ORIGINAL RESEARCH PAPER

Clinical Biochemistry

EFFECT OF HEMOLYSIS ON THE LEVELS OF TOTAL T3, TOTAL T4 &TSH : A CLINICAL CHEMISTRY PERSPECTIVE

KEY WORDS:

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ABSTRACT

Background: Thyroid function tests—including thyroid-stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4)—are essential in the evaluation of thyroid disorders. Hemolysis, a common pre-analytical error in clinical laboratories, can interfere with laboratory results through release of intracellular contents and assay disruption. Although its impact on several biochemical parameters is well-documented, limited data exist on the effect of hemolysis on thyroid hormone measurements. **Objective:** To evaluate the effect of hemolysis on thyroid function tests by comparing results from non-hemolyzed and hemolyzed serum samples derived from the same individuals. **Methods:** Venous blood samples were collected from 60 healthy adult volunteers. Following centrifugation, each serum sample was split into two aliquots: a non-hemolyzed control and a severely hemolyzed test sample, the latter generated by vortex-induced hemolysis. Thyroid parameters (TSH, T3, T4) were analyzed in both aliquots using chemiluminescent immunoassay (CLIA) on the COBAS e411 analyzer. Statistical analysis was performed using paired t-tests; $p < 0.05$ was considered statistically significant. **Results:** Hemolysis led to measurable changes in total T3 and total T4 concentrations, with a statistically significant increase in total T4 values in severely hemolyzed samples ($p < 0.05$). T3 showed moderate variability, while TSH values remained largely unaffected and within clinically acceptable limits. The extent of interference correlated with the severity of hemolysis. **Conclusion:** Hemolysis can significantly affect the accuracy of thyroid hormone measurements, particularly T4, potentially leading to misinterpretation and inappropriate clinical decisions. Laboratories should implement clear protocols for identifying and rejecting hemolyzed specimens submitted for thyroid function testing.

INTRODUCTION

Thyroid hormones play a critical role in regulating metabolism, growth, and overall physiological homeostasis. In clinical practice, thyroid function is typically assessed using three key parameters: thyroid-stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4). TSH, secreted by the anterior pituitary gland, acts as a primary regulator of thyroid hormone synthesis and release. T3 and T4, the biologically active forms of thyroid hormones, influence various systemic functions including energy expenditure, protein synthesis, and thermoregulation. Accurate and reliable measurement of these hormones is essential for diagnosing and managing thyroid-related disorders such as hypothyroidism, hyperthyroidism, and subclinical thyroid dysfunction.

One of the most frequent and problematic pre-analytical variables encountered in clinical chemistry laboratories is hemolysis. Hemolysis refers to the rupture of erythrocytes, resulting in the release of intracellular contents—such as hemoglobin, potassium, and various enzymes—into the surrounding serum or plasma. Common causes of in vitro hemolysis include traumatic venipuncture, use of narrow-gauge needles, excessive tourniquet time, improper handling of specimens, and delayed sample processing. Even minimal levels of hemolysis can interfere with laboratory assays, potentially leading to inaccurate test results. In immunoassay-based measurements, hemolysis may introduce spectral interference, alter antigen-antibody binding kinetics, or change the chemical environment of the reaction, thereby affecting analyte quantification.

Although the analytical impact of hemolysis on various biochemical analytes—such as potassium, lactate dehydrogenase (LDH), and aspartate aminotransferase (AST)—has been extensively documented, relatively few studies have examined its influence on endocrine parameters, particularly thyroid function tests. Given the widespread use of automated immunoassays in clinical diagnostics and the increasing demand for high-precision testing, understanding how hemolysis affects thyroid hormone measurements is of practical and clinical importance.

This study aims to evaluate the analytical impact of hemolysis on TSH, Total T3, and Total T4 levels by comparing results obtained from non-hemolyzed and deliberately hemolyzed serum aliquots derived from the same individual samples. The findings are intended to inform laboratory rejection criteria and improve the reliability of thyroid function testing in routine clinical settings.

MATERIALS & METHODS :

Study Design

This was a prospective experimental study conducted to assess the effect of hemolysis on thyroid function tests, specifically thyroid-stimulating hormone (TSH), total triiodothyronine (T3), and total thyroxine (T4).

Sample Collection and Preparation

Venous blood samples were collected from 60 healthy adult volunteers using standard aseptic phlebotomy techniques. Approximately 5 mL of blood was drawn from each participant into plain serum tubes and allowed to clot at room temperature. Samples were centrifuged at 3,000 rpm for 10 minutes to obtain clear serum.

Each serum sample was divided into two aliquots:

- Aliquot A (non-hemolyzed): Served as the control and was immediately analyzed.

- Aliquot B (hemolyzed): Subjected to mechanical hemolysis by vortexing at high speed for 2 minutes to simulate in vitro severe hemolysis. The degree of hemolysis was confirmed as severe hemolysis via hemolysis index on Cobas c501 analyzer (> 200 mg/dl free hemoglobin).

