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FALSE ELEVATION OF FREE THYROXINE AND TRIIODOTHYRONINE DUE TO THE PRESENCE OF ANTIBODIES TO IODOTHYRONINES

İYODOTİRONİN OTOANTİKORLARI SEBEBİ İLE YALANCI YÜKSEK TİROKSİN VE TRİİYODOTİRONİN DÜZEYLERİ

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ABSTRACT
The prevalence of autoimmune thyroid disease (AITD) has progressively increased. Circulating autoantibodies associated with AITD may affect the result of laboratory analyses and cause incorrect conclusions in the assessment of thyroid hormone status. In this report, we present a patient with Hashimoto's thyroiditis with high thyrotrophin and free thyroxine (T4) and triiodothyronine (T_3) levels due to the presence of auto-antibodies to iodothyronines. Free T_4 and free T_3 levels measured by direct immunometric assays method were high, while those estimated by measurement of the serum T_4-binding capacity were low. L-thyroxine replacement therapy was started. In patients with discrepant laboratory results, interference from autoantibodies should be considered.
Key words: Thyroxine auto-antibody; hypothyroidism; false high free thyroxine; triiodothyronine levels.

ÖZET
Otoimmun tiroid hastalığı prevalansı giderek artmaktadır. Otoimmun tiroid hastalığı ile ilişkili otoantikorlar laboratuvar sonuçlarını etkileyebilmekte ve tiroid hormon durumunu değerlendirmede yanılmalara sebep olabilmektedir. Burada iyodotironin otoantikorları sebebi ile serbest tiroksin (T_4), triiyodotironin (T_3) ve TSH düzeyleri yüksek bir hasta sunduk. Direkt immunometrik yöntemlerle ölçülen free T_4 ve T_3 düzeyleri yüksek olmasına rağmen, serum T_4 bağlama kapasitesi ölçümü ile tahmin edilen değerler düşük olduğundan hastaya L-tiroksin replasman tedavisi başlanmıştır. Bu çalışmada; nadir karşılaşılan free T_4, T_3 ve TSH düzeylerinde tutarsızlık/uyumsuzluk bulunan hastalarda ayırıcı tanı olarak iyodotironin otoantikorlarına bağlı otoimmun tiroid hastalığı görülebileceği vurgulanmıştır.
Anahtar kelimeler*:* Tiroksin otoantikoru; hipotiroidi; yalancı yüksek serbest tiroksin; tiriiodotironin düzeyleri; otoimmun tiroidit.

INTRODUCTION

The prevalence of autoimmune thyroid disease (AITD) has progressively increased (1,2). The most common cause of AITD is Hashimoto's thyroiditis. In this condition, thyroid peroxidase (TPO), thyroglobulin (TG), and rarely, thyrotropin (TSH) receptor blocking antibodies, as well as antibodies to iodothyronines can be detected (1,2,3,4). In rare situations, free T_4 (FT_4) and free T_3 (FT_4) levels, measured by routine automated immunometric methods, can give false results (5,6,7,8). In this article, a biochemically hypothyroid patient with high TSH, FT_4 and FT_3 levels due to antibodies against the iodothyronines is presented.

SUBJECTS AND RESULTS

A 59-year old female patient presented to our outpatient clinic with symptoms of fatigue and exhaustion. Previous laboratory analysis showed high serum levels

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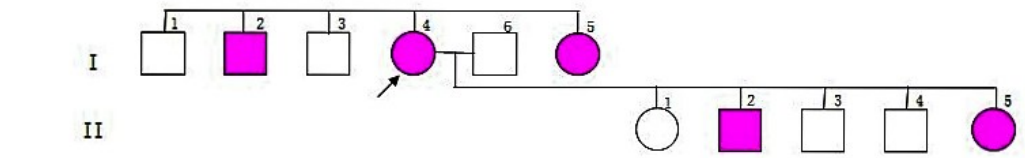
of TSH, FT_4 and FT_3. These laboratory abnormalities persisted for at least 6 years. Her blood pressure was 130/80 mmHg, heart rate 86 pulse/min and regular, temperature 36.5° C. She had a grade 2/6 systolic ejection murmur and a trace amount of pretibial non-pitting edema. Also, she has been found to have type 2 diabetes mellitus and hypertension for 12 years. Family history was negative except for diabetes mellitus. Laboratory analyses showed a TSH of 29.6 μIU/ml (normal range 0.35-5.5 μIU/ml), FT_4 3.56 ng/dl (normal range 0.74-1.79 ng/dl), FT3 >40 pg/ml (normal 2.3-4.2 pg/ml). Magnetic resonance imaging (MRI) was not compatible with a TSH secreting pituitary adenoma. TRH test was performed and after the administiration of 400 μg Protelin, intravenously, TSH and prolactin levels increased to 46.9 μIU/ml and 59.3 ng/dl at 30th min, respectively. At 60th minute, TSH and prolactin levels were found to be 34.2 μIU/ml and 27.3 ng/ml, respectively. The possibility that the patient might have resistance to thyroid hormone (RTH) or to TSH was considered. Sequencing of the *THRB* and *TSH receptor* genes showed no abnormalities.

