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Research Article

Macro-TSH in Covid-19 Patients with an Underlying Thyroid Condition: A Case Series and Literature Review

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Abstract

Introduction: The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that began in Wuhan (China), that spread rapidly worldwide, seriously affecting the population leading to a public health emergency declared by the World Health Organization. It is unknown how SARS-CoV-2 infection can affect thyroid function.

In this study, we describe the characteristics of an unusual analytical interference that may explain the false detection of hypothyroidism in patients with SARS-CoV-2 infection. This is the first time that a phenomenon of this nature has been described in patients with COVID-19 and underlying thyroid dysfunction.

Material and Methods: Observational study of patients admitted for severe COVID-19 infection and underlying thyroid disease.

Results: TSH levels presented an atypical pattern different from that of Euthyroid Sick Syndrome (ESS), confirmed by the lack of response to substitution treatment. We develop different studies to confirm or eliminate potential interferences, being diagnosed of a possible macro-TSH.

Conclusion: It is important to consider that in patients with COVID-19 and high concentrations of TSH and thyroid hormones within reference ranges with lack of response to treatment, one of the possible causes to consider is the presence of macro-TSH. The presence of macro-TSH interference in COVID-19 patients requires extreme precautions to avoid errors in diagnosis or treatment (replacement dose adjustment) in patients with hypothyroidism secondary to an underlying disease.

Keywords: COVID-19; SARS-CoV-2; Euthyroid sick syndrome; Hypothyroidism; Interference; Macro-TSH

Abbreviations

ACE2: Angiotensin-Converting–Enzyme 2; CMIA: Chemiluminescence Microparticle Immunoassay; COVID-19: Coronavirus Disease 2019; ESS: Euthyroid Sick Syndrome; FT3: Free T3; FT4**:** Free T4; HAMA: Human Antimouse Antibodies; HBR: Heterophilic Blocking Reagent; HLA: Human Leukocyte Antigen; ICUs: Intensity of Care Units; JHU: Johns Hopkins University; p38-MAPK: p38 Mitogen-Activated Protein Kinase; PI3K/Akt**:** Phosphatidylinositol 3-Kinase/Akt Pathway; PEG: Polyethylene Glycol; rT3: Reverse T3; RF: Rheumatoid Factor; RLUs: Relative Light Units; SARS: Severe Acute Respiratory Syndrome; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; SAT: Subacute Thyroiditis; TBG: Thyroxine-Binding Globulin; TBPA: Thyroxine-Binding Prealbumin; TGAb: Thyroglobulin Antibodies*;* TPOAb: Thyroperoxidase Antibodies; TRAb: TSH Receptor Antibodies; TSH: Thyroid-Stimulating Hormone; TTR: Transthyretin; WHO: World Health Organization

Introduction

In December 2019, multiple pneumonia cases were reported in Wuhan, China, which were associated with a new coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). This virus caused Coronavirus Disease 2019 (COVID-19) that spread rapidly worldwide, leading to a public health emergency declared by the World Health Organization [1-3]. Since then, the number of cases of this disease has consistently increased up to 99,517,851 positive patients and 2,135,108 deaths (source: COVID-19 Dashboard by the Center for Systems Science and Engineering at Johns Hopkins University [JHU]; on 01/25/2021). The first case of COVID-19 in Spain was recorded on January 31, 2020.

COVID-19 is characterized by some frequent signs and symptoms, including fever, shortness of breath, dry cough, sore throat, dyspnea, fatigue (which may alter the patient's phonation and movement), lack of appetite, and digestive symptoms (diarrhea and vomiting). Other less frequent symptoms have also been described, including conjunctivitis, ageusia and/or anosmia, skin rashes, and thoracic pain [3,4]. Respiratory failure associated with acute respiratory distress syndrome is the main cause of mortality [3,4]. Despite the existing reports on severe and complex complications in other organs and systems, such as the immune, digestive, circulatory, hepatic, renal, neurological, and hematological systems [3,4], there is, currently, scarce data on thyroid complications related to this infection in

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patients with and without prior conditions.

An acute disease, such as COVID-19, affects the thyroid's metabolic regulation function as a result of the direct cell damage caused by an uncontrolled viral entry and intracellular replication, as well as by the hypoxia secondary to the underlying respiratory compromise. This induces apoptosis mediated by the p38 Mitogen-Activated Protein Kinase (p38-MAPK) [5,6], which may be aggravated by the use of inotropic and vasoactive drugs that stimulate such enzymatic pathways [5,6].

