AJMB
Avicenna Journal of Medical Biochemistry
Avicenna J Med Biochem, 2023; 11(1):88-91. doi:10.34172/ajmb.2023.2420
http://ajmb.umsha.ac.ir
UMSHA Press

Mini Review

A mini-review in Thyroid Function Test: More Than Meets Our Eye

Soumik Goswami*, Mounam Chattopadhyay

Department of Endocrinology, Nilratan Sircar Medical College, The West Bengal University of Health Sciences, Kolkata, India

***Corresponding author:** Soumik Goswami, Email: dr.soumikgoswami@gmail.com

Received: January 31, 2023, **Revised:** March 16, 2023, **Accepted:** March 16, 2023, **ePublished:** May 3, 2023

Please cite this article as follows: Goswami S, Chattopadhyay M. A mini-review in thyroid function test: more than meets our eye. Avicenna J Med Biochem. 2023; 11(1):88-91. doi:10.34172/ajmb.2023.2420

Thyroid function tests (TFTs) happen to be the most commonly performed tests in endocrinology, leaving aside plasma glucose. Apparently though, it seems quite easy to interpret, but there are subtle nuances to it. If the test results are not analyzed thoroughly through clinico-biochemical tests, there are high chances of erroneous diagnosis and treatment.

Two-site immunometric assays which are also known popularly as "sandwich" assays are used generally to measure thyroid stimulating hormone (TSH). In this study, we used two sets of antibodies: one bound to a solid phase known as "capture antibody" and the other labeled as "detection antibody". The analyte forms a complex with these two antibodies, and as a result, a signal is generated which helps us measure the concentration of the analyte, namely, TSH. Sometimes certain human anti-animal antibodies (HAAAs) could be present in the serum of the individual. It is more commonly seen among those who received some sort of immunological therapy at any point in their life. They can cause both positive and negative interferences depending on their reactivity. If the HAAA is reactive both to capturing and detecting antibodies but not to the analyte, it will form a complex binding to both and generate a false high signal as if the analyte is present in high concentration in the sample. On the other hand, if the HAAA is only reactive to capture antibody and prevents binding of the analyte to it and forms a complex, it will not form any complex and will generate a falsely low signal as if the analyte is present in low concentration in the sample. Generally, human anti-mouse antibodies are found to cause such interactions. To alleviate this problem, some commercial assay systems use panels of antigens or pre-immune sera from source animals to mop up the HAAAs, but in practice, these polyspecific antibodies are quite difficult to remove from the sample (1-3).

People often taking biotin supplements at least 5 mg/d can present with altered TFT that mimics thyrotoxicosis. There are several immunoassay systems that involve the use of biotinylated antibodies and streptavidin-coated microparticles that help with the immobilization of antigen-antibody complexes for the solid phase of the assay system (4). Biotin appears to be a double-edged sword that affects thyroid hormone assays in both ways. Free T4 measurement is done in a competitive assay system. During the initial incubation, the T4 in the patient sample binds to a T4-specific antibody, labeled with ruthenium complex. Subsequently, biotinylated T4 is added that binds to residual binding sites on T4-specific antibodies. To capture the complex formed by biotinylated T4 and T4-specific antibodies, streptavidin-coated microparticles are added. The microparticles remain captured magnetically, and unbound substances are washed away. The Ru-complex liberates a voltage-generated signal (5). Given that analyte concentration and signal intensity are inversely proportional in competitive immunoassays, biotin in the sample competes with biotinylated T4 to bind to streptavidin, causing a low signal and thereby a false considerable result. In the Cobas system, the TSH test is conducted as a sandwich assay. In the first incubation, biotinylated TSH-specific antibodies and Ru-complex labeled antibodies bind to analyte TSH, forming a sandwich complex. Streptavidin-coated microparticles are added in the second incubation to capture the sandwich complexes. There is magnetic capturing of microparticles, whereas unbound substances are washed away leaving behind Ru-complex that generates voltage-mediated signals (6). In sandwich assays, the signal is proportional to the analyte TSH concentration. Biotin competes with a biotinylated sandwich complex to create a binding to streptavidin, causing a low signal and therefore a falsely low outcome

(7). Biotin levels of 20 mcg/L have been reported to falsely lower TSH levels in the Cobas e601 sandwich assay. The Roche Cobas package insert recommends that samples ought not to be taken from patients receiving doses of biotin for at least eight hours following the last biotin administration (8).

