Anti-Thyroid Peroxidase Antibodies in Patients with Primary Hypothyroidism: A Cross-Sectional Study

Buddhi Raj Pokhrel[1]*, Binit Kumar Sharma[1], Archana Thapa Chettri[1], Kritika Chaudhary[1], Jharana Shrestha[1], Binaya Tamang[1], Narayan Gautam[1], Anup Shamsher Budhathoki[2]

Affiliations

[1]Universal College of Medical Sciences and Teaching Hospital, Rupandehi, Nepal

[2]National Medical College (former), Birgunj, Nepal

Correspondence to:
Dr. Buddhi Raj Pokhrel
Department of Biochemistry
Universal College of Medical Sciences and Teaching Hospital, Rupandehi, Nepal
Email: buddhi.pokhrel@gmail.com
Contact Number: +977- 9841406400

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ABSTRACT

Introduction

Primary hypothyroidism is a major health concern worldwide and in Nepal. Anti-thyroid peroxidase (anti-TPO) antibodies indicate autoimmune thyroid involvement and serve as a key marker for Hashimoto's thyroiditis, a leading cause in iodine-sufficient regions. Regional data on anti-TPO prevalence across different stages of hypothyroidism remain limited. This study aimed to determine the prevalence of anti-TPO positivity among hypothyroid patients in Western Nepal and explore its relationship with disease stage.

Methods

This hospital-based cross-sectional study was conducted at Universal College of Medical Sciences and Teaching Hospital, Lumbini Province, Nepal, from November 2022 to October 2023. A total of 170 adults with laboratory-confirmed primary hypothyroidism were enrolled. Patients with other thyroid or autoimmune disorders, pregnancy, or medications affecting thyroid function were excluded. Serum anti-TPO ≥ 30 IU/ml was considered positive. Hypothyroidism was classified as subclinical and overt. Data analysis included descriptive statistics, Mann-Whitney U test, chi-square analysis, and binary logistic regression, with $p < 0.05$ considered significant.

Results

Median age was 45 years, with 75.3% female. Subclinical hypothyroidism predominated (84.7%). The median anti-TPO level was 11.9 IU/ml, with 32.4% testing positive. Anti-TPO positivity was significantly higher in overt hypothyroid patients, with adjusted odds 3.9 times higher than subclinical cases (95% CI: 1.6 – 9.5; $p = 0.003$).

Conclusion

Anti-TPO antibodies were present in one-third of hypothyroid patients, more frequently in overt disease. While a useful supportive marker, longitudinal studies are needed to clarify its prognostic value and natural course in the Nepalese population.

Keywords

Anti-TPO; Hypothyroidism; Overt; Subclinical

Introduction

Primary hypothyroidism is a prevalent disorder worldwide and in South Asia, including Nepal. The commonest causes of primary hypothyroidism are reported to be iodine deficiency and Hashimoto's thyroiditis, an autoimmune disorder characterized by raised anti-TPO antibodies. The global prevalence of Hashimoto's thyroiditis, the commonest cause in iodine-sufficient areas, is reported to be 5% - 10% with some areas reporting <0.5% and others >20% as well.[1, 2, 3] Data from Central Nepal reported the prevalence of Hashimoto's thyroiditis to be 48.3% among total hypothyroid disorders.[4]

Anti-thyroid peroxidase (anti-TPO) antibodies are often evaluated in hypothyroid individuals to confirm autoimmune involvement.5 Despite this, regional data on its prevalence and its association with different stages of hypothyroidism remain scarce. Most evidence is derived from Western populations, which differ in iodine status, genetics, and environment. This study aims to estimate the prevalence of anti-TPO antibodies in hypothyroid patients in Western Nepal and their association with subclinical and overt hypothyroidism.

Methods

This hospital-based cross-sectional analytical study estimated anti-TPO levels in adult patients with laboratory-confirmed primary hypothyroidism in Universal College of Medical Sciences and Teaching Hospital (UCMS-TH), Lumbini Province, Nepal, from November 3, 2022, to October 2, 2023. Patients with other thyroid illnesses, such as hyperthyroidism, thyroid malignancy, peripheral and central hypothyroidism, patients with other autoimmune diseases such as type 1 diabetes mellitus, rheumatoid arthritis, and celiac diseases, pregnant patients, and those undertaking medications that could alter TFT results (e.g., lithium, amiodarone, systemic corticosteroids) were excluded. A total of 170 patients were included in the study via purposive sampling. The sample size was calculated by using the formula, $n = Z^2 PQ/e^2$, where Z = 1.96, P = 25.58 %^{2}, Q = 100–P, and e (allowable error) = 7%. The initial calculated sample size was 149.25 ≈ 150. Adding a 10% non-response rate, the calculated n was 165. Finally, a total of 170 primary hypothyroid patients were included in the study.

