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A macro-TSH: a clinical diagnostic dilemma

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ABSTRACT

Isolated increase in thyrotropin stimulating hormone (TSH) in a clinically euthyroid patient may be caused by the formation of a macromolecule between TSH and autoantibodies causing discordant thyroid function test results. Despite the effort to eliminate interferences in immunoassays, these assays are still vulnerable to different interferences. Immunoassay interferences may cause erroneous results and lead to misdiagnosis which may subject a patient to unnecessary investigations and treatment. Immunoassays are affected by multiple substances; these may be endogenous or exogenous such as heterophile antibodies, autoantibodies, macromolecules, and human anti-mouse antibodies. This case reports a 47-year-old African woman who presented with a persistent elevated TSH with thyroid hormones within normal reference limits. She was found to have a macro-TSH which was associated with IgA paraprotein.

INTRODUCTION

An increased TSH in the presence of normal free T4 (fT4) commonly suggests subclinical hypothyroidism. However, other causes such as TSH resistance, poor compliance to thyroxine, recovery phase of non-thyroidal illness, and assay interference need to be excluded (1). Automated immunoassays are prone to various interferences resulting in erroneous results. Some of these assay interferences include heterophile antibodies, anti-mouse antibodies, and macromolecules e.g., macro-TSH. Macro-TSH is a macromolecule as a result of the complex binding of TSH to immunoglobulins, which results in increased TSH. Even though macro-TSH has poor biological activity, and circulates longer, it retains the ability to react with antibodies used in immunoassays, causing falsely elevated results (1, 2). This case reports a 47-year-old South African woman who presented with a persistent elevated TSH with thyroid hormones within normal reference limits. She was found to have a macro-TSH which was associated with IgA Kappa paraprotein. This case reports the potential interference on assays, if not identified, may have adverse effects on the patient clinical outcomes. Also, it emphasises the need for laboratory professionals to work closely with clinicians.

CLINICAL-DIAGNOSTIC CASE

A 47-year-old woman was referred to the Endocrine Unit, for evaluation of abnormal thyroid function test results. She denied any history of weight gain, hoarseness of voice, and cold intolerance. She reported no headaches and blurred vision. She reported no personal or family history of thyroid or autoimmune diseases. Examination was unremarkable.

Thyroid function tests revealed markedly elevated thyrotropin stimulating hormone (TSH) with free thyroxine (fT4) and free triiodothyronine (fT3) within reference intervals. Her anti-thyroid peroxidase antibodies were negative (Table 1). Since she was clinically euthyroid, no medication was prescribed.

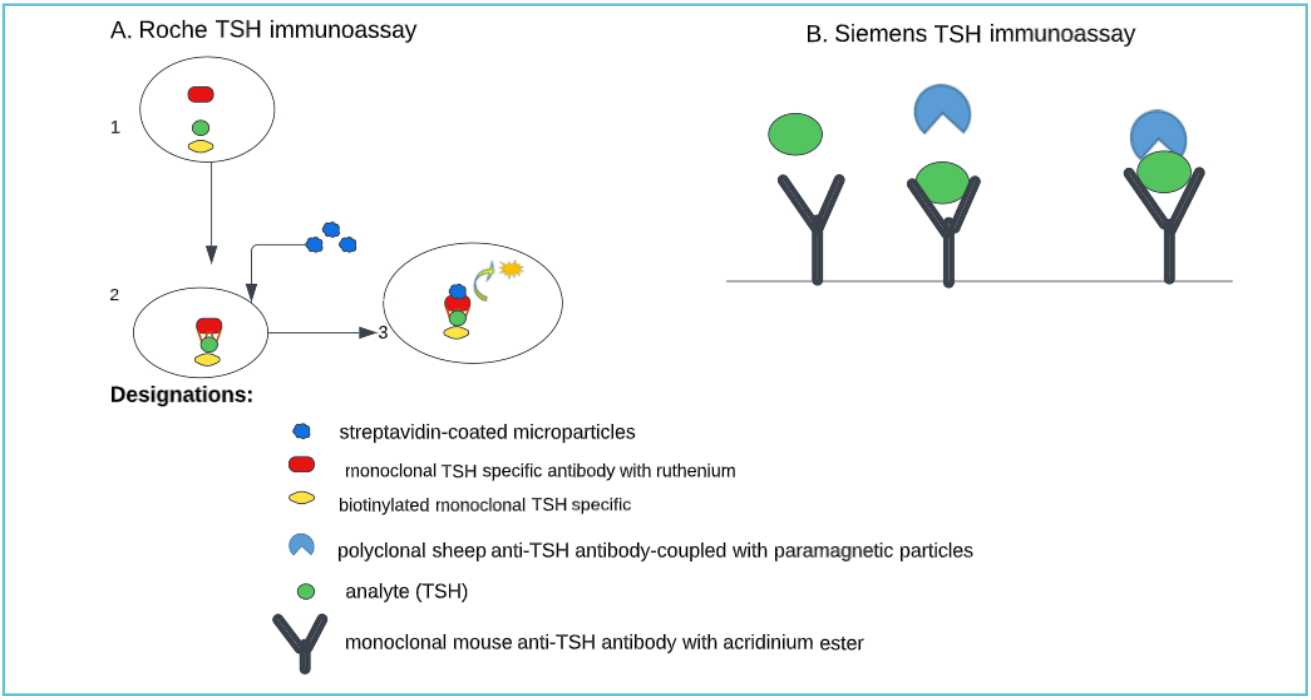
Due to discordant thyroid function results on repeated measurements with no symptoms suggestive of thyroid disorder in the patient, an interference was suspected. In our patient, thyroid function tests were measured using a Roche Cobas c602 (Roche Diagnostics, Mannheim, Germany, Figure 1A).

