# Serum Thyroglobulin as a Marker for Differential Diagnosis of Hyperthyroidism

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## ABSTRACT

**Objective:** To evaluate the role of serum thyroglobulin (TG) as a biochemical marker for differential diagnosis of common aetiologies of hyperthyroidism.

**Study Design:** Comparative cross-sectional study.

**Place and Duration of the Study:** Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from October 2023 to March 2024.

**Methodology:** One hundred and forty-eight patients with clinical or biochemical hyperthyroidism were enrolled in the study. Samples were taken for serum thyroglobulin and evaluated for different causes of hyperthyroidism as demonstrated on the thyroid scan. Serum TG levels were measured using a chemiluminescence immunoassay and levels >42 ng/ml were considered elevated. Relationship between TG levels and different causes of hyperthyroidism was determined *via* independent samples non-parametric Kruskal-Wallis test.

**Results:** The study found that 76.3% (n = 113) of patients had raised TG levels, with significant differences in median TG levels observed among the different hyperthyroidism aetiologies. Thyroiditis exhibited the highest median TG levels (202.5 ng/ml), followed by toxic adenoma (139 ng/ml) and toxic multinodular goitre (102 ng/ml), while Graves' disease had the lowest levels (34 ng/ml). Sensitivity of thyroglobulin to detect thyroid disease was 76% while specificity came out to be 100%. Positive predictive value (PPV) was 100% while negative predictive value (NPV) was 20.45%.

**Conclusion:** Serum TG levels vary markedly among different aetiologies of hyperthyroidism with the highest levels in thyroiditis and the lowest in Graves' disease.

**Key Words:** *Thyroglobulin, Hyperthyroidism, Graves' disease, Thyroiditis*.

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## INTRODUCTION

Hyperthyroidism is a state of overproduction of thyroid hormone in the body. Toxic multinodular goitre (TMNG), autoimmune aetiology (Graves' disease, GD), and toxic thyroid adenoma (TA) are the most common causes of hyperthyroidism. Subacute thyroiditis is a transient thyrotoxic state characterised by following an upper respiratory viral illness. Some other forms of thyroiditis that cause transient hyperthyroidism include Hashimoto chronic thyroiditis and postpartum thyroiditis. Factitious hyperthyroidism and drug-induced thyroiditis are among less common causes.[1] Prevalence of overt hyperthyroidism in Pakistan is 6.7% while that of subclinical hyperthyroidism is 12% which suggests a large disease burden.[2]

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Panel of tests are used to differentiate between different causes of hyperthyroidism, as it is crucial to know the aetiology for targeted treatment. These include the use of autoantibodies, for example, thyroid stimulating hormone receptor antibody (TRAb)[3] and anti-thyroid peroxidase antibodies, ultrasound of the thyroid gland, and radioactive uptake scan ($I^{123}$ or $Tc^{99}$). Thyroid scan, scintigraphic characteristics, and thyroid uptake value diagnose the cause of hyperthyroidism effectively. Graves' disease or a toxic nodular goitre is often associated with elevated uptake value and diffusely enlarged thyroid or localised nodules, while subacute thyroiditis is associated with reduced uptake value and poor thyroid gland visibility.[4] However, these tests are expensive and expose patients to radiation hazards. They also need trained personnels and advanced testing facilities.

Thyroglobulin (TG) is a large protein produced by thyroid follicular cells and stored in the follicular lumen. It is crucial for thyroid hormone synthesis. Currently, it is used as a tumour marker for monitoring and detecting the recurrence of differentiated thyroid carcinoma (DTC).[5] However, different amounts are released from thyroid tissue in other conditions which involve

stimulation of the thyroid gland by autoantibodies, destructive inflammation or disordered growth of the gland. This variability may be used to differentiate between various benign conditions. The purpose of this study was to evaluate serum TG as an effective biochemical marker for the differential diagnosis of hyperthyroidism in the Pakistani population.

## METHODOLOGY

This was a comparative cross-sectional study, conducted at the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, over a period of six months from October 2023 to March 2024 after approval from the Institutional Review Board and the Ethical Committee (IRB No: MP-CHP22-10/READ-IRB/23/1740). The sampling technique used was non-probability consecutive sampling. The sample size was calculated using the WHO sample size calculator keeping the confidence interval at 95%, the margin of error at 5%, and the probability from previous studies at 6.7.[2] A total of 148 patients with clinical or biochemical hyperthyroidism under investigation for the aetiological diagnosis who presented to Nuclear Medicine Centre (NMC) AFIP for $Tc^{99}$ scan were included in the study.

Patients of both genders and all ages were included in the study. Patients who were on medication affecting thyroid function tests (especially amiodarone and lithium) were excluded from this study as these medicines affect the metabolism of thyroid hormones. Critically ill patients and those with thyroid malignancy were also excluded.

