for production of thyroid hormones in the thyroid gland (16). It is also a major thyroid autoantigen that elicits the production of autoantibodies found in the serum of many patients with autoimmune thyroid disease as well as other autoimmune diseases (17). Inhibition of TPO due to persistent autoantibody (anti-

TPO) leads to decreased synthesis of thyroid hormones leading to autoimmune type of hypothyroidism also known as hashimoto's thyroiditis. TG is a glycoprotein homodimer produced predominantly by the thyroid gland. It acts as a substrate for the synthesis of thyroxine and triiodothyronine as well as the storage of the inactive forms of thyroid hormone and iodine. TG is secreted from the endoplasmic reticulum to its site of iodination, and subsequent thyroxine biosynthesis, in the follicular lumen occurs. Presence of anti-TPO antibodies in blood circulation has been used for clinical diagnosis of this disease in USA, Europe and many other countries but not in India. Hypothyroidism diagnosis is limited to determination of thyroid hormone and thyrotropin serum levels and evaluation of autoimmune antibodies in patients is not currently practiced in India. We therefore investigated the presence of anti-TPO antibodies in patients with hypothyroidism. The anti-TPO antibody levels were also analyzed based on the age at onset of disease as well as gender of the patients.

II. SUBJECTS AND METHODS

A. Subjects

The study plan was approved by 'Institutional Ethics Committee for Human Research (IECHR), Faculty of Medicine, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India, Ethics Committee Reg. No. ECR/85/Inst/GJ/2013 for IECHR submission no: 44/2013 dated 28th Sep 2013. The importance of the study was explained to all participants and written consent was obtained from all patients and controls (Consent form as Pro forma available in appendix). The study group included 84 hypothyroidism patients comprised of 78 females and 6 males who referred to S.S.G. Hospital, Vadodara. The diagnosis of hypothyroidism was based on thyroid profile analysis (serum T₃, T₄ and TSH levels) and patients had no other associated autoimmune diseases. A total of 62 ethnically and sex-matched unaffected individuals were included as controls in this study. The control group comprised 55 females and 7 males (Table 3.1). None of the healthy individuals had any evidence of hypothyroidism and any other diseases.

Table 3.1 Demographic characteristics of hypothyroidism patients and controls.

	Hypothyroidism Patients (n =84)		Anti-TPO positive Hypothyroidism Patients (n =50)		Controls (n=62)	
	Female (78)	Male(6)	Female (44)	Male (6)	Female (55)	Male (7)
Average age (years, Mean age ± SD)	43.25 ± 15.16	64.00 ± 09.17	43.40 ± 14.01	64.00 ± 9.17	41.27 ± 15.27	58.33 ± 2.08
Onset Age (years, Mean age ± SD)	38.72 ± 13.86	61.33 ± 9.03	38.5 ± 12.25	61.33 ± 9.03	NA	NA
Duration of disease (years, Mean age ± SD)	4.53 ± 3.71	2.67 ± 0.67	4.91 ± 3.98	2.67 ± 0.67	NA	NA

B. Estimation of anti-Thyroid Peroxidase (anti-TPO) antibodies levels

Plasma from hypothyroidism patients was examined to find the levels of anti-TPO antibodies compared to controls. Plasma samples of 84 hypothyroidism patients and 62 controls were analyzed for the presence of anti-TPO antibodies by Enzyme linked immunosorbent assay (ELISA). Approximately, 5 ml venous blood was collected from the patients and healthy subjects in K3EDTA coated vacutainers (BD, Franklin Lakes, NJ 07417, USA) and plasma was extracted. Presence of anti-TPO antibodies were assessed by ELISA method as per manufacturer’s protocol (Genway Biotech, Inc. San Diego, CA). Absorbance of all wells was measured at 450nm using 620nm as reference wavelength.

Anti-TPO antibody levels were plotted and analyzed by unpaired t-test and ANOVA using Prism 5 software (Graphpad software Inc; San Diego CA, USA, 2007). *P-values* less than 0.05 were considered as statistically significant.

