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INTERFERENCE IN IMMUNOASSAY MEASUREMENTS OF TSH AND THYROID HORMONES AFTER VACCINE FOR COVID-19 OR COVID-19 DISEASE

Abstract: Immunoassays are commonly used to measure thyroid-stimulating hormone (TSH) and thyroid hormone levels (TH). However, interference in these immunoassays may lead to the misinterpretation of patient results and incorrect therapeutic decisions. Analytical interference can occur following exposure to animals, infections, vaccination, in autoimmune diseases, or without an identified cause. Analytical interference associated with COVID-19 exposure has been observed in D-dimer measurements. A dilution test was used to confirm interference in the measurement of TSH and TH levels. In three patients, interference in TSH and TH levels was detected after COVID-19 infection or vaccination.

Conclusions: Owing to the high number of infected and vaccinated individuals, we can expect an increase in the prevalence of interference in immunometric methods.

Key words: Interference, Covid-19, TSH, Thyroid hormones

Introduction:

Immunoassays are commonly used to measure thyroid-stimulating hormone (TSH), total and free thyroxine, and triiodothyronine (TT4, FT4, TT3, and FT3) levels. However, interference in these immunoassays may lead to the misinterpretation of patient results and incorrect therapeutic decisions, with possible deleterious consequences for patients [1].

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Interfering substances can be endogenous or exogenous. Endogenous substances are polyreactive antibodies or autoantibodies (heterophile) or human anti-animal antibodies (in this report the term "heterophile" will be used for both types of antibodies). Biotin is the most common exogenous interfering substance. These interferences can result in falsely elevated or low analyte concentrations in one or more assay systems [2–7].

Heterophile antibodies can appear following exposure to animals or animal products, viral or bacterial infections, vaccination, in autoimmune diseases, or without an identified cause [8]. According to the WHO COVID dashboard, the total number of confirmed cases of COVID-19 is greater than 572 million, and as of July 26, 2022, 12,248,795,623 vaccine doses have been administered [9]. Analytical interference associated with exposure to COVID-19 has already been noted in D-dimer measurement [10]. Here, we report for the first time the development of interference in thyroid function tests after COVID-19 vaccination or COVID-19 infection in three patients.

Materials and Methods:

The patients were well known to their physicians and were attending endocrine clinics having had a diagnosis of thyroid disease. The suspicion of interference was based on a discrepancy between the clinical findings and the current and previous laboratory results. Patient 1 was diagnosed with papillary thyroid cancer, treated 4 years earlier, and had been receiving a stable dose of levothyroxine 75 mg daily for 3 years. All semi-annual thyroid function tests in the preceding three years showed normal or low TSH and normal FT4 levels. There was no change in levothyroxine dose, and the patient was clinically euthyroid. Thyroid function tests were expected to show normal or low TSH levels and normal TT4 and FT4 levels. Instead she was found to have a markedly elevated TSH (15.5 mu/L, reference range 0.3-5.5) with normal TT4 and FT4. Patient 2 had subclinical hyperthyroidism with increased TSH receptor antibodies (2.8 IU/L, cut-off value 1.8 IU/L). During the previous 18 months she had several thyroid function tests showing a serum TSH of 0.01-0.02 mIU/L (reference range 0.3-5.5), FT4 16.6-18.1 pmol/L (reference range 7-18 ng/L) and TT3 1.35-1.85 nmol/L (reference range 1.2-2.8). The patient did not receive any thyroid medications. The patient was clinically euthyroid. Thyroid function tests were expected to show low TSH levels and normal TT4 and FT4 levels. However, her serum TSH level was normal. Patient 3 was diagnosed with primary hypothyroidism and had been receiving a stable daily dose of 125 mg of levothyroxine for the previous year. Her TSH was 3.0 mIU/L, three weeks before the first COVID vaccine. She was clinically euthyroid and her thyroid function tests were expected to be normal. However, her TSH was low (0.1 mu/L, normal range 0.3-5.5) and her FT4 elevated (21.7 pmol/L, normal range 7-18). All three patients were vaccinated against COVID-19, had COVID-19, or both. No unexpected or incongruous thyroid function

