THE UNUSUAL PRESENTATION OF THYROID DISORDERS

EDITED BY: Giampaolo Papi and Alfredo Pontecorvi
PUBLISHED IN: Frontiers in Endocrinology

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88963-194-0
DOI 10.3389/978-2-88963-194-0

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

THE UNUSUAL PRESENTATION OF THYROID DISORDERS

Topic Editors:
Giampaolo Papi, Azienda USL Modena, Italy
Alfredo Pontecorvi, Catholic University of the Sacred Heart, Fondazione Policlinico Universitario A. Gemelli – IRCCS, Italy

The present Research Topic includes 10 case reports, 4 review articles and 2 original research papers.

Its aim is to provide new insights in the field of clinical-pathological manifestations of thyroid disorders, focusing on the unusual presentation of thyroiditis, thyroid dysfunction syndromes, benign and malignant nodular thyroid disease.

Multifaceted signs and symptoms, effects of new cancer drugs on the thyroid gland, uncommon cytological and histological features, new gene mutations underlying neoplasms: the authors deal with, and thoroughly discuss, these peculiar aspects.

Citation: Papi, G., Pontecorvi, A., eds. (2019). The Unusual Presentation of Thyroid Disorders. Lausanne: Frontiers Media. doi: 10.3389/978-2-88963-194-0

Table of Contents

frontiers in Endocrinology

EDITORIAL
published: 20 August 2019
doi: 10.3389/fendo.2019.00560

Editorial: The Unusual Presentation of Thyroid Disorders

Giampaolo Papi[1]* *and Alfredo Pontecorvi*[2,3]

[1] *Endocrinology Unit-Northern Area, Azienda USL Modena, Modena, Italy,* [2] *Department of Endocrinology, Catholic University of the Sacred Heart, Rome, Italy,* [3] *Endocrinology Unit, Fondazione Policlinico Universitario A. Gemelli – IRCCS, Rome, Italy*

Keywords: nodular thyroid disease, hypothyroidism, hyperthyroidism, thyroid neoplasm, intrathyroidal parathyroid carcinoma, atypical thyroid lesions

Editorial on the Research Topic

The Unusual Presentation of Thyroid Disorders

Clinical endocrinologists deal every day with patients affected by thyroid diseases and are aware of their multifaceted signs and symptoms. Thus, the "scholar" description of thyroid dysfunction syndrome referring to heart, brain, skin and eyes as the main targeted sites, sometimes does not apply to the real life. For example, evidence is growing that thyroid autoimmunity, hypothyroidism or hyperthyroidism may manifest not only through the "classical" thyroid dysfunction syndrome, but also affecting postural equilibrium (1), mood (2), or handwriting (3). Nonetheless, recent research has pointed out that medications given for non-thyroidal illnesses (i.e., immune checkpoint inhibitors for cancer therapy) may have a great impact on the thyroid gland (4), and that coexistence of thyropathies with diseases of other organs may alter the clinical features of thyroid illnesses, the medications usually given to treat them, and their dosage (5). Finally, anatomical or morphological anomalies of the gland, uncommon cytological and histological features, or new gene mutations underlying neoplasm should contribute to the atypical presentation of thyroid disorders (6).

The scope of the present Research Topic—including 10 case reports, 4 review articles and 2 original research papers—was to provide new insights in the field of clinical-pathological manifestations of thyroid disorders.

Giuliani et al. reviewed the involvement of nuclear factor-kappa B (NF-kB)—an ubiquitous transcription factor involved in inflammatory and immune responses, and also in regulation of expression of many other genes related to cell survival, proliferation, and differentiation —in thyroid autoimmunity (included Graves' orbitopathy), thyroid cancer, and thyroid-specific gene regulation. Interestingly, this review has shown that, in thyroid cancer, the increased activity of NF-kB correlates with a more aggressive pattern.

Keeping to the topic of autoimmunity, Yao et al. investigated the expression of IL-36α mRNA in peripheral blood mononuclear cells from newly diagnosed patients with Graves' disease (GD), refractory GD patients and normal controls. They concluded that IL-36α and CD4+IL-36α+T cells may be involved in the pathogenesis of GD by promoting the production of Th1, Th2, and Th17 cytokines.

Hashimoto's thyroiditis (HT) and its relationship with thyroid cancer in children are reviewed by Esposito et al. Analyzing the literature, the authors state that children with HT should be considered at higher risk for thyroid cancer development and discuss the possible reasons of such coexistence.

Benvenga et al. report increased requirement of daily doses of L-thyroxine in two patients with the atrophic variant of Hashimoto's thyroiditis and liver cirrhosis. Because of better intestinal absorption, L-T4 oral liquid formulation was able to circumvent the increased need of L-T4 in these patients.

Viola et al. introduce the subject of the unusual behavior of some thyroid cancers. They report the case of a patient presenting with structural recurrence of papillary thyroid cancer—identified

OPEN ACCESS

Edited and reviewed by:
Terry Francis Davies, Icahn School of Medicine at Mount Sinai, United States

***Correspondence:**
Giampaolo Papi
g.papi@ausl.mo.it

Specialty section:
This article was submitted to Thyroid Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *15 July 2019*
Accepted: *31 July 2019*
Published: *20 August 2019*

Citation:
Papi G and Pontecorvi A (2019) Editorial: The Unusual Presentation of Thyroid Disorders. Front. Endocrinol. 10:560. doi: 10.3389/fendo.2019.00560

by increasing levels of anti-Thyroglobulin antibodies—after 10 years from excellent response to initial treatment (total thyroidectomy and radioiodine remnant ablation).

Marina et al. have thoroughly worked up a patient with a huge high grade epitheliod angiosarcoma of the thyroid gland, which is a rare, aggressive, mesenchymal tumor with vascular differentiation. The patient is still alive at 62 month follow-up, following total thyroidectomy, resection of central and left compartment neck lymph-nodes, and chemotherapy with epirubicin and ifosfamide.

Alharbi et al. describe an unusual parathyroid carcinoma arising from a completely intrathyroidal parathyroid gland. This case should alert the Endocrinologists who deal with patients affected by symptomatic hypercalcemia and no parathyroid gland detectable in the neck, on the possibility of atypical intrathyroidal parathyroid neoplasm.

Similarly, Asa and Mete report a mammary analog secretory carcinoma (MASC), an unusual tumor of salivary gland type, presenting as thyroid nodule and mimicking papillary thyroid carcinoma. The intrathyroidal location of MASC may be explained by the occasional finding of salivary gland tissue within the thyroid. Thus, this lesion should represent a pitfall in the cytological and histological work-up of thyroid nodules.

The peculiar issue of nodule location within the thyroid gland is the topic of the paper by Pontieri et al. Assessing literature data and guidelines to plan the extension of surgery in a patient with cytologically indeterminate thyroid nodule, the authors found several studies supporting that the isthmus malignant lesions were associated with a higher rate of multifocality, capsular invasion, extrathyroidal extension and central lymph node metastases.

Paragliola et al. report two cases of apparently sporadic medullary thyroid carcinoma (MTC) associated with the variant in exon 2 of RET (Rearranged during Transfection) gene. As the most frequent RET protooncogene variants are located in exons 10, 11, and 13 through 16 of the RET gene, it is crucial to check also the unusual RET mutations arising from the exon 2, in order to identify hereditary forms of MTC wrongly classified as "sporadic."

In their thorough review, Baloch and LiVolsi explain in detail the pathologic pictures associated with clinical and/or biochemical hyperthyroidism, recalling even the rarest and unusual lesions causing thyrotoxicosis, i.e., struma ovarii, gestational trophoblastic disease, TRH- and TSH-secreting tumors, malignant neoplasms. They also focus on hyperthyroidism associated with antineoplastic agents and targeted therapies, which was the case of the two patients reported by Iadarola et al. The authors describe their experience with thyrotoxicosis induced by nivolumab, an immune checkpoint inhibitor. Thyroid dysfunction in both patients presented with a low serum level of TSH. However, endocrine evaluation showed a completely different etiology and clinical evolution.

In their research paper, Paragliola et al. evaluate the time to TSH normalization, on a specific L—T4 therapy dose regimen, in patients undergone total thyroidectomy for Graves' disease The authors have demonstrated that time to normalization of TSH may be prolonged, particularly in subjects with either longer duration of the disease before surgery, and high values of anti-TSH receptor autoantibodies (TrAb) at the diagnosis of hyperthyroidism.

Urselli et al. discuss in detail the risk to benefit ratio of treatment options in a patient affected by moderate-to-severe Graves orbitopathy with high clinical activity score, associated to uncontrolled type 2 diabetes mellitus. Based on a well-thought out choice, a regimen of low dose methylprednisolone administration plus fractionated low-dose orbital radiotherapy should be effective and better tolerated.

Finally, Sørensen et al. performed a systematic review on the impact of goiter and thyroidectomy on esophageal anatomy, esophageal physiology, and subjective swallowing dysfunction. They found that thyroidectomy relieved patients with goiter from dysphagia, within 6 months of surgery, probably via increase in the cross-sectional area of the esophagus.

Taken together, these studies have shown the multiform appearance of thyroid disorders, the complexity of clinical and therapeutic approach to thyroid patients, and the need of further research to bring to light thoroughly what hides under the tip of this intriguing iceberg.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Chiarella G, Russo D, Monzani F, Petrolo C, Fattori B, Pasqualetti G, et al. Hashimoto thyroiditis and vestibular dysfunction. *Endocr Pract.* (2017) 23:863–8. doi: 10.4158/EP161635.RA
2. Barbuti M, Carvalho AF, Köhler CA, Murru A, Verdolini N, Guiso G, et al. Thyroid autoimmunity in bipolar disorder. A systematic review. *J Affect Disord.* (2017) 221:97–106. doi: 10.1016/j.jad.2017.06.019
3. Papi G, Botti C, Corsello SM, Ciardullo AV, Pontecorvi A, Hegedüs L. The impact of Graves' disease and its treatment on handwriting characteristics. *Thyroid.* (2014) 24:1218–22. doi: 10.1089/thy.2013.0668
4. Ferrari SM, Fallahi P, Elia G, Ragusa F, Ruffilli I, Patrizio A, et al. Autoimmune endocrine dysfunctions associated with cancer immunotherapies. *Int J Mol Sci.* (2019) 20:2560. doi: 10.3390/ijms20102560
5. Virili C, Stramazzo I, Santaguida MG, Bruno G, Brusca N, Capriello S, et al. Ulcerative colitis as a novel cause of increased need for levothyroxine. *Front Endocrinol.* (2019) 10:233. doi: 10.3389/fendo.2019.00233
6. Cipri C, Vescini F, Torresan F, Pennelli G, Pelizzo MR, et al. An unusual case of medullary thyroid carcinoma and a revision of current literature. *Endocr Metab Immune Disord Drug Targets.* (2019) 19:226–9. doi: 10.2174/1871530319666181220165350

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 01 March 2019
doi: 10.3389/fendo.2019.00095

Factors Predicting Time to TSH Normalization and Persistence of TSH Suppression After Total Thyroidectomy for Graves' Disease

*Rosa Maria Paragliola[1], Vincenzo Di Donna[2], Pietro Locantore[1,2], Giampaolo Papi[1], Alfredo Pontecorvi[1,2] and Salvatore Maria Corsello[1,2]**

[1] Department of Endocrinology, Università Cattolica del Sacro Cuore, Rome, Italy, [2] Endocrinology Unit, Fondazione Policlinico Universitario "A. Gemelli' – IRCCS, Rome, Italy

Hyperthyroidism related to Graves' disease is associated with a suppression of TSH values which may persist after surgery in spite of a LT_4 replacement therapy at non-TSH-suppressing doses. The aim of this retrospective study was to evaluate the time to TSH normalization in a group of patients who underwent total thyroidectomy for Graves' disease receiving a LT_4 therapy dose regimen based on a previously published nomogram, and to identify possible correlations between the time to normalization of post-operative TSH values and preoperative clinical and biochemical parameters. 276 patients affected by Graves' disease who underwent surgery between 2010 and 2015, were retrospectively evaluated for clinical and biochemical parameters as well as post-surgical LT4 treatment regimen. Of the 276 subjects, 174 had initiated LT4 dosage corresponding to a previously published nomogram. 59 patients were excluded because their LT4 requirement (in mcg/kg/day) changed and deviated from the nomogram during the follow-up period, 15 patients were excluded because their TSH level was >4 mcU/ml during the first biochemical evaluation and 2 patients were excluded because they had low TSH levels potentially related to central hypothyroidism due to concomitant hypopituitarism. Therefore, 98 patients were included in our statistical analysis. TSH and FT4 were evaluated at the first post-operative assessment and during follow up until the normalization of TSH values was achieved, and then included in the analysis. During the first post-operative evaluation 2 months after surgery, 59/98 patients had TSH values in the normal range (0.4 to 4.0 mcU/ml), while 39/98 patients had a TSH value < 0.4 mcU/mL. The persistence of post-operative TSH levels < 0.4 mcU/ml was significantly correlated ($p = 0.022$) with longer duration of the disease. The value of anti-TSH receptor autoantibodies (TrAb) at the diagnosis of hyperthyroidism, significantly correlated ($p = 0.002$) with the time to TSH normalization in the group of patients with TSH < 0.4 mcU/ml at first control. This retrospective analysis confirms that in subjects who have undergone thyroidectomy for Graves' disease, time to normalization of TSH may be prolonged. Hence, the role of TSH as the "gold standard" to assess the appropriate LT_4 replacement therapy regimen during the initial months following surgery may need to be reconsidered.

Keywords: Graves' disease, total thyroidectomy, TSH, levothyroxine, anti TSH receptor autoantibodies

OPEN ACCESS

Edited by:
Jacqueline Jonklaas, Georgetown University, United States

Reviewed by:
Onyebuchi Okosieme, Cwm Taf University Health Board, United Kingdom
Bijay Vaidya, University of Exeter, United Kingdom

***Correspondence:**
Salvatore Maria Corsello
salvatore.corsello@unicatt.it

Specialty section:
This article was submitted to Thyroid Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *01 October 2018*
Accepted: *01 February 2019*
Published: *01 March 2019*

Citation:
Paragliola RM, Di Donna V, Locantore P, Papi G, Pontecorvi A and Corsello SM (2019) Factors Predicting Time to TSH Normalization and Persistence of TSH Suppression After Total Thyroidectomy for Graves' Disease. Front. Endocrinol. 10:95. doi: 10.3389/fendo.2019.00095

INTRODUCTION

Total thyroidectomy is considered a reasonable ablative option for selected patient affected by Graves' disease when medical therapy is unsuccessful and radioiodine ablation is not feasible or it is contraindicated (1). Since total thyroidectomy provides an immediate resolution of the hyperthyroidism, levothyroxine (LT_4) substitutive therapy must be started immediately after surgery and TSH levels must be assessed at least 6–8 weeks later in order to determine the need for dose-adjustment so to reach and maintain post-operative TSH values within the normal reference range.

Several strategies for the evaluation of the LT_4 starting dose after surgery for benign thyroid disease have been proposed (2). While body weight represents a relevant factor in the prediction of the LT_4 starting dose (3), it certainly is not the only parameter. In particular, our recently published nomogram considers other factors such as age and body mass index (BMI) (3), with LT_4 requirement ranging from 1.8 mcg/kg (for subjects <40 years and with BMI < 23 kg/m^2) to 1.4 mcg/kg (for subjects > 55 years and with BMI > 28 kg/m^2). This proposed nomogram allows to the achievement of "normal" TSH values (i.e., 0.4–4 mcU/mL) in 68% of patients at the first post-surgical evaluation (3).

Post-surgical hypothyroidism for Graves' disease represents a peculiar model. In fact, for a variable time between the disease onset and surgery, the hyperthyroidism causes a suppression of TSH values which may persist after surgery in spite of a LT_4 replacement therapy aimed at obtaining normal TSH values, as often seen in the clinical practice.

The aim of this retrospective study is to evaluate the time of TSH normalization in patients who underwent total thyroidectomy for Graves' disease and who were treated with LT_4 therapy based on our previously published nomogram (3), and to evaluate possible correlations between the time of TSH normalization and preoperative clinical and biochemical parameters.

MATERIALS AND METHODS

We retrospectively evaluated 276 patients affected by Graves' disease who underwent surgery between 2010 and 2015. Total thyroidectomy has been performed by the same surgeon in our Institution (Catholic University School of Medicine, Rome). All patients underwent pre- and post-surgical clinical and biochemical evaluations in our Institution.

Retrospective analysis of the clinical parameters included:

- gender;
- age at the diagnosis and at the time of surgery;
- duration of disease;
- medium dosage of anti-thyroid drug used for the medical treatment of hyperthyroidism and immediately before surgery;
- final histology;
- body mass index (BMI) [kg/m^2]
- LT_4 dosage (expressed in mcg/kg/day) prescribed after surgery and during each biochemical control.

TABLE 1 | Nomogram for the prediction of LT_4 (mcg/kg/day) starting dose after total thyroidectomy (Adapted by Di Donna et al.(3).

	BMI		
	≤ 23	23–28	>28
AGE			
≤40	1.8	1.7	1.6
40-55	1.7	1.6	1.5
>55	1.6	1.5	1.4

BMI: kg/m^2; age: years; LT_4 dose: mcg/kg/day.

Retrospective analysis of the biochemical parameters included:

- thyroid function test and anti-TSH receptor autoantibodies (TrAb) at the onset of hyperthyroidism and immediately before surgery;
- TSH and FT_4 at the first evaluation (2 months after surgery);
- for patients who did not reach normal TSH values at the first check, TSH and FT_4 were further performed about every 4 months until the normalization of TSH.

Thyroid ultrasound to exclude significant remnant thyroid tissue after surgery was performed using a real-time ultrasound (10–12 MHz linear transducer).

Biochemical evaluations were performed in the same laboratory of our institution. TSH, FT_4, and FT_3 results were measured by chemiluminescent assays. Analytical sensitivity of TSH, FT_3, and FT_4 is < 0.0025 mcU/mL, < 1 pg/ml and < 4 pg/ml, respectively. For TrAb, electrochemiluminescent IMA (Roche Diagnostics, Mannheim, Germany) on Cobas E 8,000 platform was used. Cut-off value suggested by the manufacturer was 1.75 U/L.

We classified patients on the basis of age and BMI and evaluated the dosage of LT_4 (mcg/kg) prescribed after surgery.

We retrospectively included only 174 patients who after surgery had started LT_4 which corresponded to the dose calculated according to our previously published nomogram (**Table 1**). Among these 174 patients:

- 59 were excluded because their LT4 requirement (in mcg/kg/day) changed and deviated from the nomogram during the follow-up period, for example, for significant weight variations or for clinical reasons which suggest to increase or to reduce the posology. In other words, we have considered in the statistical analysis only the patients in which the LT4 requirement remained unchanged over time. In this way, we can reasonably suppose that changes in TSH levels are not related to change in LT4 requirement;
- 15 patients were excluded because their TSH level was > 4 mcU/ml during the first biochemical evaluation;
- 2 patients were excluded because they had low TSH levels potentially related to central hypothyroidism due to concomitant hypopituitarism. In these patients, affected by empty sella and pituitary macroadenoma, pituitary function

test revealed a hypopituitarism (TSH, GH and gonadotropin deficiency and panhypopituitarism, respectively).

After these selection criteria, 98/174 patients were included in the statistical analysis. "Normal TSH reference range" was considered between 0.4 and 4 mcU/ml. For patients who had a TSH value in the normal TSH reference range at the first post-operative biochemical evaluation, we did not consider further biochemical data during the follow-up. On the contrary, for patients who had TSH values < 0.4 mcU/ml at the first post-operative biochemical evaluation, we considered the following biochemical data until "normalization" of TSH in the reference range was achieved. It is important to underline that we considered only patients who did not change LT_4 requirement (mcg/kg/day) during their follow-up period. On the basis of these data, we evaluated the time between surgery and TSH normalization.

Statistical Analysis

Demographic, clinical and biochemical characteristics at the baseline of the enrolled patients and the results of the post-operative evaluations were described by Mean and Standard Error for continuous variables and by frequencies and percentages for categorical variables.

A multivariate logistic regression analysis was performed to identify predictive factors of lack of TSH normalization at the first biochemical evaluation. Factors included in the analysis as explanatory variables included gender, age at diagnosis, preoperative FT4 values, TrAb values at the diagnosis, time between the onset of disease and surgery, dosage of anti-thyroid drug during the medical treatment, dosage of anti-thyroid drug before surgery, type of anti-thyroid drug and histology.

A multivariate linear regression analysis was then performed in order to study possible associations between independent variables (including gender, age at diagnosis, preoperative FT4 values, TrAb values at the diagnosis, time between the onset of disease and surgery, dosage of anti-thyroid drug during the medical treatment, dosage of anti-thyroid drug before surgery, type of anti-thyroid drug and histology) and the duration of TSH normalization in patients who did not reach "biochemical euthyroidism" at their first control.

All statistical analyses were performed using STATA version 13.1 (Copyright 1985–2013 StataCorp LP, 4905 Lakeway Drive, College Station, Texas 77845 USA) and a $p < 0.05$ was considered statistically significant.

RESULTS

A total of 98 Caucasian patients (89 female and 9 male) were included in the study. Following the diagnosis of hyperthyroidism, patients were treated with medical therapy for a mean of 35 months before surgery. The most commonly used drug was methimazole (87/98, 94.6%). Final histology showed benign disease in 91/98 patients (92.86%) and an incidentally discovered very low-risk carcinoma in 7/98 patients (7.14%). In all 7 cases, the tumor was a well-differentiated classical papillary carcinoma pT1a Nx and, in consideration of the "low risk," TSH suppression was not considered necessary.

TABLE 2 | Clinical characteristics of the enrolled patients.

	Mean	standard error
Age at diagnosis (years)	36.13	1.17
Age at surgery (years)	38.84	1.19
Time between the onset of disease and surgery (months)	35.11	3.34
Dose of MTM used during the treatment (mg)	10	0.13
Dose of MTM used immediately before surgery (mg)	7.5	0.16
Dose of PTU used during the treatment (mg)	125	0.11
Dose of PTU used immediately before surgery (mg)	150	0.09
Body mass index at the time of surgery	24.39	0.37
LT4 dose after surgery (mcg/kg/day)	1.67	0.01
TrAb at the diagnosis (U/L)	13.29	1.31
FT4 before surgery (pg/ml)	13.78	0.67
FT3 before surgery (pg/ml)	4.7	0.27

MTM, methimazole; PTU, propylthiouracil; Anti-TSH receptors autoantibodies normal values are < 1.75 U/L, as suggested by the manufacturer.

The daily mean dosage of LT_4 used in the post-operative phase was 1.67 mcg/kg.