The typical thyroid alteration caused by this infection is the Euthyroid Sick Syndrome (ESS) [7] or also known as thyroid function in nonthyroidal illness. It can be described as abnormal findings on thyroid function tests that occur in the setting of a nonthyroidal illness, without preexisting hypothalamic-pituitary and thyroid gland dysfunction. Any of them have low serum concentrations of both Free Thyroxine (FT4) and Free Triiodothyronine (FT3), and their serum Thyroid-Stimulating Hormone (TSH) concentration also may be low. Previously, these patients were thought to be euthyroid, and the term ESS was used to describe the laboratory abnormalities. After recovery from an nonthyroidal illness, these thyroid function test result abnormalities should be completely reversible. This mechanism plays a significant physiological role in cell defense, increasing cellular tolerance to hypoxia by suppressing the activation of the p38-MAPK cascade and promoting tissue repair through controlled activation of the phosphatidylinositol 3-kinase/Akt pathway (PI3K/Akt) [8].

Thyroid function assessment in patients with COVID-19 is challenging, especially in patients admitted to Intensive Care Units (ICUs) and underlying thyroid dysfunction, given the large number of interferences introduced by acutely or chronically ill and external pharmacological factors, which may affect the results (especially due to alterations in the levels of thyroxine transport protein, Thyroxine-Binding Globulin [TBG]) [9] and the thyroid function tests.

Almost all patients with COVID-19 present low levels of Free Thyroxine (FT4), Free Triiodothyronine (FT3), and Thyroid-Stimulating Hormone (TSH) [10-14]. These findings may be related to the onset of transient central hypothyroidism mediated by the cytokines involved in the systemic inflammation triggered by this infection, which would have a defensive function by slowing tissue metabolism.

Thyroid function should not be assessed in seriously ill patients unless there is a strong suspicion of thyroid dysfunction.

In this study, we describe the characteristics and the clinical and analytical implications of an unusual analytical interference that may explain the false detection of hypothyroidism in patients with SARS-CoV-2 infection. This is the first time that a phenomenon of this nature has been described in patients with COVID-19 and underlying thyroid dysfunction.

Material and Methods

We studied three patients with severe SARS-COV-2 and prior thyroid conditions that were admitted during March and April 2020 (Table 1). Two patients had a history of thyroid papillary carcinoma (total thyroidectomy and postoperative hypothyroidism), and other patient had Hashimoto's thyroiditis. All patients required replacement therapy with levothyroxine and strict hormonal control. All patients were euthyroid before being admitted.

In the first analysis performed at admission, the hormonal profiles of patients 1, 2, and 3 suggested ESS. An atypical pattern in TSH increase was observed in the following hormonal tests (Table 2). It is noteworthy that thyroid function studies were repeated in patients admitted to the ICU, given their long stay and underlying thyroid pathology.

Laboratory and clinical investigations by the chemiluminescence immunoassay method revealed high TSH levels (the results were confirmed on several times) with no other demonstrable hormonal or clinical findings. Since the results obtained seemed paradoxical (high TSH values), we performed the assays suggested by the National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines to study potential interferences in immunochemical determinations [15].

It is crucial to identify causes of transient TSH elevation. A falsely high TSH level may be seen in some assays because of the presence of heterophilic antibodies, Human *Anti-Mouse Antibodies* (HAMA) and Rheumatoid Factor (RF) [16]. One of the rare causes of an elevated TSH concentration is macro-TSH. Macro-TSH is a macromolecule that includes TSH and an anti-TSH antibody [16-20].

Automated immunoassays used to evaluate thyroid function are vulnerable to different types of interference that can affect clinical decisions.

Potential interferences in thyroid function testing should always be suspected whenever clinical or biochemical discrepancies arise. Their identification usually relies on additional laboratory tests, including assay method comparison, dilution procedures, blocking reagents studies, and polyethylene glycol precipitation by listing some of the more common methods.

