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ПРИКАЗ БОЛЕСНИКА / CASE REPORT
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Challenges in interpretation of thyroid hormone test results

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SUMMARY

Introduction In interpreting thyroid hormones results it is preferable to think of interference and changes in concentration of their carrier proteins.

Outline of Cases We present two patients with discrepancy between the results of thyroid function tests and clinical status. The first case presents a 62-year-old patient with a nodular goiter and Hashimoto thyroiditis. Thyroid function test showed low thyroid-stimulating hormone (TSH) and normal to low fT4. By determining thyroid status (TSH, T4, fT4, T3, fT3) in two laboratories, basal and after dilution, as well as thyroxine-binding globulin (TBG), it was concluded that the thyroid hormone levels were normal. The results were influenced by heterophile antibodies leading to a false lower TSH level and suspected secondary hypothyroidism. The second case, a 40-year-old patient, was examined and followed because of the variable size thyroid nodule and initially borderline elevated TSH, after which thyroid status showed low level of total thyroid hormones and normal TSH. Based on additional analysis it was concluded that low T4 and T3 were a result of low TBG. It is a hereditary genetic disorder with no clinical significance.

Conclusion Erroneous diagnosis of thyroid disorders and potentially harmful treatment could be avoided by proving the interference or TBG deficiency whenever there is a discrepancy between the thyroid function results and the clinical picture.

Keywords: thyroid hormone assays; interference; thyroid-binding globulin

INTRODUCTION

Measurement of thyrotropin (TSH), total and free thyroxine (T4, fT4) and triiodothyronine (T3, fT3) enables the assessment of thyroid function [1–4].

It has been observed, in some cases, that values of thyroid hormones are not in accordance with the clinical assessment, which raised suspicion of interference. At present there are many more reports on thyroid assays interference [1, 5, 6], particularly in TSH assays, and emphases of possible erroneous results [1, 6].

In highly sensitive immunoassays, with one or two antibodies, radioimmunoassay (RIA), chemiluminescent magnetic immunoassay (CMIA) and/or chemiluminescent immunoassay (CLIA), presence of endogenous circulating antibodies against different antigens can cause false increase or decrease of thyroid hormones [1, 7]. These abnormal values can lead to unnecessary change of daily dose replacement therapy or to complex additional diagnostics, i.e. thyroid function suppression tests and scintigraphy [1].

In circulation, a majority (>95%) of thyroid hormones is reversibly bound to carrier proteins: thyroxin-binding globulin (TBG), transthyretin (TTR or prealbumin) and albumin [7]. Hereditary or acquired variations in concentration and/or affinity of carrier proteins can cause significant changes in levels of total thyroid hormones in available commercial assays [8]. However, these changes do not result in hypo or hyperthyroidism because concentrations of free hormones remain unchanged [7].

CASE REPORT

Case 1

The first case presents a 62-year-old patient with multinodular goiter, without disorders of thyroid function. A fine needle aspiration biopsy was performed and cytologic finding was chronic lymphocytic thyroiditis Hashimoto. In March 2014, the thyroid function tests showed the following: TSH 0.4 mIU/L, fT4 14.2 ng/L. In June, the TSH was 0.2 mIU/L, fT4 10.4 ng/L. TPO antibodies were positive, and apart from oscillating hypertension, the patient did not experience other problems. Additional tests were performed in July. The thyroid status (TSH, T4, fT4, T3, fT3) was determined in two laboratories (RIA and CMIA assays), basal and after five-fold dilution (the results are shown in Table 1). After that, TBG, total serum proteins, albumin, and serum protein electrophoresis were determined, as shown in Table 2. It was concluded that values of thyroid hormones are proper and that concentration of TBG is normal. The results were influenced by the presence of heterophile antibodies, which led to falsely lower TSH and suspicion of secondary hypothyroidism.

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Table 1. Thyroid function tests of the first patient

Parameter/ method	TSH mIU/L	T4 nmol/L	T3 nmol/L	fT4 pmol/L; ng/L	fT3 pmol/L; ng/L	Calcitonin
CMIA	0.59 (0.35–4.94)	107.2 (66–181)	1.61 (1.3–3.1)	16.4 (9–19)	5.11 (2.6–5.7)	6.3 ng/ml
1:5	0.32	83	1.51			
RIA	0.3 (0.3–5.5)	136.9 (55–155)	2.1 (1.2–3.0)	13 (7–18)	2.7 (2–4.25)	
1:5	3.72	139	1.4			

CMIA – chemiluminescent magnetic immunoassay; RIA – radioimmunoassay; TSH – thyroid-stimulating hormone

Table 2. Values of protein electrophoresis and thyroxine-binding globulin (TBG) of the first patient

Albumin	39.4
Alpha 1	2.1
Alpha 2	9.3
Beta 1	6.7
Beta 2	2.1
Gamma	16.1
A/G	1.25
TBG	366 (259–574 nmol/L) CLIA
Proteins	71 g/L

