ORIGINAL ARTICLE

The Frequency of Thyroid Autoantibodies is Higher in the Upper Range of Normal Thyrotropin Values

Heydarian P[a], Azizi F[b].

[a]Department of Endocrinology and Metabolism, Tehran University of Medical Sciences, Sina Hospital, [b] Endocrine Research Center, Shaheed Beheshti University of Medical Sciences, Tehran, I.R. Iran

If the upper bound of serum thyrotropin (TSH) reference range is decreased from 5.0 to 3.0 mIU/L, a large number of patients in a tertiary care practice setting would be reclassified from "normal" to having "biochemical" hypothyroidism. The predictive value of very subtle, hypothyroidism is unknown. In this paper we investigated the frequency of positive thyroid autoantibodies in the upper range of normal TSH values in the general population.

Materials and Methods: Between December 1999 and September 2000, 1434 people aged >20 years selected from families living in Tehran District-13 were entered for evaluation. Serum TSH (normal range: 0.3-3.5 mIU/L), antithyroperoxidase (TPOAb) and antithyroglobulin (TgAb) antibodies were measured. "Reference sample" was defined by excluding those with a personal history of goiter, hypothyroidism, thyrotoxicosis, thyroid nodule, neck surgery or radiotherapy, hospital admission or taking of thyroid or antithyroid drugs, estrogens or androgens, and lithium during the previous month, pregnant women, abnormal TSH values, and TPOAb+ or TgAb+.

Results: Mean serum TSH was significantly lower in the "reference sample" as compared to the total population. The frequency of TPOAb+ and TgAb+ was significantly higher in those with TSH values of 2.6-3.5 mIU/L as compared to those with TSH ≤2.5 mIU/L both in men and women. Once sequential rising values of TSH were entered into the binary logistic regression model, TSH values greater than 2.5 mIU/L could predict cases that were positive for TPOAb+ and/or TgAb+. Female sex and goiter were the two other significant predictors.

Conclusion: In the general population studied, there is a significant positive correlation between the frequency of positive thyroid autoantibodies and TSH values in the upper range of normal values. Studies are recommended to demonstrate the possible benefit of monitoring or therapy for those with TSH values in the upper range of normal values and positive thyroid autoantibodies.

Key Words: Thyrotropin, Thyroid autoantibodies, Antithyroperoxidase, Antithyroglobulin, Goiter

Correspondence: Peimaneh Heydarian, Department of Endocrinology and Medicine, Tehran University of Medical Sciences, Sina Hospital, Tehran 11367-46911, I.R.Iran
E-mail: heidarip@sina.tums.ac.ir

Introduction

There is much concern that current serum thyrotropin (TSH) reference ranges, which are standard population-based ranges, extend too high. It has been discussed that if the upper bound of serum TSH concentration reference range is decreased from 5.0 to 3.0 mIU/L, a large number of patients in a tertiary care practice setting would be reclassified from "normal" to having "biochemical" hypothyroidism. The proposed change would

result in 1 in 5 patients without known thyroid disease being classified as having biochemical hypothyroidism. Before extending the diagnosis of hypothyroidism, studies are needed that demonstrate the benefit of therapy for very mild subclinical hypothyroidism.[1] The Whickham cohort follow up study found that individuals with a serum TSH > 2.0 mIU/L at their primary evaluation had an increased odds ratio of developing hypothyroidism over the next 20 years, especially if thyroid autoantibodies were positive.[2]

Humoral and cellular immune responses are both involved in autoimmune disorders of the thyroid gland. Although, there is evidence that antithyroperoxidase (TPOAb) and antithyroglobulin (TgAb) antibodies are not responsible for initial thyroid damage,[3-7] there are substantial data related to the pathogenic importance of these antibodies.[8-18]TPOAb are more likely to be of pathogenetic importance than TgAb, and may exaggerate or perpetuate thyroid injury.[14-18]

Considering the known association between thyroid autoantibodies and autoimmune thyroid disorders and the long-term risk of developing hypothyroidism, we investigated the frequency of positive thyroid autoantibodies in the upper range of normal TSH values in a sample of the general population.