An interesting association could be observed between the use of heparin and free thyroid hormone levels. As we all know, heparin is being used extensively in modern-day medical and surgical practice mainly for thromboprophylaxis. The effect was first described in vivo by Schatz et al among patients undergoing dialysis. The free thyroid hormone levels rose promptly up to five folds within 15 minutes of intravenous injection of heparin (9). Heparin activates endothelial lipoprotein lipase that causes the release of non-esterified fatty acids (NEFA) from fat stores. The non-esterified fatty acids compete with thyroid hormones for binding with thyroid-binding globulin (TBG). They can also displace other ligands from the sites on albumin that limit their free concentrations. This artifact is most prominent at serum NEFA levels of greater than 2-3 mmol/L, hypertriglyceridemia, and hypoalbuminemia. This effect is observed across assay platforms, including equilibrium dialysis, ultracentrifugation, and the like. If the thyroid assay has to be conducted in the heparinized subject, the sample needs to be drawn at least 10 hours after the last heparin injection and with minimum delay in sample transport, processing, and analysis (10).

Nowadays, different tyrosine kinase inhibitors are used for the treatment of hematologic and solid malignancies. Sunitinib most commonly is found to lead to aberrant TFTs. The patient can develop hypothyroidism after treatment-induced destructive thyroiditis. The inhibition of VEGFR and PDGFR pathway for producing thyroid gland ischemia adds to the insult. Some propose the possibility of the inhibition of normal iodine uptake. Interestingly those who develop hypothyroidism might tend to have their cancer better controlled through the common antiangiogenic effects affecting both the thyroid and the malignant lesion (11).

There is another clinical entity known as familial dysalbuminemic hyperthyroxinemia which is an autosomal dominant disorder caused by an abnormal variant of albumin that leads to elevated free thyroxine and TSH. The condition is difficult to be discerned clinically as seldom there is a symptom associated with it. They are quite predominant among the Hispanic population. They are detected incidentally on TFT. The condition does not need to be treated; nevertheless, its identification is of utmost importance because the improper treatment of elevated free thyroxine with anti-thyroid drugs can lead to iatrogenic hypothyroidism or untoward side effects. Monitoring the condition with sole-free T4 could be misleading as it has been found to be falsely elevated (1).

Thyroid tests should be interpreted and better not ordered unless there are compelling indications to do so during fasting or critical illness. In these conditions, the TSH is blunted centrally, and there is a low formation of T3 in circulation. In addition, the binding of T3 and T4 to TBG is also interrupted. These changes create a profound diagnostic dilemma known as a euthyroid sick syndrome or non-thyroidal illness. There is no organic permanent disease in the hypothalamic-pituitary-thyroid axis which would recover eventually after the critical illness is over. There is a reduction in the activity of Deiodinases 1 and 2 which leads to reduced peripheral T3 formation from T4 (12). In addition, increased activity of Deiodinases 3 generates reverse T3 (13). In mild forms only T3 declines, and reverse T3 rises. With more severe forms of the euthyroid sick syndrome, T4 and ultimately TSH can also reduce. The fall in supply of T3 during critical illness can be conceived as an energy-sparing or nitrogen-sparing adaptation. The IL6 generated in these situations leads to the formation of reactive oxygen species that reduce intracellular thiols, leading to impairment of Deiodinases 1 and 2 sparing and Deiodinases 3 which is predominantly dependent on extracellular thiols (14). Hence, before the diagnosis of hyperthyroidism by facing a suppressed TSH in lab reports, one needs to be highly meticulous before institutional therapy (15).

Macro-TSH is a macromolecule that is formed by the combination of TSH molecule and autoimmune anti-TSH Ig. Its renal clearance is reduced for its size. It retains the immunoreactivity and causes a rare laboratory interference, leading to falsely high TSH values in the face of normal T4 levels, and the patient remains entirely asymptomatic. The samples from patients having macro-TSH in circulation recovered dramatically when they were over five times. Negative results were reported when the samples were treated with anti-animal and anti-heterophile blocking reagents. Serum rheumatoid factors were also normal. The TSH did not recover more than 20% when subjected to polyethylene glycol precipitation (14). The Roche and Perkin-Elmer DELFIA assays are more susceptible to macro-TSH interference, whereas the ADVIA Centaur assay is less sensitive to such interference (16,17) Macro-TSH is diagnosed when a macro-TSH/ hypothyroid mixture gives lower TSH values after the incubation in assays known to be adequately sensitive to macro-TSH. The gold standard test for the isolation of macro-TSH is gel filtration chromatography which is costly and not readily available (18).