Baseline clinical and biochemical characteristics of enrolled patients are presented in **Table 2** and the results of the statistical analyses are described in **Table 3**.

During the first post-operative evaluation, 59/98 patients (60.2%) had TSH values in the normal range (between 0.4 and 4.0 mcU/ml), while the remaining 39/98 patients (39.8%) had a TSH value < 0.4 mcU/ml (**Table 2**). For patients who had a TSH values < 0.4 mcU/ml, we considered the following biochemical data (TSH and FT_4 every 4 months) until "normalization" of TSH in the reference range was achieved. During the TSH-suppressive phase, patients were not clinically thyrotoxic. It is important to underline that LT_4 replacement dose in this group of patients is the same established by the nomogram and the final stable dose of LT4 (mcg/kg/day) after TSH normalization is the same used during TSH suppression period. Therefore, the biochemical TSH "normalization" can be considered not dependent by LT_4 replacement dose changes.

The multivariate logistic regression analysis, aimed at identifying predictive factors of lack of TSH normalization at the first biochemical evaluation, showed that a longer duration of the disease before surgery significantly correlated ($p = 0.022$) with the persistence of TSH levels < 0.4 mcU/ml (**Table 3**). In patients with normal TSH levels at the first biochemical control the mean time between the onset of disease and surgery was 30.08 months (SE 3.26). For patients with TSH < 0.4 mcU/mL at the first biochemical control, the mean time between the onset of disease and surgery was 42.87 months. Therefore, patients who reached "biochemical euthyroidism" at the time of the first laboratory evaluation (2 months after surgery) had a shorter duration between disease onset and surgery (**figure 1**).

Results by multivariate linear regression also showed that the value of TrAb at the diagnosis of Graves' disease, significantly correlated ($p = 0.002$) with the time to TSH normalization in the group of patients who had TSH < 0.4 mcU/ml at the first control

TABLE 3 | Factors predicting the lack of TSH normalization at the first biochemical valuation.

	p-value
Gender	0.229
Age at diagnosis	0.589
Preoperative FT4 values	0.248
TrAb values at the diagnosis	0.223
Time between the onset of disease and surgery	**0.022**
Dosage of anti-thyroid drug during the medical treatment	0.783
Dosage of anti-thyroid drug before surgery	0.356
Type of anti-thyroid drug	0.283
Histology	0.172

Bold values indicate a significant correlation.

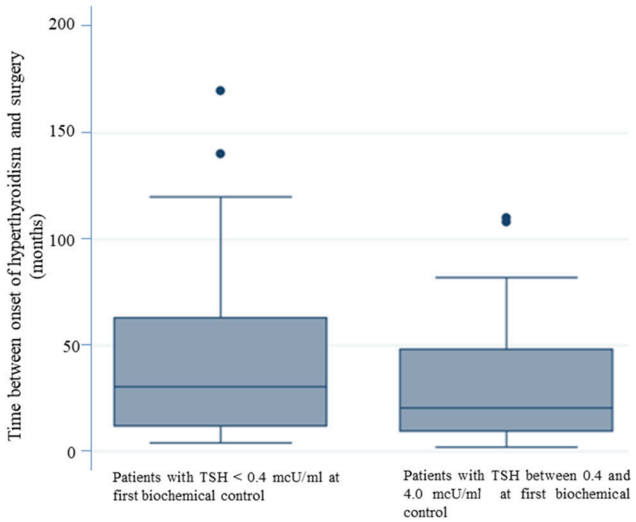


FIGURE 1 | The longer duration of the disease before surgery correlates with the persistence of TSH levels < 0.4 mcU/ml. Patients who reached "biochemical euthyroidism" 2 months after surgery, had a shorter duration of disease between the onset of hyperthyroidism and surgery.

(**Table 4**, **figure 2**). The mean value of TrAb at the diagnosis in this group was 14.27 U/L.

DISCUSSION

This retrospective analysis represents the first study in the literature evaluating the time to normalization of TSH values in patients who underwent total thyroidectomy for Graves' disease. Furthermore, the relationship between time to TSH normalization and clinical and biochemical variables has been evaluated. Indeed, while common clinical practice suggests that, using a standard post-surgery LT_4 dosage, TSH normalizes more slowly in thyroidectomized patients for Graves' disease, to our knowledge no studies have been performed to better characterize this phenomenon and to identify possible predictive factors. Our study confirmed the delay in post-surgical TSH

TABLE 4 | Variables associated with the duration of TSH normalization in patients who did not reach "biochemical euthyroidism" at first post-operative biochemical control.

	p-value
Gender	0.629
Age at diagnosis	0.076
Preoperative FT4 values	0.678
TrAb values at the diagnosis	**0.002**
Time between the onset of disease and surgery	0.957
Dosage of anti-thyroid drug during the medical treatment	0.567
Dosage of anti-thyroid drug before surgery	0.691
Type of anti-thyroid drug	0.627
Histology	0.615

Bold values indicate a significant correlation.

normalization following thyroidectomy due to Graves' disease and an association between this phenomenon and patients' clinical (duration of hyperthyroidism before surgery) and biochemical (TrAb values) pre-surgical parameters was found. About the possible correlation between pre and post-operative TSH levels, in our opinion, the presence of hyperthyroidism and the influence of the antithyroid drugs make pre-operative TSH not reliable. To obtain a model in which TSH normalization is independent of LT_4 dosage variations, we considered in our statistical analysis only patients who did not modify their LT_4 requirement (mcg/kg/day) during biochemical follow-up. A reasonable TSH reference range which can be applied to monitor LT_4 substitutive therapy is between 0.4 and 4 mcU/mL. Indeed, several studies that evaluated "normal" TSH values in the general population reported that the lower TSH limit (2.5th percentile) lies between 0.2 and 0.4 mcU/mL, while upper limits (97.5th percentile) vary between 2.4 and 4.2 mcU/mL, and appear to be related to ethnicity or geographic location (4).

In the present study only about 60% of patients with Graves' disease achieved normal TSH values at the first post-operative biochemical evaluation. Therefore, the performance of the previously published nomogram for the estimation of LT_4 replacement therapy (3), which was retrospectively evaluated in this group, resulted to be lower than that observed in patients thyroidectomized for other benign diseases. Furthermore, considering that patients with TSH > 4 mcU/mL have been previously excluded from the statistical analysis, all the patients who did not reach normalization of TSH during first post-operative biochemical evaluation, had TSH values < 0.4 mcU/mL.

The longer duration of disease before surgery represents a risk factor for not reaching normalization of TSH at the time of the first biochemical evaluation.

A similar behavior can be observed in patients who, after having undergone surgery for differentiated thyroid cancer and are then being treated with LT_4 suppressive dose, switch to a substitutive dose regimen. This phenomenon has been described in a recent study which showed long-term pituitary suppression

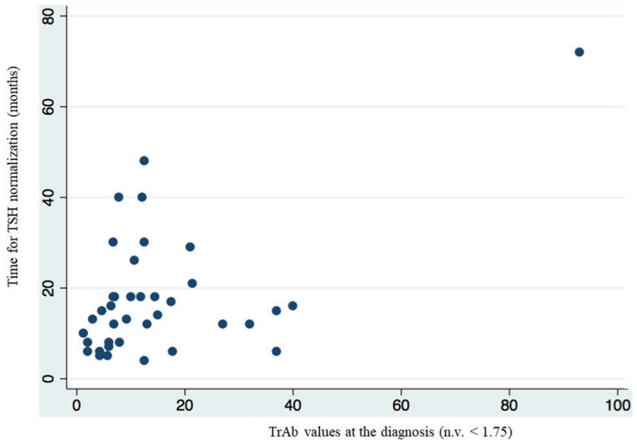


FIGURE 2 | Multivariate linear regression analysis showed a correlation between higher value of TrAb at the diagnosis and longer time of TSH normalization in the group of patients who had TSH < 0.4 mcU/ml at the first biochemical evaluation.

in patients who had been treated with TSH suppressive dose of LT_4 for a long period following surgery for differentiated thyroid carcinoma (5). In this cohort, even 6 months appear to be not enough for TSH secretion to be restored and to allow the evaluation of thyroid hormone status by measuring serum TSH. The authors suggested that this phenomenon may be due to a long-term suppressive effect exerted by the "excess" of LT_4, which delays the recovery of the feedback mechanism (5). Similarly, in our group of patients, the persistence of suppressed TSH values can be explained by considering the prolonged TSH suppression induced by the hyperthyroidism.

It is well known, as shown by previous studies, that in the medical treatment of Graves' disease, serum TSH levels may remain low or suppressed for several months to years, despite normalization of FT_3 and FT_4 (6). Several mechanisms have been proposed to explain the persistence of TSH suppression after therapy. A systematic review, evaluating 18 articles on this topic, proposed different possible mechanisms (7). Some studies, demonstrated the evidence of a "thyrotroph atrophy," hypothesizing that this can contribute to the prolonged suppression (8). The histological evaluation of the pituitary performed post-mortem in patients who died during thyrotoxicosis, shows a pronounced decrease or loss of immunoreactivity to TSH, without morphological differences between Graves' disease or Plummer's disease or between genders. However, the loss of pituitary TSH immunoreactivity was found to be reversible in patients with hyperthyroidism after medical treatment, as demonstrated in 16 additionally concurrently studied patients who were in thyrotoxicosis but have been successfully treated with medical therapy and subsequently had normal thyroid function or ypothyroidism (9).

Other studies involved the autocrine and paracrine feedback exerted by TSH at pituitary and hypothalamic levels (10) [ultra-short and short feedback, respectively (11)]. In fact, TSH receptors have been identified on folliculo-stellate cells of the pituitary as well as astroglial cells of the hypothalamus. These receptors are recognized by their specific autoantibodies and are able to downregulate TSH secretion directly or by reducing TRH, independently from thyroid hormone levels (11). The role of immuno-mediated processes, involving the TrAb, has been emphasized by other Authors (12–14). This mechanism probably involves also deiodinase activity, as demonstrated in hyperthyroid sera with TrAb in which the activity of type 2 deiodinase is increased (15).

The time of recovery of TSH has been evaluated in several studies (12, 13, 16–18). Clinical data suggest that TSH recovery is most likely to occur within the first 6 months after treatment, with recovery being achieved in approximately 70% of patients (7). The larger cohort study retrospectively evaluated patients for as long as 30 months, showing that 85.7% of patients have recovered TSH at 30 months (13). In our study, TSH recovery has been reached in 78.9% of patients until 20 months after surgery. The mean time of TSH normalization was about 17 months and normalization was obtained maintaining the same LT_4 dosage calculated in mcg/kg/day.

Interestingly, the majority of the studies have been conducted in preclinical models, and clinical studies have been limited to patients who reached the remission from Graves' disease by medical therapy or after radioiodine ablation. Instead, data from patients who underwent total thyroidectomy for Graves' disease are lacking. In our opinion, total thyroidectomy is the most accurate model to confirm the delayed recovery of TSH after remission from Graves' disease since it completely and instantaneously removes the confounding effect of any residual thyroid function on post-surgical hormonal assessment. Furthermore, this model allows comparing the trend of TSH values matching them with those of patients who underwent surgery for other disease. To demonstrate this different trend in Graves' disease, we selected only patients who were treated with a LT_4 dosage targeted to obtain a TSH level in the substitutive dosage reference range.

An interesting finding deduced by our evaluation is the possible role of TrAb in predicting the time of TSH recovery. In fact, the levels of TrAb at the diagnosis of Graves' disease significantly affects the time of TSH normalization during the post-surgical follow-up. Higher TrAb levels at the diagnosis of hyperthyroidism are significantly associated with a longer time of TSH recovery. In our study, all patients with persistence of TSH suppression during the first post-operative evaluations, mean TrAb values at the diagnosis were about 8-fold higher than the normal reference range (14.5 U/L, n.v. < 1.75). There is evidence reported in literature that TrAb play a role in regulating TSH secretion. In fact, Graves' disease is the only cause of hyperthyroidism based on an autoimmune etiology and, with the discovery of TSH receptors within the brain, it has been proposed that TrAb may be involved in the regulation of negative feedback (12). The first observations have been reported in Guinea pigs, in which lower TSH levels have been detected after the injection of IgG from patients with Graves' ophthalmopathy (19). In a clinical study evaluating the thyroid status during treatment with thionamides, higher TrAb values are significantly correlated with suppressed TSH (20), as confirmed also by other Authors(14). An inverse relationship between TrAb

and TSH levels may be observed both in euthyroid rats and in euthyroid humans, remarking an active role by autoantibodies in suppressing TSH (13, 14).

A significant limitation of our study, related to the retrospective evaluation, is the lack of TrAb values at the time of TSH normalization. However, the positive correlation between TrAb values at the diagnosis of hyperthyroidism and persistently suppressed TSH levels after surgery, suggest the possibility of a more complex mechanism, involving a persistent activity of TrAb on negative feedback, until several months after surgery. This phenomenon should be analyzed by prospective studies on this topic.

CONCLUSIONS

This retrospective analysis confirms that TSH normalizes slowly in patients who achieve remission for Graves' disease. To our knowledge, this represents the first study on this topic, focused on post-surgery hypothyroidism, while other previous studies were focused on the trend of TSH levels after medical therapy or radioiodine ablation. In our analysis, the persistence of reduced TSH levels is associated with a longer duration of disease before surgery, and the time to TSH normalization correlates with higher TrAb levels at the diagnosis of hyperthyroidism. Aside from possible physio-pathological hypotheses on the regulation of the hypothalamus-pituitary-thyroid axis, the results of our study offer useful suggestions for clinical practice. In fact, in patients affected by Graves' disease, the TSH value alone cannot be considered as the "gold standard" in evaluating the efficacy of the LT_4 replacement therapy during the first months after surgery. It is, therefore, reasonably to avoid the reduction of LT_4 therapy in presence of reduced TSH levels when patients present clinically with "euthyrodism." Furthermore, TSH should be monitored periodically considering that the mean time to TSH normalization in the majority of these patients is about 17 months.

ETHICS STATEMENT

All participants gave their written informed consent to participate to the study.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

We are grateful to the memory of Dr. Paolo Campanella who helped us in statistical analysis.

REFERENCES

1. Elfenbein DM, Schneider DF, Havlena J, Chen H, Sippel RS. Clinical and socioeconomic factors influence treatment decisions in Graves' disease. *Ann Surg Oncol.* (2015) 22:1196–9. doi: 10.1245/s10434-014-4095-6
2. de Carvalho GA, Paz-Filho G, Mesa Junior C, Graf H. Management of endocrine disease: pitfalls on the replacement therapy for primary and central hypothyroidism in adults. *Eur J. Endocrinol.* (2018) 178:R231–44. doi: 10.1530/EJE-17-0947
3. Di Donna V, Santoro MG, de Waure C, Ricciato MP, Paragliola RM, Pontecorvi A, et al. A new strategy to estimate levothyroxine requirement after total thyroidectomy for benign thyroid disease. *Thyroid.* (2014) 24:1759–64. doi: 10.1089/thy.2014.0111
4. Spencer CA, Hollowell JG, Kazarosyan M, Braverman LE. National health and nutrition examination survey III thyroid-stimulating hormone (TSH)-thyroperoxidase antibody relationships demonstrate that TSH upper reference limits may be skewed by occult thyroid dysfunction. *J Clin Endocrinol Metab.* (2007) 92:4236–40. doi: 10.1210/jc.2007-0287
5. Kim HI, Kim TH, Kim H, Kim YN, Jang HW, Kim JH, et al. Delayed TSH recovery after dose adjustment during TSH-suppressive levothyroxine therapy of thyroid cancer. *Clin Endocrinol.* (2017) 87:286–91. doi: 10.1111/cen.13344
6. Ross DS. Serum thyroid-stimulating hormone measurement for assessment of thyroid function and disease. *Endocrinol Metab Clin North Am.* (2001) 30:245–64. doi: 10.1016/S0889-8529(05)70186-9
7. Yu H, Farahani P. Thyroid stimulating hormone suppression post-therapy in patients with Graves' disease: a systematic review of pathophysiology and clinical data. *Clin Invest Med.* (2015) 38:E31–44. doi: 10.25011/cim.v38i1.22574
8. Fischer HR, Hackeng WH, Schopman W, Silberbusch J. Analysis of factors in hyperthyroidism, which determine the duration of suppressive treatment before recovery of thyroid stimulating hormone secretion. *Clin Endocrinol.* (1982) 16:575–85. doi: 10.1111/j.1365-2265.1982.tb03174.x
9. Scheithauer BW, Kovacs KT, Young WF Jr, Randall RV. The pituitary gland in hyperthyroidism. *Mayo Clin Proc.* (1992) 67:22–6. doi: 10.1016/S0025-6196(12)60272-9
10. Motta M, Sterescu N, Piva F, Martini L. The participation of "short" feedback mechanisms in the control of ACTH and TSH secretion. *Acta Neurol Psychiatr Belg.* (1969) 69:501–7.
11. Prummel MF, Brokken LJ, Wiersinga WM. Ultra short-loop feedback control of thyrotropin secretion. *Thyroid.* (2004) 14:825–9. doi: 10.1089/thy.2004.14.825
12. Brokken LJ, Wiersinga WM, Prummel MF. Thyrotropin receptor autoantibodies are associated with continued thyrotropin suppression in treated euthyroid Graves' disease patients. *J Clin Endocrinol Metab.* (2003) 88:4135–8. doi: 10.1210/jc.2003-030430
13. Chung YJ, Lee BW, Kim JY, Jung JH, Min YK, Lee MS, et al. Continued suppression of serum TSH level may be attributed to TSH receptor antibody activity as well as the severity of thyrotoxicosis and the time to recovery of thyroid hormone in treated euthyroid Graves' patients. *Thyroid.* (2006) 16:1251–7. doi: 10.1089/thy.2006.16.1251
14. Brokken LJ, Scheenhart JW, Wiersinga WM, Prummel MF. Suppression of serum TSH by Graves' Ig: evidence for a functional pituitary TSH receptor. *J Clin Endocrinol Metab.* (2001) 86:4814–7. doi: 10.1210/jcem.86.10.7922
15. Molnar I, Szentmiklosi JA, Somogyine-Vari E. Hyperthyroidism in patients with Graves' ophthalmopathy, and thyroidal, skeletal and eye muscle specific type 2 deiodinase enzyme activities. *Exp Clin Endocrinol Diab.* (2017) 125:514–21. doi: 10.1055/s-0043-113831
16. Uy HL, Reasner CA, Samuels MH. Pattern of recovery of the hypothalamic-pituitary-thyroid axis following radioactive iodine therapy in patients with Graves' disease. *Am J Med.* (1995) 99:173–9. doi: 10.1016/S0002-9343(99)80137-5
17. Woeber KA. Relationship between thyroid stimulating hormone and thyroid stimulating immunoglobulin in Graves' hyperthyroidism. *J Endocrinol Invest.* (2011) 34:222–4. doi: 10.1007/BF03347070

18. Chiovato L, Fiore E, Vitti P, Rocchi R, Rago T, Dokic D, et al. Outcome of thyroid function in Graves' patients treated with radioiodine: role of thyroid-stimulating and thyrotropin-blocking antibodies and of radioiodine-induced thyroid damage. *J Clin Endocrinol Metab.* (1998) 83:40–6.doi: 10.1210/jcem.83.1.4492
19. Dandona P, El Kabir DJ. On the effect of thyrotropin and immunoglobulins related to Graves' disease on thyrotropin synthesis and secretion. *Clin Endocrinol.* (1978) 9:321–7. doi: 10.1111/j.1365-2265.1978.tb02217.x
20. Ng ML, Tan TT, Roslan BA, Rajna A, Khalid BA. Usefulness and limitations of thyrotropin measurements as a first-line test for follow-up of Graves' patients. *Ann Acad Med Singapore.* (1993) 22:569–72.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

CASE REPORT
published: 17 January 2019
doi: 10.3389/fendo.2018.00810

Active Moderate-to-Severe Graves' Orbitopathy in a Patient With Type 2 Diabetes Mellitus and Vascular Complications

*Francesca Urselli, Gilda Pontieri, Livia Peschi, Alessia Liccardi, Anna Rita Ruggiero and Bernadette Biondi**

Department of Clinical Medicine and Surgery, University of Naples Federico II, Naples, Italy

Background: Graves' orbitopathy (GO) is the main extrathyroidal manifestation of Graves' disease (GD). Diabetes mellitus (DM) has been reported to be a risk factor in patients with GO. Moreover, GO can be more frequent and severe in type 2 diabetes patients. High doses of intravenous glucocorticoids represent the first line treatment of moderate-to-severe and active GO according to the international guidelines. However, this therapy is contraindicated in uncontrolled diabetes and in patients with increased cardiovascular risk. Some anti-diabetic drugs can exacerbate GO. We reported the clinical case of an active and moderate-to-severe GO in a patient with uncontrolled type 2 DM and vascular complications.

Case Report: A 61-years-old patient came to our ambulatory for a recurrence of GD and a moderate-to-severe bilateral GO. The patient had uncontrolled type 2 DM during insulin therapy and a history of micro and macrovascular complications. At the physical examination, the clinical activity score was 5 and the severity of GO was moderate-to-severe. A blood sample showed overt hyperthyroidism and the persistence of anti-TSH receptor antibodies (TRAb) during treatment with methimazole. A computed tomography scan showed a moderate-to-severe bilateral exophthalmos. We discuss the benefit/risk of treatment of GO in our patient.

Conclusion: The available guidelines do not focus on the treatment of diabetic patients with uncontrolled diabetes and severe vascular complications, therefore our patient represents a difficult therapeutic challenge. The screening of thyroid function and the evaluation of GO could be useful in diabetic patients with autoimmune thyroid disease to perform a correct treatment of these disorders.

Keywords: hyperthyroidism, diabetes mellitus, Graves' orbitopathy, glucocorticoids, methotrexate, tocilizumab, radiotherapy

OPEN ACCESS

Edited by:
Giampaolo Papi, Azienda Unità Sanitaria Locale di Modena, Italy

Reviewed by:
Mario Rotondi, University of Pavia, Italy
Rosario Le Moli, Università degli Studi di Catania, Italy

****Correspondence:***
Bernadette Biondi
bebiondi@unina.it

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *02 November 2018*
Accepted: *24 December 2018*
Published: *17 January 2019*

Citation:
Urselli F, Pontieri G, Peschi L, Liccardi A, Ruggiero AR and Biondi B (2019) Active Moderate-to-Severe Graves' Orbitopathy in a Patient With Type 2 Diabetes Mellitus and Vascular Complications. Front. Endocrinol. 9:810. doi: 10.3389/fendo.2018.00810

INTRODUCTION

Graves' orbitopathy (GO) is an autoimmune disorder and represents the main extrathyroidal manifestation of Graves' disease (GD). It can also occur in patients with euthyroid or hypothyroid autoimmune thyroid disorders. The anatomical and histological findings of GO consist in soft tissues enlargement of the orbits due to an inflammatory infiltration (including T-cells, B-cells,

and mast cells) of retro-orbital adipose tissue and extra-ocular muscles and proliferation of the connective tissue (1, 2).