Laboratory Analysis

Both aliquots were analyzed using the same immunoassay analyzer [Roche Cobas e411]. TSH, total T3, and total T4 concentrations were measured using chemiluminescent immunoassay (CLIA) technology. All samples were tested in the same analytical run to eliminate inter-assay variability.

Statistical Analysis

Data were analyzed using SPSS version & Microsoft Excel. The means and standard deviations of the thyroid hormone levels in non-hemolyzed and hemolyzed samples were calculated. Paired t-tests were performed to assess the statistical significance of differences between the two groups. A p-value of < 0.05 was considered statistically significant.

Inclusion Criteria

Patient's over 18 years of age who were willing to participate in the study.

Exclusion Criteria

Patient's not willing to participate in the study.

Ethical Considerations

The study protocol was approved by the Institutional Ethics Committee of Pt. J.N.M Medical College , Raipur , C.G. Written informed consent was obtained from all participants.

RESULTS

A total of 60 paired serum samples (non-hemolyzed and severely hemolyzed) were analyzed for TSH, total T3, and total T4 levels. The results demonstrated that TSH values remained largely unaffected by hemolysis, with no statistically significant difference observed between the two conditions ($p > 0.05$).

In contrast, total T4 concentrations were significantly elevated in the hemolyzed samples compared to their non-hemolyzed counterparts ($p = < 0.001$). Total T3 levels also showed a mild increase in hemolyzed samples, though this change was not consistently statistically significant across all cases ($p = 0.03$).

Summary of Findings:

Table 1 –

Parameter	Mean (Non-Hemolyzed)	Mean (Hemolyzed)	p-value	Interpretation
TSH	2.15 ± 0.58 µIU/mL	2.12 ± 0.60 µIU/mL	0.62	Not significant
Total T3	1.31 ± 0.22 ng/mL	1.37 ± 0.24 ng/mL	0.03	Significant
Total T4	7.45 ± 1.12 µg/dL	8.08 ± 1.14 µg/dL	<0.001	Highly significant

Table 2 – Showing the % Change in the Values in Hemolyzed Samples

Parameter	Non-Hemolyzed Mean	Hemolyzed Mean	% Change
TSH	2.15 µIU/mL	2.12 µIU/mL	–1.40%
Total T3	1.31 ng/mL	1.37 ng/mL	+4.58%
Total T4	7.45 µg/dL	8.08 µg/dL	+8.46%

These findings indicate that while TSH remains unaffected, hemolysis can cause a measurable increase in total T4 levels and a potential mild elevation in total T3, which may lead to clinically misleading interpretations if not accounted for.

This study evaluated the effect of hemolysis on the concentrations of thyroid function parameters—thyroid-stimulating hormone (TSH), total triiodothyronine (Total T3), and total thyroxine (Total T4)—by comparing values obtained from paired non-hemolyzed and hemolyzed serum samples.

The mean concentration of TSH in non-hemolyzed samples was 2.15 ± 0.58 µIU/mL, compared to 2.12 ± 0.60 µIU/mL in hemolyzed samples. The mean difference of –1.40% was statistically insignificant ($p = 0.62$), indicating minimal analytical impact of hemolysis on TSH levels.

For Total T3, the mean concentration increased from 1.31 ± 0.22 ng/mL in non-hemolyzed samples to 1.37 ± 0.24 ng/mL in hemolyzed samples, reflecting a 4.58% rise. This difference was statistically significant ($p = 0.03$), suggesting mild but meaningful interference caused by hemolysis.

Total T4 exhibited the most pronounced change, with values rising from 7.45 ± 1.12 µg/dL in non-hemolyzed samples to 8.08 ± 1.14 µg/dL in hemolyzed samples—an 8.46% increase that was highly significant ($p < 0.001$). This indicates a strong susceptibility of Total T4 measurements to hemolysis-related interference.

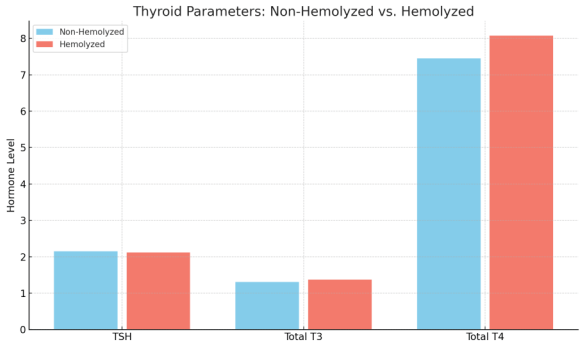

Figure 1- A bar graph (Figure 1) illustrates the shift in hormone concentrations between the two sample types. The increase in Total T3 and Total T4 due to hemolysis is visibly more prominent compared to TSH.