Methods to detect different types of interference in the thyroid function tests

TSH assays: Patients' full thyroid profile was determined using the Architect i4000SR system (Abbott Diagnostics) with a Chemiluminescence Microparticle Immunoassay (CMIA) technology for serum samples previously centrifuged at 3500 rpm for 10 minutes. The Enzyme Immunoassay (EIA) system for human TSH Architect is a two-step immunoassay to determine the presence of Thyroid Stimulating Hormone (TSH) in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex. In the first step, sample, anti-β TSH antibody coated paramagnetic microparticles and TSH Assay Diluent are combined. TSH present in the sample binds to the anti-TSH antibody coated microparticles. After washing, anti-α TSH acridinium labeled conjugate is added in the second step. Pre-trigger and trigger solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as Relative Light Units (RLUs). A direct relationship exists between the amount of TSH in the sample and the RLUs detected by the optical Architect iSystem. The Architect TSH assay is designed to have a functional sensitivity of ≤ 0.01 μU/mL, which meets the requirements of a third generation TSH assay and the analytical sensitivity (low-linearity) is defined in the ARCHITECT TSH assay parameters as 0.0025 μU/mL. A normal

Table 1: Clinical characteristics of COVID-19 patients.

Data	Patient 1	Patient 2	Patient 3
Age	65	56	73
Gender	Male	Female	Female
Thyroid disease	Papillary thyroid microcarcinoma Postsurgical hypothyroidism	Papillary thyroid microcarcinoma Postsurgical hypothyroidism	Autoimmune hypothyroidism in replacement therapy
Thyroxine replacement therapy (Dose Eutirox®)	88 µg	137 µg	100 µg
Thyroxine ajusted dose	125 µg	150 µg	150 µg
CURB-65 Score (Hospital admission)[a]	5	2	2
Days UCI	12	150	29
Treatment COVID-19[b]	Tocilizumab Kaletra[c]	Kaletra[c]	Tocilizumab Kaletra[c]

a. CURB-65: Confusion, Urea nitrogen, respiratory rate, blood pressure, 65 years of age and older. *b.* The patients were treated with hydroxychloroquine and azithromycin. *c.* Lopinavir + Ritonavir.

Table 2: Determination of TSH and FT4 in patient 2 by different assays.

Analyzer	TSH (normal reference range)	FT4 (normal reference range)
Architect i4000 (Abbott Diagnostics)	40.036 µU/mL (0.5-4.00)	0.83 ng/dL (0.8-2.00)
ADVIA Centaur XP (Siemens Healthcare Diagnostics)	10.54 µU/mL (0.5-4.80)	1.13 ng/dL (0.9-1.76)

Note: Results from the other patients were not available due to the safety measures required for COVID-19 positive samples.

range of 0.5 µU/mL to 4.0 µU/mL.

In an external laboratory, the concentrations of TSH and FT4 in sera of patient number 2 was measured with the ADVIA Centaur XP Healthcare Diagnostics) (Table 2). The results from the other patients from the other patients were not available due to the safety measures required for COVID-19 positive samples. The immunoassays for TSH were standardized to the Second International Reference Preparation (WHO80/558).

Heterophilic Antibody Interference: Heterophilic Blocking Reagent (HBR) of Scantibodies Laboratory INC was used to counteract the effect of HAMA (human anti-mouse antibodies). HBR blocks false positive reactions from a panel of HAMA, human anti-goat antibodies, human anti-sheep antibodies, human anti-rabbit antibodies and FR. The broad specificity means that Scantibodies' HBR is suitable as a "universal reagent" for 2-site immunometric assays using any of the commonly employed antibodies as solid-phase or labeled reagents. The effects of HAMA blockers were calculated as TSH concentrations with HAMA blockers / TSH concentrations without HAMA blockers × 100%. The study was performed on the serum sample from patient 2 (Table 3). Results from the other patients were not available due to the safety measures required for COVID-19 positive samples.

Polyethylene glycol (PEG) precipitation: For determining the presence of macro-TSH, all samples were subjected to the traditional method of immune complex precipitation with Polyethylene Glycol ([PEG] Merck® PEG-6000 at 25%); which is typically used for macroprolactinemia screening, described by Hattori et al. [21] and validated by Fahie–Wilson [22] and Olukoga [23,24,16]. In brief, serum samples (200 µl) were treated with 200 µl of 25% PEG (final concentration of PEG; 12.5%) to precipitate γ-globulin fractions and followed by free TSH determination in the supernatant. Control serum samples were also subjected to PEG precipitation. Results were expressed as monomeric TSH recovery percentage. The PEG-precipitable TSH(%), which may represent the amount of macro-TSH was calculated as follows: (total TSH-free TSH) / total TSH×100.

Table 3: Determination of TSH in patient 2 with Heterophilic Blocking Reagent (HBR) of Scantibodies Laboratory INC.