To screen for laboratory interference, the measurements of TSH and fT4 from the same serum sample were repeated on Siemens Advia Centaur (Siemens Medical Solutions Diagnostics, USA) which utilizes a two-site sandwich immunoassay using direct chemiluminometric technology (Figure 1B). TSH levels were significantly lower compared to Roche platform (Table 1) while fT4 remained within reference intervals; this further strengthened the suspicion of the presence of interference.

We further performed serial dilutions (1/2, 1/5, 1/10, and 1/20) of the serum using a diluent recommended by the manufacturer. We obtained non-linear results which suggested the presence of assay interference (Table 2). Unfortunately, non-linear recovery cannot differentiate the heterophile antibodies from macro-TSH and rheumatoid factor interferences.

To exclude macro-TSH, the serum of the patient was subjected to polyethylene glycol (PEG) 6000 solution (25% w/v) using a well described procedure commonly used for macroprolactinemia. Briefly, in this procedure an equal amount of the patient sample and PEG solution was mixed and incubated for 10 minutes at room temperature. Then the mixture was centrifuged at 10900 rpm (9430 g) speed for precipitation to occur and the supernatant was analysed. The post-PEG TSH was 2.92 mIU/L (reference interval, 0.27-4.20 mIU/L, 2.9% recovery) suggesting that the interfering antibodies were in the macro-form.

Figure 1 The schematic representation of differences between Roche Cobas (A) and Siemens's (B) TSH assays



A. *TSH in the sample is incubated with biotinylated monoclonal TSH-specific antibody and a monoclonal TSH specific antibody labelled with ruthenium (1) to form a sandwich complex (2). Streptavidin-coated microparticles are added, a complex becomes bound to the solid phase via interaction of biotin and streptavidin inducing chemiluminescent emission (3).*

B. *TSH binds firstly to a monoclonal mouse anti-TSH antibody with acridinium and secondly to a polyclonal sheep anti-TSH antibody-coupled with paramagnetic particles, measuring the concentration using the chemiluminometric technology.*

Table 1 Measurements of thyroid function test on different instruments

	On admission (Roche)	1-month later (Roche)	1-month later (Siemens)	5-months later (Roche	1-year later (Roche)	Reference intervals
Test	**Results**					
TSH	>100.0	>100.0	7.40	>100.0	>100.0	0.27-4.20 mIU/L
fT4	17.2	14.3	14.2	12.9		12.0-22.0 pmol/L
fT3	4.9			4.7		3.1-6.8 pmol/L
Anti-TPO Abs		5				<34 U/mL

TSH, thyroid-stimulating hormone, fT4, thyroxine, fT3, free triiodothyronine, Anti-TPO Abs, Anti-thyroid peroxidase antibodies.