The most common clinical features of GO are periorbital edema, conjunctival chemosis, exophthalmos, diplopia, corneal ulcerations, and upper-eyelid retraction; optic nerve compression can occur in severe cases (1–3).

There are standardized criteria for assessing the activity (active or inactive) and severity (mild, moderate-to-severe, and very severe) of GO to address its management (1, 3).

Smoking and thyroid dysfunction are well-recognized risk factors for GO, according to the guidelines of the European Group on Graves' Orbitopathy (EUGOGO) and the American Thyroid Association (1, 3). Therefore, in all patients with GD (also without GO) it is necessary to recommend not smoking. The restoration of euthyroidism is essential to avoid the exacerbation of GO (1, 3).

Diabetes mellitus (DM) has also been reported to be a risk factor in patients with GO (4). The association of autoimmune thyroid disease and type 1 diabetes mellitus is denominated as autoimmune polyglandular syndrome type 3 variant or APS3 (5). Moreover, GO can be more frequent and severe in type 2 diabetes mellitus (T2DM) patients (6).

We reported the clinical case of an active and moderate-to-severe GO in a patient with uncontrolled T2DM and vascular complications. As the first line treatment for moderate-to-severe and active GO, the EUGOGO guidelines recommend high doses of intravenous glucocorticoids (methylprednisolone); however, this therapy is contraindicated in uncontrolled diabetes and in patients with associated important cardiovascular risk factors (1).

The guidelines do not focus on the treatment of diabetic patients with severe vascular complications, therefore, the choice of a possible therapy for this category of patients is a difficult task.

CASE PRESENTATION

A 61-years-old patient came to our ambulatory for a recurrence of GD and a moderate-to-severe bilateral GO (**Figure 1**). He referred that GO developed at the first manifestation of GD in 2014 and worsened over time**.** The patient had type 2 DM with a metabolic glycemic decompensation (HbA1c 8.5%) during insulin therapy. He had been treated with thiazolidinediones (TZDs) many years before.

The patient had a history of micro and macrovascular diabetes complications, diabetic nephropathy with microalbuminuria and mild non-proliferative diabetic retinopathy. He mentioned that he had undergone an aorto-coronary bypass for ischemic heart disease and a foot amputation of the first metatarsal for diabetes.

At the physical examination, the thyroid was enlarged and the assessment of the GO activity showed the swelling of eyelids and caruncle and chemosis, confirming an active GO (CAS≥3) according to the European Thyroid Association guidelines. Severity of GO was moderate-to-severe for the presence of exophthalmos ≥3 mm above normal, diplopia, and severe soft tissue involvement**.** A blood sample showed overt hyperthyroidism and the persistence of thyrotropin receptor antibodies (TRAb) during treatment with anti-thyroid drugs

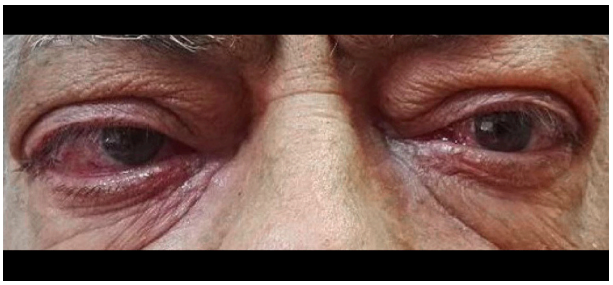


FIGURE 1 | Clinical features of Graves' orbitopathy in our patient.

TABLE 1 | Thyroid function at our first evaluation.

		Reference range
TSH	0.01 μU/ML	(0.41–4.30)
FT3	7.6 PG/ML	(3.0–4.70)
FT4	23.1 pmol/l	(9–20)
TRAB	26.8 U/L	(<1.75)
ABTG	780 U/ML	(0–60)

(**Table 1**). Thyroid ultrasound showed an increased thyroid volume and vascularization. A computed tomography scan showed a bilateral exophthalmos with a thickening of the ocular extrinsic musculature.

We analyzed the literature data to choose the best treatments of GO for our diabetic patient.

DISCUSSION

Type 1 diabetes mellitus (T1DM) is a risk factor for GD for the associated common genetic susceptibility. Moreover, the incidence of optic neuropathy (a very severe GO) is higher in patients with GO and type 1 DM (33.3%), compared to patients with GO without DM (3.9%). These data could be explained by the low oxygenation of the optic nerve and his compression by the expansion of extraocular muscles due to microvascular complications of DM (4, 7). A strong link between GD and type 1 DM, but not with type 2 DM was reported in an Italian study (6). However, GO was more severe in patients with long term T2DM and associated microvascular and macrovascular complications. Insulin-like growth factor I (IGF1) bioavailability was found to be increased in patients with T2DM and GO because of the insulin-resistance; the compensatory hyperinsulinemia probably reduced the IGF-1 binding proteins 1 and 2 (6). Furthermore, an overexpression of IGF-1 receptor was found in orbital pre-adipocytes/fibroblast. All these factors could contribute to the increase adipogenesis, inflammation, and overproduction of hyaluronan in orbital tissue (6).

Treatment of T2DM can affect the onset and progression of GO. In fact, TZDs, which are potent agonists of peroxisome proliferator activated receptor-γ (PPAR-γ), can exacerbate

GO. PPAR-γ is located in the adipose tissue and can increase retrobulbar fat and stimulate the TSH receptor expression in orbital fibroblasts (6, 8). Therefore, TZDs can contribute to the pathogenesis of GO in T2 diabetic patients.

On the contrary, a recent *in vitro* study (carried out on fibroblast cultures taken from GO patients undergoing orbital decompression) demonstrated a possible therapeutic effects of biguanides by inhibiting hyaluronan synthesis and pro-inflammatory molecule production in orbital fibroblasts (9). Moreover, metformin and phenformin significantly inhibit the adipogenic pathway during the differentiation of orbital fibroblasts (9).

The current guidelines do not recommend specific therapeutic options for GO in patients with uncontrolled DM and vascular complications, even in those with cardiovascular comorbidity (1). Intravenous high-dose of glucocorticoids, alone or in association with ciclosporin, are considered the treatment of choice for moderate-to severe active GO for their efficacy (1). However, these drugs are contraindicated in uncompensated diabetes and in patients with severe hypertension and coronary heart disease. Orbital radiotherapy, which is recommended as a second-line treatment for GO can be used alone or in combination with glucocorticoids (1). This treatment improves diplopia and ocular motility and is considered relatively safe. However, diabetic retinopathy and uncontrolled hypertension represent an absolute contraindication for this treatment because of the increased worsening of retinopathy (1, 10, 11).

The off-label use of rituximab (RTX) is also considered a second-line treatment by the EUGOGO guidelines (1). RTX is a monoclonal antibody anti-CD20 commonly used in the treatment of autoimmune disease and B cell lymphomas. Two randomized clinical trials evaluated the efficacy of RTX (12, 13) and showed conflicting results. RTX was effective in patients with active moderate-to-severe GO compared with intravenous glucocorticoids in the study by Salvi et al. in which 32 patients with thyroid-associated orbitopathy were treated with intraorbital low doses of RTX vs. high doses of systemic glucocorticoids (12). These results highlight the efficacy of RTX in reducing orbitopathy CAS and severity. However, important side effects (such as myocardial infarction and arrhythmias) have been reported in cardiac patients (14). Moreover, some cases of optic neuropathy have been described in patients with GO treated with RTX (15).

There are some data on the potential use of methotrexate (MTX), an immune suppressive drug that inhibits folic acid synthesis and is used for the treatment of several autoimmune diseases. MTX may have a potential role in GO for its immunosuppressive properties (16). One study showed its efficacy in the treatment of GO as a corticosteroid-sparing agent in patients previously treated with prednisone (17).

A successful treatment with tocilizumab has been reported in two patients with GO (18). Tocilizumab is a IgG monoclonal antibody targeting the IL-6 receptor; it is used for the treatment of rheumatoid arthritis not-responsive to conventional anti-rheumatic drugs. As demonstrated by *in vitro* studies, pro-inflammatory cytokine IL-6 and TSH receptors mutually stimulate their expression in orbital fibroblasts of patients with GO (19, 20). Tocilizumab interrupts the inflammatory process by blocking the IL-6 receptors (20); this IL-6 signal inhibition can have positive effects in diabetic subjects. TSH and IGF-1 work interdependently in the GO pathogenesis, and antibodies recognizing and activating the IGF-IR signaling have been detected in patients with GD (21). Moreover, an inverse correlation was found between IGF-IR and the CAS (22). Teprotumumab, a IGF-1 receptor inhibitory antibody, has been evaluated in a recent multicenter double-masked randomized placebo-controlled trial in patients with GO. This treatment improved proptosis and reduced the CAS (22). However, hyperglycemia was observed in some patients with diabetes (22).

All of these options were not considered suitable for our patient because of the potential side-effects. Moreover, thyroidectomy was contraindicated for the severe cardiac conditions.

Our patient had a mild non-proliferative diabetic retinopathy. Therefore, we discussed with him about the benefit/risk of treatment of GO and decided to perform low-doses of intravenous glucocorticoids (1,500 mg in 6-weeks) administration (after monitoring glycemic and cardiac conditions) only in the event of further exacerbation of his ocular symptoms, eventually associated with fractionated low-dose orbital radiotherapy (1 Gy per week over a 20-weeks period) (10, 23, 24). This scheme of 1 Gy per week over a 20-weeks period was effective and better tolerated compared to 2 Gy daily over 2-weeks in patients with moderately severe GO, showing lower rates of side effects compared to high RX dose (23).

CONCLUSIONS

Diabetes mellitus can be considered a risk factor for GO in patient with Graves' disease or autoimmune thyroid disorders. Considering the high prevalence and severity of GO in patients with T1 and T2DM, the screening of thyroid function and the evaluation of orbitopathy is useful in diabetic patients to perform a correct treatment of these disorders. Caution should be placed for the choice of anti-diabetic drugs in patients with GO and DM.

Based on the above mentioned considerations, our patient represents a difficult therapeutic challenge. In the future, international guidelines could help clarify the therapeutic options for GO in patients with uncontrolled DM and associated vascular complications.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Bartalena L, Baldeschi L, Boboridis K, Eckstein A, Kahaly GJ, Marcocci C, et al. The 2016 European Thyroid Association/European Group on graves' orbitopathy guidelines for the management of graves' orbitopathy. *Eur Thyroid J.* (2016) 5:9–26. doi: 10.1159/000443828
2. Bahn RS. Graves' ophthalmopathy. *N Engl J Med.* (2010) 362:726–38. doi: 10.1056/NEJMra0905750
3. Ross DS, Burch HB, Cooper DS, Greenlee MC, Laurberg P, Maia AL, et al. 2016 American Thyroid Association guidelines for diagnosis and management of hyperthyroidism and other causes of thyrotoxicosis. *Thyroid* (2016) 26:1343–421. doi: 10.1089/thy.2016.0229
4. Kalmann R, Mouritz MP. Diabetes mellitus: a risk factor in patients with Graves' orbitopathy. *Br J Opthalmol.* (1999) 83:463–5.
5. Hansen MP, Matheis N, Kahaly GJ. Type 1 diabetes and polyglandular autoimmune syndrome: a review. *World J Diabetes* (2015) 6:67–79. doi: 10.4239/wjd.v6.i1.67
6. Le Moli R, Muscia V, Tumminia A, Frittitta L, Buscema M, Palermo F, et al. Type 2 diabetic patients with Graves' disease have more frequent and severe Graves' orbitopathy. *Nutr Metab Cardiovasc Dis.* (2015) 25:452–7. doi: 10.1016/j.numecd.2015.01.003
7. Unger RH, Foster DW. Diabetes mellitus. In: Rifkin H, Porte D, editors. *Ellenberg and Rifkin's Diabetes Mellitus: Theory and Practice,* 4th ed. New York, NY: Elsevier (1990).
8. Lee S, Tsirbas A, Goldberg RA, McCann JD. Thiazolidinedione induced thyroid associated orbitopathy. *BMC Ophthalmol.* (2007) 7:8. doi: 10.1186/1471-2415-7-8
9. HanYE, Hwang S, Kim JH, Byun JW, Yoon JS, Lee EJ. Biguanides metformin and phenformin generate therapeutic effects via AMP-activated protein kinase/extracellular-regulated kinase pathways in an *in vitro* model of Graves' orbitopathy. *Thyroid* (2018) 28:528–36. doi: 10.1089/thy.2017.0338
10. Tanda ML, Bartalena L. Efficacy and safety of orbital radiotherapy for Graves' orbitopathy. *J Clin Endocrinol Metab.* (2012) 97:3857–65. doi: 10.1210/jc.2012-2758
11. Marcocci C, Bartalena L, Rocchi R, Marinò M, Menconi F, Morabito E, et al. Long-term safety of orbital radiotherapy for Graves' ophthalmopathy. *Clin Endocrinol Metab.* (2003) 88:3561–6. doi: 10.1210/jc.2003-030260
12. Salvi M, Vannucchi G, Currò N, Campi I, Covelli D, Dazzi D, et al. Efficacy of B-cell targeted therapy with rituximab in patients with active moderate to severe Graves' orbitopathy: a randomized controlled study. *J Clin Endocrinol Metab.* (2015) 100:422–31. doi: 10.1210/jc.2014-3014
13. Stan MN, Garrity JA, Carranza Leon BG, Prabin T, Bradley EA, Bahn RS. Randomized controlled trial of rituximab in patients with Graves' orbitopathy. *J Clin Endocrinol Metab.* (2015) 100:432–41. doi: 10.1210/jc.2014-2572
14. Savino G, Mandarà E, Gari M, Battendieri R, Corsello SM, Pontecorvi A. Intraorbital injection of rituximab versus high dose of systemic glucocorticoids in the treatment of thyroid-associated orbitopathy. *Endocrine* (2015) 48:241–7. doi: 10.1007/s12020-014-0283-1
15. Stan MN, Salvi M. Rituximab therapy for Graves' orbitopathy – lessons from randomized control trials. *Eur J Endocrinol.* (2017) 176:R101–9. doi: 10.1530/EJE-16-0552
16. Rubinov A, Zommer H, Aghazadeh H, Weis E. Role of methotrexate in thyroid-related orbitopathy. *Can J Ophthalmol.* (2017) 53:34–8. doi: 10.1016/j.jcjo.2017.07.009
17. Rivera-Grana E, Lin P, Suhler EB, Rosenbaumet JT. Methotrexate as a corticosteroid-sparing agent for thyroid eye disease. *J Clin Ophthalmol.* (2015) 6:422. doi: 10.4172/2155-9570.1000422
18. Russel D, Wagner L, Seiff S. Tocilizumab as a steroid sparing agent for the treatment of Graves' orbitopathy. *Am J Ophthalmol.* (2017) 7:146–8. doi: 10.1016/j.ajoc.2017.07.001
19. Kumar S, Schiefer R, Coenen MJ, Bahn RS. A stimulatory thyrotropin receptor antibody (M22) and thyrotropin increase interleukin-6 expression and secretion in Graves' orbital preadipocyte fibroblasts. *Thyroid* (2010) 20:59–65. doi: 10.1089/thy.2009.0278
20. Jyonouchi SC, Valyasevi RW, Harteneck DA, Dutton CM, Bahn RS. Interleukin-6 stimulates thyrotropin receptor expression in human orbital preadipocyte fibroblasts from patients with Graves' ophthalmopathy. *Thyroid* (2001) 11:929–34. doi: 10.1089/1050725017532 10984
21. Marinò M, Rotondo Dottore G, Ionni I, Lanzolla G, Sabini E, Ricci D, et al. Serum antibodies against the insulin-like growth factor-1 receptor (IGF-1R) in Graves' disease and Graves' orbitopathy. *J Endocrinol Invest.* (2018) 41:1–10. doi: 10.1007/s40618-018-0943-8
22. Smith TJ, Kahaly GJ, Ezra DG, Fleming JC, Dailey RA, Tang RA, et al. Teprotumumab for thyroid-associated ophthalmopathy. *N Engl J Med.* (2017) 376:1748–61. doi: 10.1056/NEJMoa1614949
23. Kahaly GJ, Rosler HP, Pitz S, Hommel G. Low- versus high-dose radiotherapy for Graves' ophthalmopathy: a randomized, single blind trial. *J Clin Endocrinol Metab.* (2000) 85:102–8. doi: 10.1210/jcem.85.1.6257
24. Johnson KT, Wittig A, Loesch C, Esser J, Sauerwein W, Eckstein AK. A retrospective study on the efficacy of total absorbed orbital doses of 12, 16 and 20 Gy combined with systemic steroid treatment in patients with Graves' orbitopathy. *Graefes Arch Clin Exp Ophthalmol.* (2010) 248:103–9. doi: 10.1007/s00417-009-1214-3

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

CASE REPORT
published: 17 January 2019
doi: 10.3389/fendo.2018.00813

Nivolumab Induced Thyroid Dysfunction: Unusual Clinical Presentation and Challenging Diagnosis

*Carmine Iadarola[1], Laura Croce[1,2], Erica Quaquarini[2,3], Cristina Teragni[3], Sara Pinto[1], Antonio Bernardo[3], Rodolfo Fonte[1], Michele Marinò[4], Mario Rotondi[1] and Luca Chiovato[1]**

[1] Unit of Internal Medicine and Endocrinology, ICS Maugeri IRCCS, University of Pavia, Pavia, Italy, [2] University of Pavia, Dottorato in Medicina Sperimentale, Pavia, Italy, [3] Unit of Medical Oncology, ICS Maugeri IRCCS, University of Pavia, Pavia, Italy, [4] Endocrinology Unit I, Department of Clinical and Experimental Medicine, University Hospital of Pisa, University of Pisa, Pisa, Italy

In recent years, immune checkpoint inhibitors (ICIs) had a great impact in cancer therapy. ICIs display a peculiar toxicity profile, which is characterized by autoimmune-like manifestations against multiple organs, including endocrine glands. We hereby report the case history of two patients who experienced nivolumab-induced endocrine immuno-related adverse events (irAEs). Thyroid dysfunction in both patients presented with a low serum level of TSH. However, endocrine evaluation showed a completely different etiology and clinical evolution. The two patients' histories indicate that nivolumab can cause a large spectrum of thyroid and endocrine dysfunctions resulting in cumbersome diagnostic problems. In these peculiar patients the evaluation of endocrine experts is warranted.

Keywords: thyroid, graves' disease, nivolumab, immune checkpoint inhibitors, PD-1, autoimmune, hyperthyroidism, hypothyroidism

OPEN ACCESS

Edited by:
Alfredo Pontecorvi, Catholic University of Sacred Heart, Italy

Reviewed by:
Marialuisa Appetecchia, Istituti Fisioterapici Ospitalieri (IRCCS), Italy
Pasqualino Malandrino, University of Catania, Italy

***Correspondence:**
Luca Chiovato
luca.chiovato@icsmaugeri.it

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *06 September 2018*
Accepted: *27 December 2018*
Published: *17 January 2019*

Citation:
Iadarola C, Croce L, Quaquarini E, Teragni C, Pinto S, Bernardo A, Fonte R, Marinò M, Rotondi M and Chiovato L (2019) Nivolumab Induced Thyroid Dysfunction: Unusual Clinical Presentation and Challenging Diagnosis. Front. Endocrinol. 9:813. doi: 10.3389/fendo.2018.00813

INTRODUCTION

Immune checkpoint molecules expressed in tumor microenvironment play a crucial role in anti-tumor immunity evasion. This notion has had a great impact on cancer therapy. Currently, three classes of immune checkpoint inhibitors (ICIs) are available for the treatment of different advanced solid tumors: the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitor (ipilimumab), the programmed cell death protein-1 (PD-1) inhibitors (nivolumab and pembrolizumab) and the programmed cell death-ligand 1 (PD-L1) inhibitors (atezolizumab and durvalumab). Another CTLA-4 inhibitor, tremelimumab, is still under evaluation in clinical trials.

Immunological tolerance to self-antigens is largely warranted by immune checkpoint molecules. In view of their immunomodulating properties, it is not surprising that ICIs display a peculiar toxicity profile, which is characterized by autoimmune-like manifestations against multiple organ/systems, including the gastrointestinal tract, skin, and endocrine glands. These immunological side-effects are commonly referred as immune-related adverse events (irAEs).Due to the increasing use of ICIs in tumor patients, several endocrine irAEs have been described. These include autoimmune thyroiditis, hypophysitis, primary adrenal insufficiency, and autoimmune diabetes mellitus (1–3).

We hereby report the case of two peculiar patients who experienced nivolumab-induced endocrine irAEs, both presenting with a low serum level of TSH. Interest for these patients stems in the first case from the complex work-up, which proved necessary to reach the correct diagnosis, and, in the second case, from the unusual type of thyroid disease being responsible for the low serum TSH.