Informed consent was obtained from all patients. History was taken according to a self-designed proforma, which included patient demographic data, signs and symptoms of hyperthyroidism, medical and surgical history, medication history, family history of thyroid disease, and smoking history. Hyperthyroidism was confirmed *via* hormonal profile (raised triiodothyronine and tetraiodothyronine, suppressed TSH). Based on the $Tc^{99}$ scan, they were categorised into four major groups: Graves' disease, toxic adenoma, thyroiditis, and toxic multinodular goitre. Sample was taken for serum thyroglobulin (TG) in plain tube, centrifuged at 3,500 rpm for five minutes, and analysed on a chemiluminescence immunoassay analyser. TG levels >42 ng/ml were considered to be raised.

The data were analysed using Statistical Package for Social Sciences (SPSS) version 23. Test of normality (Kolmogorov-Smirnov) indicated that the data were non-parametric. Medians and interquartile ranges were calculated for the quantitative variable (serum thyroglobulin) and frequencies and percentages were determined for qualitative variables (age, gender, family history of thyroid disease, smoking history, clinical symptoms of hyperthyroidism, and presence of goitre). Mann-Whitney U test and Chi-square tests were performed to compare the variables between different groups. The non- parametric Kruskal-Wallis test was applied to identify significant differences in TG levels across the four groups, with pairwise comparisons conducted using the Dunn test. A p-value of $<0.05$ was considered statistically significant. Sensitivity and specificity were calculated using a 2 by 2 contingency table for serum thyroglobulin as a diagnostic biomarker for hyperthyroidism.

## RESULTS

Seventy-eight (52.7%) females and 70 (47.3%) males participated in the study. The mean age of participants was 48.9 years. A total of 29.1% had a family history of thyroid disorders. Out of the total 148 patients, 113 (76.3%) had raised TG levels. The median TG was higher in patients with a family history of thyroid disease and in those with clinical symptoms of hyperthyroidism ($p = 0.002$ and 0.02, respectively). However, the distribution of serum TG was the same across both genders, ages, and smoking status. The frequency of elevated TG was higher in patients aged over 40 years, those with a family history, and those with clinical symptoms compared to their counterparts. The highest levels of TG were observed in patients with thyroiditis (median 202.5 ng/ml), while Graves' disease had the lowest levels (median 34). Table I shows the medians, interquartile ranges, and p-values for TG levels across various categories of hyperthyroidism. The differences in TG levels among the four groups were statistically significant ($p < 0.05$).

Figure 1 shows the box plot representation of TG medians and interquartile ranges in various groups. Table II shows pairwise comparisons of TG levels among the 4 groups and the normal study on thyroid scans. Significant differences (p-value $<0.05$) were observed between the normal study and MNG, the normal study and toxic adenoma, the normal study and thyroiditis, Graves' disease and MNG, Graves' disease and toxic adenoma, and Graves' disease and thyroiditis. No significant differences were noted between Graves' disease and normal patients or between all thyroiditis and nodular diseases.

Figure 2 shows receiver operating characteristic (ROC) curves for the four categories of hyperthyroidism. The area under the curve (AUC) for thyroiditis, toxic adenoma, toxic MNG and Graves' disease was 0.73, 0.66, 0.55, and 0.16, respectively. The sensitivity of serum TG for detecting hyperthyroidism (all causes) was 76.3% and the specificity was 100%. The positive predictive value was 100%, while the negative predictive value was 20.45%.

## DISCUSSION

Serum thyroglobulin (TG) is a well-established tumour marker used to indicate residual or recurrent thyroid cancer and the presence of functioning thyroid tissue.[6] This study adds to this body of knowledge by demonstrating the utility of serum TG as a biomarker for the differential diagnosis of hyperthyroidism. It shows that serum TG can effectively distinguish between various causes of hyperthyroidism, such as thyroiditis, Graves' disease, toxic adenoma, and toxic multinodular goitre. The results align with the established understanding that elevated serum TG levels indicate thyroid gland stimulation or damage.

Significant differences in median TG levels were observed across different hyperthyroidism aetiologies. Patients with thyroiditis demonstrated the highest median TG levels (202.5 ng/mL), likely due to destructive inflammation releasing large amounts of TG from damaged thyroid follicles.