Materials and Methods

The study was conducted by the Endocrine Research Center of the Shaheed Beheshti University of Medical Sciences within the framework of the Tehran Lipid and Glucose Study (TLGS). [19] Briefly, in the first phase of the TLGS, a cluster random sample of 15005 people aged ≥3 years selected from 7146 families living in Tehran Urban District-13, who were under coverage of three university related health centers participated in a cardiovascular risk assessment program conducted in the TLGS research unit located in the area, from February 1999 to August 2001. All adults aged >20 years who participated in the TLGS between December 1999 and September 2000, including 1434 people, were entered in a thyroid assessment program, published in detail previously.[20]

Participants completed a standard thyroid questionnaire including demographic data; personal history of goiter, hypothyroidism, thyrotoxicosis and thyroid nodule; neck surgery or radiotherapy; hospital admission or history of taking drugs affecting thyroid function tests during the previous month; and pregnancy status in married females. Thyroid size was graded according to WHO grading system to grade 0, 1 and 2 and the total goiter rate was defined as the prevalence of grades 1 and 2.[21]

Fasting serum TSH was measured by radioimmunoassay-immunoradiometric assay (TSH RIA Kit, Kavoshyar, Iran), TPOAb and TgAb by immunoenzymometric assay (Anti TPO IEMA WELL Kit, Radim, Italy) and (TGAB IEMA WELL Kit, Radim, Italy) respectively. Urinary iodine concentration was measured for a random subsample of 160 people by digestion. The normal range was 0.3-3.5 mIU/L for TSH, ≤100 IU/mL for TPOAb, and ≤150 IU/mL for TgAb. The inter- and intra-assay coefficients of variation were 5.2-9.1% and 2.4-5.6% for TSH; 6.9-12.5% and 7.4-11.8% for TPOAb; and 7.0-11.0% and 4.3-9.1% for TgAb, respectively.

Statistical analysis was performed using SPSS version 11.5. Age groups were defined as 21-24, 25-34, 35-44, 45-54, 55-64, and ≥65 years. Chi squared test for trend was used to determine the pattern of goiter and thyroid autoantibody prevalences in relation to different age groups. T-test was used to compare mean TSH between two sexes and between autoantibody positive and negative

groups. Kappa agreement coefficient was used to assess the agreement between TPOAb and TgAb in being positive or negative. The frequency of TPOAb+ and TgAb+ was reported according to serum TSH values divided into four strata: ≤2.5, 2.6-3.5, 3.6-10 and >10 mIU/L. For categorical or nominal variables, Fisher's exact test or χ^2 test was used. Binary logistic regression models (Forward conditional method) were used to assess the significance of different variables in predicting TSH >2.5 mIU/L and TPOAb+ and/or TgAb+ cases. The level of significance was set at 0.05.

Results

Of the original sample of 1434 people, thyroid questionnaires and blood sampling were completed for 1426 people. Excluding people with personal history of goiter, hypothyroidism, thyrotoxicosis, thyroid nodule, neck surgery or radiotherapy, hospital admission or taking thyroid or antithyroid drugs, estrogens or androgens, and lithium during the previous month, and pregnant women; a "disease-free sample" of 1145 people remained. Excluding those with TSH values out of normal range, and those with TPOAb+ or TgAb+ from the "disease-free sample" a "reference sample" of 901 people (501 men and 400 women) was defined.

Mean (±SD) age was 43.2±14.2 yr in men (n=639) and 40.5±13.1 yr in women (n=787). Total goiter rate was 33.0% in women vs. 15.5% in men (p<0.001). The prevalence of WHO grade 2 goiters was 15.1% in women and 3.0% in men. Total goiter rate and WHO grade 2 goiters decreased by age in both sexes. Goiter prevalences decreased one decade earlier in men, starting from the age group of 35-44 years in men but 45-54 years in women. Median urinary iodine concentration was 18.5 μg/dL.

Mean (95% CI) TSH was 1.25 mIU/L (1.11-1.39) in the total sample, higher in women compared to men [1.05 mIU/L (0.98-1.13) vs. 0.84 mIU/L (0.79-0.90), P <0.001]. Mean (95% CI) TSH was higher in TPOAb+ compared to TPOAb- women [2.52 mIU/L (1.68-3.36) vs. 1.22 mIU/L (1.09-1.34), P<0.001], in TgAb+ compared to TgAb- women [2.33 mIU/L (1.64-3.02) vs. 1.18 mIU/L (1.07-1.28), P <0.001], and in TPOAb+ compared to TPOAb- men [1.87 mIU/L (1.04-2.70) vs. 0.96 mIU/L (0.73-1.20), P <0.05]. In the "reference sample", mean TSH (95% CI) was 0.94 mIU/L (0.89-0.99) and remained higher in women than in men [1.05 mIU/L (0.98-1.13) vs. 0.85 mIU/L (0.80-0.90), P <0.001]. Mean TSH was significantly lower in the "reference sample" compared to the total sample (P<0.001).