CASE REPORTS

Case 1

A 64-year-old woman, with an uneventful past medical history, was diagnosed with stage IIIB (cT3pN3M0), epithelial-growth-factor-receptor (EGFR) wild type, KRAS mutated lung adenocarcinoma. The patient underwent 6 cycles of first line chemotherapy with cisplatin/gemcitabine obtaining a stable disease as best response. After 6 months, tumor progression was identified, as assessed by whole-body 18-Fluorodeoxyglucose positron emission tomography (FDG-PET) scan showing liver, bone, pleural and node metastasis. Nivolumab, 3 mg/kg every 2 weeks, was started. For dyspnea, the patient was also addressed to 3D conformational mediastinal radiotherapy for a total of 30 Gy in 12 fractions. During radiotherapy, nivolumab was temporally stopped for 1 month. While pre-nivolumab thyroid function was normal, 3 months after starting the therapy a low serum TSH level was found (TSH < 0.01 mU/L), associated with an FT4 level in the mid normal range (1.3 ng/dl: n.r. 0.89–1.76). Thyroid antibody (Ab) tests, including TSH-receptor Ab, were negative. At ultrasound examination, the estimated thyroid volume was in the upper normal range (18 ml) and gland parenchyma was normo-echoic. Due to these unclear findings, a laboratory assessment of other pituitary axes was requested, which showed low levels of serum cortisol (1.8 mcg/dl; n. r. 6.02–18.4) and ACTH (<5.0 pg/ml; n. r. 7.2–63.3), and inappropriately low for a menopausal state serum levels of LH (0.46 mUI/ml; n. r. 1.7–8.6) and FSH (7.14 mUI/ml; n.r. 1.5–12.4). The serum levels of GH (5.3 ng/mL; n. r. <10 ng/ml) and IGF-1 (162 ng/ml; 75th centile for sex and age) were in the normal range. Drug history indicated that the patient had not received corticosteroid therapy for the last 6 months. The patient was apparently symptomless regarding adrenocortical deficiency, and her blood pressure and serum electrolytes were normal. Adrenal stimulation with 1-24 ACTH (250 mcg i.v.) yielded a partial increase in serum cortisol levels (basal = 1.7 mcg/dl; 30 min = 8.1 mcg/dl; 60 min =10.4 mcg/dl). These data suggested a condition of partial hypopituitarism with impairment of at least the adreno-cortical and gonadal axes, possibly due to a nivolumab-induced hypophysitis, which however was not evident at magnetic resonance imaging (MRI) of the pituitary gland. The patient was started on a replacement dose of cortisone acetate (12.5 mg at 8:00 a.m.; 5 mg at 2:00 p.m.; and 5 mg at 6:00 p.m.) while thyroid function was monitored with no specific treatment. One month later, serum TSH was slightly below the normal range (0.29 mU/L; n. r. = 0.35–4.2) in spite of a subnormal level of serum FT4 (0.74 ng/dl: n. r. 0.89–1.76). A 99 mTc-pertechnetate scintigraphy was performed showing normal and reduced areas of radionuclide uptake. The pituitary-thyroid axis was further checked with a TRH test. This provocative test was performed 5 months after the initiation of nivolumab and showed a blunted TSH response (basal = 0.7 mU/L, 20 min = 5.13 mU/L, 60 min = 3.44 mU/L). Thyroid replacement was not started and 1 month later serum TSH (1.0 mU/L n. r. = 0.35–4.2) and FT4 (0.97 ng/dl; n. r. = 0.89–1.76) were both in the normal range. Nine months later, the patient was still receiving glucocorticoids replacement therapy, while thyroid function remained normal with no specific treatment. The thyroid hormone profile of the patient is shown in **Figure 1**. Nivolumab therapy was continued since she achieved a response, which was partial for bone, pleural and node metastasis and complete for liver ones. No other irAEs occurred during treatment.

Taken together the above clinical, laboratory, and instrumental data indicate that the patient had both a painless thyroiditis and an autoimmune hypophysits. Painless thyroiditis presented with an early phase of subclinical thyrotoxicosis, which was followed by a later phase of hypothyroidism (low FT4) and by a subsequent complete recovery of thyroid function. During the hypothyroid phase, TSH was inappropriately low-normal and its response to TRH was blunted. It is reasonable to believe that the failed raise of serum TSH can be attributed the concomitant presence of autoimmune hypophysitis.

Case Report 2

A 66-year-old male patient presented with a diagnosis of left lung adenocarcinoma for which he underwent superior-left lobectomy and local lymphadenectomy. Thereafter, he received adjuvant chemotherapy with cisplatin and vinorelbine, as well as local radiation therapy. Twelve months later, the patient experienced a relapsing disease, as assessed by whole-body FDG-PET, which showed disseminated metastatic disease involving lung, liver and bone. Docetaxel *plus* nintedanib therapy was performed for 8 months till liver and lung progression was observed. At this point, nivolumab (3 mg/kg i.v. every 2 weeks) was started. Pre-treatment serum levels of TSH, FT4 and FT3 were in the normal range; tests for anti-thyroglobulin (TgAb) and anti-thyroid-peroxidase (TPO-Ab) antibodies were negative. After the second administrations of nivolumab, the patient complained of palpitations and tremors. Biochemical assessment showed an undetectable serum TSH (<0.01 mU/L) associated with elevated levels of FT3 (5.71 pg/ml; n.r. = 2.0–4.4). The serum level of FT4 was in the upper-normal range (FT4 1.36 ng/dl; n. r. = 0.89–1.76). Tests for TRAb, TPO-Ab and Tg-Ab were negative. In the month before, the patient did not receive any iodinated contrast media nor corticosteroid therapy. In basal conditions, other pituitary and peripheral hormones (ACTH, cortisol, GH, IGF-1, PRL, FSH, LH, testosterone) were normal. Adrenal stimulation with 1-24 ACTH (250 mcg i.v.) yielded a normal increase in serum cortisol levels (basal = 6.1 mcg/dl; 30 min = 16.4 mcg/d; 60 min = 21.3 mcg/dl). Thyroid ultrasound showed a multinodular goiter (estimated volume = 34 ml) with a normo-echoic pattern of the parenchyma and a normal pattern of vascularization. Fine-needle aspiration was performed on the two dominant nodules which yielded cytological benign findings.

The patient was initially treated with beta-blocker drugs only, but in the subsequent follow-up a worsening T3-toxicosis

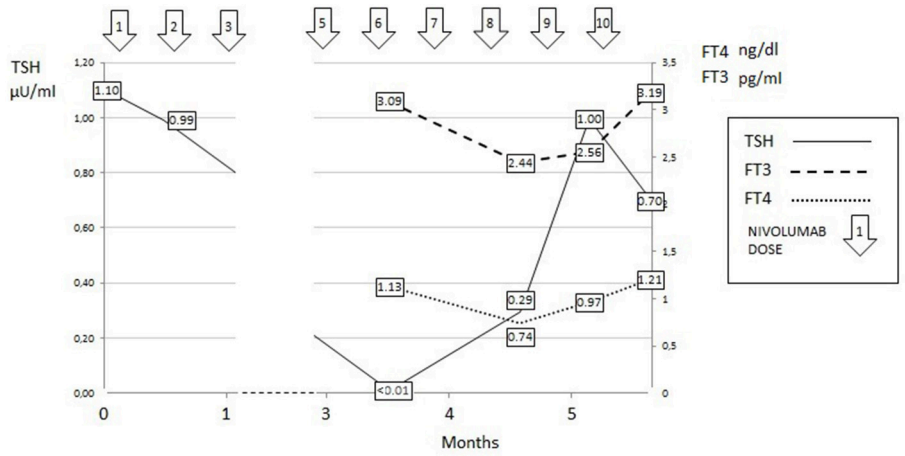


FIGURE 1 | Thyroid hormone profile of Case 1.

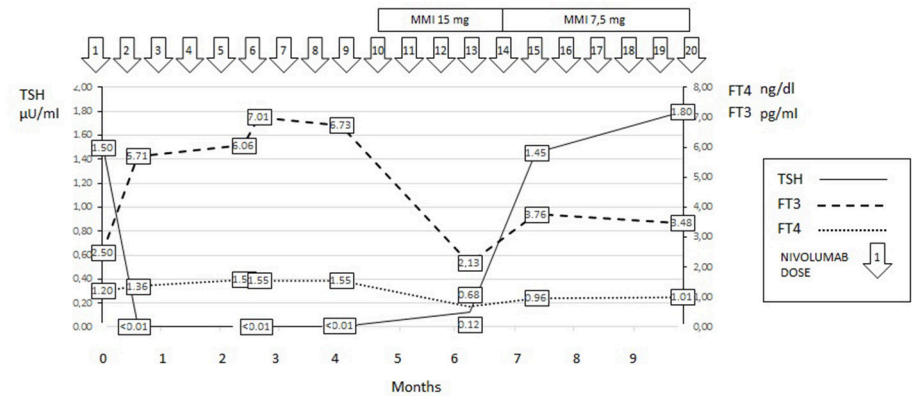


FIGURE 2 | Thyroid hormone profile of Case 2.

was evident. At this time, a 99 mTc scintigraphy revealed a diffuse thyroid uptake of the radionuclide suggesting Graves'-like hyperthyroidism. Methimazole (MMI) therapy was started at a dose of 15 mg/day. In the subsequent 3 months, the MMI dose was tapered and the patient is currently euthyroid under a maintenance dose of 7.5 mg/day of the drug. TRAb tests remained persistently negative. The thyroid hormone profiles of the patient are shown in **Figure 2**. Nivolumab therapy was continued and is still ongoing with no further progression of the neoplastic disease.

Written informed consent was obtained from both patients for the publication of this case reports.

DISCUSSION

The unusual case histories of two patients who developed thyroid dysfunction while receiving nivolumab therapy for metastatic lung cancer are reported. The development of thyroid dysfunction in patients receiving anti-cancer treatment with nivolumab has been repeatedly reported. As reviewed by Barroso-Sousa et al. (1), the prevalence of hypothyroidism in nivolumab treated patients is as high as 6.5% and a low serum level of TSH, suggesting thyrotoxicosis, is reported in nearly 2.5% of them. When the cause of low serum TSH was specifically investigated, as in the study by Yamauchi et al. (4) reporting five such patients, destructive (painless) thyroiditis was found to be responsible for the thyrotoxic state. A similar diagnosis was rendered in other isolated case reports (1, 2, 5). Although clearly described, hypophysitis in the course of nivolumab treatment is less frequently reported, with prevalence of 0.3% of treated patients as assessed by a further analysis of reviewed series (1). However, it should be emphasized that, at difference with the hypophysis-thyroid and -gonadal axes, the isolated hypophysis-adrenal axis failure secondary to ICIs is rarely reversible, requiring appropriate treatment (6).

The clinical presentation of the first patient was particularly intriguing due to the concomitant occurrence of destructive thyroiditis and hypophysitis. Indeed, after the initial thyrotoxic phase, the course of FT4, being characterized by a transient reduction (hypothyroidism) followed by a complete normalization in the absence of any specific

treatment, was typical of destructive thyroiditis. However, serum TSH did not increase during the hypothyroid phase, reasonably due to a concomitant pituitary failure. This case highlights how nivolumab-induced irAEs may simultaneously involve more than one endocrine gland. Indeed, the concomitant presence of primary hypothyroidism and secondary adrenal failure was previously described in several case reports (5, 7–10).

The second reported patient demonstrates that nivolumab can also induce Graves'-like hyperthyroidism. To the best of our knowledge, this is the first description of such an occurrence. The development of Graves' disease was previously reported in a patient treated with ipilimumab, which, unlike nivolumab, is a CTLA-4 inhibitor, and in another case receiving tremelilumab, another CTLA-4 inhibitor (11–13). In contrast with these previous observations, in which Graves' disease was accompanied by positive tests for TRAb, this antibody was persistently negative in our patient. This is an intriguing aspect of nivolumab-induced Graves'-like hyperthyroidism in our patient, but is in line with the debated role of thyroid autoantibodies in the pathogenesis of PD-1 inhibitor-induced thyroid dysfunction. Indeed, some studies report a close relationship between thyroid antibodies and PD-1 inhibitor-induced thyroid dysfunction (13) while others do not (14, 15). Studies in larger series will be required to fully elucidate this issue.

In conclusion, one of our unusual patients highlights the possibility that nivolumab can concomitantly induce different autoimmune endocrine diseases, thus making the diagnosis and the decision of specific treatments a clinical challenge. The second case indicates that Graves' like hyperthyroidism can also occur in nivolumab treated patients, even in the absence of circulating TSH-receptor antibody. The complexity of both case reports suggests that patients with nivolumab-induced thyroid dysfunction should be always referred to an endocrine expert for a thorough evaluation.

ETHICS STATEMENT

This study was carried out with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. This study didn't involve approval from the ethical committee since it was exclusively retrospective.

AUTHOR CONTRIBUTIONS

CI, LaC, EQ, CT, SP, AB, and RF followed patients in the clinical setting. LuC, MR, and MM designed the manuscript. All the authors agreed with the final version of the manuscript.

REFERENCES

1. Barroso-Sousa R, Barry WT, Garrido-Castro AC, Hodi FS, Le Min, Krop IE, et al. Incidence of endocrine dysfunction following the use of different immune checkpoint inhibitor regimens a systematic review and meta-analysis. *JAMA Oncol*. (2018) 4:173–82. doi: 10.1001/jamaoncol.2017.3064
2. Barroso-Sousa R, Ott PA, Hodi FS, Kaiser UB, Tolaney SM, Min L. Endocrine dysfunction induced by immune checkpoint inhibitors: practical recommendations for diagnosis and clinical management. *Cancer* (2018) 124:1111–21. doi: 10.1002/cncr.31200
3. Torino F, Barnabei A, Paragliola R, Baldelli R, Appetecchia M, Corsello SM. Thyroid dysfunction as an unintended side effect of anticancer drugs. *Thyroid* (2013) 23:1345–66. doi: 10.1089/thy.2013.0241
4. Yamauchi I, Sakane Y, Fukuda Y, Fujii T, Taura D, Hirata M, et al Clinical features of nivolumab-induced thyroiditis: a case series study. *Thyroid* (2017) 27:894–901. doi: 10.1089/thy.2016.0562
5. Kastrisiou M, Kostadima F, Kefas A, Zarkavelis G, Kapodistrias N, Ntouvelis E, et al Nivolumab-induced hypothyroidism and selective pituitary insufficiency in a patient with lung adenocarcinoma: a case report and review of the literature. *ESMO Open* (2017) 2:e000217. doi: 10.1136/esmoopen-2017-000217
6. Corsello SM, Salvatori R, Barnabei A, De Vecchis L, Marchetti P, Torino F. Ipilimumab-induced endocrinopathies: when to start corticosteroids (or not). *Cancer Chemother Pharmacol*. (2013) 72:489–90. doi: 10.1007/s00280-013-2213-y
7. Zeng MF, Chen L, Ye HY, Gong W, Zhou LN, Li YM, et al. Primary hypothyroidism and isolated ACTH deficiency induced by nivolumab therapy: case report and review. *Medicine* (2017) 96:e8426. doi: 10.1097/MD.0000000000008426
8. Oda T, Sawada Y, Okada E, Yamaguchi T, Ohmori S, Haruyama S, et al. Hypopituitarism and hypothyroidism following atrioventricular block during nivolumab treatment. *J Dermatol*. (2017) 44:e144–5. doi: 10.1111/1346-8138.13797
9. Takebayashi K, Ujiie A, Kubo M, Furukawa S, Yamauchi M, Shinozakiet H, et al. Isolated adrenocorticotropic hormone deficiency and severe hypercalcemia after destructive thyroiditis in a patient on nivolumab therapy with a malignant melanoma. *J Clin Med Res*. (2018) 10:358–62. doi: 10.14740/jocmr3257w
10. Kuru S, Khan N, Shaaban H. Acute hypophysitis secondary to nivolumab immunotherapy in a patient with metastatic melanoma. *Int J Critic Illness Injury Sci*. (2017) 7:177–80. doi: 10.4103/IJCIIS.IJCIIS_15_17
11. Azmat U, Liebner D, Joehlin-Price A, Agrawal A, Nabhan F. Treatment of ipilimumab Induced graves' disease in a patient with metastatic melanoma. *Case Rep Endocrinol*. (2016) 2016:2087525. doi: 10.1155/2016/2087525
12. Gan EH, Mitchell AL, Plummer R, Pearce S, Perros P. Tremelimumab-induced graves hyperthyroidism. *Eur Thyroid J*. (2017) 6:167–70. doi: 10.1159/000464285
13. Osorio JC, Ni A, Chaft JE, Pollina R, Kasler MK, Stephens D, et al. Antibody-mediated thyroid dysfunction during T-cell checkpoint blockade in patients with non-small-cell lung cancer. *Ann Oncol*. (2017) 28:583–9. doi: 10.1093/annonc/mdw640
14. Delivanis DA, Gustafson MP, Bornschlegl S, Merten MM, Kottschade L, Withers S, et al. Pembrolizumab-induced thyroiditis. Comprehensive clinical review and insights into underlying involved mechanisms. *J Clin Endocrinol Metab*. (2017) 102:2770–80. doi: 10.1210/jc.2017-00448
15. de Filette J, Jansen Y, Schreuer M, Everaert H, Velkeniers B, Neyns B, et al. Incidence of thyroid-related adverse events in melanoma patients treated with pembrolizumab. *J Clin Endocrinol Metab*. (2016) 101:4431–9. doi: 10.1210/jc.2016-2300

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

CASE REPORT
published: 13 December 2018
doi: 10.3389/fendo.2018.00750

Is the Isthmus Location an Additional Risk Factor for Indeterminate Thyroid Nodules? Case Report and Review of the Literature

*Gilda Pontieri[1], Francesca Urselli[1], Livia Peschi[1], Alessia Liccardi[1], Anna Rita Ruggiero[1], Emilia Vergara[2], Claudio Bellevicine[3], Giancarlo Troncone[3], Maurizio De Palma[4] and Bernadette Biondi[1]**

[1] Department of Clinical Medicine and Surgery, University of Naples Federico II, Naples, Italy, [2] Dipartimento Assistenziale Integrato di Oncoematologia, Diagnostica per Immagini e Morfologica e Medicina Legale, A.O.U. Federico II, Naples, Italy, [3] Department of Public Health, University of Naples Federico II, Naples, Italy, [4] Dipartimento Chirurgico Generale e Polispecialistico, Chirurgia 2, AORN Cardarelli, Naples, Italy

OPEN ACCESS

Edited by:
Giampaolo Papi,
Azienda Unità Sanitaria Locale di Modena, Italy

Reviewed by:
Francesco Romanelli,
La Sapienza University of Rome, Italy
Dario Giuffrida,
Istituto Oncologico del Mediterraneo, Italy
Roberto Vita,
Università degli Studi di Messina, Italy

***Correspondence:**
Bernadette Biondi
bebiondi@unina.it

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: 04 October 2018
Accepted: 27 November 2018
Published: 13 December 2018

Citation:
Pontieri G, Urselli F, Peschi L, Liccardi A, Ruggiero AR, Vergara E, Bellevicine C, Troncone G, De Palma M and Biondi B (2018) Is the Isthmus Location an Additional Risk Factor for Indeterminate Thyroid Nodules? Case Report and Review of the Literature. Front. Endocrinol. 9:750. doi: 10.3389/fendo.2018.00750

Background: The management of indeterminate thyroid lesions is controversial. The American Thyroid Association (ATA) guidelines suggest a conservative approach for low risk indeterminate thyroid lesions (TIR3A).

Case Report: We report a clinical case of a young girl who had TIR3A in a thyroid nodule located in the isthmus. After considering clinical and ultrasound (US) risk factors, we assessed literature data and guidelines to plan the extension of surgery. We found several studies supporting that the isthmus malignant lesions were associated with a higher rate of multifocality, capsular invasion, extrathyroidal extension, and central lymph node (LN) metastases. These data could predict a more aggressive behavior and a poor prognosis of the isthmus thyroid cancer compared to differentiated thyroid cancer, originating in the thyroid lobes. On the basis of these literature data and considering the familial risk for thyroid cancer of our patient, we decided to perform a total thyroidectomy. The histological examination revealed a follicular variant of papillary carcinoma located in the isthmus with capsular invasion.

Conclusion: The isthmus location could be an additional risk factor to consider for a correct surgical approach in indeterminate thyroid lesions and thyroid cancer at fine-needle aspiration (FNA). We suggest that a careful ultrasonography should be carried out in patients with isthmus nodules. Total thyroidectomy should be performed in aggressive nodular disease. Prospective studies are needed to establish the best treatment for these lesions.

Keywords: indeterminate thyroid lesion, isthmus nodule, multifocality, capsular invasion, extrathyroidal extension, lymph nodes metastasis, total thyroidectomy, isthmusectomy

INTRODUCTION

The management of the indeterminate thyroid nodule in clinical practice is still a matter of debate. The American Thyroid Association (ATA) guidelines suggest a conservative strategy for TIR3A (low risk indeterminate lesions according to the Bethesda System) by repeating the fine-needle aspiration (FNA) and performing molecular tests (when available) (1). If the repetition of FNA cytology confirms a TIR3A, a surveillance program with a clinical and ultrasound (US) follow-up or surgical excision for a definitive diagnosis may be performed depending on the clinical risk factors, sonographic features, patient preference, and results of the molecular tests (1). On the other hand, surgery is recommended as a first choice treatment for high risk indeterminate lesions (TIR3B) (1). This approach is justified by the higher risk of malignancy in TIR3B vs. TIR3A, although this risk differ in the international subclassifications of indeterminate thyroid nodules (1–3). The risk of malignancy was <10 and 15–30%, respectively for TIR3A and TIR3B in a recent Italian consensus for the reporting of thyroid cytology (ICCRTC) (4). A meta-analysis including six retrospective studies confirmed that this classification allows clinicians to distinguish between low and high risk malignancy in indeterminate lesions (5). Lobectomy is recommended as the initial approach when surgery is indicated in patients with a solitary indeterminate thyroid nodule. Total thyroidectomy may be preferred in patients with large nodules (>4 cm), indeterminate nodules with sonographic or cytological findings suspicious for malignancy, positivity for specific mutations associated with thyroid carcinoma, familial thyroid cancer, and history of radiation exposure (1, 2).

The incidence of thyroid carcinoma in the isthmus ranges between 3 and 9.2% (6, 7).

We report the clinical case of a young girl with an isolated indeterminate thyroid nodule (TIR3A) located in the isthmus. No standard treatment is recommended when thyroid lesions result as TIR3A or papillary thyroid cancer (PTC) in the isthmus. Current guidelines do not support the surgical management of thyroid cancer with procedures other than thyroid lobectomy, near-total, and total thyroidectomy. Isthmusectomy is not reported in the ATA guidelines as an appropriate surgical procedure for differentiated thyroid cancer. International guidelines have not formulated specific recommendations for a correct surgical approach of the isthmus nodule, neither for carcinoma nor for indeterminate nodules.

Therefore, we performed a review of the literature to assess the aggressiveness and prognosis associated with these lesions.

CASE REPORT

An 18 year old woman was referred to our outpatient clinic of Endocrinology, University-Hospital of Naples Federico II because of hypothyroidism due to Hashimoto's thyroiditis. Blood samples showed high levels of thyroperoxidase and thyroglobulin antibodies and normal calcitonin serum levels. The patient was euthyroid with normal serum levels of thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), and free thyroxine (FT4) during replacement therapy with L-T4. At physical examination, a palpable nodule of ∼2 cm in size was detected in the isthmus of the thyroid. There were no palpable cervical lymph-nodes. An US evaluation confirmed an isolated lesion located in the isthmus, showing an isoechoic solid nodule with smooth margins; its size was 18 × 13 × 6 mm with intra and perilesional vascularity (**Figure 1A**). Therefore, a FNA was performed and cytological results revealed a TIR3A lesion. The cytological specimen showed an increased cellularity with some microfollicular structures in the background of scant colloid (**Figure 1B**). Thus, we assessed the risk factors associated with the isolated TIR 3A nodule of our patient. According to the ATA guidelines we repeated the FNA which confirmed the same result (TIR3A). The second US (after 6 months) showed that there were no clear signs suggesting malignancy such as microcalcifications or taller than wide-shaped nodules. However, we found a small hypoechoic cranial component in the nodule with blurred margins and elastography revealed an increased stiffness in this cranial component. No nodules were detected in the contralateral lobes by US; cervical lymph nodes were normal. Among the possible risk factors, our patient referred a familial history of thyroid cancer. Her mother was submitted to total thyroidectomy for a follicular variant of PTC twenty years ago; our subsequent evaluation showed that she was disease free at the moment.