**Table I: Spectrum of serum thyroglobulin values in various causes of hyperthyroidism.**

| Thyroid scan | Median (ng/ml) | Serum Thyroglobulin | | | | Raised TG Count (Percent) | Normal TG Count (Percent) | p-value** |
|---|---|---|---|---|---|---|---|---|
| | | 25 Percentile | 75 Percentile | IQR | p-value* | | | |
| Toxic multinodular goitre | 102 | 59 | 180 | 121 | <0.001 | 43 (89.6%) | 5 (10.4%) | <0.001 |
| Graves' disease | 34 | 15 | 65 | 50 | | 13 (44.8%) | 16 (55.2%) | |
| Thyroiditis | 202.5 | 105 | 272 | 167 | | 32 (88.9%) | 4 (11.1%) | |
| Toxic adenoma | 139 | 97 | 244 | 147 | | 25 (96.2%) | 1 (3.8%) | |

**calculated via Kruskal-Wallis test. **calculated via Pearson's Chi-Square test.*

**Table II: Comparison of thyroglobulin levels in various groups.**

| Group 1 - Group 2 | Standard error | Standard test statistics | p-value* |
|---|---|---|---|
| Normal study - Graves' disease | 16.33 | 1.22 | >0.99 |
| Normal study - Toxic multinodular goitre | 15.55 | 4.23 | <0.001 |
| Normal study - Toxic adenoma | 16.56 | 4.82 | <0.001 |
| Normal study -Thyroiditis | 15.95 | 5.40 | <0.001 |
| Graves' disease - Toxic multinodular goitre | 10.07 | 4.55 | <0.001 |
| Graves' disease - Toxic adenoma | 11.56 | -5.17 | <0.001 |
| Graves' disease - Thyroiditis | 10.68 | -6.19 | <0.001 |
| Toxic multinodular goitre - Toxic adenoma | 10.42 | -1.34 | >0.99 |
| Toxic multinodular goitre - Thyroiditis | 9.44 | -2.15 | 0.315 |
| Toxic adenoma - Thyroiditis | 11.02 | 0.56 | >0.99 |

**Calculated via Dunn test.*

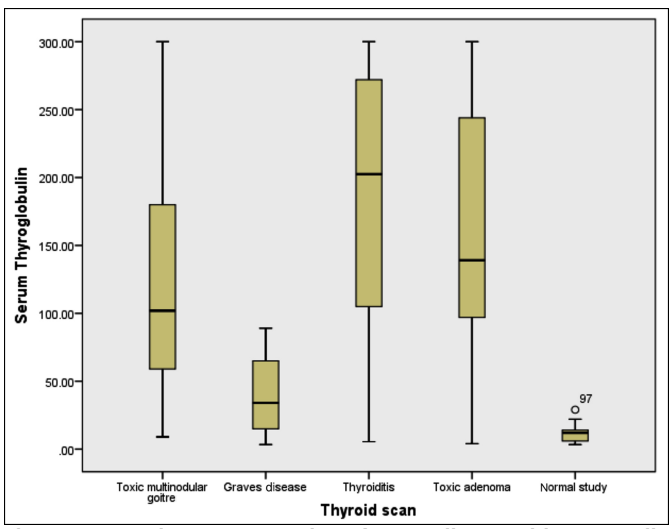


**Figure 1: Box plot representation of TG medians and interquartile ranges in various groups.**

Toxic adenoma and toxic multinodular goitre also showed elevated levels, but to a lesser extent than thyroiditis, indicating increased thyroid activity due to nodular hyperfunction. Graves' disease had the lowest median TG levels (34 ng/mL), reflecting uniform overactivity of the thyroid gland rather than localised damage.

These findings are consistent with the mechanism of TG release, where destructive processes/ inflammation (as in thyroiditis) result in higher TG levels compared to hyperfunctioning nodules or diffuse stimulation (as in toxic adenoma and Graves' disease). It is often a diagnostic challenge for physicians to differentiate between Graves' disease and thyroiditis based on thyroid scans, as both show patchy diffuse uptake. Uptake may also be increased or decreased based on the stage of thyroiditis. The large difference between the median TG levels in Graves' disease and thyroiditis suggests that serum TG is a useful marker to differentiate between these two conditions.