Ethical clearance for the study was obtained from the Institutional Review Committee (IRC) of UCMS-TH (UCMS/IRC/137/22). Both verbal and written informed consent were obtained from all patients before data collection. Data were collected using a structured proforma. This included patient ID, socio-demographic details, body mass index (BMI), smoking and alcohol history, comorbidities, and laboratory parameters (serum fT_3, fT_4, TSH, and anti-TPO antibodies). Anti-TPO antibody levels were measured using the same serum sample used for routine TFT parameters. The sample was analyzed immediately after confirming thyroid status and obtaining informed consent, using a chemiluminescent assay (Maglumi 2000). Primary hypothyroidism was defined as loss of thyroid function due to destruction of the thyroid gland, leading to increased TSH.[5] Primary hypothyroidism was classified as subclinical (elevated TSH with normal fT_4) and overt (elevated TSH with low fT_4) hypothyroidism.[5] The following reference ranges were used: fT_3: 2.0-4.2 pg/ml, fT_4: 8.9-17.2 pg/ml, and TSH: 0.3-4.5 μIU/ml. Anti-TPO positivity was defined as ≥30 IU/ml, per manufacturer's instructions.

Data were entered into Microsoft Excel and analyzed using SPSS version 20. The Shapiro-Wilk test of continuous variables showed significant deviation from normal distribution. Hence, the data were expressed as their median and inter-quartile range (IQR) values. Categorical data were expressed in both frequency and percentage. The Mann-Whitney test and Chi-square analysis were performed to compare numerical and categorical variables between the hypothyroid groups. Binary logistic regression was used to estimate the unadjusted and adjusted odds ratios (OR) for anti-TPO positivity with 95% CI. A two-sided p-value of <0.05 was considered statistically significant.

Results

The study included 170 participants with primary hypothyroidism. The median age was 45 years (IQR: 32.7-52.0), and the majority were

females (n =128; 75.3%). Most patients had subclinical hypothyroidism (n = 144; 84.7%), while 26 (15.3%) had overt hypothyroidism. The participants' socio-demographic, anthropometric, and clinical characteristics are shown in Table 1.

Table 2 presents the thyroid function test (TFT) parameters of the study participants. Serum TSH and anti-TPO levels were significantly higher in overt hypothyroid patients, whereas fT_3 and fT_4 levels were significantly lower.

Table 3 presents the unadjusted and adjusted odds of anti-TPO positivity in hypothyroid groups. The odds of anti-TPO positivity were significantly higher in patients with overt hypothyroidism compared to those with subclinical hypothyroidism, both in unadjusted (OR: 3.5; 95% CI: 1.5–8.4) and adjusted analyses (OR: 3.9; 95% CI: 1.6–9.5). The variables adjusted for are listed in the table.

Table 1: Distribution of sociodemographic, anthropometric and clinical parameters

		Total (n=170)	**Hypothyroid Group**		**P-value**
			Subclinical (n=144)	**Overt** (n = 26)	
Age (years)		45.0 (32.7–52.0)	45.5 (33.0–52.0)	43.5 (29.5–50.5)	0.566*
BMI (kg/m^2)		23.7 (20.7–27.2)	23.7 (20.4–27.6)	24.8 (21.8–27.9)	0.132*
Sex	Male	42 (24.7%)	37	5	0.482**
	Female	128 (75.3%)	107	21	
Religion	Hindu	148 (87.1%)	126	22	0.687**
	Non/Hindu[1]	22 (12.9%)	18	4	
Alcohol	Yes	11 (6.5%)	10	1	0.554**
	No	159 (93.5%)	134	25	
Smoking	Yes	12 (7.1%)	11	1	0.487**
	No	158 (92.9%)	133	25	
Comorbidities[2]	Yes	77 (45.3%)	70	7	0.041**
	No	93 (54.7%)	74	19	

Numerical data expressed in median (IQR). Categorical data expressed in n (%). *P-values from the Mann-Whitney test. ** P-values obtained from Chi-squared analysis. $P < 0.05$ was considered statistically significant.