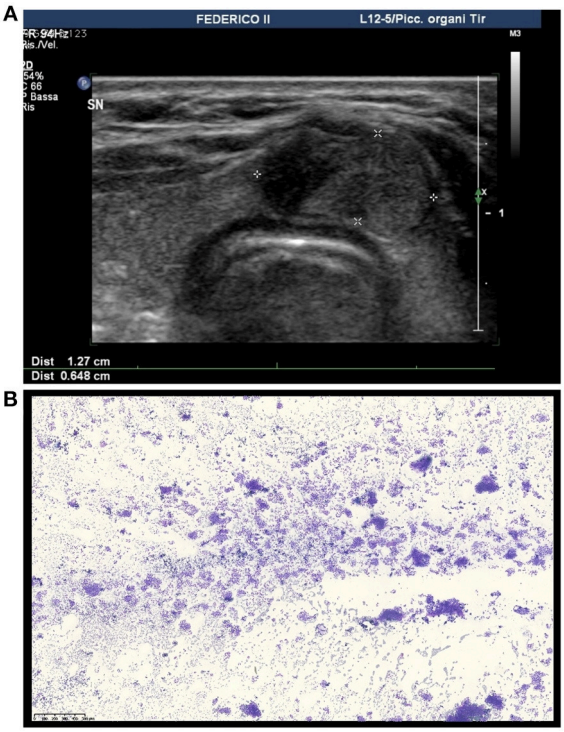


FIGURE 1 | Ultrasound and cytological features of the thyroid nodule in our patient. **(A)** Ultrasound image showing an isoechoic solid nodule with a hypoechoic cranial component with blurred margins located in the isthmus of the thyroid. **(B)** Medium power magnification showing a hypercellular smear featuring thyrocytes arranged in microfollicular structures (DiffQuik staining, 100X).

On this basis, we decided that a surgical treatment was indicated for our patient and assessed the risk/benefit of total thyroidectomy vs. isthmusectomy.

DISCUSSION

We assessed literature data on the relationship between the site of the thyroid lesions and the complications associated with the extension of surgery to decide whether an isthmusectomy was a sufficient surgical option in our patient or if a more extensive surgery was needed. A few studies have reported the results of isthmusectomy in thyroid cancer (7–9) and examined the postoperative complications of total thyroidectomy vs. isthmusectomy or isthmus-lobectomy. Some reports have suggested that when PTC is confined in the isthmus, thyroid isthmusectomy could be the first surgical choice because of the reduced risk of damaging the recurrent laryngeal nerve and parathyroid glands (10, 11). In fact, this surgical option allows surgeons to maintain the dissection on the anterior surface of the trachea, avoiding the exploration of the trachesophageal groove or the identification of the recurrent laryngeal nerve. Moreover, the parathyroid glands are not exposed during this surgical procedure. One prospective study including nine patients with indeterminate thyroid nodules, or nodules suspicious of malignancy located in the isthmus or pyramidal lobe, reported that isthmusectomy was a safe procedure in patients with a maximum diameter of 30 mm (12). On the other hand, some reports have found no significant difference in recurrent laryngeal nerve injury between lobectomy and total thyroidectomy in a group of patients with microcarcinoma, although the risk of permanent hypoparathyroidism was higher in the total thyroidectomized group than in the lobectomized group (1.7 vs. 0%, respectively) (8).

Regarding the prognosis of thyroid cancer, no significant difference for recurrent disease between lobectomy and total thyroidectomy was detected in patients with low and intermediate risk of structural disease recurrence (13, 14). One large retrospective study from Memorial Sloan Kettering Cancer

TABLE 1 | Literature data of main features of isthmus vs. non isthmus thyroid cancer.

Authors	Lee et al. (15)	Hahn et al. (16)	Karatzas et al. (17)	Goldforb et al. (18)	Lee et al. (6)	Song et al. (19)	Wang et al. (20)	Xiang et al. (21)
No. of patients	190	2,623 (144 analyzed in the study)	575	281	1,973	194	3,577	949
Patients with isthmus DTC	**7.3%**	**2.2%** (48 analyzed in the study)	**9.3%**	**4.2%**	**9.2%**	**45**	**2%**	**7.3%**
Surgical procedure in isthmus DTC vs. in non isthmus DTC	**TT+CDN vs.** TT+CDN	**81.3% TT+CND 18.7% TT+CND+LND vs.** 85.4% TT+CND 14.6% TT+CND+LND	**TT+CND**[*1] **vs.** TT+CND[*1]	**TT ± CND+LND**[*2] **vs.** –	**90.6% TT+CND ipsilateral 9.4% TT+CND+LND vs**. 81.8% TT+CND ipsilateral 18.2% TT+CND+LND	**86.7% TT+CND 13.3% TT+ CND+ LND vs.** 83.3% TT+CND 16.7% TT+CND+LND	**TT+ CND vs.** –	**SubTT+CND+LND**[*2] **vs.** Loboisthmusectomy or TT+CND+LND[*2]
Patients with multifocality in isthmus DTC vs. in non isthmus DTC	**64.3 vs**. 40.3%	**54.2 vs**. 45.8%	**51.9 vs.** 35.7%	**67 vs**. –	**48.6 vs.** 39.8%	–	–	–
Patients with capsular invasion in isthmus DTC vs. in non isthmus DTC	–	–	**25.9 vs.** 22.1%	**33 vs.** –	**70.2 vs.** 60.8%	**46.7 vs.** 4.4%	–	–
Patients with ETE in isthmus DTC vs. in non isthmus DTC	**100 vs.** 54%	**83.3 vs.** 65.6%	–	–	–	–	**11% vs.** –	–
Patients with CLN mts in isthmus DTC vs. in non isthmus DTC	**71.4 vs.** 44.6%	**68.8 vs.** 58.3%	**29.6 vs.** 16.3%	**50% vs.** –	**40.3 vs.** 42.1%	**71.1 vs.** 40.3%	**46.6% vs.** –	**44 vs.** 28.2%
Patients with LLN mts in isthmus vs. in non isthmus DTC	**14.3 vs.** 11.9%	**16.7 vs.** 14.6%		8% **vs.** –	**9.4 vs.** 18.2%		–	**4 vs.** 4.6%

DTC, differentiated thyroid cancer; TT, total thyroidectomy; ETE, extrathyroidal extension; CLN, central lymph nodes; LLN, lateral lymph nodes; CND, central lymph node dissection; LND, lateral lymph node dissection; mts, metastasis; [*1]*if mts detected by FNA or palpation;* [*2]*if mts detected by US or FNA. Bold values correspond to patients with isthmus DTC.*

Center, from a database of 1,810 patients, assessed the outcome of patients treated with thyroid isthmusectomy alone for localized well-differentiated thyroid cancer during a 20-year period. The authors reported that only 19 patients with PTC (1%) were suitable for isthmusectomy, between 1986 and 2005, because of an isolated lesion of the thyroid isthmus without evidence of extraglandular spread. The regional and distant recurrence-free survival were 100% in this study (10). All of these literature data could support the concept that isthmusectomy could be suitable in patients with solitary nodules, confined to the isthmus without evidence of extraglandular extension to avoid the dissection of the recurrent laringeal nerve and parathyroid glands.

We also carried out a review of the literature to assess if the isthmus location could represent an additional risk factor to plan the extension of surgery. Therefore, we analyzed the risk of multifocality, capsular invasion, extrathyroidal extension, and lymph node (LN) metastasis as pathological features associated with the isthmus cancer compared to the carcinoma originating in the thyroid lobe (**Table 1**). We found several studies reporting that thyroid malignant lesions located in the isthmus were more aggressive and associated with poor prognosis (6, 15, 22–24).

Literature data reported a higher rate of multifocality in the isthmic PTC compared to cancers located in other parts of the thyroid gland (18). In fact, since the isthmus is a very small portion of the parenchyma and its location is in the center of the thyroid, it is reasonable that isthmus cancer could spread into one of the two lobes. Moreover, the isthmus thickness is 2–6 mm, therefore, capsular invasion and extrathyroidal extension could be more frequent in the isthmus cancer than in those originating in the thyroid lobes (16). Literature data confirmed that capsular invasion and extrathyroidal extension were independent of tumor size in the isthmus cancers because these findings were also frequent in the isthmus microcarcinoma (17).

We found several studies also demonstrating a higher frequency of LN metastases in thyroid cancer located in the isthmus compared to non-isthmus carcinoma (19, 21). Thyroid isthmus has a different lymphatic drainage compared to the thyroid lobe and in particular, lymphatic isthmic vessels usually drain into the prelaryngeal, pretracheal, and paratracheal LN. Prelaryngeal LN are also called Delphian, from "the Oracle of Delphi," a greek legend, predicting an unfavorable prognosis (22). We also found studies reporting a more frequent involvement of the central compartment in patients with isthmus carcinoma, probably due to the particular isthmus lymphatic drainage. Interestingly, some authors (17) showed that there was no statistically significant difference between LN metastasis with isthmus cancer <10 mm and >10 mm suggesting that the isthmus location could be a risk factor for central LN involvement, regardless of tumor size. Therefore, many authors considered total thyroidectomy with bilateral central LN compartment dissection as an appropriate surgical approach for patients with isthmic PTC, due to the high rate of bilateral central LN metastasis. (6, 20, 25). Moreover, a limited experience from an Italian group suggest the possible effectiveness of radioiodine (RAI) ablation in patients with isthmic thyroid cancer (26).

These findings could suggest that the isthmus location could be an additional risk factor for indeterminate thyroid nodules and, therefore, total thyroidectomy could represent the most appropriate therapeutic strategy in patients with thyroid cancer located in the isthmus because of its more frequent multifocality, capsular invasion, extrathyroidal extension, and LN metastasis.

However, our accurate analysis found important limits of the literature studies on the isthmus nodules because the majority of the available data are retrospective and heterogeneous differing in terms of patient's age and sex, tumor size, and extension of surgery. Moreover, a small sample of malignant lesions have been included in these reports.

Our young patient had a familial history of thyroid cancer. The ATA guidelines recommend that total thyroidectomy may be the preferred treatment of indeterminate thyroid lesions in presence of a familial history of thyroid carcinoma because of the increased risk of malignancy (1). Several authors demonstrated that familial PTC is a distinct and isolated entity rather than a casual association of the same disease in a family. In fact, some studies reported an increased incidence of PTC when parents or siblings were affected by PTC with a particular risk among sisters and, an appearance, at an earlier age in the second generation (27, 28). Moreover, a "genetic anticipation" of PTC was found, even in the presence of only two family members affected by thyroid malignancy. Indeed, tumors in the second generation of the family were diagnosed at a younger age and were more aggressive, showing a multifocality and a higher rate of local and distant metastasis (29).

Therefore, considering the familial history, US risk factors and the literature regarding the isthmus cancer we decided to perform a total thyroidectomy in our patient. The surgical treatment was planned at the surgical center of our hospital (where a high volume of thyroidectomies per year is performed).

The histological examination revealed a follicular variant of papillary carcinoma. The tumor was located in the isthmus with capsular invasion; no additional LN were sampled. The immunohistochemical panel was positive for Galectin-3, HBME-1, and negative for CD56. According to the American Joint Committee on Cancer (AJCC) 8th edition, it was classified as T1bNx. One year after treatment, our patient is now disease free with undetectable levels of thyroglobulin and thyroglobulin antibodies and without any complications from surgery, although we did not perform RAI ablation. It is important to emphasize that, although a recent meta-analysis reported an association between HT and PTC, thyroid cancer in this setting was associated with a better prognosis, for the earlier discover (30).

CONCLUDING REMARKS

We suggest that a careful US should be carried out in patients with isthmic nodules to accurately assess the dimensions of the nodules, the involvement of the thyroid lobes, signs of capsular invasion and /or extrathyroidal extension and characteristics of LNs. According to the literature results, an isthmusectomy could be the procedure of choice in patients with isolated isthmus lesion <30 mm without evidence of multifocality, extraglandular spread or LN involvement. Total thyroidectomy could be performed in

invasive nodular disease, molecular tests positive for mutations that are associated with an aggressive behavior and in patients with familial thyroid cancer or history of radiation exposure.

Prospective studies are needed to establish the best treatment for isthmus indeterminate thyroid nodules or cancer.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 american thyroid association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association guidelines task force on thyroid nodules and differentiated thyroid cancer. *Thyroid* (2016) 26:1–133. doi: 10.1089/thy.2015.0020
2. Pacini F, Basolo F, Bellantone R, Boni G, Cannizzaro MA, De Palma M, et al. Italian consensus on diagnosis and treatment of differentiated thyroid cancer: joint statements of six Italian societies. *J Endocrinol Invest.* (2018) 41:849–76. doi: 10.1007/s40618-018-0884-2
3. Cibas ES, Ali SZ. The 2017 bethesda system for reporting thyroid cytopathology. *Thyroid* (2017) 27:1341–6. doi: 10.1089/thy.2017.0500
4. Nardi F, Basolo F, Crescenzi A, Fadda G, Frasoldati A, Orlandi F, et al. Italian consensus for the classification and reporting of thyroid cytology. *J Endocrinol Invest*. (2014) 37:593–9. doi: 10.1007/s40618-014-0062-0
5. Trimboli P, Crescenzi A, Giovanella L. Performance of italian consensus for the classification and reporting of thyroid cytology (ICCRTC) in discriminating indeterminate lesions at low and high risk of malignancy. A systematic review and meta-analysis. *Endocrine* (2018) 60:31–35. doi: 10.1007/s12020-017-1382-6
6. Lee YS, Jeong JJ, Nam KH, Chung WY, Chang HS, Park CS. Papillary carcinoma located in the thyroid isthmus. *World J Surg.* (2010) 34:36–9. doi: 10.1007/s00268-009-0298-6
7. Sugenoya A, Shingu K, Kobayashi S, Masuda H, Takahashi S, Shimizu T, et al. Surgical strategies for differentiated carcinoma of the thyroid isthmus. *Head Neck* (1993) 15:158–60. doi: 10.1002/hed.2880150212
8. Kwon H, Jeon MJ, Kim WG, Park S, Kim M, Song DE, et al. A comparison of lobectomy and total thyroidectomy in patients with papillary thyroid microcarcinoma: a retrospective individual risk factor-matched cohort study. *Eur J Endocrinol.* (2017) 176:371–8. doi: 10.1530/EJE-16-0845
9. Maser C, Donovan P, Udelsman R. Thyroid isthmusectomy: a rarely used but simple, safe, and efficacious operation. *J Am Coll Surg.* (2007) 204:512–4. doi: 10.1016/j.jamcollsurg.2006.12.018
10. Nixon IJ, Palmer FL, Whitcher MM, Shaha AR, Shah JP, Patel SG, et al. Thyroid isthmusectomy for well-differentiated thyroid cancer. *Ann Surg Oncol*. (2011) 18:767–70. doi: 10.1245/s10434-010-1358-8
11. Huang H, Liu SY, Ni S, Zhang ZM, Wang XL, Xu ZG. Treatment outcome of papillary carcinoma confined to the thyroid isthmus. *J Cancer Ther*. (2016) 7:963–9. doi: 10.4236/jct.2016.712093
12. Skilbeck C, Leslie A, Simo R. Thyroid isthmusectomy: a critical appraisal. *J Laryngol Otol.* (2007) 121:986–9. doi: 10.1017/S0022215106005238
13. Nixon IJ, Ganly I, Patel SG, Palmer FL, Whitcher MM, Tuttle RM, et al. Thyroid lobectomy for treatment of well differentiated intrathyroid malignancy. *Surgery* (2012) 151:571–9. doi: 10.1016/j.surg.2011.08.016
14. Vaisman F, Shaha A, Fish S, Michael Tuttle R. Initial therapy with either thyroid lobectomy or total thyroidectomy without radioactive iodine remnant ablation is associated with very low rates of structural disease recurrence in properly selected patients with differentiated thyroid cancer. *Clin Endocrinol.* (2011) 75:112–9. doi: 10.1111/j.1365-2265.2011.04002.x
15. Lee YC, Na SY, Chung H, Kim SI, Eun YG. Clinicopathologic characteristics and pattern of central lymph node metastasis in papillary thyroid cancer located in the isthmus. *Laryngoscope* (2016) 126:2419–21. doi: 10.1002/lary.25926
16. Hahn SY, Han BK, Ko EY, Shin JH, Ko ES. Ultrasound findings of papillary thyroid carcinoma originating in the isthmus: comparison with lobe-originating papillary thyroid carcinoma. *AJR Am J Roentgenol.* (2014) 203:637–42. doi: 10.2214/AJR.13.10746
17. Karatzas T, Charitoudis G, Vasileiadis D, Kapetanakis S, Vasileiadis I. Surgical treatment for dominant malignant nodules of the isthmus of the thyroid gland: a case control study. *Int J Surg.* (2015) 18:64–8. doi: 10.1016/j.ijsu.2015.04.039
18. Goldfarb M, Rodgers SS, Lew JI. Appropriate surgical procedure for dominant thyroid nodules of the isthmus 1 cm or larger. *Arch Surg.* (2012) 147:881–4. doi: 10.1001/archsurg.2012.728
19. Song CM, Lee DW, Ji YB, Jeong JH, Park JH, Tae K. Frequency and pattern of central lymph node metastasis in papillary carcinoma of the thyroid isthmus. *Head Neck* (2016) 38:412–6. doi: 10.1002/hed.24009
20. Wang J, Sun H, Gao L, Xie L, Cai X. Evaluation of thyroid isthmusectomy as a potential treatment for papillary thyroid carcinoma limited to the isthmus: a clinical study of 73 patients. *Head Neck* (2016) 38:1510–4. doi: 10.1002/hed.24270
21. Xiang D, Xie L, Xu Y, Li Z, Hong Y, Wang P. Papillary thyroid microcarcinomas located at the middle part of the middle third of the thyroid gland correlates with the presence of neck metastasis. *Surgery* (2015) 157:526–33. doi: 10.1016/j.surg.2014.10.020
22. Chai YJ, Kim SJ, Choi JY, Koo do H, Lee KE, Youn YK. Papillary thyroid carcinoma located in the isthmus or upper third is associated with Delphian lymph node metastasis. *World J Surg.* (2014) 38:1306–11. doi: 10.1007/s00268-013-2406-x
23. Lim ST, Jeon YW, Suh YJ. Correlation between surgical extent and prognosis in node-negative, early-stage papillary thyroid carcinoma originating in the isthmus. *World J Surg.* (2016) 40:344–9. doi: 10.1007/s00268-015-3259-2
24. Lei J, Zhu J, Li Z, Gong R, Wei T. Surgical procedures for papillary thyroid carcinoma located in the thyroid isthmus: an intention-to-treat analysis. *Onco Targets Ther.* (2016) 9:5209–16. doi: 10.2147/OTT.S106837
25. Vasileiadis I, Boutzios G, Karalaki M, Misiakos E, Karatzas T. Papillary thyroid carcinoma of the isthmus: total thyroidectomy or isthmusectomy? *Am J Surg.* (2018) 216:135–9. doi: 10.1016/j.amjsurg.2017.09.008
26. Campennì A, Giovanella L, Siracusa M, Stipo ME, Alibrandi A, Cucinotta M, et al. Is malignant nodule topography an additional risk factor for metastatic disease in low-risk differentiated thyroid cancer? *Thyroid* (2014) 24:1607–11. doi: 10.1089/thy.2014.0217
27. Hemminki K, Eng C, Chen B. Familial risks for nonmedullary thyroid cancer. *J Clin Endocrinol Metab.* (2005) 90:5747–53. doi: 10.1210/jc.2005-0935
28. Moses W, Weng J, Kebebew E. Prevalence, clinicopathologic features, and somatic genetic mutation profile in familial versus sporadic nonmedullary thyroid cancer. *Thyroid* (2011) 21:367–71. doi: 10.1089/thy.2010.0256
29. Capezzone M, Marchisotta S, Cantara S, Busonero G, Brilli L, Pazaitou-Panayiotou K, et al. Familial non-medullary thyroid carcinoma displays the features of clinical anticipation suggestive of a distinct biological entity. *Endocr Relat Cancer*. (2008) 15:1075–81. doi: 10.1677/ERC-08-0080
30. Resende de Paiva C, Grønhøj C, Feldt-Rasmussen U, von Buchwald C. Association between hashimoto's thyroiditis and thyroid cancer in 64,628 patients. *Front Oncol*. (2017) 7:53. doi: 10.3389/fonc.2017.00053

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 03 December 2018
doi: 10.3389/fendo.2018.00737

The Pathology of Hyperthyroidism

Virginia A. LiVolsi and Zubair W. Baloch*

Department of Pathology and Laboratory Medicine, University of Pennsylvania Medical Center, Philadelphia, PA, United States

This article reviews those pathologic lesions which are associated with clinical and/or biochemical hyperthyroidism. Beginning with the descriptive pathology of classical Graves' disease and the less common toxic nodular goiter and hyper-functioning thyroid nodules, this paper describes the effects of non-thyroidal hormones, glandular function (including pituitary and hypothalamic lesions), ectopic production of thyroid stimulating proteins by non-thyroidal neoplasms, exogenous drug reactions causing hyper-function and finally conditions associated with a mechanic- destructive cause of hyperthyroidism.

Keywords: hyperthyroidism, thyrotoxicosis, non-hyperthyroid, Graves' disease, hyperfunctioning nodules, ectopic hyperthyroidism, drug reactions, mechanico-destructive

INTRODUCTION

Hyperthyroidism is a clinical syndrome characterized by hypermetabolic state due to the increased free serum thyroxine (T4) and/or free triiodothyronine (T3). There are many known factors and pathologies both inherent to the thyroid gland as well of non-thyroidal origin that lead to hyperthyroidism. It can result from hyperplasia and overstimulation of thyroid epithelium, acute destruction of thyroid follicles, and follicular epithelium due to various forms of thyroiditis or metastatic tumors. In addition, various drugs and antineoplastic agents can lead to thyroid dysfunction. In this review we provide a pathologist's perspective on various pathologic features that can be encountered in thyroids of patients with clinical hyperthyroidism.

TOXIC GOITER

This condition can be divided into diffuse and nodular types.