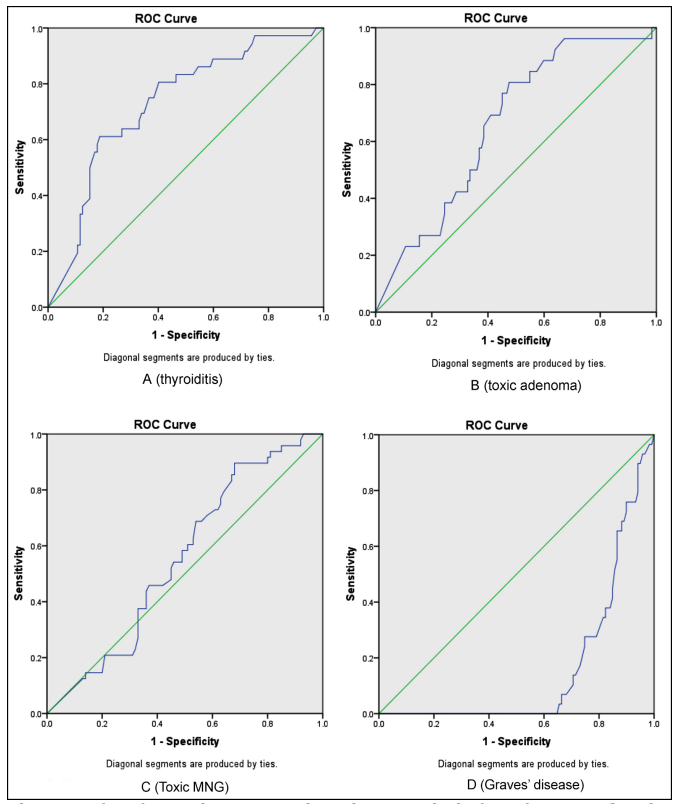


**Figure 2: (A-D) Receiver operating characteristic (ROC) curves for the four categories of hyperthyroidism. The area under the curve (AUC) for thyroiditis, toxic adenoma, toxic MNG, and Graves' disease were 0.73, 0.66, 0.55, and 0.16, respectively.**

Previous studies, such as that by Faruque *et al.* reported similar results, with the highest TG levels in subacute thyroiditis (median 116 ng/ml), followed by toxic nodular goitre (median 56.3 ng/ml) while Graves' disease had the lowest median TG levels.[7] Their results showed that serum TG was higher in patients over 40 years of age. However, this study found that age had no effect on TG levels, nor did gender. Another study by Pacini *et al*. showed similar

findings, with the highest median TG in subacute thyroiditis, followed by MNG and toxic adenoma.[8]

In contrast to the results of this study, Ramanathan *et al.* found the highest median TG levels in patients with toxic multinodular goitre (356 ng/ml), followed by Graves' disease (150.40 ng/ml), and the lowest in subacute thyroiditis (7.42 ng/ml).[9] This discrepancy may be due to the progression of thyroiditis, where TG levels are high in the early stages due to the release of preformed TG but lower in the later stages due to diminished thyroid gland function.

Hidaka *et al.* suggested that significantly higher TG levels in postpartum thyroiditis (PPT) could differentiate it from postpartum Graves' disease when thyroid-stimulating immunoglobulin (TSI) measurement is unavailable and RAIU is contraindicated.[10] According to their suggestion, measuring TG serially can help identify the thyrotoxicosis background since women with PPT have much higher levels than they had prior to the disease's onset.

Baseline TG was found to be considerably greater in individuals with Graves' ophthalmopathy (GO) than in those without it, Khamisi *et al.* conjectured that TG could be a warning indicator of GO.[11] The authors proposed that the level of stressor affecting the thyroid and retro-orbital tissues may be represented by the amount of circulating TG. Another interesting utility of TG appeared in a study by Bonefacic *et al.* when patients with MNG and toxic adenoma were evaluated for serum TG before and after radioiodine therapy.[12] A significant decline in levels was observed one year after therapy, suggesting that TG can be used not only for diagnosis but also as a prognostic marker in thyroid disorders.

Antithyroglobulin antibodies (TGAb) pose interference in immunometric TG assays, which can produce falsely low TG results.[13] Heterophile antibodies present in some individuals can overestimate serum TG levels.[14] This forms a limitation of this study which could affect the diagnostic performance of TG. This limitation is not of major significance because TGAb are present more frequently in cases of differentiated thyroid carcinoma (25% of all patients) and less often in other disorders.[15] Future studies should address these limitations to enhance the reliability of serum TG as a diagnostic marker for hyperthyroidism. Larger sample sizes and multi-centred trials may help validate TG's diagnostic performance in thyroid disorders.

## CONCLUSION

This study highlights the effectiveness of serum thyroglobulin as a chemical biomarker for diagnosing the cause of hyperthyroidism. Elevated TG levels vary significantly across different thyroid conditions, with thyroiditis showing the highest and Graves' disease the lowest. These differences emphasise TG's diagnostic value in distinguishing between the causes of hyperthyroidism.

**ETHICAL APPROVAL:**
The protocol of this research was approved by the Institutional Review Board and Ethical Committee of the Armed Forces Institute of Pathology, Rawalpindi (IRB no. MP-CH-P22-10/READ-IRB/23/1740).

**PATIENTS' CONSENT:**
Informed written consent was obtained from all the patients.

**COMPETING INTEREST:**
The authors declared no conflict of interest.

**AUTHORS' CONTRIBUTION:**
FJQ: Data collection, processing, analysis, literature search, and manuscript writing.
MA: Project conception and design, interpretation of results, and statistical analysis.
MQAK: Study design, critical review, and final approval of manuscript.
MY: Project development, drafting of the manuscript, and interpretation of data.
ZSD: Acquisition of data, processing, and literature review.
SS: Data review, critical analysis, and interpretation of results.
All authors approved the final version of the manuscript to be published.

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