The frequency of TPOAb+ was 15.9% in women compared to 8.3% in men (P<0.001) and the frequency of TgAb+ was 21.5% in women compared to 11.0% in men (P<0.001). There was significant agreement between the frequencies of TPOAb+ and TgAb+ in both sexes (P<0.001). The only significant age-related pattern was the increment in the frequency of TPOAb+ in women starting from the age group of 45-54 year (P<0.05). Compared to nongoitrous women, goitrous women had a significant higher frequency of TPOAb+ (22.7% vs. 12.5%, P<0.005), and a higher frequency of TgAb+ approximating the significance level (26.2% vs. 19.2%, P=0.078).

As there was no significant difference in the frequency of positive thyroid autoantibodies in the different TSH strata between

Table 1. Frequency of TPOAb+ and TgAb+ according to TSH concentration

TSH (mIU/L)	Men % (n=639)		Women % (n=787)		Overall % (n=1426)	
	TPOAb+	TgAb+	TPOAb+	TgAb+	TPOAb+	TgAb+
≤2.5 (n=1313)	7.0	10.1	13.9	19.3	10.7	15.0
28.6*24.7*28.323.329.429.42.6-3.5 (n=77)						
54.2*54.2*52.647.460.080.03.6-10 (n=24)						
>10 (n=12)	50.0	-	50.0	70.0	50.0*	58.3*
Total (n=1426)	8.3	11.0	15.9	21.5	12.5	16.8

TPOAb+: antithyroperoxidase antibody >100 IU/mL; TgAb+: antithyroglobulin antibody>150 IU/mL; TSH: thyrotropin; *P<0.001 compared to TSH ≤2.5 mIU/L; Empty cell show no people in that subgroup

the total sample and the "disease-free sample", the results were reported for the total sample.

The frequency of TPOAb+ and TgAb+ was significantly higher in those with TSH values of 2.6-3.5 mIU/L compared to those with TSH ≤2.5 mIU/L (P<0.001) both in men and women, and there was no significant difference in the frequency of positive thyroid autoantibodies between the two subgroups with elevated TSH values of 3.6-10 and >10 mIU/L (Table 1). The frequency of positive thyroid autoantibodies was significantly higher in TSH >3.5 mIU/L compared to TSH values of 2.6-3.5 mIU/L (P<0.05).

In the binary logistic regression model; female sex, TPOAb+ and TgAb+ could significantly predict TSH >2.5 mIU/L (Table 2). Once sequential rising values of TSH were entered into the binary logistic regression model for predicting TPOAb+ and/or TgAb+, TSH values greater than 2.5 mIU/L could predict cases that were positive for TPOAb+ and/or TgAb+[odds ratio (95% CI)=3.13 (2.10-4.68), P<0.001]; and female sex and goiter were the two other significant predictors in the model (Table 3).

Table 2. Binary logistic regression model for predicting TSH >2.5 mIU/L

	Odds ratio (95% CI)	-2 Log LR	P
Female sex	2.74 (1.71-4.40)	20.7	<0.001
TPOAb+	2.75 (1.69-4.50)	15.7	<0.001
TgAb+	1.92 (1.20-2.09)	7.0	<0.01

TSH: thyrotropin; TPOAb+: antithyroperoxidase antibody >100 IU/mL vs. <100 IU/mL; TgAb+: antithyroglobulin antibody>150 IU/mL vs. <150 IU/mL

Table 3. Binary logistic regression model for predicting TPOAb+ and/or TgAb+

	Odds ratio (95% CI)	-2 Log LR	P
TSH >2.5 mIU/L	3.13 (2.10-4.68)	29.8	<0.001

Female sex	1.79 (1.36-2.36)	18.0	<0.001
Goiter	1.39 (1.04-1.85)	5.0	<0.05

TPOAb+: antithyroperoxidase antibody >100 IU/mL vs. <100 IU/mL; TgAb+: antithyroglobulin antibody>150 IU/mL vs. <150 IU/mL; TSH: thyrotropin; Goiter: goiter Grades 1 and 2 vs. Grade 0