After obtaining written consents, blood samples were collected from the patient and first degree relatives for further testing as approved by the Institutional Review Board. TPO and TG antibodies were detected (Fujirebro, Japan) in the serum of the proband (I-4) indicating the presence of AITD. As shown in figure 1, she also had low total T_4 and reverse T_3 (rT_3) measured by the Elecsys (Roche, Indianapolis, IN) platform and low free T_4 index, (FT_4I) estimated by measuring the serum T_4-binding capacity. The latter measurements suggested hypothyroidism, a diagnosis confirmed by a high serum TSH concentration. Antibodies to T_4, T_3 and rT_3 were also identified by precipitation with polyethylene glycol radioiodinated tracers of these iodothyronines added to the subject's serum. These antibodies were not detected in her unaffected brother (I-3) (Fig. 1).



AGE (years)	72	63	61	57	50	37	35	33	28	23	REFERENCE RANGES
TT_4 μg/dl	8.4	9.0	8.7	**4.4**	8.6	8.0	8.7	8.0	8.7	7.7	5 - 11.6
TT_3 ng/dl	132	112	148	132	123	163	138	151	178	144	90 - 180
TrT_3 ng/dl	28.3	21.5	27.2	**< 2**	29.0	18.6	24.7	26.7	23.8	20.0	16 - 36
FT_4I	9.4	**11.5**	9.0	**2.8**	9.7	8.5	9.7	8.7	9.4	8.2	6.0 - 10.5
TSH μU/ml	0.6	2.2	1.9	**27**	2.5	**4.3**	2.4	3.4	3.1	3.1	0.4 - 3.6
TG ng/dl	112	13	5	50	12	10	16	9	9	11	2 - 38
TPO ab	-	**1:1,280**	-	**1:128,000**	**1:1,280**	-	**1:20,480**	-	-	**1:1,280**	-
TG ab	-	**1:160**	-	**1:16,000**	**1:160**	-	-	-	-	-	-
T_4 ab			1.9	**14.5**							<3
T_3 ab			2.1	**12.0**							<3
rT_3 ab			2.0	**9.3**							<3

Figure 1: Pedigree of the family including thyroid function tests and circulating antibody levels. Females are represented by circles and males by squares. The proband is indicated with an arrow. Test results are aligned with each symbol. Abnormal values are represented in bold numbers; high in red and low in blue.

Four first degree relatives, two siblings (I-2 and I-5) and two of their children (II-2 and II-5) had also AITD based on the presence of TPO antibodies (Fig. 1). All had normal tests of thyroid function, except for a mild increase in the FT_4I in her brother (I-2; Fig. 1).

Treatment of the proband with L-thyroxine was initiated and the dose was adjusted according to her serum TSH levels. Serum free hormone levels were not taken into consideration for dose adjustement. Nine months later, on 75 mcg of L-thyroxine, TSH level was 2.74 mU/L.

CONCLUSIONS

We report herein a patient with Hashimoto's thyroiditis and high free T_4 and T_3 levels due to T_4 and T_3 auto-antibodies. In recent years, the prevalence of the AITD has been increasing worldwide and these auto-antibodies may affect laboratory analysis and cause difficulties in assessing the thyroid status. In autoimmune thyroiditis, the iodothyronines within the thyroglobulin molecule behave as haptens bound to a carrier and thus elicit an immune response to generate antibodies (8,9). Antibodies develop against the specific iodothyronine epitopes in the TG molecule. It has been reported that the incidence of T_4 or T_3 autoantibodies is 1.8% (10) and that these autoantibodies can coexist with TPO autoantibodies (11). Erregragui et al. reported that autoantibodies against the hormone-forming site included in P3 peptide of thyroglobuline (12). Depending on the immunometric method being used these antibodies can result in free and total iodothyronine levels that are falsely high or low (4,5,6,7,13). In our patient, this led to the suspicion of TSH producing adenoma, RTH and resistance to TSH, all having been excluded by specific tests.

In patients with discrepancies in the clinical status and thyroid function tests, iodothyronine auto-antibodies

should be measured. If present, TSH levels should be used to adjust the hormone replacement dose.

CONFLICT OF INTERESTS
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