III. RESULTS AND DISCUSSION

A. Anti-TPO antibody levels in hypothyroidism patients and controls

It was found that 59.52% (n=50) of total hypothyroidism patients (n=84) had anti-TPO antibodies in their circulation suggesting that autoimmunity may play an important role in the pathogenesis of the disease; moreover, these autoimmune hypothyroidism patients had significantly increased anti-TPO antibody levels as compared to controls ($p < 0.0001$) (Figure 3.1). From table 3.1 it is evident that hypothyroidism and autoimmune hypothyroidism are commoner in females (n=78 and n=44, respectively) than in male (n=6 and n=6, respectively). Furthermore, there is clear evidence from the data that females in age group 21-40 years (n= 43 and n=25) are more susceptible than in age groups 41-60 years (n= 27 and n=15) and >60 years (n= 8 and n=4) for hypothyroidism and autoimmune hypothyroidism respectively. However, all male hypothyroidism patients (n=6) were found to have autoimmune hypothyroidism (n=6) and belonged to age group of >60 years.

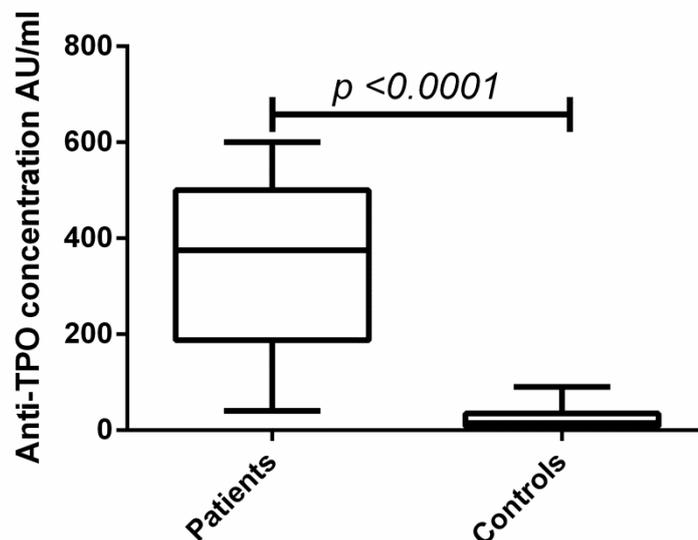


Figure 3.1 Anti-TPO antibody levels in autoimmune hypothyroidism patients and controls. Patients showed significantly higher levels of anti-TPO antibody as compared to controls (Mean \pm SEM: 333.3 \pm 24.06 N=50 for patients vs 22.97 \pm 2.925 N=62 for controls; $p < 0.0001$).

B. Anti-TPO antibody levels in autoimmune hypothyroidism patients and Gender biasness

Similarly, anti-TPO antibody levels in autoimmune hypothyroidism patients were also evaluated for gender biasness. No significant difference for anti-TPO levels in females and males were observed ($p > 0.05$) (Figure 3.2).

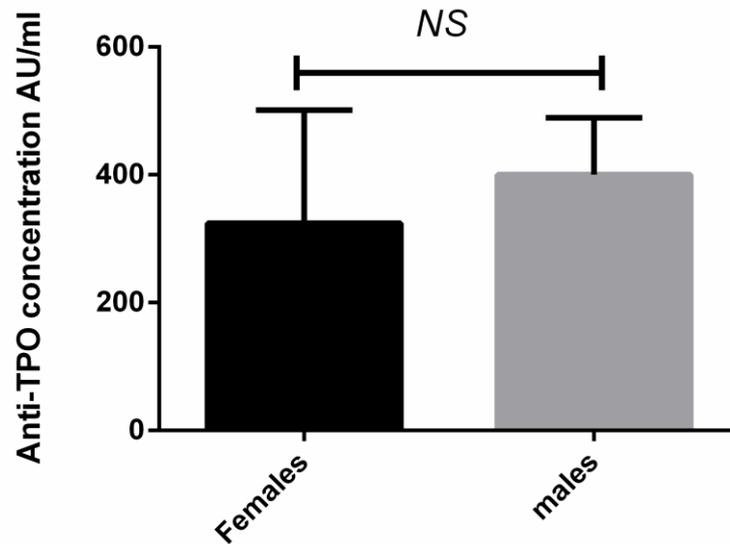


Figure 3.2 Anti-TPO antibody levels in autoimmune hypothyroidism patients based on gender. No significant difference was observed for anti-TPO antibody levels between females and males (Mean \pm SEM: 324.2 \pm 26.69 N=44 vs 400.0 \pm 36.51 N=6; $p = 0.3109$, NS: Non Significant).