Table 2 TSH results after serial dilutions analyzed on Roche instrument

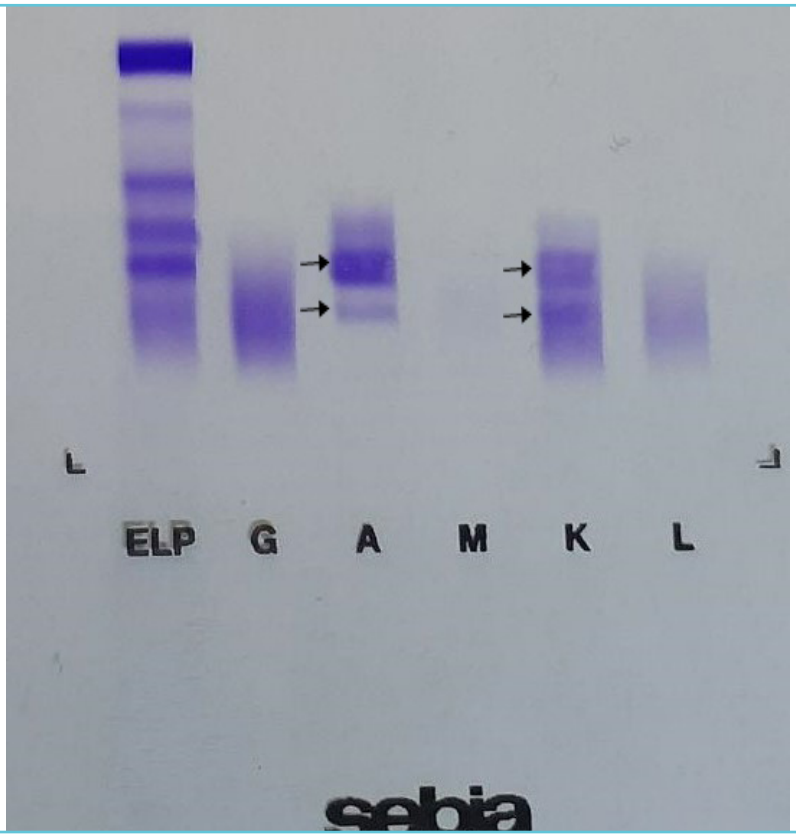
Test	Without Dilution	1/2 Dilution	1/5 Dilution	1/10 Dilution	1/20 Dilution	Reference Intervals
TSH	>100	>100	103.2	113.6	115.6	0.27-4.20 mIU/L

Table 3 Biochemical results before and after polyethylene glycol (PEG) precipitation

Test	Before PEG	After PEG	Reference Intervals (RI)
TSH	99.54	2.92	0.27-4.20 mIU/L
Prolactin	510.7	136.0	4.8- 23.3 µg/L (Post-PEG 3.5-18 µg/L)

TSH, thyroid-stimulating hormone, PEG, polyethylene glycol.

Figure 2 Serum immunofixation image depicting two IgA Kappa monoclonal gammopathy (arrows)



The recovery of TSH post-PEG was calculated as (post-PEG TSH ÷ pre-PEG TSH) x 100%. Because we suspected the presence of a high molecular weight interfering substance might affect other hormones, we ordered prolactin, since macroprolactin is commonly encountered in clinical practice compared to other hormones. Prolactin concentrations were markedly high 510.7 µg/L (4.8- 23.3) using Roche Cobas e602 (Table 3). She was not pregnant or breastfeeding or any medication known to increase prolactin. The post-PEG prolactin was 136 µg/L (post-PEG intervals: 3.5- 18) which is 26.6 % recovery (Table 3). No further investigation was done since the patient was not complaining of the headaches and had no blurred vision that would have suggested a prolactinoma.

To further characterize interfering antibodies, immunoglobulins were ordered. The immunoglobulins results show markedly elevated IgA: 9.39 g/L (0.7-4 g/L) while IgG: 12.32 g/L (7-16 g/L) and IgM: 0.40 g/L (0.4- 2.3 g/L) were within the reference intervals. These results suggested the presence of TSH-IgA macro-TSH interferences. This was further confirmed with the immunofixation which confirmed the presence of two IgA Kappa paraproteins (Figure 2).

DISCUSSION

Interferences in the measurement on immunoassays are common [1, 2]. Since interferences are common, grossly increased TSH should be investigated when the isolated elevation is not consistent with the rest of thyroid function tests and the clinical presentation.

Commonly, immunoassays are interfered with by endogenous and exogenous substances. Substances such as heterophile antibodies, human anti-mouse/animal antibodies, anti-streptavidin antibodies, mega doses of biotin, and rheumatoid factors have been implicated [2].

The increased use of biotin supplementation, especially mega doses has created huge challenges on laboratory assays [3]. The effect of biotin on immunoassays has been recognised by manufacturers. In particular, Roche assays have been improved to tolerate higher biotin levels without significant interferences. In our patient, the new versions TSH, fT4 and fT3 assays were utilised making biotin interference unlikely. Also, high biotin levels cause a negative interference on TSH [3], which was not the case in our patient. In addition to biotin interference, heterophile antibodies needs to be considered. Heterophile antibodies are classified as a group of natural antibodies and autoantibodies, and have ability to react with heterogeneous and poorly defined antigens of different compounds. They either affect the antigen binding to the antibody in immunoassay or act as an antigen due to polyreactive nature [2]. To elucidate the presence of heterophile antibodies, firstly, the sample can be analysed on the different platform. Secondly, the serial dilution to demonstrate nonlinear results in the presence of interfering antibodies. Thirdly, the test is analysed after incubation with heterophile blocking agents/tubes [4], these agents aid in removal of heterophile antibodies.