Patient 2	**TSH** (normal reference range)
HBR (Scantibodies Laboratory INC)	47.70 µU/mL (0.5-4.00)

Note: Results from the other patients were not available due to the safety measures required for COVID-19 positive samples.

Although the values used to define the presence of macro-TSH vary from 70% to 90% (higher percentage, higher probability), but this is the first study in patients with thyroid pathology and SARS-CoV-2 infection, we opted to use an arbitrary lower cut-off point (> 65%) [25,26] in order to avoid missing any sample with possible interference.

Serial dilution study of TSH: Subsequently, samples were processed after dilutions [15] (1:2, 1:5, and 1:10) using the recommended diluent (Architect Multiassay Manual Diluent) to verify the lack of parallelism against a control sample (linearity decreased as the dilution increased and the concentration of interfering factor reduced) (Table 1).

Gel filtration chromatography: The definitive test to detect the macro-TSH is gel filtration chromatography, but this technique was not used because it is expensive and is not available in most centers [18]. Currently, the most widely used technique to detect macro-TSH is polyethylene glycol precipitation.

Results

During their hospital stay, three study patients required ICU admission and invasive therapy due to respiratory deterioration. In this period, their thyroid profile showed alterations characterized by a persistent increase in TSH levels and a reduction in FT4 and FT3 levels, which were initially considered suggestive of ESS, without further confirmatory studies. However, the subsequent increase in TSH levels was unexpected, as it is not characteristic of the typical ESS pattern. Levothyroxine doses were readjusted based on the TSH levels after ruling out malabsorption and/or dosage errors.

Based on the obtained results, we performed additional studies

Table 4: Results after PEG 6000 precipitation and serial dilutions.

Data	Patient 1	Patient 2	Patient 3
TSH (0,500-4,000 µU/mL)	15.950	40.036	28.062
T4L (0,800- 2,000 ng/dL)	0.880	0.830	0.670
T3L (1,700- 4,000 pg/mL)	1.360	1.810	1.830
TSH Post-PEG 6000 (monomeric-TSH)	5.516	15.008	6.430
% recovery	34.6%	37.5%	22.9%
Control TSH Post-PEG 6000 % recovery	91.2%	88.5%	83.4%
Dilutions			
1:2	13.033	41.525	28.063
1:5	17.147	43.423	26.699
1:10	16.009	39.009	26.464
Control dilutions	**Undiluted** 47.70	**1:2** 21.60 43.20	**1:5** 8.58 42.90
Estimated Glomerular Filtration rate (eGFR) (CKD-EPI-equation) mL/min/1,73m²	>90 G1 Normal	>90 G1 Normal	>90 G1 Normal

The same analytical checks were developed in each patient in the successive samples received, observing the same pattern and confirming the presence of macro-TSH during admission.

to confirm or eliminate potential interferences. As a result, TSH concentration was determined by a Polyethylene Glycol precipitation test (PEG-6000) at 25%, similar to the macroprolactin study [21], and a highly reduced percentage was obtained (TSH in controls was 87.70 ± 3.23 % and in the samples of our patients 31.66 ± 6.31 %) (Table 4). Today, the most commonly used technique for detecting macro TSH [18].

Using the same blood samples for which the TSH levels were found to be high, the PEG method found TSH concentrations with values of 6.430 µU/mL in the patient 3 (a decrease > 70%) and 15,008 µU/mL in the patient 2 (a decrease < 70%), respectively.

Furthermore, potential antibody interferences can also be detected or confirmed by serial dilution of samples. Hence, we performed serial dilution of the samples collected from the patients (1:2, 1:5, and 1:10) and proceeded to analyze the TSH concentrations. Results were not linear in any of the samples (Table 4) and suggested the presence of a molecule of high molecular weight, which would affect TSH determination (Figure 1 and Table 5).

Other useful techniques for sample processing are the heterophilic antibody blocking agents (HBR of Scantibodies Laboratory INC) (Table 3) and exclusion chromatography, which was not available due to the safety measures required for COVID-19-positive samples.

Renal function should also be considered, as TSH-IgG complexes, which have a high molecular weight, disappear slowly from circulation. Also, their levels increase upon renal failure due to decreased clearance of macro-TSH [21] (decreased glomerular filtration) (Table 4).

Discussion

Thyroid endocrine alterations in patients diagnosed with symptomatic SARS-CoV-2 infection remain underrated due to their unknown physiopathological mechanism.