[1]Non-Hindu: Muslim (n =19); Buddhist (n = 3).

[2]Co-morbidities: DM, hypertension, allergy, epilepsy, psychiatric illness, GI ulcer, and arthritis.

Table 2: Distribution of laboratory parameters in study participants

		Total (N=170)	Hypothyroid Group		P-value
			Subclinical (n=144)	Overt (n=26)	
fT_3 (pg/ml)		2.5 (2.1 – 3.1)	2.8 (2.2 – 3.2)	1.9 (1.7 – 2.0)	< 0.001
fT_4 (pg/ml)		7.4 (1.2 -10.8)	9.3 (1.2 – 11.4)	5.8 (1.0 – 8.1)	0.012
TSH (µIU/ml)		7.2 (5.8 – 11.6)	6.8 (5.6 – 9.3)	55.9 (19.7 – 100.0)	< 0.001
Anti-TPO (IU/ml)		11.9 (3.7 – 88.7)	10.4 (3.6 – 41.7)	85.8 (5.5 – 870.8)	0.017
Anti-TPO*	**Positive**	55 (32.4%)	40 (27.78%)	15 (57.69%)	0.005
	Negative	115 (67.6%)	104 (72.22%)	11 (42.31%)	

P-values of numerical lab parameters from the Mann-Whitney U test. *Anti-TPO < 30 was considered negative as per the manufacturer's instructions. Column percentage used. P-values from Chi-square analysis for categorical variables. P < 0.05 was considered statistically significant.

Table 3: Odds of anti-TPO positivity in hypothyroid groups (unadjusted and adjusted analyses)

		Anti TPO		Unadjusted		Adjusted	
		Positive (n=55)	Negative (n=115)	OR (95% CI)	P-value	OR (95% CI)	P-value
Hypothyroid group	Subclinical	40	104	Reference	0.004	Reference	0.003
	Overt	15	11	3.5 (1.5 – 8.4)		3.9 (1.6 – 9.5)	
Sex (Male)	No	38	90	Reference	0.197	Reference	0.100
	Yes	17	25	1.6 (0.8 – 3.3)		1.9 (0.9 – 4.4)	
Age (15 years)*	-			0.8 (0.6 – 1.2)	0.340	0.7 (0.5 – 1.2)	0.235
BMI (5 kg/m²)**	-			1.0 (0.6 – 1.6)	0.958	0.9 (0.7 – 1.2)	0.760
Comorbidities	No	33	60	Reference	0.338	Reference	0.937
	Yes	22	55	0.7 (0.4 -1.4)		0.9 (0.5 – 2.1)	
Alcohol	No	52	107	Reference	0.710	Reference	0.422
	Yes	3	8	0.8 (0.2 – 3.0)		0.5 (0.1 – 2.4)	
Smoking	No	50	108	Reference	0.477	Reference	0.317
	Yes	5	7	1.5 (0.5 -5.1)		1.9 (0.5 – 7.4)	

P-values and 95% CI from binary logistic regression. P < 0.05 was considered statistically significant.
*OR of age rescaled to 15 years. **OR of BMI rescaled to 5 kg/m².

Discussion

This study assessed anti-TPO levels and the prevalence of anti-TPO positivity among patients with primary hypothyroidism. The median anti-TPO level was 11.9 IU/mL, and approximately one-third of the patients (32.4%) were anti-TPO positive. Among patients with overt hypothyroidism, 57.69% were anti-TPO positive, which was significantly greater than the proportion observed in subclinical hypothyroidism (27.78%).

Previous studies from Nepal have reported considerable variability in the prevalence of anti-TPO positivity. Reports from central Nepal have documented higher prevalence rates in patients with primary hypothyroidism, ranging from 45% to 62.2%.[4,6,7] Anti-TPO positivity in both subclinical and overt hypothyroidism has also been reported to be higher than that observed in the present study.[6-9] Similarly, studies from western Nepal (Gandaki Province), have shown a wide range of anti-TPO positivity, from 38.3% to as high as 82%[10-11] These variations likely reflect not only regional differences but also methodological differences across studies, including laboratory techniques, assay platforms, and cut-off values used to define anti-TPO positivity. For example, one study from central Nepal reporting a prevalence of 62.2% employed an ELISA-based method with a different cut-off value (10 IU/ml) for anti-TPO positivity than that used in the present study (30 IU/ml).[7] International studies likewise demonstrate substantial variability in anti-TPO positivity, with reported prevalences generally higher than those observed in our cohort.[12,13] Differences in assay methods, cut-off thresholds, and geographic factors should therefore be carefully considered when interpreting and comparing these findings.