CLIA – chemiluminescent immunoassay

Table 3. Thyroid function tests of the second patient

Parameter/method	TSH mIU/L	T4 nmol/L	T3 nmol/L	fT4 pmol/L; ng/L	fT3 pmol/L; ng/L
CMIA	2.02 (0.35–4.94)	48 (66–181)	0.67 (1.3–3.1)	11.8 (9–19)	3.36 (2.6–5.7)
1:5	1.31	43.5	0.63		
RIA	1.0 (0.3–5.5)	68.2 (55–155)	2.0 (1.2–3.0)	14.4 (7–18)	2.8 (2–4.25)
1:5	2.23	102.1	0.5		

Table 4. Values of protein electrophoresis and TBG of the second patient

Albumin	38.7
Alpha 1	2.7
Alpha 2	2.0
Beta 1	9.1
Beta 2	4.9
Gamma	17.3
A/G	1.13
TBG	95 (259–574 nmol/L)
Proteins	73 g/L

Table 5. Thyrotropin-releasing hormone (TRH) test (200 mcg TRH i.v.) of the second patient

Time	0′	60′	90′
TSH mIU/L	2.31	16.07	12.07

Case 2

A 40-year-old patient had been examined and followed because of 3 mm thyroid nodule and borderline elevated TSH level. During the follow-up, thyroid nodule size increased to 7 mm, with TSH 2.3 mIU/L, fT4 13.1 ng/L, calcitonin less than 2 ng/ml. Since May 2014, she had complained to palpitations while resting, shortness of breath and fatigue at the slightest exertion and syncope. Results showed TSH level of 2.17 mIU/L, T4 45 nmol/L, T3 <7 nmol/L, thyroid nodule of 11 × 9.6 mm, with colloidal appearance without pathological features. Pituitary MRI finding was normal. Based on this, additional analyses were performed, whose results are shown in Tables 3 and 4. The thyrotropin-releasing hormone (TRH) test was also done. It was concluded that TSH response during the TRH stimulation was normal, and normal TSH level with low T4 and T3 concentrations were the consequences of low TBG. Thus, the conclusion was that this was a hereditary genetic disorder with no clinical significance, and treatment was not required.

DISCUSSION

Determination of TSH is considered to be the "gold standard" for the evaluation of thyroid function and often serves as the initial test [2, 3]. Since decisions on treatment and additional diagnostics are based on it, it is necessary to consider the presence of heterophile antibodies and interference when there is a mismatch between TSH, fT4, and the clinical aspect of the patient [1, 5]. The first clinical case had a multinodular goiter, heterogenous due to chronic lymphocytic thyroiditis. Repeated testing of thyroid status showed low–normal TSH and normal and borderline fT4, which may indicate secondary hypothyroidism. In our laboratories, RIA, CLIA, and CMIA are available thyroid assays. Ever since the 60s, when first implemented, RIA uses a radioactive isotope as antibody and/or antigen tracer in the assay. However, due to radioactivity, highly sensitive assays that use enzymes or chemiluminescent particles as tracers started to get used more frequently. Ever since 1987, when Brennan et al. [5] first described falsely higher TSH values because of endogenous anti-mouse antibodies, there have been more similar reports. Heterophilic antibodies, also known as human anti-animal antibodies, are defined as antibodies directed against immunoglobulins of animal origin [6, 9, 10]. They can interfere in wide range of immunoassays i.e. in those for viral antigens, ferritin, tumor markers. The best known heterophilic antibodies are human anti-mouse antibodies (HAMA) that can react with the monoclonal murine antibodies present in many immunoassays [1]. Heterophilic antibodies may exist in cancer patients after infusion of monoclonal antibodies of murine origin in diagnostic and/or therapeutic purposes [11, 12]. These antibodies could also be present after vaccination or a blood transfusion, after use of pharmaceutical products of animal origin or in contact with animals from the environment, not pets, so they can be detected more frequently in farmers and veterinarians [13, 14]. The prevalence of persons with heterophile antibodies ranges from 1% to 80% in general population [15]. These antibodies are also found in various autoimmune diseases [11]. "Sandwich" assays are usually most sensitive to HAMA interference [1]. Samples of patients expressing HAMA may give falsely increased or decreased readings in the immunoassays. False positive values as a result of HAMA interference is the most common

type of interference described in TSH assays [1]. In case of our patient, due to absence of expected linearity in CMIA values, though more sensitive, we accepted RIA results after dilution ensuring greater reliability and precision. Diluting in 1:5 proportion is the best way to "imitate" serum environment. However, there is no data what dilution is best suited. Higher RIA readings, consistent with pathology of the thyroid gland, although still within normal ranges, confirm the presence of heterophile antibodies that falsely lowered readings in the CMIA assay. This is a less common type of TSH assay interference, but not of less clinical importance, since the patient did not require additional functional tests and imaging methods. In the literature, cases of false-positive TSH readings are more common [15]. In case of unnecessary treatment of wrongly diagnosed hypothyroidism, based on high TSH level, the replacement therapy can lead to iatrogenic thyrotoxicosis.