Table 5: Evolution of the thyroid profile in the study patients. Parameters and units.

Patient 1			
Date	**TSH**	**FT4**	**FT3**
10/11/19	0.598		
01/14/19[a]	0.462	1.24	
HA: 03/29/20[b]	0.915	0.67	<1.07
04/08/20	15.95	0.88	1.36
04/16/20[c]	15.079	0.92	1.91
04/21/20	16.02	0.95	2.01
05/29/20	0.015	1.49	
[a]**TPOAb**: 0.49; [b]**TRAb**: 1.09; [c]**TPOAb**: 0.33			
Patient 2			
Date	**TSH**	**FT4**	**FT3**
06/05/19[a]	0.544	1.18	
HA: 04/03/20	0.303	0.66	1.43
04/27/20[b]	40.036	0.83	1.81
05/07/20	25,348	0.89	2.48
05/22/20[c]	1.263	1.0	
[a]**TGAb**: 1.16; [b]**TPOAb**: 1.43; [c]**TGAb**: 0.34 and **TG**: 0.66			
Patient 3			
Date	**TSH**	**FT4**	**FT3**
08/20/18	0.832		
03/13/20	5.363	0.84	1.27
HA: 03/24/20	3.198	0.58	1.27
04/14/20	28.62	0.67	1.83
04/17/20[a]	22.44	0.82	1.52
04/30/20	10.54	1.52	2.71
06/16/20	0.702	0.87	2.78
07/13/20	0.964		
[a]**TPOAb**: 2.71			

Normal reference range: TSH: 0.5-4.0 µU/mL; FT4: 0.8-2.0 ng/dL; FT3: 1.7-4.0 pg/mL; TPOAb: 0.0-5.61 U/mL; TGAb: 0.0-4.11 U/mL; TG: 1.4-78.0 ng/dL; TRAb: <2 U/L; HA: Hospital Admission.

Viral infection causes a significant generalized inflammatory response and a considerable increase in the levels of circulating cytokines (mainly IL-2, IL-7, TNF-α, IFN-α, IFN-γ, NF-κB, and IL-6) [8-23,26,27], which may cause pulmonary [3] and thyroid damage in patients suffering from this infection.

Brancatella et al. have recently reported the first case of Subacute Thyroiditis (SAT) [28-30] following SARS-CoV-2 infection [27], suggesting the thyroid gland as a target organ of SARS-CoV-2. It is well known that SAT is generally preceded by an upper respiratory pathway infection, as it has been reported during the outbreaks of echovirus, coxsackievirus, paramyxovirus, adenovirus, orthomyxovirus, Epstein-Barr virus, hepatitis E virus, human immunodeficiency virus, cytomegalovirus, dengue virus, and rubella virus [31]. Additionally, it is noteworthy that thyroid hormones also seem to prevent the efficacy of certain viruses, such as herpes simplex virus, due to the stimulation of intracellular defense immune mechanisms mediated by natural

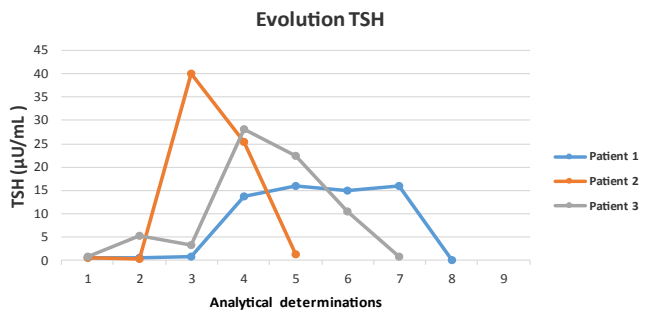

Figure 1: Evolution TSH in the patients of the study.

killer cells, as well as the immunostimulant effect associated with interferons [9].

During the 2002 Coronavirus Strain (SARS-CoV) outbreak, damage in the follicular cells of thyroid glands was detected in autopsies of infected patients [32]. Wei et al found a significant decrease in thyrotrope cells [32], suggesting that the endocrine cells of the adenohypophysis may be damaged by the coronavirus, partly explaining why TSH secretion cannot be stimulated by the negative feedback cycle with decreased T3 and T4 concentrations.