Diffuse Toxic Goiter

Most patients with classical hyperthyroidism caused by autoantibodies against the TSH receptors, stimulating thyroid follicular cell receptors show an enlarged hypervascular thyroid without obvious nodularity. This condition known in North America as Graves' disease and in Europe as Basedow disease is a disorder of young usually female patients who present with heat intolerance, tachycardia, tremors, weight loss and orbitopathy (1–6). This disease is characterized by thyroid enlargement with smooth capsule, non-nodular growth, and increased vascularity. Histologically one notes the presence of a diffuse papillary and follicular hyperplasia and varying degrees of lymphocytic infiltration into the thyroid stroma (7–9) (**Figures 1**, **2**). In contrast to classic Hashimoto's disease, the lymphocytes do not infiltrate the follicular cells. The latter often are enlarged and can show cytoplasmic eosinophilia. The nuclei of these cells can also be enlarged and can in extreme cases mimic the nuclei of papillary thyroid carcinoma (10–12). However, the nuclei in Graves' disease tend to maintain a rounded shape and to have internal structure with minimal if any clearing (10–12).

There has been controversy regarding whether the presence of Graves' disease can lead to the development of papillary carcinoma and if the two coexist, does the carcinoma behave more

OPEN ACCESS

Edited by:
Noriyuki Koibuchi, Gunma University, Japan

Reviewed by:
Cesidio Giuliani, Università degli Studi G. d'Annunzio Chieti e Pescara, Italy
Giampaolo Papi, Azienda Unità Sanitaria Locale di Modena, Italy

****Correspondence:***
Virginia A. LiVolsi
linus@pennmedicine.upenn.edu

Specialty section:
This article was submitted to Thyroid Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *07 August 2018*
Accepted: *20 November 2018*
Published: *03 December 2018*

Citation:
LiVolsi VA and Baloch ZW (2018) The Pathology of Hyperthyroidism. Front. Endocrinol. 9:737. doi: 10.3389/fendo.2018.00737

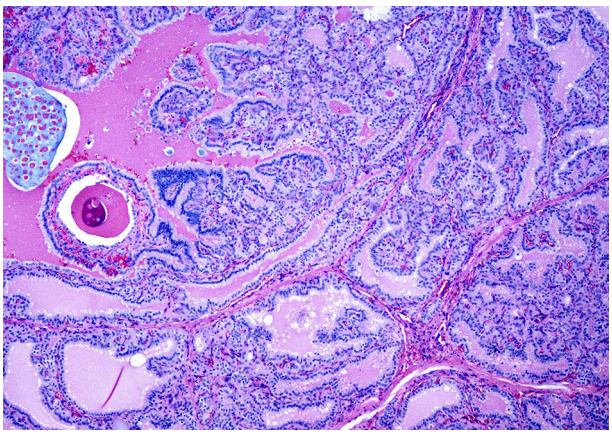


FIGURE 1 | A case of Graves' disease on low power showing exuberant papillary hyperplasia.

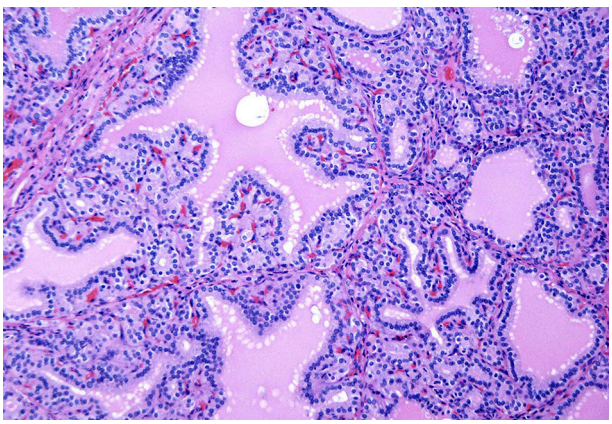


FIGURE 2 | A case of Graves' disease on medium power showing cells with round nuclei and even chromatin pattern lining the papillae.

aggressively than similar tumors not arising in this background setting. Several studies and our own experience have shown that the data needs to be evaluated systematically (13–17). If a papillary microcarcinoma is discovered incidentally in a surgically removed hyperthyroid gland, the prognosis is excellent. If a clinically evident tumor is identified in a Graves' patient, then the pathological characteristics of that lesion (size, extent, mutlifocality) will determine the prognosis. It does not appear that the background gland influences the prognosis adversely (18).

Toxic Nodular Goiter

As the name implies is an enlarged gland with multiple nodules of varying sizes. Usually the nodules show the papillary and follicular hyperplasia although the lymphocytic stromal infiltration may be within the nodules and in the non-nodular thyroid (9, 19). The correlation between the histology of the nodules and radioactive iodide scan results is fair to poor since nodules with histologic evidence of hyperfunction often are warm or cool on scan. Toxic nodular goiter tends to occur in older individuals and affects males as well as females. In some older patients, the clinical manifestations of the hyperthyroidism may not be related to the thyroid at all; many of these individuals show symptoms related to cardiac disease, frequently atrial fibrillation. This disorder, sometimes referred to as "apathetic hyperthyroidism" needs to be considered by treating clinicians and appropriate laboratory testing will lead to the correct diagnosis (20–22).

HYPERTHYROIDISM ASSOCIATED WITH HYPERFUNCTIONING THYROID TUMORS

Most autonomously functioning thyroid tumors are benign that is follicular adenomas or hyperplastic nodules. These lesions are also designated as 'autonomous nodules" and have been given the acronym "Plummer's disease" (23, 24).

Benign hyperfunctional adenomas (*AKA Toxic Adenoma*) are clonal, autonomously functioning follicular proliferations that produce supra-physiological amounts of thyroid hormones causing TSH suppression. These are more common in women and usually present at an older age. Usually, a radioisoptope scan confirms the preoperative diagnosis and most are not subjected to fine-needle aspiration (FNA). However, in rare cases a FNA is performed by a clinician or surgeon without the knowledge of thyroid function tests. In such cases, the FNA specimen is usually cellular and most likely will be diagnosed as a follicular neoplasm (Bethesda Category IV).

On surgical excision the toxic adenoma grossly shows a distinct capsule and may be centrally cystic. These lesions can also show a papillary pattern of growth without nuclear features of papillary carcinoma. The autonomously functioning nodule usually occurs in young females. This lesion also termed "papillary hyperplastic nodule" (25) (a term coined by the late Dr. Austin Vickery) is an encapsulated or at least circumscribed area in the thyroid composed of exuberant papillary structures often with some follicle formation in the cores of the papillae; the lesions are often centrally cystic and the papillae tend to point toward the center of the nodule. Importantly the nuclei lining these papillary structures are round, have internal structure and are often polarized within the cells (**Figures 3**, **4**). Lymphocytes are rarely found within these lesions (11, 12). Most of these nodules are clonal proliferations and are therefore considered adenomas (26–29). (The term "papillary adenoma" would be an appropriate one for these lesions; however, this term is shunned since it has been used to described encapsulated papillary carcinomas in older literature) (30). Although the great majority of these hyperplastic nodules are not associated with clinical hyperthyroidism, about 15–20% of affected patients do have symptomatic hyperfunction and about another 30% have biochemical hyperthyroid indices (25, 31, 32).

Rarely, malignant tumors of the thyroid may be associated with hyperthyroidism. These are usually but not always follicular carcinomas; some are encapsulated follicular variants of papillary carcinoma (33–35). Although the tumors may lead to hyperfunction while still confined within the gland, many of the

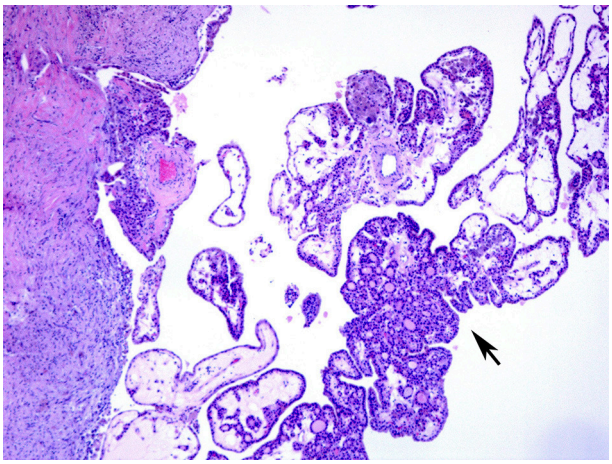


FIGURE 3 | A case of papillary hyperplastic nodule on low power showing cystic nodule with papillary architecture (arrow).

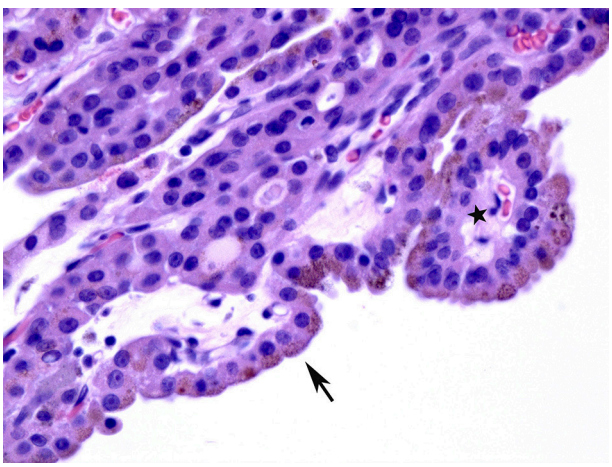


FIGURE 4 | A case of papillary hyperplastic nodule on high power showing oncocytic cells lining the papillary structures (arrow).

affected patients have metastatic disease. Some authors indicate that tumor burden correlates with the degree of hyperthyroidism (36–41).

Another interesting thyroid carcinoma that can present with hyperthyroidism is the rare diffuse follicular variant of papillary carcinoma (42). A tumor that is most often found in young females who present with goiter, the clinical picture resembles classic Graves' disease or toxic goiter. About 25% of these lesions will show hyperthyroidism and abnormal thyroid function tests. Treatment of the cancer will lead to resolution of the metabolic abnormality (40–44).

HASHITOXICOSIS

This term originally coined about 40 years ago by the Mayo Clinic group describes patients who present clinically with hyperthyroidism but whose glands show the histology of chronic lymphocytic thyroiditis including oxyphilia (Hürthle cell metaplasia) (45). This histologic presentation is also often seen in children and very young usually teenage patients who present with thyroid hyperfunction. Often these patients go through a phase of euthyroidism and subsequently hypothyroidism over a period of decades (46–48).

SECONDARY AND TERTIARY HYPERTHYROIDISM

When hyperthyroidism is associated with lesions of the pituitary gland or the hypothalamus, it is considered secondary and tertiary hyperthyroidism respectively. In comparison to primary hyper thyroidism, these clinical conditions are extremely rare (*<1% of hyperthyroidism*). The lesion in the pituitary gland is most frequently multifocal thyrotroph hyperplasia rather than a thyroid stimulating hormone (TSH) producing adenoma. Lesions of the hypothalamus producing thyrotropin releasing hormone (TRH) can stimulate the pituitary thyrotrophes to hyper secrete thyroid stimulating hormone (TSH) and subsequently to influence thyroid gland to produce excess thyroid hormone. Lesions of the hypothalamus responsible for this excess TRH include tumors, granulomatous disease (i.e., sarcoid) and other mass producing lesions (49–53).

HYPERTHYROIDISM DUE TO STRUMA OVARII

The presence of thyroid tissue within the ovary is usually seen in benign cystic teratomas also known as dermoid cysts of the ovary (54). The thyroid in these lesions is often part of a multi-tissue proliferation, that is tissues from all three embryological germ layers are represented. When thyroid tissue is the only or majority of tissue (>50%) in a teratoma (often termed mono dermal teratoma) it is diagnosed as struma ovarii. In most cases the thyroid either appears normal or shows changes consistent with colloid goiter. In rare instances, the thyroid will appear hyperplastic or even show lymphocytic infiltration mimicking thyroiditis. Rarely neoplasms originating in the thyroid gland including papillary carcinoma, follicular carcinoma or even poorly differentiated carcinoma can arise in a background of struma ovarii. Most of these tumors do not involve the ovarian surface and do not spread (these tumors have been designated by some authors as "proliferating struma") (55). Unusual situations have been described wherein the struma ovarii or tumors therein may hyper secrete thyroid hormone and lead to clinical hyperthyroidism (56–58).

HYPERTHYROIDISM ASSOCIATED WITH ECTOPIC PRODUCTION OF THYROTROPIN (THYROID STIMULATING HORMONE- (TSH)) AND THYROTROPIN RELEASING HORMONE (TRH)

Rare reported cases of non-endocrine malignant tumors secreting TSH or TRH have been reported. The most common

histology is that of hepatocellular carcinoma. The tumor produces these stimulatory hormones and when tumor is entirely removed the levels of hormones drop and hyperthyroidism regresses (59, 60).

HYPERTHYROIDISM ASSOCIATED WITH TROPHOBLASTIC DISEASE

Gestational trophoblastic disease including hydatidiform mole and choriocarcinoma is associated with marked elevation of beta human chorionic gonadotropin (beta HCG). Because the beta subunit of HCG is identical in chemical structure to one of the subunits of TSH, the HCG elevation can mimic elevated TSH and stimulate the thyroid to produce excess thyroid hormone. Although it is rare to see tissue from the thyroid in these patients, it is expected that the gland would show a hyperplastic appearance with papillae and cellular enlargement. Lymphocytic infiltration would be absent. Treatment of the gestational trophoblastic disease by uterine evacuation followed by chemotherapy usually leads to resolution of the hyperthyroid state (61–64).

DRUG ASSOCIATED HYPERTHYROIDISM

A variety of classes of pharmaceutical agents can cause thyroid dysfunction. It is beyond the purpose of this review to engage in a lengthy discussion of the clinical disorders caused by these drugs. Some of these interfere with metabolism of iodine, others with the production of thyroid hormone and its conversion to active moieties, and still others do not produce abnormalities in thyroid function but cause chemical interference with thyroid function test measurements. Many drugs can affect thyroid function (phenytoin and derivatives, therapies associated with interleukin administration usually in oncology settings) (65–67). The pathologic counterparts for these include lymphocytic infiltration of the gland with or without fibrosis (68).

Those drugs that cause hyperthyroidism are fewer and they usually exert their effect through interference with the metabolism of iodine. It is rare to see pathological specimens from these patients; the exceptions is the cardiac drug, amiodarone, interleukin containing regimens for chemotherapy and most recently PDL 1 or immune checkpoint inhibitors; these will be discussed below.

Amiodarone Associated Thyroid Dysfunction (AATD)

The literature notes that there are two types of thyroid lesions that are associated with amiodarone, an iodine containing compound used to treat cardiac arrhythmias. Amiodarone induced thyrotoxicosis (AIT) is classified as type I and type II, the former occurs in patients with underlying thyroid disease such as nodular goiter, autonomous nodular goiter or Graves' disease, whereas, Type II is caused by iodine-led destruction of the thyroid follicular epithelium in a normal thyroid gland. Because amiodarone is vital to control the cardiac problems, it is often not possible to wean the patient from the medication or to change to another drug. The first line of therapy in amiodarone induced thyrotoxicosis is treatment with Thionamides in AIT I and glucocorticoids in AIT II. Thyroid excision is undertaken in patients who do not respond to medical therapy in order to treat the hyperthyroidism which often worsens cardiac symptoms (69–72).

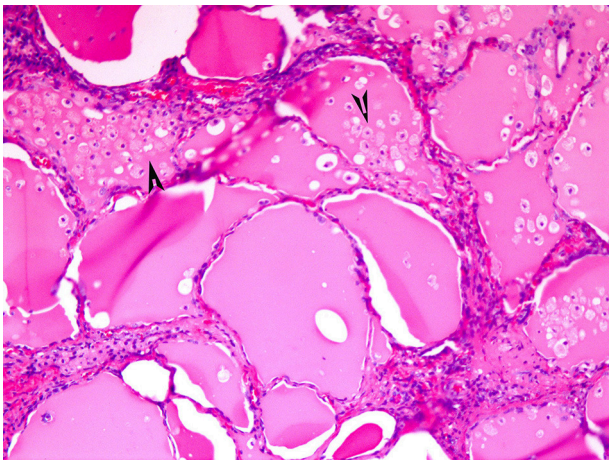


FIGURE 5 | Amiodarone associated follicular cell damage. Low and high power showing large thyroid follicles filled with colloid and numerous histiocytes (arrow heads, 3A,B).

If the gland is already pathologically abnormal (nodular thyroid goiter, Graves' disease), the pathology of the resected gland shows follicular disruption with histiocytes infiltrating the follicular epithelium and colloid (**Figures 5**, **6**). Rarely, inflammatory cells are noted within the thyroid parenchyma (Type I). On the other hand if the thyroid is histologically normal (Type II), the pathologic lesions show much milder follicular damage (73–75). These changes are similar to those seen in amiodarone induced pulmonary and liver toxicity (76, 77). Ultrastructural studies of both lung and thyroid tissues have shown lysosomal and mitochondrial inclusions in follicular cells consistent with follicle cell destruction (77). However, this simple explanation is not the only reason for the thyroid dysfunction. For example, co-cultures of amiodarone with human thyrocytes have shown the production of interleukin 6 and the drug also decreases the sodium-iodide symporter mRNA in the follicular cells (78).

The removal of the thyroid in amiodarone induced hyperthyroidism results in resolution of the hyperfunction and reversion of the cardiac disorder to baseline (75, 79).

Hyperthyroidism Associated With Antineoplastic Agents and Targeted Therapies

Thyroid dysfunction can occur in 20–50% patients receiving antineoplastic agents and targeted therapies. High dose IL-2 therapy can lead to hyperthyroidism in 7% of patients. In

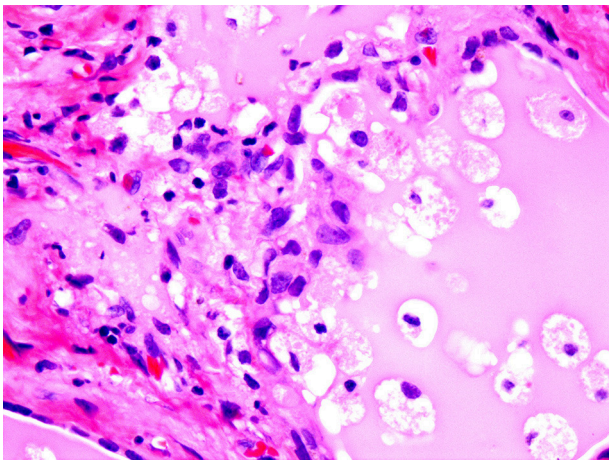


FIGURE 6 | Same as **Figure 5**.

rare instances, interferon-alpha treatment can lead to classic Graves' disease and even Graves' opthalmopathy; and these condition can persist even after the cessation of therapy (67, 80). At present, several tyrosine kinase inhibitors (TK1) are being used to treat different types of malignant neoplasms. TKI can lead to various forms of toxicities including those related to endocrine organs. Transient hyperthyroidism can occur during TKI therapy and is often due to destructive thyroiditis (67, 80).

Immune check-point inhibitors with their antitumor activity have shown to improve the survival rates of non-small cell lung carcinoma, melanoma, bladder and renal carcinoma, and ovarian carcinoma. A small number of patients undergoing treatment with immune-checkpoint inhibitors such as anti PD-1/anti-PDL-1 can develop hyperthyroidism (81).

MECHANICO- DESTRUCTIVE CAUSES OF HYPERTHYROIDISM (NON-HYPERTHYROID THYROTOXICOSIS)

The term mechanico-destructive hyperthyroidism (non-hyperthyroid thyrotoxicosis) has been coined by us to describe those conditions in which relatively rapid destruction of thyroid tissue followed by release of stored thyroid hormone from colloid as follicles or destroyed produces hyperfunction. Both benign and malignant conditions can be associated with this type of hyperthyroidism. An important clinical clue to the possibility of one of these disorders is that in contrast to more common causes of hyperthyroidism, radionucleotide scans show uptakes in the range of 1% or less. This reflects the destruction of the thyroid gland by the inflammatory or neoplastic process; the follicular epithelium is destroyed and cannot take up the radioactive isotope.

Subacute Thyroiditis ("Granulomatous Thyroiditis"; De Quervain Thyroiditis)

This condition is believed to be associated with systemic and or thyroid infection usually viral in nature, is often a painful cause of hyperthyroidism. Patients with this disorder will often present with neck pain which may be referred to the jaw or the chest. In the initial phases of this disease symptoms of hyperthyroidism are often clinically evident. As the gland is replaced by the inflammatory granulomatous process, the follicular epithelium is destroyed, follicles of ruptured and stored thyroid hormone within the colloid is released into the circulation. Unlike usual Graves' disease however the thyroid cannot take up iodide and produce more hormone. Thus, a phase of hypothyroidism is noted until healing occurs (82–84).

MALIGNANT NEOPLASMS CAUSING HYPERTHYROIDISM

Malignant neoplasms which are rapidly growing can be associated with this mechanic-destructive type of hyperthyroidism. The tumors most often identified are *anaplastic thyroid carcinoma, malignant lymphoma usually primary in the thyroid and of large cell type and poorly differentiated metastatic cancers* involving the thyroid (breast carcinoma and lung carcinoma most commonly). Histologically one sees the highly malignant tumor freely infiltrating the thyroid, destroying and replacing the tissue, with rupture of the follicles and release of thyroid hormone containing colloid. The rapidity of the process can lead to market elevation of thyroid hormone and a toxic state simulating thyroid storm (85–89).

In affected patients, there is often near complete destruction of the gland and the eventual development of hypothyroidism. Patients need to be supplemented with thyroid hormone to maintain a euthyroid metabolic state; if treatment of the tumor is successful, regeneration of thyroid follicles may occur from the residual thyroid tissue and as in subacute thyroiditis, normalization of thyroid function may occur (86–89).

CONCLUSION

This review has described the pathology and clinicopathologic correlations of unusual lesions of the thyroid and extrathyroidal tissues which can show clinical manifestations of hyperthyroidism. Although most of these conditions are rare especially when compared to Graves' disease or toxic nodular goiter, it is important for both the clinician and pathologist to be aware of them as diagnostic considerations.

AUTHOR CONTRIBUTIONS

VL and ZB have equally contributed to the literature review, drafting the manuscript and obtaining microscopic photographs. Both authors have reviewed the final version of this manuscript before submitting it to topic editors of the journal.