Repeating analysis on another analyzer by a different manufacturer often reveals the presence of interference. Assays from alternative manufacturers are mostly likely to use different antibodies and targeting at different epitopes [5]. In our case we analysed the same patient sample on a different instrument and TSH results were different, providing the clue to possible interference.

Another approach to screen for interference is through serial dilution using recommended manufacturer specific diluents. Nonlinear serial dilution provides a clue of the presence of the interference [6]. We observed a nonlinear dilution pattern, indicating assay interference.

This method is not perfect, only 60% of patients may show lack of linearity [5, 6].

Macromolecules such as macroprolactin and macro-TSH have been described as causes of immunoassay interference [7, 8]. Macro-TSH is estimated to have prevalence between 0.6-1.6% [6]. When monomeric TSH combines with immunoglobulin (Ig), commonly IgG forms macro-TSH. Unlike the monomeric, bioactive form which is 28 kDa, macro-TSH has a large molecular weight of approximately 150 kDa. Because of the molecular weight, the renal clearance of macro-TSH is markedly reduced, hence circulating longer. The immunoreactivity of the TSH-Ig complex is retained [6, 9, 10]. Currently, the available immunometric assays will not be affected to the same degree by macro-TSH as shown as shown in our patient (Siemens TSH was 7mU/L, while Roche TSH was >100mU/L). Therefore, macro-TSH has the ability to react with antibodies used for measurement causing elevated TSH. However, the biological activity of macro-TSH is low, and the patient remains clinically euthyroid [9]. The pitfall of this procedure is that interferences from other molecules such as heterophile antibodies and rheumatoid factors may give similar results. In addition, linear dilutions have been reported in the presence of macro-TSH. We were able to demonstrate non-linear recovery in our patient. Unfortunately, we were not able to exclude the presence of heterophile antibodies due to unavailability and cost of heterophile blocking tubes.

The common and easy method to screen for macroprolactin is the use of polyethylene glycol (PEG) precipitation which has been adapted to screen for macro-TSH [11]. Briefly, the PEG solution is added to patient serum. Even though PEG precipitation is easy to use, the low recovery needs to be confirmed by gel filtration chromatography. However, the latter is costly and not widely accessible. Compared to other methods, PEG precipitation is commonly performed because of ease of use, cost effective, and correlates well to gel chromatography which is considered a gold standard for macroprolactin [12] but it remains to be seen if same result will be achieved for macro-TSH. At this stage, there is no single method that reliably identifies macro-TSH. Therefore, various diagnostic strategies are often applied to rule out the presence of macro-TSH [13].

Once macro-TSH was detected in this patient, we searched for the involvement of other hormones. Since prolactin is a frequently detected macroform, it was performed of which was found to be elevated as well. The patient had no clinical features that suggested hyperprolactinaemia and not on medication known to increase prolactin. The post-PEG was higher above the upper limits of post-PEG reference intervals suggesting true hyperprolactinaemia even though 26.6% which is less than the 40% cut-off commonly used. Studies are now advocating for use of post-PEG reference intervals rather than recovery percentage after precipitation to establish the presence of true hyperprolactinaemia or macroprolactin [14]. At this stage no reference intervals for post-PEG TSH have been established. Despite the absence of a protocol to screen macro-TSH, Mills et al. has suggested that macro-TSH should be suspected when TSH is >10mIU/L with thyroid hormones within the reference intervals [8]. However, this approach may be costly since the prevalence of macro-TSH is low.

CONCLUSIONS

In conclusion, this case demonstrated the importance of considering interferences when biochemical results, especially analyzed on immunoassays are discordant with the clinical presentation before costly and potentially invasive investigations are performed. In addition, strong interaction between clinicians and

laboratory professionals is necessary to identify interferences and costly investigations.

TAKE-HOME MESSAGES/ LEARNING POINTS

1. Isolated TSH with normal thyroid hormones should trigger the suspicion of the interference, especially in the clinical euthyroid patient.
2. Some immunoassays are unable to differentiate macroTSH from the bioactive TSH molecule.
3. MacroTSH is a rare phenomenon that should be exclude to avoid unnecessary management and possible invasive investigations. Therefore, collaboration between clinician and laboratory professional is vital.
4. Measurement of TSH after addition of PEG precipitation can reveal the presence of macroTSH. This method has been used for diagnosis of macroprolactin due to low cost and high accessibility.

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Author disclosures & contributions

Written informed consent was obtained from patient for publication of this case.

Ethical clearance was obtained from the Medical Human Research Ethics Committee, University of the Witwatersrand - clearance certificate no. M210758.

X Nkuna wrote the first draft of the manuscript. X Nkuna, Z Dire, and SP Khoza edited the submitted manuscript. All authors reviewed and approved the final manuscript for publication.

The authors declare that they have no competing interests.

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