Discussion

The area studied is sufficiently iodine repleted according to WHO epidemiological criteria.[21]

Ten years ago, before implementation of universal salt iodization, Tehran was a hyperendemic area with a goiter prevalence of more than 50% in children aged 6 to 12 years of age.[22]The study showed that the goiter prevalence was critically influenced by age and sex, and, in both sexes, decreased during adulthood. The decline was sharper in men than in women. The above results are compa- rable with previous reports from endemic areas.[23]

In most previous reported studies, mean serum TSH was not significantly different between men and women.[24]National Health and Nutrition Examination Survey (NHANES III) showed a significantly higher mean serum TSH in females than males for both the total population and the disease-free population, but not in the reference popula- tion. Disease-free population excluded those reporting thyroid disease, goiter, or taking thyroid medication, and the reference popula- tion was the disease free population exclud- ing those who were pregnant, taking estro- gen, androgen or lithium, or with measurable thyroid antibodies or chemical evidence of hypothyroidism or hyperthyroidism.[25] In our study, mean serum TSH remained higher in women than men even in the "reference sample", and female sex could predict TSH >2.5 mIU/L in the binary logistic regression model.

Our results are comparable to previous reports showing an increase in the frequency of thyroid autoantibodies in women with age. The frequency was lower in men and had no age trend.[24,26,27]

High TgAb can be detected in about 10% of normal adults, and in up to 15% of women over 60 years of age.[28,29] The concentrations are high in about 90% of patients with chronic autoimmune thyroiditis and in 50 to 60% of patients with Graves' hyperthyroidism.[30] High TPOAb can be detected in about 10% of normal adults, and in up to 30% of older adults.[29,31] The concentrations are high in more than 90% of patients with chronic autoimmune thyroiditis and in up to 75% of patients with Graves' hyperthyroidism.[31] Among patients with autoimmune thyroid disease, more have high TPOAb concentrations than have high TgAb concentrations, and only rare patients have high TgAb and normal TPOAb concentrations.

There is evidence both against and favoring a pathogenetic role for these autoantibodies. Evidence against a pathogenetic role for TgAb includes their presence in normal subjects and patients with monoclonal gammopathy with no evidence of thyroid disease,[3-4] and the lack of correlation between serum TgAb concentration and disease activity in patients with chronic autoimmune thyroiditis and animals with experimental autoimmune thyroiditis.[4-5]Evidence favoring a pathogenetic role of TgAb includes the oligoclonality of the antibodies in patients with autoimmune thyroid disease, compared with their polyclonal nature in normal subjects,[8-9] the deposition of immune complexes of Tg and anti-Tg antibodies along basal membranes,[10] the increased likelihood of postpartum thyroiditis in women with high TgAb concentrations during pregnancy,[11-12] and the production of autoimmune thyroiditis by passive transfer of TgAb in animals.[13] TPOAb are more likely to be of pathogenetic importance than TgAb, for several reasons. They fix complement and may directly damage thyroid cells.[14] TPOAb

concentrations are positively correlated with the activity of chronic autoimmune thyroiditis.[15] More patients with thyroiditis have high TPOAb concentrations than have high TgAb concentrations.[16] TPOAb are cytotoxic in vitro, as detected by antibody-dependent cytotoxicity tests,[17] and thus are likely to be involved in the development of hypothyroidism.[18] On the other hand, there is evidence that these antibodies are not responsible for initial thyroid damage. Infusion of serum with high concentrations of complement- fixing TPO antibodies into monkeys did not cause thyroid damage,[6] and infants who received these antibodies from their mothers via the placenta do not have permanent hypothyroidism.[7] In short, TPOAb may exaggerate or perpetuate thyroid injury, but probably do not initiate it.

The 20-year follow up of the Whickham cohort showed that the annual risk of spontaneous hypothyroidism was higher in those women who had serum TSH concentrations > 2 mIU/L and higher concentrations of antithyroid microsomal antibodies at first survey.[2] In NHANES III, mean serum TSH was insignificantly lower in the reference population compared to the total population but people with risk factors had significantly higher mean TSH concentrations than those in the reference population, i.e. without risk factors.[25] In the general population of our sufficiently iodine-replete area, mean serum TSH was significantly lower in the "reference sample" compared to the total sample and there was a significant positive correlation between the frequency of positive thyroid autoantibodies and TSH values in the upper bound of normal range.