One can also be presented with elevated levels of free T4 but devoid of suggestive symptoms. Family or genetic analysis is used to differentiate patients with TBG mutations from acquired cases of TBG deficiency that is most commonly caused by the use of androgens, anabolic steroids, glucocorticoids, severe hepatocellular disease, severe nonthyroidal illness, protein losing nephropathies, and enteropathies. Though the free T4 is mildly elevated, the total T4 is low. The long arm of the X-chromosome (Xq22.2) contains the *TBG* (*SERPINA7*) gene and is made up of four coding exons. Hereditary TBG deficiencies

could be seen in approximately 1:3000 individuals, and since hemizygous (XO) males are fully affected by X-linked TBG defects, deficiencies are categorized as either a complete deficiency or partial deficiency based on the male's serum TBG concentration. Heterozygous females with complete deficiency typically exhibit half the normal serum TBG concentration. Accordingly, one has to rule out thyroid hormone resistance and TSH-producing adenoma (19).

Nevertheless, another important cause of the elevation of both thyroid hormones and TSH (sometimes inappropriately normal) is resistance to thyroid hormones. Generally, thyroid hormone receptor beta mutation affects the binding to intracellular T3. It is inherited in an autosomal dominant pattern and is mainly presented with signs of hypothyroidism in tissues expressing TR beta (e.g., pituitary and liver) and overactivity-mediated thyrotoxic features in tissues expressing TR alpha (e.g., cardiac muscles). TR alpha mutations are rarely presented with mental retardation, short stature, and cardiac hypofunction (20).

Thyroid dysfunction is known to exist alongside diabetes mellitus. T2DM suppresses the TSH levels and impairs the conversion of thyroxine (T4) to triiodothyronine (T3) in the peripheral tissues. Poorly managed T2DM can lead to insulin resistance and hyperinsulinemia, which causes thyroid tissue proliferation and increases nodule formation and goiter size. Moreover, metformin, the cornerstone of oral hypoglycemic therapy, is known to reduce TSH by regulating hypothalamic AMPK, thyroid volume, and nodule size (21).

Hence, keeping all this in mind, an observant endocrinologist should take thyroid function reports not too casually but with a pinch of salt and delve deeper into them, particularly in doubtful situations before arriving at a diagnosis to avoid unnecessary therapeutic decisions.

Authors' Contribution

Conceptualization: Soumik Goswami.
Data curation: Soumik Goswami, Mounam Chattopadhyay.
Formal analysis: Soumik Goswami, Mounam Chattopadhyay.
Investigation: Soumik Goswami, Mounam Chattopadhyay.
Methodology: Soumik Goswami, Mounam Chattopadhyay.
Project administration: Soumik Goswami, Mounam Chattopadhyay.
Resources: Soumik Goswami, Mounam Chattopadhyay.
Supervision: Soumik Goswami, Mounam Chattopadhyay.
Validation: Soumik Goswami, Mounam Chattopadhyay.
Visualization: Soumik Goswami, Mounam Chattopadhyay.
Writing–original draft: Soumik Goswami, Mounam Chattopadhyay.
Writing–review & editing: Soumik Goswami, Mounam Chattopadhyay.

Competing Interests

The authors have no conflict of interests.

Ethical Approval

Not applicable.

Funding

None of the authors received any financial support or grant for this article.