The Angiotensin II Converting Enzyme (ACE2) is the main entry receptor for SARS-CoV and SARS-CoV-2 cellular invasion, facilitating direct damage by the virus during infection [33]. Li et al recently reported that the ACE2 receptor is expressed at high concentrations in the thyroid gland [33,34], suggesting that this gland is a potential target for direct viral damage.

The underlying mechanism for thyroid and hypophysial function damage is still unknown, but it is generally believed to be caused by a direct glandular viral infection, which is evidenced by the wide intracellular distribution of viral nucleic acid and the post-viral inflammatory reaction in genetically predisposed subjects [32,35,36]. Human Leukocyte Antigen (HLA) haplotypes, especially HLA-Bw35, but also HLA-B67, HLA-B15/62, and HLA-Drw8, have been reported as predisposing factors for thyroiditis [31,35]. Indirect damage, i.e. damage caused by the host's immune hyperactivity as a consequence of a systemic inflammatory response, is considered to be another cause of the disease.

Thyroid impairment caused by SARS-CoV-2 has a dynamic evolution [36], progressing toward gradual recovery.

In this way, a decrease in TSH and other thyroid hormones was observed compared to the normal range [36], but, surprisingly, the decrease was lower than in patients without COVID-19.

Evidence suggests that these patients develop transient central hypothyroidism [37]. It is likely that the changes in thyroid function, characterized by decreased thyrotropin-releasing hormone mRNA levels in the hypothalamic paraventricular nucleus (possibly cytokine-dependent) observed in patients with this severe disease, are protective because they avoid excessive tissue catabolism. Abnormalities have also been observed in TSH glycosylation, which could decrease their bioactivity [38].

As a result, T3 or T4 replacement therapy could be expected to have a favorable effect on mortality. However, this was not the case. In randomized trials on severely ill patients with decreased T3 and T4 levels, T3 or T4 replacement did not affect mortality as opposed to the control group. Based on this evidence, a new randomized, double-blind, placebo-controlled trial (Thy-Support, ClinicalTrials.gov Identifier: NCT04348513) will be conducted to study the effect of high (intravenous) doses of T3 for improving recovery of critically ill COVID-19 patients [8].

It is known that about 15%–20% of hospitalized patients and 50% of patients admitted to the ICU have low T4 levels (low T4 syndrome) due to decreased concentration of one or more transport proteins (TBG, transthyretin [TTR] or Thyroxine-Binding Prealbumin [TBPA], and albumin). As TBG is the main transport protein, serum T4 decrease could be caused by an alteration in abnormal glycosylation binding or dissociation during circulation [39].

Moreover, most hospitalized patients have low T3 concentrations (low T3 syndrome). About 80% of circulating T3 is produced by peripheral 5′-deiodination of T4, a reaction catalyzed by 5′-monodeiodinases (D1 and D2) in different organs (muscle, liver, and kidney). Since 5′-monodeiodination decreases in any non-thyroid disease, even a mild one, the 5'-monodeiodinase (D3) activity, which converts T4 into reverse T3 (rT3) [40], will be predominant.

The changes in thyroid function will vary depending on the severity of the disease. Hence, low FT3 concentration has been correlated with a longer hospital stay, ICU admission, mechanical ventilation requirement [41], and increased mortality of patients with acquired pneumonia [26,42].

FT4 levels have also been correlated with the outcomes of critical patients. Values < 0.3 ng/dL have been associated with mortality rates > 85% [43].

It has been suggested that inactivation of T4 to T3 conversion during the disease course may be a beneficial way of adaptation.

This study demonstrates the presence of TSH increase in patients with SARS-CoV-2 infection and an underlying thyroid condition, caused by the presence of an autoimmune complex formed by the association of TSH with G-IgG immunoglobulin called macro-TSH, an analytical alteration that has not been described in the existing literature. As the size of a TSH molecule is 28 kDa, it is freely filtrated through the renal glomerulus. However, when the IgG-TSH complex is formed, its size increases to approximately 180 kDa, which impedes renal filtration and causes its accumulation, resulting in a slower renal clearance.

The IgG-TSH molecule is biologically inactive but variably immunoreactive depending on the method used [17]. The multiple concentrations observed with all the available analytical methods may be because when macro complexes are formed, TSH epitopes are more or less exposed, resulting in a different reactivity based on the design of each assay. Precipitation with polyethylene glycol has been used to detect this interference, with a quite low TSH percentage being recovered, suggesting the presence of a molecule with higher molecular weight, such as HAMA or any other antibody [44], interfering in TSH determination. We believe that if TSH levels >10 µU/L are higher than 65% after polyethylene glycol precipitation, the presence of macro-TSH should be suspected.