C. Anti-TPO antibody levels in different age of onset groups of autoimmune hypothyroidism patients

When effect of onset age on anti-TPO antibody levels in autoimmune hypothyroidism was analyzed between different age groups, there was no significant difference between levels of anti-TPO antibodies in the circulation of female patients among different age groups 21-40 years, 41-60 years and 61-70 years (Figure 3.3).

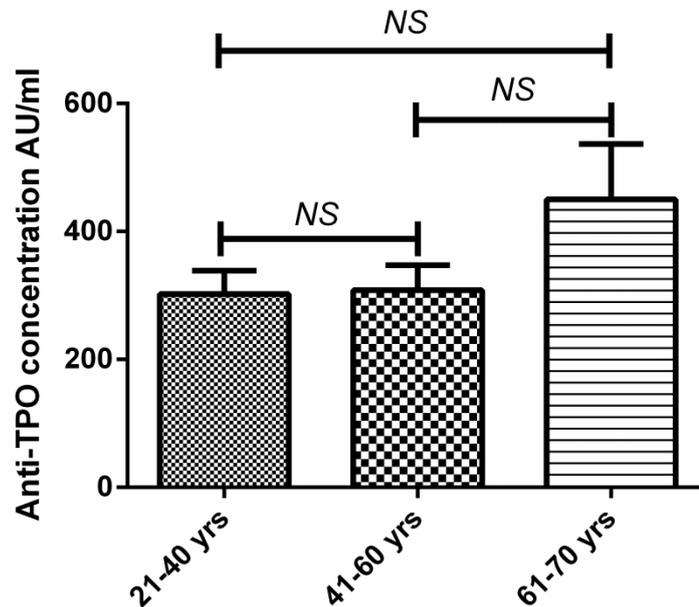


Figure 3.3 Anti-TPO antibody levels in autoimmune hypothyroidism females and onset of age. No significant difference was observed for anti-TPO antibody levels between females belonging to three different age groups, 21-40 yrs, 41-60 yrs and >60yrs with Mean \pm SEM: 302.0 \pm 36.73 N=25, 308.0 \pm 39.38 N=15 and 450.0 \pm 150.0 N=4 respectively.

In Summary, the present study showed low iodine intake is not the sole etiological candidate for hypothyroidism disorder in India as approximately half of the hypothyroidism patients included in this study were found to have presence of anti-TPO antibodies (Table 3.1) (Prevalence of autoimmune hypothyroidism 59.52%). However, presence of this autoantibody is considered as consequences of autoimmune hypothyroidism and not the cause of this disease, but no clear evidence for the same is yet available (18). Also, in a recent study revealing prevalence of thyroid diseases in eight cities of India, presence of anti-TPO antibodies was shown to be conclusive for the disease (19); however, unlike our study, prevalence of autoimmune hypothyroidism from the study has not been reported.

No relationship between disease onset age of the patient and level of anti-TPO antibodies was found in present study, which is in accordance with the previous study (19). There is clear evidence from the data that females in age group 21-40 years are more susceptible to hypothyroidism and autoimmune hypothyroidism than any other age group. Hence, research in this field is directed to discover the underlying pathogenesis mechanism that can unfold the gender specific biasness of this disease but no success has been achieved till date.

IV. CONCLUSION

Our findings suggest that iodine deficiency is not the only etiological factor responsible for increased number of hypothyroidism cases in India. Autoimmunity as an etiological factor also has its role to play in pathogenesis of this disease leading to autoimmune hypothyroidism. Hence, to understand clinical features and therapeutic requirements of patients having different type of the hypothyroidism, *per se* iodine deficiency hypothyroidism or autoimmune hypothyroidism, it is necessary that, in India also, physician recommend performing clinical test for anti-TPO antibodies evaluation along with T3, T4 and TSH test.

V. REFERENCES

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