References

1. Koulouri O, Moran C, Halsall D, Chatterjee K, Gurnell M. Pitfalls in the measurement and interpretation of thyroid function tests. Best Pract Res Clin Endocrinol Metab. 2013;27(6):745-62. doi: 10.1016/j.beem.2013.10.003.
2. Santhana Krishnan SG, Pathalapati R, Kaplan L, Cobbs RK. Falsely raised TSH levels due to human anti-mouse antibody interfering with thyrotropin assay. Postgrad Med J. 2006;82(973):e27. doi: 10.1136/pmj.2006.049809.
3. Levinson SS, Miller JJ. Towards a better understanding of heterophile (and the like) antibody interference with modern immunoassays. Clin Chim Acta. 2002;325(1-2):1-15. doi: 10.1016/s0009-8981(02)00275-9.
4. Diamandis EP, Christopoulos TK. The biotin-(strept)avidin system: principles and applications in biotechnology. Clin Chem. 1991;37(5):625-36.
5. Roche Diagnostics. Cobas FT4 II assay package insert. Roche Diagnostics, GmbH 2013– 05, V2.0 English.
6. Rulander NJ, Cardamone D, Senior M, Snyder PJ, Master SR. Interference from anti-streptavidin antibody. Arch Pathol Lab Med. 2013;137(8):1141-6. doi: 10.5858/arpa.2012-0270-CR.
7. Kwok JS, Chan IH, Chan MH. Biotin interference on TSH and free thyroid hormone measurement. Pathology. 2012;44(3):278-80. doi: 10.1097/PAT.0b013e3283514002.
8. Elston MS, Sehgal S, Du Toit S, Yarndley T, Conaglen JV. Factitious Graves' disease due to biotin immunoassay interference-a case and review of the literature. J Clin Endocrinol Metab. 2016;101(9):3251-5. doi: 10.1210/jc.2016-1971.
9. Schatz DL, Sheppard RH, Steiner G, Chandarlapaty CS, de Veber GA. Influence of heparin on serum free thyroxine. J Clin Endocrinol Metab. 1969;29(8):1015-22. doi: 10.1210/jcem-29-8-1015.
10. Stevenson HP, Archbold GP, Johnston P, Young IS, Sheridan B. Misleading serum free thyroxine results during low molecular weight heparin treatment. Clin Chem. 1998;44(5):1002-7.
11. Ahmadieh H, Salti I. Tyrosine kinase inhibitors induced thyroid dysfunction: a review of its incidence, pathophysiology, clinical relevance, and treatment. Biomed Res Int. 2013;2013:725410. doi: 10.1155/2013/725410.
12. Peeters RP, Wouters PJ, Kaptein E, van Toor H, Visser TJ, Van den Berghe G. Reduced activation and increased inactivation of thyroid hormone in tissues of critically ill patients. J Clin Endocrinol Metab. 2003;88(7):3202-11. doi: 10.1210/jc.2002-022013.
13. Everts ME, de Jong M, Lim CF, Docter R, Krenning EP, Visser TJ, et al. Different regulation of thyroid hormone transport in liver and pituitary: its possible role in the maintenance of low T3 production during nonthyroidal illness and fasting in man. Thyroid. 1996;6(4):359-68. doi: 10.1089/thy.1996.6.359.
14. Verhoye E, Van den Bruel A, Delanghe JR, Debruyne E, Langlois MR. Spuriously high thyrotropin values due to anti-thyrotropin antibodies in adult patients. Clin Chem Lab Med. 2009;47(5):604-6. doi: 10.1515/cclm.2009.138.
15. Van den Berghe G. Non-thyroidal illness in the ICU: a syndrome with different faces. Thyroid. 2014;24(10):1456-65. doi: 10.1089/thy.2014.0201.
16. Vieira JG, Maciel RM, Hauache OM, Nishida SK, Boelter DM, Pinheiro MF. [Unexpected high values of TSH: the presence of high molecular weight forms (macro TSH) must be investigated]. Arq Bras Endocrinol Metabol. 2006;50(3):445-9. doi: 10.1590/s0004-27302006000300006. [Portuguese].
17. Mendoza H, Connacher A, Srivastava R. Unexplained high thyroid stimulating hormone: a "BIG" problem. BMJ Case Rep. 2009;2009:bcr01.2009.1474. doi: 10.1136/

bcr.01.2009.1474.
18. Loh TP, Kao SL, Halsall DJ, Toh SA, Chan E, Ho SC, et al. Macro-thyrotropin: a case report and review of literature. J Clin Endocrinol Metab. 2012;97(6):1823-8. doi: 10.1210/jc.2011-3490.
19. Berger HR, Creech MK, Hannoush Z, Watanabe Y, Kargi A, Weiss RE. A novel mutation causing complete thyroid binding globulin deficiency (Tbg-Cd Mia) in a male with coexisting Graves' disease. AACE Clin Case Rep. 2017;3(2):e134-e9. doi: 10.4158/ep161421.cr.
20. Refetoff S, Weiss RE, Usala SJ. The syndromes of resistance to thyroid hormone. Endocr Rev. 1993;14(3):348-99. doi: 10.1210/edrv-14-3-348.
21. Kalra S, Aggarwal S, Khandelwal D. Thyroid dysfunction and type 2 diabetes mellitus: screening strategies and implications for management. Diabetes Ther. 2019;10(6):2035-44. doi: 10.1007/s13300-019-00700-4.