REFERENCES

1. Carnell NE, Valente WA. Thyroid nodules in Graves' disease:classification, characterization, and response to treatment. *Thyroid* (1998) 8:571–6. doi: 10.1089/thy.1998.8.571
2. Gossage A, Munro D. The pathogenesis of Graves' disease. *Clin Endocrinol Metab*. (1985) 14:299–330. doi: 10.1016/S0300-595X(85)80036-0
3. Burman K, Baker J. Immune Mechanisms in Graves' disease. *Endocr Rev*. (1985) 6:183–223. doi: 10.1210/edrv-6-2-183
4. Karoutsou E, Polymeris A. Pathogenesis of Graves' disease focusing on Graves' ophthalmopathy. *Endocr Regul.* (2011) 45:209–20. doi: 10.4149/endo_2011_04_209
5. Leovey A, Bako G, Sztojka I, Szabo J, Kalman K, Szabo T. The common incidence of Basedow's-Graves' disease and chronic lymphocytic thyroiditis. *Radiobiol Radiother*. (1984) 25:769–74.
6. McKenna TJ. Graves' disease. *Lancet* (2001) 357:1793–6. doi: 10.1016/S0140-6736(00)04906-0
7. Hirota Y, Tamai H, Hayashi Y, Matsubayashi S, Matsuzuka F, Kuma K, et al. Thyroid function and histology in forty-five patients with hyperthyroid Graves' disease in clinical remission more than ten years after thionamide drug treatment. *J Clin Endocrinol Metab.* (1986) 62:165–9. doi: 10.1210/jcem-62-1-165
8. Mizukami Y, Matsubara F. [Clinicopathological study on the chronic thyroiditis and Graves' disease –relationship between histological classification and function (author's transl)]. *Nihon Naika Gakkai Zasshi* (1980) 69:321–9. doi: 10.2169/naika.69.321
9. Spjut H, Warren W, Ackerman L. Clinical-pathologic study of 76 cases of recurrent Graves' disease, toxic (nonexophthalmic) goiter, and nontoxic goiter. *Am J Clin Pathol.* (1957) 27:367–92. doi: 10.1093/ajcp/27.4.367
10. Albores-Saavedra J, Wu J. The many faces and mimics of papillary thyroid carcinoma. *Endocrine Pathol.* (2006) 17:1–18. doi: 10.1385/EP:17:1:1
11. Baloch ZW, LiVolsi VA. Cytologic and architectural mimics of papillary thyroid carcinoma. Diagnostic challenges in fine-needle aspiration and surgical pathology specimens. *Am J Clin Pathol.* (2006) 125(Suppl.):S135–44. doi: 10.1309/YY72M308WPEKL1YY
12. LiVolsi VA. Papillary neoplasms of the thyroid. Pathologic and prognostic features. *Am J Clin Pathol.* (1992) 97:426–34. doi: 10.1093/ajcp/97.3.426
13. Bitton RN, Sachmechi I, Tabriz MS, Murphy L, Wasserman P. Papillary carcinoma of the thyroid with manifestations resembling Graves' disease. *Endocr Pract.* (2001) 7:106–9. doi: 10.4158/EP.7.2.106
14. Braga M, Graf H, Ogata A, Batista J, Hakim NC. Aggressive behavior of papillary microcarcinoma in a patient with Graves' disease initially presenting as cystic neck mass. *J Endocrinol Invest.* (2002) 25:250–3. doi: 10.1007/BF03343999
15. Lucas Martin A, Sanmarti Sala A. [Association of Graves-Basedow disease with thyroid papillary carcinoma:a pathogenic relationship?]. *Med Clin*. (1987) 89:664–5.
16. Pujadas R, Fernandez F, Camacho L, Foz M. [Association of Graves-Basedow disease with thyroid papillary carcinoma:a pathogenic relationship?]. *Med Clin.* (1987) 88:786.
17. Valenti TM, Macchia E, Pisa R, Bucalo ML, Russo V, Colletti I, et al. Toxic adenoma and papillary thyroid carcinoma in a patient with Graves' disease. *J Endocrinol Invest.* (1999) 22:701–4. doi: 10.1007/BF03343633
18. Wei S, Baloch ZW, LiVolsi VA. Thyroid carcinoma in patients with Graves' disease:an institutional experience. *Endocrine Pathol*. (2015) 26:48–53. doi: 10.1007/s12022-014-9343-6
19. Studer H, Peter H, Gerber H. Toxic nodular goitre. *Clin Endocrinol Metab*. (1985) 14:351–72. doi: 10.1016/S0300-595X(85)80038-4
20. Thomas FB, Mazzaferri EL, Skillman TG. Apathetic thyrotoxicosis: A distinctive clinical and laboratory entity. *Ann Intern Med.* (1970) 72:679–85. doi: 10.7326/0003-4819-72-5-679
21. Johnson PC, Kahil ME. Apathetic hyperthyroidism. A type of masked thyrotoxicosis. *Tex Med.* (1967) 63:59–62.
22. Wu W, Sun Z, Yu J, Meng Q, Wang M, Miao J, et al. A clinical retrospective analysis of factors associated with apathetic hyperthyroidism. *Pathobiology* (2010) 77:46–51. doi: 10.1159/000272954
23. Miller JM. Plummer's disease. *Med Clin North Am.* (1975) 59:1203–16. doi: 10.1016/S0025-7125(16)31968-X
24. Messina G, Viceconti N, Trinti B. Diagnostic items and treatment of Plummer's disease:a study on 180 patients. *La Clinica Terapeutica* (1998) 149:191–5.
25. Vickery AL Jr. Thyroid papillary carcinoma. Pathological and philosophical controversies. *Am J Surg Pathol.* (1983) 7:797–807. doi: 10.1097/00000478-198307080-00009
26. Apel RL, Ezzat S, Bapat BV, Pan N, LiVolsi VA, Asa SL. Clonality of thyroid nodules in sporadic goiter. *Diagn Mol Pathol.* (1995) 4:113–21. doi: 10.1097/00019606-199506000-00007
27. Deleu S, Allory Y, Radulescu A, Pirson I, Carrasco N, Corvilain B, et al. Characterization of autonomous thyroid adenoma:metabolism, gene expression, and pathology. *Thyroid* (2000) 10:131–40. doi: 10.1089/thy.2000.10.131
28. Aeschimann S, Kopp PA, Kimura ET, Zbaeren J, Tobler A, Fey MF, et al. Morphological and functional polymorphism within clonal thyroid nodules. *J Clin Endocrinol Metabol.* (1993) 77:846–51.
29. Kopp P, Kimura ET, Aeschimann S, Oestreicher M, Tobler A, Fey MF, et al. Polyclonal and monoclonal thyroid nodules coexist within human multinodular goiters. *J Clin Endocrinol Metabol.* (1994) 79:134–9.
30. Meissner W, Warren S. *Tumors of the Thyroid Gland.* Washington, DC: Armed Forces Institute of Pathology (1969).
31. Khurana KK, Baloch ZW, LiVolsi VA. Aspiration cytology of pediatric solitary papillary hyperplastic thyroid nodule. *Arch Pathol Lab Med.* (2001) 125:1575–8. doi: 10.1043/0003-9985(2001)125<1575:ACOPSP>2.0.CO;2
32. Khayyata S, Barroeta JE, LiVolsi VA, Baloch ZW. Papillary hyperplastic nodule:pitfall in the cytopathologic diagnosis of papillary thyroid carcinoma. *Endocr Pract*. (2008) 14:863–8. doi: 10.4158/EP.14.7.863
33. Sasaki J, Odaka Y, Kato R, Tada T, Yagawa K, Kowata T, et al. [Hyperfunctioning follicular carcinoma of the thyroid. A case report]. *Nippon Geka Gakkai Zasshi* (1988) 89:286–91.
34. Michigishi T, Mizukami Y, Shuke N, Satake R, Noguchi M, Aburano T, et al. An autonomously functioning thyroid carcinoma associated with euthyroid Graves' disease. *J Nucl Med.* (1992) 33:2024–6.
35. Ardito G, Vincenzoni C, Cirielli C, Guidi ML, Corsello MS, Modugno P, et al. Papillary thyroid carcinoma mimicking an autonomous functioning nodule. *Eur J Surg Oncol.* (1997) 23:569. doi: 10.1016/S0748-7983(97)93397-7
36. Niepomniszcze H, Suarez H, Pitoia F, Pignatta A, Danilowicz K, Manavela M, et al. Follicular carcinoma presenting as autonomous functioning thyroid nodule and containing an activating mutation of the TSH receptor (T620I) and a mutation of the Ki-RAS (G12C) genes. *Thyroid* (2006) 16:497–503. doi: 10.1089/thy.2006.16.497
37. Siddiqui AR, Karanauskas S. Hurthle cell carcinoma in an autonomous thyroid nodule in an adolescent. *Pediatr Radiol.* (1995) 25:568–9. doi: 10.1007/BF02015798
38. Smith M, McHenry C, Jarosz H, Lawrence AM, Paloyan E. Carcinoma of the thyroid in patients with autonomous nodules. *Am Surg.* (1988) 54:448–9.
39. Tfayli HM, Teot LA, Indyk JA, Witchel SF. Papillary thyroid carcinoma in an autonomous hyperfunctioning thyroid nodule:case report and review of the literature. *Thyroid* (2010) 20:1029–32. doi: 10.1089/thy.2010.0144
40. Siddiqi IN, Friedman J, Barry-Holson KQ, Ma C, Thodima V, Kang I, et al. Characterization of a variant of t(14;18) negative nodal diffuse follicular lymphoma with CD23 expression, 1p36/TNFRSF14 abnormalities, and STAT6 mutations. *Mod Pathol.* (2016) 29:570–81. doi: 10.1038/modpathol.2016.51
41. Vinciguerra GL, Noccioli N, Bartolazzi A. Diffuse follicular variant of papillary thyroid carcinoma:a case report with a revision of literature. *Rare Tumors* (2016) 8:6536. doi: 10.4081/rt.2016.6536
42. Sobrinho-Simões M, Soares J, Carneiro F, Limbert E. Diffuse follicular variant of papillary carcinoma of the thyroid: report of eight cases of a distinct aggressive type of thyroid tumor. *Surg Pathol.* (1990) 3:189–203.
43. Cha YJ, Chang HS, Hong SW. Diffuse follicular variant of papillary thyroid carcinoma in a 69-year-old man with extensive extrathyroidal extension: a case report. *J Korean Med Sci*. (2013) 28:480–4. doi: 10.3346/jkms.2013.28.3.480
44. Mizukami Y, Nonomura A, Michigishi T, Ohmura K, Noguchi M, Ishizaki T. Diffuse follicular variant of papillary carcinoma of the thyroid. *Histopathology* (1995) 27:575–7. doi: 10.1111/j.1365-2559.1995.tb00331.x
45. Fatourechi V, McConahey WM, Woolner LB. Hyperthyroidism associated with histologic Hashimoto's thyroiditis. *Mayo Clin Proc.* (1971) 46:682–9.

46. Mori T. [Hashitoxicosis and Hashimoto's disease with the symptoms of thyrotoxicosis]. *Nihon Rinsho* (1980) 38:1677–83.
47. Nabhan ZM, Kreher NC, Eugster EA. Hashitoxicosis in children:clinical features and natural history. *J Pediatr.* (2005) 146:533–6. doi: 10.1016/j.jpeds.2004.10.070
48. Wasniewska M, Corrias A, Salerno M, Lombardo F, Aversa T, Mussa A, et al. Outcomes of children with hashitoxicosis. *Horm Res Paediatr.* (2012) 77:36–40. doi: 10.1159/000334640
49. Waldhausl W, Bratusch-Marrain P, Nowotny P, Buchler M, Forssmann WG, Lujf A, et al. Secondary hyperthyroidism due to thyrotropin hypersecretion:study of pituitary tumor morphology and thyrotropin chemistry and release. *J Clin Endocrinol Metab.* (1979) 49:879–87. doi: 10.1210/jcem-49-6-879
50. Gomez JB, Diaz MA, Jerez ML. [Tertiary hyperthyroidism. Criteria of evaluation]. *Rev Med.* (1973) 17:231–9.
51. Kourides I, Ridgway E, Weintraub B, Bigos S, Gershengorn M, Maloof F. Thyrotropin-induced hyperthyroidism:use of alpha and beta subunit levels to identify patients with pituitary tumors. *J Clin Endocrinol Metab.* (1977) 45:534–43. doi: 10.1210/jcem-45-3-534
52. Dorfman SG. Hyperthyroidism. Usual and unusual causes. *Arch Internal Med.* (1977) 137:995–6. doi: 10.1001/archinte.1977.03630200005005
53. Tolis G, Bird C, Bertrand G, McKenzie J, Ezrin C. Pituitary hyperthyroidism. *Am J Med.* (1978) 64:177–181. doi: 10.1016/0002-9343(78)90202-4
54. Prat J, Cao D, Carinelli SG, Nogales FF, Vang R, Zaloudek CJ. *WHO Classification of Tumours of Female Reproductive Organs*. Lyon: IARC (2014).
55. Devaney K, Snyder R, Norris HJ, Tavassoli FA. Proliferative and histologically malignant struma ovarii:a clinicopathologic study of 54 cases. *Int J Gynecol Pathol.* (1993) 12:333–43. doi: 10.1097/00004347-199310000-00008
56. Yang Q, Yang X, Liu ZZ, Jiang YX, Li JC, Su N, et al. Sonographic and pathologic features of struma ovarii. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao* (2015) 37:309–14. doi: 10.3881/j.issn.1000-503X.2015.03.012
57. Yassa L, Sadow P, Marqusee E. Malignant struma ovarii. *Nat Clin Pract Endocrinol Metabol.* (2008) 4:469–72. doi: 10.1038/ncpendmet0887
58. Wei S, Baloch ZW, LiVolsi VA. Pathology of struma ovarii: a report of 96 Cases. *Endocrine Pathol.* (2015) 26:342–8. doi: 10.1007/s12022-015-9396-1
59. Helzberg JH, McPhee MS, Zarling EJ, Lukert BP. Hepatocellular carcinoma:an unusual course with hyperthyroidism and inappropriate thyroid-stimulating hormone production. *Gastroenterology* (1985) 88:181–4. doi: 10.1016/S0016-5085(85)80152-9
60. Carri J, Peral F, Surreco M, Lujan A, Leguizamon R, Martinez G, et al. [Fibrolamellar hepatocellular carcinoma:a clinical report with paraneoplastic hyperthyroidism (apropos of a case)]. *Acta Gastroenterol Latinoam.* (1989) 19:155–64.
61. Berdjis N, Baldauf A, Kittner T, Manseck A, Wirth M. [Paraneoplastic hyperthyroidism in a patient with metastasizing teratocarcinoma and excessively high HCG]. *Aktuelle Urol.* (2003) 34:407–9. doi: 10.1055/s-2003-43174
62. Kohler S, Tschopp O, Jacky E, Schmid C. Paraneoplastic hyperthyroidism. *BMJ Case Rep.* (2011) 2011. doi: 10.1136/bcr.04.2011.4163
63. Oosting SF, de Haas EC, Links TP, de Bruin D, Sluiter WJ, de Jong IJ, et al. Prevalence of paraneoplastic hyperthyroidism in patients with metastatic non-seminomatous germ-cell tumors. *Ann Oncol.* (2010) 21:104–8. doi: 10.1093/annonc/mdp265
64. Solomon CG, Dluhy RG. Paraneoplastic endocrine syndromes. *Curr Ther Endocrinol Metab.* (1994) 5:537–42.
65. Hein MD, Jackson IM, Review:thyroid function in psychiatric illness. *Gen Hosp Psychiatry* (1990) 12:232–44. doi: 10.1016/0163-8343(90)90060-P
66. Visser WE, de Rijke YB, van Toor H, Visser TJ. Thyroid status in a large cohort of patients with mental retardation:the TOP-R (Thyroid Origin of Psychomotor Retardation) study. *Clin Endocrinol.* (2011) 75:395–401. doi: 10.1111/j.1365-2265.2011.04089.x
67. Krouse RS, Royal RE, Heywood G, Weintraub BD, White DE, Steinberg SM, et al. Thyroid dysfunction in 281 patients with metastatic melanoma or renal carcinoma treated with interleukin-2 alone. *J Immunother Emphasis Tumor Immunol.* (1995) 18:272–8. doi: 10.1097/00002371-199511000-00008
68. Rosenbaum A, Maruta T, Richelson E. Drugs that alter mood:lithium. *Mayo Clin Proc.* (1979) 54:401–7.
69. Amico J, Richardson V, Alpert B, Klein I. Clinical and chemical assessment of thyroid function during therapy with amiodarone. *Arch Intern Med.* (1984) 144:487–90. doi: 10.1001/archinte.1984.00350150071023
70. Alves L, Rose E, Cahill T. Amiodarone and the thyroid. *Ann Intern Med.* (1985) 102:412. doi: 10.7326/0003-4819-102-3-412_1
71. Gammage M, Franklyn J. Amiodarone and the thyroid. *Q J Med.* (1987) 238:83–6.
72. Bogazzi F, Bartalena L, Gasperi M, Braverman LE, Martino E. The various effects of amiodarone on thyroid function. *Thyroid* (2001) 11:511–9. doi: 10.1089/105072501300176471
73. Smyrk T, Goellner J, Brennan M, Carney J. Pathology of the thyroid in amiodarone assoicated thyrotoxicosis. *Am J Surg Pathol.* (1987) 11:197–204. doi: 10.1097/00000478-198703000-00004
74. Saad A, Falciglia M, Steward DL, Nikiforov YE. Amiodarone-induced thyrotoxicosis and thyroid cancer:clinical, immunohistochemical, and molecular genetic studies of a case and review of the literature. *Arch Pathol Lab Med.* (2004) 128:807–10. doi: 10.1043/1543-2165(2004)128<807:ATATCC>2.0.CO;2
75. Elnaggar MN, Jbeili K, Nik-Hussin N, Kozhippally M, Pappachan JM. Amiodarone-induced thyroid dysfunction:a clinical update. *Exp Clin Endocrinol Diabetes* (2018) 126:333–41. doi: 10.1055/a-0577-7574
76. Marchlinski F, Gansler T, Waxman H, Josephson M, Amiodarone pulmonary toxicity. *Ann Intern Med.* (1982) 97:839–45. doi: 10.7326/0003-4819-97-6-839
77. Dake M, Madison J, Montgomery C, Shellito JE, Hinchcliffe WA, Winkler ML, et al. Electron microscopic demonstration of lysosomal inclusion bodies in lung, liver, lymph nodes and blood leukocytes of patients with amiodarone pulmonary toxicity. *Am J Med.* (1985) 78:506–512. doi: 10.1016/0002-9343(85)90346-8
78. Yamazaki K, Mitsuhashi T, Yamada E, Yamada T, Kosaka S, Takano K, et al. Amiodarone reversibly decreases sodium-iodide symporter mRNA expression at therapeutic concentrations and induces antioxidant responses at supraphysiological concentrations in cultured human thyroid follicles. *Thyroid* (2007) 17:1189–200. doi: 10.1089/thy.2007.0215
79. Bartalena L, Bogazzi F, Chiovato L, Hubalewska-Dydejczyk A, Links TP, Vanderpump M. 2018 European Thyroid Association (ETA) guidelines for the management of amiodarone-associated thyroid dysfunction. *Eur Thyroid J.* (2018) 7:55–66. doi: 10.1159/000486957
80. Hamnvik OP, Larsen PR, Marqusee E. Thyroid dysfunction from antineoplastic agents. *J Natl Cancer Inst.* (2011) 103:1572–87. doi: 10.1093/jnci/djr373
81. Guaraldi F, La Selva R, Sama MT, D'Angelo V, Gori D, Fava P, et al. Characterization and implications of thyroid dysfunction induced by immune checkpoint inhibitors in real-life clinical practice:a long-term prospective study from a referral institution. *J Endocrinol Invest.* (2018) 41:549–56. doi: 10.1007/s40618-017-0772-1
82. Pichler R, Wolfl S, Bogner S, Sulzbacher H, Shamiyeh A, Maschek W. [Subacute thyroiditis with cell destruction and temporary hyperthyroidism in Graves'disease–case report]. *Acta Medica Austriaca.* (2002) 29:137–40. doi: 10.1046/j.1563-2571.2002.02008.x
83. Woolf PD. Painless thyroiditis as a cause of hyperthyroidism:subacute or chronic lymphocytic? *Arch Internal Med.* (1978) 138:26–7. doi: 10.1001/archinte.1978.03630250010006
84. Janssen OE. [Atypical presentation of subacute thyroiditis]. *Dtsch Med Wochenschr.* (2011) 136:519–22. doi: 10.1055/s-0031-1274535
85. De Ridder M, Sermeus AB, Urbain D, Storme GA. Metastases to the thyroid gland-a report of six cases. *Eur J Intern Med.* (2003) 14:377–9. doi: 10.1016/S0953-6205(03)90005-7
86. Shimaoka K. Thyrotoxicosis due to metastatic involvement of the thyroid. *Arch Intern Med.* (1980) 140:284–5. doi: 10.1001/archinte.1980.00330140142050

87. Shimaoka K. VanHerle AJ, Dindogru A, Thyrotoxicosis secondary to involvement of the thyroid with malignant lymphoma. *J Clin Endocrinol Metab*. (1976) 43:64–8. doi: 10.1210/jcem-43-1-64
88. Chung AY, Tran TB, Brumund KT, Weisman RA, Bouvet M. Metastases to the thyroid:a review of the literature from the last decade. *Thyroid* (2012) 22:258–68. doi: 10.1089/thy.2010.0154
89. Li B, Tang Y, Xiao D, Zhong M, Coexistence of thyroid metastasis carcinoma in the background of HT and primary thyroid mucosa-associated lymphoid tissue B cell lymphoma in a thyroid gland. *Ann Hematol.* (2010) 89:1053–6. doi: 10.1007/s00277-010-0906-4

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

SYSTEMATIC REVIEW
published: 20 November 2018
doi: 10.3389/fendo.2018.00679

The Impact of Goiter and Thyroid Surgery on Goiter Related Esophageal Dysfunction. A Systematic Review

Jesper Roed Sorensen[1], Steen Joop Bonnema[2], Christian Godballe[1] and Laszlo Hegedüs[2]*

[1] Department of ORL–Head and Neck Surgery and Audiology, Odense University Hospital, Odense, Denmark, [2] Department of Endocrinology and Metabolism, Odense University Hospital, Odense, Denmark

Background: Patients with goiter referred for thyroidectomy report swallowing difficulties. This might be associated with esophageal compression and deviation as this is present in a significant number of patients. Studies on how goiter and subsequently its treatment affect the esophagus are sparse and point in various directions. Our aim was to investigate, through a systematic review, the impact of goiter and thyroidectomy on esophageal anatomy, esophageal physiology, and subjective swallowing dysfunction.