TBG deficiency can be misinterpreted, most often, as hypothyroidism [7]. Deficit of thyroid hormone protein carriers should be considered in inadequately low total concentrations of thyroid hormones, as opposed to normal TSH levels in clinically euthyroid patients, as was the case of our second patient [7, 8]. She had a nodular goiter of variable ultrasonografic size, although surveys were not always conducted by the same radiologist and/or endocrinologist. At first, the results of THS level tests indicated to subclinical hyperthyroidism, after which normal TSH level was registered with lower levels of total thyroid hormones with a negative calcitonin. Symptoms to which the patient complained resembled symptoms and signs of hypermetabolism, which required more detailed examinations. Results obtained by CMIA method, as more sensitive, showed that there were normal TSH, fT4 and fT3 basal and after dilution. Low TBG was the cause of lower total thyroid hormone levels, which was the reason for TBG deficiency diagnosis. Thyroid function tests in patients with TBG deficiency show normal TSH, normal fT4, low T4, and occasionally low T3 [7]. The most important clinical aspect is recognition of this condition and avoidance of unnecessary and potentially harmful replacement therapy [7, 16, 17, 18]. In our patient, suspicion of secondary hypothyroidism was rejected due to a good TSH response in TRH test. MRI of the pituitary gland was unnecessary. The initiation of levothyroxine replacement therapy was fortunately avoided knowing that it could only aggravate our patient's symptoms. TBG deficiency can be an acquired or a hereditary disorder. Causes of acquired TBG deficiency are hyperthyroidism, nephrotic syndrome, chronic renal failure, chronic liver disease, severe systemic diseases, malnutrition, etc. [7, 19]. Complications commonly associated with its deficit are the result of the primary misbalance. Two forms of hereditary TBG deficiency were identified: (a) a complete deficit (TBG-CD) with a total loss, and (b) partial (incomplete) deficit (TBG-PD) with reduced or changed structure of TBG [7, 18, 20, 21]. The prevalence of TBG-CD is 1:15,000 and TBG-PD 1:4,000 live births. In the study on disorders of TBG deficient patients with impaired thyroid function tests prevalence of complete and partial TBG deficiency is 1:2,500 or 1:200. TBG deficiency is more common in certain populations. It can be identified in all age groups as well as at birth. TBG is a polypeptide synthesized in the liver and coded by one copy SERPINA7 gene located on the long arm Xq22 [22, 23, 24]. Thus, hereditary TBG deficiency is gender linked, as men can have a complete or partial deficit, while in women there can only be only less TBG [10, 19, 20]. Our patient was advised to recommend testing to close relatives.

Any reduction of TBG causes an increase in concentration of free thyroid hormones. Consequences are inhibition of the TSH secretion and resulting decreased secretion of thyroid hormones from the thyroid gland. Concentrations of total serum thyroid hormones decrease until concentrations of free thyroid hormones return to normal. This balance is achieved extremely fast. In chronic conditions, reduced extrathyroid "pool" of thyroid hormones may lead to a small, transient reduction of circulating free thyroid hormones and therefore transient TSH stimulation of the gland. Since TBG deficiency is not an acute condition, hypothyroidism does not occur [7].

We have presented two patients with discrepancy between the results of thyroid function tests and clinical status, which raised suspicion of possible interference by heterophilic antibodies or TBG deficiency. Exact causes have been proven by repeated tests in different laboratories and after the dilution. In this way, erroneous diagnosis of thyroid dysfunction was avoided as well as inappropriate and unnecessary diagnostics and potentially harmful treatments. Thus, cooperation between clinicians and biochemists is very important.

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Изазов у тумачењу резултата анализе тироидних хормона

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КРАТАК САДРЖАЈ

Увод При тумачењу резултата тироидних хормона треба мислити на интерференцију и промене концентрације њихових протеинских носача.

Прикази болесника Приказали смо два пацијента код којих постоји несклад између вредности тироидних хормона и клиничког статуса. Први случај представља шездесетдвогодишњу пацијенткињу са полинодозном струмом и Хашимото тироидитисом. Тестови тироидне функције показали су низак ТСХ и нисконормалан *fT*4. Одређивањем тироидног статуса (ТСХ, *T*4, *fT*4, *T*3, *fT*3) у две лабораторије, базно и после разблажења и тироксин-везујућег глобулина закључено је да постоје уредне вредности тироидних хормона и нормална концентрација *TBG*. На резултате је утицало присуство хетерофилних антитела, што је довело до лажно нижих вредности ТСХ и сумње на секундарни хипотироидизам. Прегледана је и четрдесетдвогодишња пацијенткиња која се прати због тироидног нодуса променљиве величине и прво лако повишеног ТСХ, затим ниских укупних тироидних хормона и уредног ТСХ. На основу додатних анализа закључено је да су низак *T*4 и *T*3 последица ниског *TBG*. У питању је наследни генетски поремећај, без клиничког значаја.

Закључак Доказивањем интерференције и дефицита тироксин-везујућег глобулина када постоји несклад између резултата тироидних хормона и клиничког статуса, избегава се погрешно постављање дијагнозе тироидних поремећаја и потенцијално штетни ефекти терапије.

Кључне речи: ТСХ есеји; интерференција; тироксин-везујући глобулин

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