Considering the known association between thyroid autoantibodies and autoimmune thyroid disorders, and the risk of developing hypothyroidism in long term,[14,15,18] studies are recommended to demonstrate the possible benefit of monitoring or therapy for those with TSH values in the upper range of normal range who have positive thyroid autoantibodies.

Acknowledgement

This study was supported by a grant from the Endocrine Research Center, Shaheed Be- heshti University of Medical Sciences. The authors are grateful to coworkers of the Tehran Thyroid Study:

Habib Emami, M.Sc., for epidemiological data collection; Mehdi Hedayati, Ph.D., for laboratory data collection; Jafar Ghanbili, Ph.D., for laboratory data collection; Yadollah Mehrabi, Ph.D., for analysis assistance; Mojgan Padyab, B.Sc., for analysis assistance

References

1. Fatourechi V, Klee GG, Grebe SK, Bahn RS, Brennan MD, Hay ID, et al. Effects of reducing the upper limit of normal TSH values. JAMA. 2003 Dec 24;290 (24):3195-6.
2. Vanderpump MP, Tunbridge WM, French JM, Appleton D, Bates D, Clark F, et al. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. Clin Endocrinol (Oxf). 1995 Jul;43 (1):55-68.
3. Yativ N, Buskila D, Blank M, Burek CL, Rose NR, Shoenfeld Y. The detection of antithyroglobulin activity in human serum monoclonal immunoglobulins (monoclonal gammopathies). Immunol Res. 1993;12 (4):330-7.
4. Rose NR, Kong YC, Okayasu I, Giraldo AA, Beisel K, Sundick RS. T-cell regulation in autoimmune thyroiditis. Immunol Rev. 1981;55:299-314.
5. Miescher P, Gorstein F, Benacerraf B, Gell PG. Studies on the pathogenesis of experimental immune thyroiditis. Proc Soc Exp Biol Med. 1961 May;107:12-6.