In our study, anti-TPO levels were significantly higher in patients with overt hypothyroidism compared with those with subclinical hypothyroidism. After adjusting for sex, age, BMI, alcohol consumption, and smoking, the odds of anti-TPO positivity were nearly fourfold higher (OR 3.9) in the overt hypothyroid group. However, this association has not been consistently demonstrated in earlier studies. Marasini et al. reported no significant difference in median anti-TPO titres between subclinical and overt hypothyroidism.[9] Similarly, a study from Western Nepal showed no significant association of anti-TPO positivity between subclinical and overt hypothyroidism.[10] A study from Central Nepal, however, showed a significant association of anti-TPO positivity with overt hypothyroidism, a finding concordant with our results.[6] Differences in cut-off values may partly explain these discrepancies. The latter study used a cut-off of 34 IU/mL, whereas the earlier study applied a much lower threshold of 9 IU/mL, which may have inflated anti-TPO positivity in the subclinical group. Additionally, subclinical hypothyroidism is not a uniform clinical entity but encompasses early autoimmune thyroiditis, transient thyroid dysfunction, iodine-related changes, and non-autoimmune causes. Cross-sectional studies, including ours, may therefore capture patients at different points along this continuum, resulting in variable patterns of anti-TPO positivity.

In our study, anti-TPO positivity was more frequent in patients with overt hypothyroidism. However, this observation requires cautious interpretation. Elevated anti-TPO levels should not be equated directly with greater disease severity or regarded as evidence of progression from subclinical to overt hypothyroidism. Anti-TPO antibodies are known to be present in healthy euthyroid individuals, patients with other autoimmune conditions, including autoimmune thyroid diseases (AITDs), and in pregnant women.[5, 14] Studies have suggested that anti-TPO antibodies in Hashimoto's thyroiditis may serve primarily as markers of immune activation rather than acting as direct pathogenetic effectors [5, 15] In this context, the higher anti-TPO levels and positivity observed in the overt hypothyroid group in our study likely reflect more sustained or established autoimmune activity rather than an antibody-driven disease process. This interpretation is further supported by the observation that more than 40% of patients with overt hypothyroidism in our cohort were anti-TPO negative. Although iodine status was not assessed in this study, the widespread implementation and success of the universal salt iodization (USI) program in Nepal, along with prior evaluations of urinary iodine status in Western Nepal, suggest that iodine deficiency is less likely to be a major contributing factor in this population.[16, 17] At the same time, other studies have reported that thyroid

autoantibodies may independently predict the future development of both hypothyroidism and hyperthyroidism.[18] Studies have also shown that anti-TPO levels are positively associated with pro-inflammatory markers and negatively associated with health-related quality of life (HRQoL). [19,20] These associations, however, remain largely observational and are difficult to translate into a clear biological or clinical distinction between subclinical and overt hypothyroidism. Taken together, anti-TPO positivity appears to reflect the presence and persistence of autoimmune thyroid activity rather than the degree of thyroid dysfunction itself. This limits its usefulness in routine practice for reliably differentiating between subclinical and overt hypothyroidism. Therefore, until more definitive evidence emerges, anti-TPO positivity should be considered a supportive marker rather than a standalone tool for assessing disease stage or severity.

There are several limitations to this study. Its cross-sectional design, small number of overt hypothyroid patients, single-center setting, and lack of iodine assessment limit the ability to draw causal conclusions and may affect generalizability. Larger, multicenter, longitudinal studies are needed to improve generalizability, clarify causal relationships, and better understand the clinical significance and natural course of anti-TPO positivity.

Conclusions

The majority of the patients were subclinical hypothyroid. Anti-TPO antibodies were present in 32.4%. The odds of anti-TPO positivity were significantly higher in the overt hypothyroid group than in subclinical hypothyroid patients.

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Conflict Of Interest

The authors declare no competing interests related to this study.

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