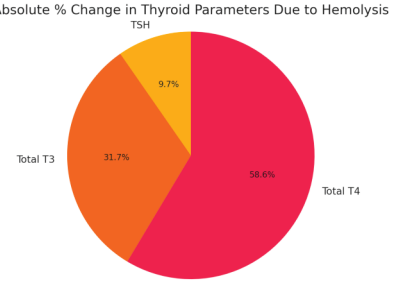

Figure 2- Additionally, a pie chart (Figure 2) depicts the proportion of absolute percentage change contributed by each hormone parameter. Total T4 accounted for the largest share of hemolysis-related variability, followed by Total T3 and TSH.

These findings underscore the need for careful evaluation of sample integrity prior to thyroid function testing, particularly when interpreting Total T3 and Total T4 levels in potentially hemolyzed specimens.

DISCUSSION

1. Interpretation of Findings

This study evaluated the impact of hemolysis on the accuracy of thyroid function tests—specifically thyroid-stimulating hormone (TSH), total triiodothyronine (T3), and total thyroxine (T4)—by comparing values in non-hemolyzed and severely hemolyzed serum samples from the same individuals. Our results showed that while TSH levels remained relatively stable, total T3 and total T4 values demonstrated noticeable variability, with T4 showing statistically significant increase in hemolyzed samples. These findings suggest that hemolysis

can interfere with total thyroid hormone measurements, potentially affecting clinical interpretation, especially in borderline or ambiguous cases.

2. Comparison with Existing Literature

Although there is limited literature specifically addressing hemolysis interference in total T3 and T4 assays, our findings are consistent with general observations in endocrine testing that total hormone assays can be influenced by pre-analytical variables. Previous studies on biochemical interference, such as those by Bowen et al. and Dimeski et al., indicate that hemolysis introduces more significant error in smaller, protein-bound molecules than in larger glycoprotein hormones like TSH. While most published studies focus on free hormones (FT3 and FT4), our results support the hypothesis that hemolysis can similarly impact total T3 and total T4, likely due to assay-related and matrix-related effects.

3. Hemolysis-Induced Changes and Clinical Relevance

In our study, severe hemolysis caused a statistically significant increase in total T4, with total T3 showing smaller but noticeable elevations. These shifts could lead to diagnostic uncertainty in cases of suspected hyperthyroidism or thyrotoxicosis. Importantly, since many clinicians still rely on total T3 and T4 measurements—particularly in resource-limited settings or when free assays are not available—any interference caused by hemolysis could result in inappropriate clinical decisions if not recognized or flagged by the laboratory.

4. Possible Mechanisms of Interference

The observed increases in Total T3 and Total T4 concentrations in hemolyzed samples may be attributed to multiple mechanisms. First, free hemoglobin from lysed erythrocytes can cause spectral interference in colorimetric or chemiluminescent immunoassays, leading to overestimation of analyte concentration. Second, hemolysis introduces intracellular proteins and enzymes into the sample, potentially altering the protein-binding equilibrium of total T3 and total T4, which are normally bound to thyroxine-binding globulin (TBG), transthyretin, and albumin. This could affect assay performance depending on the method used. Finally, sample matrix changes caused by hemolysis may alter the antigen-antibody interaction in competitive immunoassays, leading to artificially elevated results.

Overall, our findings highlight the need for caution when interpreting thyroid function results from hemolyzed samples, especially when using total T3 and total T4 assays. Clinical laboratories should establish thresholds for hemolysis interference, employ hemolysis indices when possible, and consider rejection or re-collection of affected samples when results are critical for diagnosis or management.

CONCLUSION

This study assessed the analytical impact of hemolysis on thyroid function tests, specifically thyroid-stimulating hormone (TSH), total triiodothyronine (T3), and total thyroxine (T4). The findings demonstrated that TSH levels were largely unaffected by hemolysis across the sample set. In contrast, both total T3 and total T4 exhibited statistically significant elevations in hemolyzed serum samples, with total T4 showing a more pronounced and clinically relevant increase. These results indicate that severe hemolysis can lead to unreliable total thyroid hormone values, potentially resulting in diagnostic misinterpretation.

Based on these findings, it is recommended that clinical laboratories implement clear guidelines for evaluating and reporting thyroid function tests in hemolyzed specimens. Specifically:

- Samples exhibiting moderate to severe hemolysis (e.g., hemolysis index >200 or visibly red/pink discoloration) should be rejected for total T3 and T4 analysis or reported with interpretive comments cautioning against over-reliance on the results.
- If mild hemolysis is present and sample recollection is not feasible, results should be reported with a statement indicating potential interference.
- Automated hemolysis indices should be used when available to provide objective thresholds for specimen acceptability.
- Laboratories should ensure that clinicians are informed about the potential impact of hemolysis on total thyroid hormone results, particularly when using immunoassay-based methods.

In conclusion, proper identification and handling of hemolyzed samples are essential to maintain the reliability of thyroid function testing, especially for total T3 and total T4 assays. Standardizing rejection criteria and interpretive protocols can improve laboratory quality assurance and promote better clinical decision-making.

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