6. Roitt IM, Doniach D. Human autoimmune thyroiditis: serological studies. Lancet. 1958 Nov 15;2 (7055):1027-33.
7. Dussault JH, Letarte J, Guyda H, Laberge C. Lack of influence of thyroid antibodies on thyroid function in the newborn infant and on a mass screening program for congenital hypothyroidism. J Pediatr. 1980 Mar;96 (3 Pt 1):385-9.
8. Dietrich G, Kazatchkine MD. Normal immunoglobulin G (IgG) for therapeutic use (intravenous Ig) contain antiidiotypic specificities against an immunodominant, disease-associated, cross-reactive idiotype of human anti-thyroglobulin autoantibodies. J Clin Invest. 1990 Mar;85 (3):620-5.
9. Caturegli P, Kuppers RC, Mariotti S, Burek CL, Pinchera A, Ladenson PW, et al. IgG subclass distribution of thyroglobulin antibodies in patients with thyroid disease. Clin Exp Immunol. 1994 Dec;98 (3):464-9.
10. Jordan SC, Buckingham B, Sakai R, Olson D. Studies of immune-complex glomerulonephritis mediated by human thyroglobulin. N Engl J Med. 1981 May 14;304 (20):1212-5.
11. Roti E, Emerson CH. Clinical review 29: Postpartum thyroiditis. J Clin Endocrinol Metab. 1992 Jan;74 (1):3-5.
12. Lazarus JH, Hall R, Othman S, Parkes AB, Richards CJ, McCulloch B, et al. The clinical spectrum of postpartum thyroid disease. QJM. 1996 Jun;89 (6):429-35.
13. Nakamura RM, Weigle WO. Transfer of experimental autoimmune thyroiditis by serum from thyroidectomized donors. J Exp Med. 1969 Aug 1;130 (2):263-85.
14. Guo J, Jaume JC, Rapoport B, McLachlan SM. Recombinant thyroid peroxidase-specific Fab converted to immunoglobulin G (IgG) molecules: evidence for thyroid cell damage by IgG1, but not IgG4, autoantibodies. J Clin Endocrinol Metab. 1997 Mar;82 (3):925-31.
15. Yoshida H, Amino N, Yagawa K, Uemura K, Satoh M, Miyai K, et al. Association of serum antithyroid antibodies with lymphocytic infiltration of the thyroid gland: studies of seventy autopsied cases. J Clin Endocrinol Metab. 1978 Jun;46 (6):859-62.
16. Mariotti S, Anelli S, Ruf J, Bechi R, Czarnocka B, Lombardi A, et al. Comparison of serum thyroid microsomal and thyroid peroxidase autoantibodies in thyroid diseases. J Clin Endocrinol Metab. 1987 Nov;65 (5):987-93.
17. Raspe E, Costagliola S, Ruf J, Mariotti S, Dumont JE, Ludgate M. Identification of the thyroid Na+/I-cotransporter as a potential autoantigen in thyroid autoimmune disease. Eur J Endocrinol. 1995 Apr;132 (4):399-405.
18. Chiovato L, Bassi P, Santini F, Mammoli C, Lapi P, Carayon P, et al. Antibodies producing complement-mediated thyroid cytotoxicity in patients with atrophic or goitrous autoimmune thyroiditis. J Clin Endocrinol Metab. 1993 Dec;77 (6):1700-5.
19. Azizi F. Tehran Lipid and Glucose Study: study methodology and summarized findings. Tehran: Endocrine Research Center. Shaheed Beheshti University of Medical Sciences; 2001.
20. Heydarian P, Azizi F. Thyroid dysfunction and autoantibodies 10 years after implementation of universal salt iodization: Tehran Thyroid Study. Iranian J Endocrin Metab. 2003 Winter;4 (4):229-41. (Persian).
21. ICCIDD/UNICEF/WHO. Assessment of iodine deficiency disorders and monitoring their elimination. A guide for programme managers. 2nd ed. Geneva: WHO; 2001.
22. Azizi F. Success in prevention of iodine deficiency disorder in Iran. Ir J Nucl Med. 1995;3:1-3. (Persian).
23. DelangeF. Intrinsic and extrinsic variables: Iodine deficiency. In: Braverman LE, Utiger RB, editors. Werner & Ingbar's The Thyroid: A fundemental and clinical text. 8th ed. Philadelphia: Lippincott Williams & Wilkins; 2000. p. 295-316.
24. Gittoes NJI, Franklyn JA, Sarne DH, RefetoffS, Sheppard MC. Thyroid function tests. In: DeGroot LJ, Jameson JL, editors. Endocrinology. 4th ed. Philadelphia: W.B. Saunders Company; 2001. P. 1361-98.
25. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, et al. Serum TSH, T (4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab. 2002 Feb;87 (2):489-99.
26. Prentice LM, Phillips DI, Sarsero D, Beever K, McLachlan SM, Smith BR. Geographical distribution of subclinical autoimmune thyroid disease in Britain: a study using highly sensitive direct assays for autoantibodies to thyroglobulin and thyroid peroxidase. Acta Endocrinol (Copenh). 1990 Nov;123 (5):493-8.
27. Roti E, Gardini E, Minelli R, Bianconi L, Braverman LE. Prevalence of anti-thyroid peroxidase antibodies in serum in the elderly: comparison with other tests for anti-thyroid antibodies. Clin Chem. 1992 Jan;38 (1):88-92.
28. Spencer CA, Takeuchi M, Kazarosyan M, Wang CC, Guttler RB, Singer PA, et al. Serum thyroglobulin autoantibodies: prevalence, influence on serum thyroglobulin measurement, and prognostic significance in patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab. 1998 Apr;83 (4):1121-7.
29. Mariotti S, Sansoni P, Barbesino G, Caturegli P, Monti D, Cossarizza A, et al. Thyroid and other

organ-specific autoantibodies in healthy centenarians. Lancet. 1992 Jun 20;339 (8808):1506-8.
30. Pinchera A, Mariotti S, Vitti P, Tosi M, Grasso L, Pacini F, et al. Interference of serum thyroglobulin in the radioassay for serum antithyroglobulin antibodies. J Clin Endocrinol Metab. 1977 Nov;45 (5): 1077-88.
31. Mariotti S, Caturegli P, Piccolo P, Barbesino G, Pinchera A. Antithyroid peroxidase autoantibodies in thyroid diseases. J Clin Endocrinol Metab. 1990 Sep;71 (3):661-9.