The TSH values measured by the polyethylene glycol precipitation test were lower, although not markedly lower and the patients had polyethylene glycol-precipitation TSH ratios greater than 65%, but no greater than 75% as is expected by the studies presented by Hattori et al, possibly due to infection SARS-COV-2 and that this arbitrary cut-off point was obtained in patients with no known pathology [25]. This variability is very possible that is secondary to the infection by SARS-COV-2 and / or its severity.

Normal / decreased TSH and FT4 levels were obtained with a HBR tube. HAMA and rheumatoid factor, which can act like heterophile antibodies, were undetectable in these patients.

At present, the most widely used technique to detect macro-TSH is precipitation with polyethylene glycol, which has displaced gel filtration chromatography as it is an expensive technique not available in most Hospitals [18].

These antibodies can also be detected by serial dilution of samples through this interference, obtaining non-proportional results.

The clinical significance of macro-TSH is currently not known. Because TSH measurements are uncertain in these situations, the patients who have macro-TSH should be followed up clinically or maybe with FT4 levels. On the other hand, macro-TSH should be kept in mind when higher than expected levothyroxine dosages are needed in order to achieve the therapeutic target of serum TSH in patients with thyroidectomy [19,20].

Macro-TSH was screened by adding PEG to serum in order to precipitate γ-globulin fractions [25,45,46] being its prevalence is not well known. Macro-TSH seems to be a heterogeneous entity which can be suspected when normal FT4 levels are associated to elevated TSH levels, and when substances that commonly interfere with TSH assays, including heterophilic antibodies, human anti-mouse/animal antibodies and rheumatoid factors have been excluded [47]. Macro-TSH can also occur in patients with TSH concentrations within reference ranges [48].

To date, the bulk of data on macro TSH prevalence have been provided by studies by Hattori et al. [25,45,46]. This study was conducted in a group of 681 patients with subclinical hypothyroidism and no underlying thyroid disease of interest and using an arbitrary cut-off point for TSH precipitable with PEG (> 75%), these authors found a prevalence of macro TSH of 1.6% after gel filtration [45].

Subsequently, in other study with 1901 sera from patients with subclinical hypothyroidism, in whom the presence of HAMA had been excluded, the prevalence of macro TSH decreased to 0.8% [25]. Mills et al. reported a similar prevalence (0.6%) [49] in patients with TSH levels greater than 10 μU/L. We should keep in mind that even commercial TSH immunoassay systems cannot be completely free from interference by HAMA.

There are no data on cohorts of patients with thyroidectomy, and even less, with concomitant SARS-COV-2 infection in the pandemic that we are suffering.

In the study by Giustin et al. [50], PEG-precipitable TSH was 39.3% ± 1.9% in patients who had undergone total thyroidectomy for thyroid cancer. These percentages appear to be somewhat lower than those reported in 681 samples from subjects with a clinical diagnosis of subclinical hypothyroidism (21% to 100%, on average 63%) [45]. Furthermore, in this group of patients, according to the arbitrary cut-off point of ≥80%, the prevalence of macro TSH (3.1%) was higher than that reported in subclinical hypothyroidism [25,45,49]. It is not clear if this difference is due to the different cohort of patients or to the lack of gel filtration or HAMA tests [50].

Loh et al. [51] reported that the majority of macro TSH patients were women without symptoms of thyroid disorders, except for one with clinical features of hyperthyroidism, and Hattori et al. [25] stated that macro TSH is predominant in samples from elderly patients and that autoimmune mechanisms could be involved in the generation of macro TSH [45] therefore COVID-19 could be a trigger of the autoimmune mechanisms.

In our opinion, more data are needed to attribute macro TSH in SARS-COV-19 patients and underlying thyroid pathology. The macro-TSH should not be ruled out in patients with SARS-COV-2 infection because there is no high precipitation with PEG. A lower recovery with PEG (> 65%) could be compatible in patients with COVID-19. There are many unanswered questions in patients with COVID-19 and this article could lead to new unexplored research.

Detection of macro TSH should be performed in particular conditions such as positive COVID-19 patients and underlying thyroid pathology, such as Kadoya et al indicated in patients with sleep disorders [52].