test results were noted before the COVID-19 vaccination or infection. COVID-19 infection was defined as symptoms and signs consistent with those of COVID-19 infection and confirmed by a positive COVID-19 test (lateral flow or PCR) during the symptomatic phase. All three patients underwent routine thyroid function tests as part of the management of thyroid disease during an office visit. In all three cases, the results of the thyroid function tests were contradictory to their euthyroid clinical status or to the previously recorded pattern, which triggered further investigation of serum samples for interference. The patient data are presented in Table 1. Dilution tests (1:5 and 1:10 using assay buffer for TSH, and TT4, and normal saline for FT4) were performed between the end of December 2021 and beginning of May 2022. Control patients were subjects in whom interference was suspected, but not proven. No patient was taking biotin supplements at the time of testing.

TSH was measured using IRMA (INEP, Belgrade, Serbia, reference range 0.3-5.5 mIU/L, intra-assay CV (mean 6.04 mIU/l) 2.1%, inter-assay CV mean (6.07 mIU/l) 7.1%). TT4 was measured using RIA (INEP, Belgrade, Serbia, reference range 55-155 nmol/L, intra-assay CV (mean 68 nmol/L) 3.3%, inter-assay CV (mean 81.9 nmol/L) 4.28 %). FT4 was measured by RIA (Cisbio, France, reference range 7-18 ng/L, intra-assay CV (mean 14.55 ng/L) 5.1%, inter-assay CV (mean 14.35 ng/L) 5.06%). The presence of interference was confirmed by serial dilutions of 1:5 and 1:10). For FT4 dilution, normal saline was used as previously recommended [11].

Measurements from each of the serial dilutions were compared to those from the 1:1 dilution, and Z-scores were estimated using within-run CV for the range corresponding to each measurement. Thus, a measurement x_k from the 1:k dilution would use the CV corresponding to the range containing $x_{1/k}$. The SD of the measurements was calculated as $SD_k = x_k \times CV_k$. The variance for the difference between the measurement for the 1:k dilution and the 1:1 dilution was calculated as $SD^2 = SD_k^2\ SD_1^2$ and the Z-score as $Z = (x_k - x_1)/SD$, where SD_1 and x_1 are the SD and measured values of the undiluted sample, respectively. Results were designated as discordant if the absolute value of the Z-score for comparison exceeded 4 [12].

All procedures were performed in accordance with the ethical standards of the institutional and/or national research committee and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Due to the retrospective nature of the investigation and the lack of patient identification, IRB approval was not necessary.

Results:

The results of the dilution tests are presented in Table 2. In all patients, the dilution tests showed interference in the measurement of TSH and FT4 levels, and in two patients, in the measurement of TT4. In one patient, interference with TT4 determination was marginal.

Discussion:

Prior to the COVID-19 pandemic, the incidence of interference in thyroid function tests due to heterophilic antibodies was estimated at between 0.4% and 6% or more, depending on the assay and the analyte considered [13]. In patients with COVID-19, interference in D-dimer measurements has already been described [10]. We report three patients who displayed interference with the immunometric measurement of serum TSH or thyroxine between 55 and 294 days after COVID-19 vaccination or infection. Interference was suspected because of the discordance between the patients' clinical status and biochemical results. Exposure to the COVID-19 vaccine or virus was the common denominator, and preceded the abnormal thyroid function test in all cases. Although causation cannot be confirmed, this is likely the case. The frequency of interference in thyroid function tests after COVID-19, how it affects different analytes and assays, and its duration and likelihood following different COVID-19 variants and vaccines are unknown. Owing to the high prevalence of COVID-19 and the high number of vaccinated individuals, misleading thyroid function tests may be more common than previously thought and can potentially adversely affect patient management. Therefore, raising awareness of this possibility is important, and we would recommend that discrepancies between the clinical status and biochemical results of thyroid function tests should trigger testing of the sample for interference, especially in patients who have been exposed to COVID-19 or vaccinations against it. Further studies are needed to determine whether there is a causative relationship between COVID-19 infection and/or vaccination and thyroid function test interference, its true incidence, and the most efficient means of detecting it.

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