Our study has several limitations:

1. The study group includes few subjects with underlying thyroid disease and positive COVID-19.
2. Other laboratory procedures such as gel filtration chromatography were not performed because it is an expensive technique and is not available in most centers, with PEG precipitation currently being the most widely used technique.
3. The PEG-precipitable TSH cutoff was arbitrarily set very similar to the Hattori study in patients with subclinical hypothyroidism.
4. Results from the other patients were not available due to the safety measures required for COVID-19 positive samples..

Key Points

1. To date, patients with COVID-19 and underlying thyroid disease have not been studied.
2. The patients were perfectly controlled before suffering the coronavirus infection.
3. Thyroid function returned to normal when the patient recovered from COVID-19.
4. No correlation between thyroid study and clinical pathology. Without criteria for ESS.
5. All patients were treated differently (different drugs). Therefore, we can rule out drug interference.
6. Levothyroxine dose adjustment did not produce the desired effect, despite being patients with prolonged admissions. TSH levels

were not normalized with levothyroxine therapy; this suggested that the secretion of TSH and the formation, dissociation, and clearance of the TSH-anti-TSH autoantibody complexes were equilibrated, at the elevated levels of macro TSH and normal levels of free TSH.

7. It is important to closely monitor patients diagnosed with thyroid cancer or other thyroid pathology to detect possible interference with SARS-COV-2 in the thyroid study, and to avoid overdosing or treating patients incorrectly.

8. Macro-TSH is currently detected quickly and easily with PEG test and the cut-off point is arbitrary, but we consider that if precipitation is greater than 65% and with discrepancies in the clinical and laboratory results (TSH > 10μU/L) the patients should be closely monitored.

9. Therefore, patients with subclinical hypothyroidism who have serum TSH levels above 10 μU/L may be candidates for the screening of macro TSH, especially those who have extremely high levels of TSH.

10. Diagnosis of macro TSH is clinically important because it may change the therapeutic strategy.

11. The nature of the non-IgG-associated macro TSH remains to be elucidated, but it might be an aggregate of highly glycosylated TSH analogous to non-IgG-associated macro PRL.

In conclusion, free TSH levels after PEG precipitation are on average 30-40% lower than serum TSH levels in subjects without a history of thyroid carcinoma or other significant thyroid pathology. The presence of macro TSH could be a more common phenomenon than is thought in patients with COVID-19 and underlying thyroid disease. Patients suspected of having macro TSH should be followed for a long time, due to the hypothesis of a long persistence of the immunoglobulin G-TSH complex [46]. It is necessary to evaluate more serum samples in similar patients and avoid unnecessary exogenous hyperthyroxinemia.

Our data demonstrate that, in assessing the adequacy of FT4 dosage after total thyroidectomy in thyroid cancer patients, the individual balance of FT4 and macro TSH must also be considered.

Although the prevalence of macro TSH was higher in older people, it has also been observed in patients of childbearing age. Therefore, it is very important to distinguish elevated serum TSH levels caused by macro TSH from those caused by monomeric TSH in patients with subclinical hypothyroidism. At present, there are no TSH immunoassay platforms that do not cross-react with macro TSH. Therefore, we recommend that when hormone replacement therapy for subclinical hypothyroidism is considered, screening for macro TSH should be performed [25].

In 1995, Tamaki [53] was the first author to suggest the presence of high molecular weight TSH as the cause of transient high TSH levels. This was subsequently confirmed by other researchers [54]. The actual prevalence of this macro-TSH interference in adults or newborns is unknown because it has been poorly described in the existing literature [26,53,54], but it is considered to be very low (0.17%–1.6%) [54].

For this reason, TSH determination is not adequate for assessing thyroid function in these patients because the concentration measured does not reflect the actual biologically active concentration. Moreover, this interference may not always be identified, especially when the TSH increase is moderate, and it can be confused with ESS. Hence, if the presence of this complex is confirmed, assessment of the patient's thyroid function by TSH determination is not valid.

In summary, it is important to consider that in severely ill patients with COVID-19 and high concentration of TSH and thyroid hormones within reference ranges or lack of response to treatment, one of the possible causes to be considered is the presence of macro-TSH. Its detection is recommended to optimize thyroid treatment.

Ethical considerations

Ethical approval was not received because of the retrospective nature of the case report.

Ethical approval was not received due to the descriptive nature of the cases reports.

Author contributions

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Competing interests

The funding organizations played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the manuscript; or in the decision to submit the manuscript for publication.

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