Methods: The search period covered 1 January 1975 to 1 July 2018, using the scientific databases PubMed and EMBASE. Inclusion criteria were adult patients with goiter who were either observed or underwent thyroidectomy. Search terms were variations of the terms for goiter, esophagus, swallowing, and dysphagia. From an initial 3,040 titles, 55 full text evaluations led to the final inclusion of 27 papers. Seventeen papers investigated, prospectively, the impact of thyroidectomy on the esophagus, while five observational and five retrospective studies were also included.

Results: Esophageal anatomy impairment: Esophageal deviation occurred in 14% and esophageal compression in 8–27% of goiter patients. The prevalence increased with goiter size and with the extent of substernal extension. The smallest cross-sectional area of the esophagus increased by median 34% after thyroidectomy. Esophageal physiology changes: Goiter patients had increased esophageal transit time, positively correlated with goiter size, but unrelated to esophageal motility disturbances. Decrease in the upper esophageal sphincter pressure occurred early after surgery, and normalized within 6 months. Swallowing related patient-reported outcomes: Evaluated by validated questionnaires, swallowing symptoms worsened in the early period after thyroidectomy, but improved after 6 months, as compared to baseline.

Conclusions: Thyroidectomy relieved patients with goiter from dysphagia, within 6 months of surgery probably via increase in the cross-sectional area of the esophagus. Attention to the impact by goiter on the esophagus is needed, and balanced and individualized information about the potential benefits and risks of thyroid surgery is crucial in the management of patients with goiter.

Keywords: goiter, thyroidectomy, esophagus, swallowing, thyroid dysfunction, patient-reported outcomes, systematic review

OPEN ACCESS

Edited by:
Giampaolo Papi, Azienda Unità Sanitaria Locale di Modena, Italy

Reviewed by:
Michele Minuto, Università degli Studi di Genova, Italy
Rocco Bruno, Independent Researcher, Matera, Italy

****Correspondence:***
Jesper Roed Sorensen
jesper.roed.sorensen@rsyd.dk

Specialty section:
This article was submitted to Thyroid Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *24 September 2018*
Accepted: *29 October 2018*
Published: *20 November 2018*

Citation:
Sorensen JR, Bonnema SJ, Godballe C and Hegedüs L (2018) The Impact of Goiter and Thyroid Surgery on Goiter Related Esophageal Dysfunction. A Systematic Review. Front. Endocrinol. 9:679. doi: 10.3389/fendo.2018.00679

INTRODUCTION

Nodularity of the thyroid gland–caused by delicate interactions between genetic and environmental factors such as low iodine intake and cigarette smoking-is common and, depending on definition and type of imaging, affect 20–76% of the population (1–4). The vicinity of the thyroid to vital structures in the neck and upper mediastinum, i.e., the trachea, esophagus, nerves, and blood vessels, combined with a tendency to gradually increase in size, leads to a variety of potential clinical manifestations of the goiter. These include compromised respiration and dysphagia, as well as globus and choking sensation, as some of the most prevalent symptoms (5–9). The positive correlation between goiter size and tracheal compression, and the relief following thyroidectomy is well accepted (10). However, studies on how goiter and subsequently its treatment affect the esophagus are sparse and point in various directions.

Patients with goiter referred for thyroidectomy report swallowing difficulties (11–15) and these may be associated with esophageal compression and deviation, which are abnormalities found to be present in a significant number of patients (8, 16). Thyroidectomy seems to improve both swallowing difficulties (12, 17, 18) and quality of life (19–21) in patients with goiter. The latter improves already 3 months after surgery (19), whereas at least 6 months need to pass before improvements in swallowing symptoms can be expected (11, 22). Comparison of the obviously inhomogeneous studies of the esophagus is almost impossible, which makes it difficult to offer patients with goiter evidence-based information on the surgical effect regarding esophageal compression, dysphagia, and globus sensation.

The aim of the present study was, through a systematic review, to investigate the impact of goiter and thyroidectomy on esophageal anatomy and physiology, and swallowing related patient reported outcomes.

METHODS

Study Design, Search Terms, and Extraction of Data

We identified all available English language publications regarding the relationship between goiter and the esophagus, covering the period from 1 January 1975 to 1 July 2018. The search terms used in the scientific databases PubMed and EMBASE were variations of the terms for goiter, esophagus, swallowing, and dysphagia, with MESH terms being Goiter, Esophagus, Deglutition, and Deglutition disorders.

Initially, we screened publication titles and then selected abstracts of relevance. Each identified paper was screened in accordance with the inclusion criteria being: data obtained in humans; age $\geq$18 years; individuals should undergo thyroidectomy or be offered observation alone; any study should include a minimum of 15 patients. The reference list of each paper was screened for missed publications. The data extracted included number of patients, gender, study design, blinding, inclusion, and exclusion criteria, summary of results, and the presence of major confounders as well as limitations and biases. Biases were assessed for the individual studies using the "Cochrane Risk of Bias tool" (23).

RESULTS

Study Selection and Characteristics

From PubMed and EMBASE 3,040 relevant titles were included, supplemented by 20 articles obtained from the reference lists of included papers (**Figure 1**). We excluded 3,005 papers after review of titles and abstracts, leaving 55 for full text evaluation. Twenty-eight full text papers failed to meet the inclusion criteria, resulting in 27 papers for the final analyses.

Applying the "Cochrane Risk of Bias tool" across studies (23), we found a considerable risk of bias as many studies failed to describe inclusion procedures, include control groups, randomize for treatment, or blind observers or participants (**Table 1**). As a consequence, we made no attempt to perform a meta-analysis. Most of the included studies (**Tables 2**, **3**) were small, with 60 or fewer individuals in 14 of the 27 studies. Only one study applied randomization (15) and three studies used some blinding of participants, personnel or the evaluated outcomes (25, 30, 31). There were five observational studies, 17 studies investigating the impact of thyroidectomy, and five retrospective studies with two studies investigating thyroidectomy (**Tables 2**, **3**). As evident from the heterogenous studies, the level of evidense range from low to moderate (**Table 4**).

Esophageal Anatomy Abnormalities

Affection of the esophagus was a common finding among patients with goiter (**Table 2**). Four studies evaluated, by chest X-ray, CT, or MRI, the prevalence of esophageal compression and/or deviation in patients undergoing thyroidectomy (8, 16, 24, 25). A study of 198 patients found esophageal deviation in 14% of individuals, and esophageal compression in even fewer (8%) (16). A higher prevalence of esophageal compression was found in another study of only 23 patients, with six patients (26%) having esophageal compression (8). A third study found 24 of 26 patients (95%) with substernal goiter to have esophageal midline deviation (24). All the above studies had shortcomings, such as unclear definitions of either esophageal deviation and/or compression.

A more recent study (25), with well-defined definitions of both esophageal deviation and compression, evaluated 65 well-characterized goiter patients undergoing neck MRI before and 6 months after thyroidectomy. In that study, the median esophageal deviation decreased significantly from 4 mm (range: 0–23 mm) to 3 mm (range: 0–10 mm). More importantly, the median smallest cross-sectional area of the esophagus (SCAE) increased from 95 mm^2 (range: 47–147 mm^2) at baseline to 137 mm^2 (range: 72–286 mm^2) after surgery, corresponding to a median increase of 34% (range, −17 to 253%) in SCAE (25). The anterior-posterior depth of the esophagus did not change significantly following surgery, while the median-lateral width of the esophagus achieved a small but significant increase from a median of 15 mm (range: 10–21 mm) to 17 mm (range: 12–24) after surgery (**Table 2**). The esophagus had a small midline

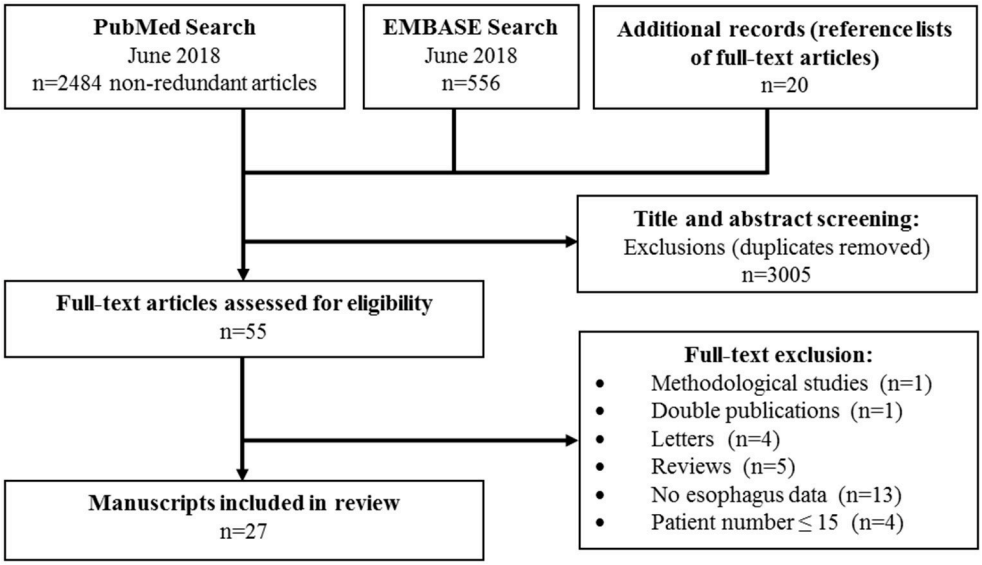


FIGURE 1 | Flow diagram of manuscript selection of final inclusion and final inclusion of full-text manuscripts.

deviation, and this persisted after surgery. More importantly, there was an increase in the cross-sectional area of the esophagus following surgery, and a change from a more round to a more ellipsoid shape. The preoperative volumes of the thyroid and the SCAEs were significantly inversely correlated, with reduction in SCAE of 0.35 mm^2 for every 10% increase in goiter volume. In addition, the SCAE increased by 1% for every 10% increase in the weight of the removed thyroid tissue (25). This implies that patients with the initially largest goiters also experienced the most pronounced improvements in SCAE, following thyroid surgery.

In relation to the impact of individual nodules on esophageal anatomy, one study found globus sensation to be present in 48% of patients. Nodules >3 cm in diameter, and/or located anteriorly and horizontally to the trachea were more often present in patients with globus sensation than in patients with smaller nodules or nodules located laterally to the trachea (26).

Esophageal Physiology Changes

The swallowing function had been investigated by various objective methods (**Table 2**). Using scintigraphic methods for assessment, an increase in the esophageal transit time by 2–7 s were demonstrated in patients with a large goiter (>7 cm long or >3 cm in depth, evaluated by ultrasound), as compared to smaller goiters (27). Another study, also using nuclear medicine techniques, found 39% of goiter patients to have increased esophageal transit time (28).

A study of 36 patients identified a decrease in the upper esophageal sphincter (UES) pressure, using water manometry, in the early period after total thyroidectomy (13). However, these findings were normalized 18–24 months after surgery in a subsequent study of the same patient cohort (29). Another study of 33 patients, found normal esophageal motility, both before and 6 month after surgery (30). Only the UES basal pressure increased significantly from 71 to 88 mmHg 6 month after surgery, the latter value being within the normal limits (30). In our study, using the Chicago classification version 3.0 for assessing motility disturbances (42), only one of the 33 patients with a benign goiter (3%) had a minor peristaltic disorder preoperatively, defined as >50% weak or failed coordinated esophageal muscle contractions during 10 swallowing events (30). Two patients (6%) fulfilled these criteria 6 months after surgery. Importantly, as patient characteristics as well as the manometric examination techniques (i.e., water manometry and high resolution esophageal manometry), differed between the studies, a direct, comparison across studies is not meaningful. Nevertheless, it seems that decrease in the UES pressure potentially may be present in the early period after surgery as the goiter is removed. This issue is of no greater concern beyond 6 months after surgery.

A few studies have investigated the hyoid bone movement, epiglottic tilting, and laryngeal mobility in goiter patients (31–35). A study of 54 patients demonstrated a preoperative association between abnormal laryngeal mobility and the prevalence of dysphagia in patients with goiter (31). In the immediate weeks after surgery the laryngeal mobility was reduced, with recovery at 6 months postoperatively (32). Another study found the hyoid bone movement to be unchanged after surgery in an investigation of 34 patients (33). In a study with 54 patients divided into two groups using neither randomization nor blinding, trans-axillary thyroidectomy was associated with a lower degree of neck muscle adhesion and a greater hyoid bone movement, as compared with conventional thyroidectomy (34). The prevalence of reflux laryngitis was also found lower in patients with cervical compared to substernal goiter, at 21 vs. 42%, respectively (35).

TABLE 1 | Risk of bias summary in the 27 studies included for review.

References	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete data	Selective reporting	Other bias
Shin et al. (16)	?	?	?	?	?	?	?
Netterville et al. (8)	?	?	?	?	?	?	?
Mackle et al. (24)	?	+	?	?	?	?	?
Brinch et al. (25)	?	?	+	+	?	?	+
Nam et al. (26)	−	+	?	?	+	+	?
Jorgensen et al. (27)	?	?	?	?	?	?	?
Glinoer et al. (28)	?	?	?	?	?	?	?
Scerrino et al. (13)	?	?	?	?	?	?	?
Scerrino et al. (29)	?	?	?	?	−	?	?
Sorensen et al. (30)	?	?	+	+	?	?	?
Arakawa-Sugueno et al. (31)	−	−	−	+	?	?	?
Gohrbandt et al. (32)	−	−	−	−	+	?	?
Fiorentino et al. (33)	?	?	?	?	−	?	?
Hyun et al. (34)	?	?	?	?	+	+	−
Rodrigues et al. (35)	−	−	−	−	?	?	?
Sabaretnam et al. (12)	?	?	?	?	?	−	−
Greenblatt et al. (17)	?	?	?	?	?	?	?
Maung et al. (18)	?	?	?	?	?	?	?
Lombardi et al. (11)	?	?	?	?	−	?	?
Holler and Anderson (36)	?	?	?	?	+	+	+
Tae et al. (22)	?	−	?	?	?	?	?
Lombardi et al. (37)	?	?	?	?	+	?	?
Lombardi et al. (15)	?	?	?	?	?	?	?
Grover et al. (38)	?	?	?	?	?	?	?
Burns and Timon (39)	+	?	?	?	+	?	?
Krekeler et al. (40)	?	?	?	?	+	+	?
Pereira et al. (41)	+	+	−	−	?	?	?

?, Unclear risk of bias; +, low risk of bias; −, high risk of bias.

Swallowing Related Patient-Reported Outcomes

Three studies, using validated swallowing specific questionnaires, have investigated the patients' perception of swallowing before and after thyroidectomy (**Table 3**) (12, 17, 18). By using the "swallowing related quality of life questionnaire," eight (12) and nine (17) of 11 swallowing related domains were affected in studies of 116 and 224 patients with goiter, respectively, all of whom were referred for thyroidectomy. Using the validated Glasgow-Edinburgh throat scale, only two of 12 items improved at 3 months after surgery, while six items had improved after 12 months (18).

Nine studies, the largest including more than 100 patients, used the non-validated swallowing impairment score or modified versions hereof (11, 13, 15, 22, 29, 34, 36–38). Prior to thyroidectomy, the prevalence of dysphagia varied between 48 and 78% (11, 13, 36). At 1 week, 1 month, and 3 months after thyroidectomy, three studies found increased prevalence (i.e., worsening) of swallowing symptoms compared to the preoperative level (11, 22, 37), while a single study of 53 patients found a decreased level of dysphagia (15). However, at 6 months or later after surgery, patients had fewer symptoms than at baseline (11, 22, 29), although a subgroup had persistent swallowing complaints (38). Studies using other examination techniques, such as qualitative analyses, supported these findings (39–41). The impact on swallowing symptoms seemed uninfluenced by the surgical technique or whether hemi- or total thyroidectomy was performed (15, 17, 18, 22).

Two recent studies, carried out by our group on the same patient cohort, assessed the impact of thyroidectomy and esophageal dysfunction by use of the well-validated disease-specific ThyPRO questionnaire (25, 30). Goiter patients had a significantly affected mean Goiter Symptom score of 40 points at baseline (maximum of 100 points equals severe symptoms), which improved significantly to a mean of 10 points postoperatively (25) (minimum of 0 points equals no symptoms) (**Table 2**). All 11 items of the Goiter Symptom scale improved 6 months after surgery. The items related to swallowing difficulties, such as "discomfort swallowing," underwent a significant score reduction from a mean of 1.8 points (maximum

TABLE 2 | Studies investigating the effect of goiter on the esophagus anatomy abnormalities and the esophageal physiology changes.

References	Design	*n*	Cohort	Methods	Results
Shin et al. (16)	RE	198	Age: 59 (22–89) years. Goiter: 143 g	CXR, CT	Esophageal compression/deviation: 8%/14% of patients with goiter
Netterville et al. (8)	RE	23	Age: 59 (32–91) years. Goiter: 148 (38–426) g	CXR, CT, MRI	Esophageal compression or deviation: 27% of patients with substernal goiter
Mackle et al. (24)	OB	26	Age: mean 57 years. Goiter: NA	CT	Esophageal deviation: 95% of patients with substernal goiter
Brinch et al. (25)	TH	64	Age: 52 (21–77) years. Goiter: 57 (14–642) mL	MRI, ThyPRO	SCAE increased from 95 mm^2 (47–147) to 137 (72–286) mm^2 in combination with decrease in goiter symptoms from 40 to 10 points at 6 months after surgery
Nam et al. (26)	OB	175	Age: 54 ± 12 years. Goiter: Single nodule	US	Globus sensation: 48 %. Nodules >3 cm and nodules located horizontally and anteriorly to the trachea are associated with globus sensation
Jorgensen et al. (27)	OB	74	Age: 40 (20–74) years. Goiter: NA	Scintigraphy	Increased MTT for patients with large goiters compared to healthy controls, and patients with small goiters, or enlarged atrium
Glinoer et al. (28)	OB	148	Age: 42 ± 13 years. Goiter: 33 ± 22 g	Scintigraphy	39% of patients with goiter had abnormal MTT MTT prolonging correlates with substernal goiter
Scerrino et al. (13)	TH	36	Age: 49 (26–65) years. Goiter: <60 mL	Manometry, MSIS	Swallowing impairment: Baseline: 78% of patients, after 1 month: 64% of patients. Decrease in UES 1 month after total thyroidectomy
Scerrino et al. (29)	TH	36	Age: 48 ± 6 years. Goiter: <60 mL	Manometry, MSIS	Swallowing impairment: 42 % of patients 18–24 months after surgery. UES approaches preoperative values 18–24 months after surgery
Sorensen et al. (30)	TH	33	Age: 60 ± 12 years. Goiter: 50 (8–607) g	Manometry, ThyPRO	UES increases after surgery 6 months after surgery in combination with decrease in goiter symptoms from 39 (2–61) to 5 (0–52) points at 6 months after surgery
Arakawa-Sugueno et al. (31)	TH	54	Age: NA Goiter: NA	Vide-endoscopic evaluation	Seven days after thyroidectomy, dysphagia in 87 % of patients with abnormal laryngeal mobility (ALM) and 44% of patients with normal laryngeal mobility (NLM). 60 days after surgery dysphagia in 67% of ALM patients and 25% of NLM patients
Gohrbandt et al. (32)	TH	53	Age: 52 ± 13 years. Goiter: NA	US	Reduced laryngeal mobility for at least 6 months after surgery for men, while women have fully recovered at 6 months after surgery
Fiorentino et al. (33)	TH	34	Age: 51 (21–77) years. Goiter: 58 ± 18 mL	VFSS	No change in hyoid elevation, epiglottic tilting or stasis of food bolus after thyroidectomy
Hyun et al. (34)	TH	47	Age: NA Goiter: NA	Swallowing movement, SIS	Transaxillary endoscopic thyroidectomy less SIS score, lower muscle adhesion, and greater hyoid bone movement than following regular thyroidectomy
Rodrigues et al. (35)	RE	113	Age: NA Goiter: >40 mL	Chart-review	Reflux laryngitis; cervical goiter: 21% vs. substernal goiter: 42% Digestive-compressive symptoms; cervical goiter: 77% vs. substernal goiter: 85%

RE, Retrospective; OB, Observational; TH, Thyroidectomy; NA, Not available; CXR, Chest X-ray; ThyPRO, Thyroid-specific Patient-reported Outcome; SCAE, Smallest cross-sectional area of the esophagus; US, Ultrasound; MTT, Mean esophageal transit time; MSIS, Modified Swallowing Impairment Score; UES, Upper esophageal sphincter pressure; VFSS, Videoflurographic swallowing study; SIS, Swallowing Impairment Score.

of 4 points) to 0.3 points, postoperatively. Similarly, the mean score for "difficulty swallowing" decreased significantly from 1.2 to 0.2 points after surgery, while "Globus sensation" decreased from 2.3 to 0.7 points.

DISCUSSION

Studies using objective measures of the effect of goiter on the esophagus are limited. Nevertheless, the existing literature suggests that presence of goiter is associated with both esophageal deviation and compression, and that this is relieved by surgery (8, 16, 24, 25). In addition, goiter patients may show a prolonged passage and some impact on the esophageal peristaltic movement and/or sphincter pressure, as determined by either nuclear medicine techniques or various forms of esophageal manometry (13, 27, 28, 30). Importantly, surgery does not cause deterioration in the above mentioned parameters.

Objective analyses of the esophagus support the high prevalence of preoperative swallowing difficulties as reported by 48–78% of the patients (11, 13, 36). Evaluated by patient reported outcomes, reports on swallowing symptoms during the first 3 months after surgery have shown conflicting results (11, 13, 15, 22, 37). In fact, some studies have shown deterioration until 6 months after surgery (11, 22, 37). More consistently, all studies demonstrate significant improvement of the swallowing

TABLE 3 | Studies investigating the effect of goiter on the swallowing-related patient reported outcomes.

References	Design	*n*	Cohort	Methods	Results
Sabaretnam et al. (12)	TH	224	Age: mean 38 years. Goiter: mean 85 g	SWAL-QOL	SWAL-QOL: 8 of 11 domains affected before surgery all of which improved 6 months after surgery.
Greenblatt et al. (17)	TH	116	Age: 49 ± 13 years. Goiter: 36 ± 34 g	SWAL-QOL	SWAL-QOL: 9 of 11 domains affected before surgery with improvement in 8 of the 9 domains after surgery. No difference between hemi- and total thyroidectomy.
Maung et al. (18)	TH	41	Age: mean 48 years. Goiter: ≥WHO gr. II	GETS	GETS: 3 months after surgery: 2 of 12 items improved 12 months after surgery: 6 of 12 items improved
Lombardi et al. (11)	TH	110	Age: 47±13 years. Goiter: 33 ± 22 g	SIS	SIS ≥1 before: 47% of patients. After 1 week: 74% of patients. After 1 month: 64% of patients. After 3 months: 48% of patients. After 1 year: 20% of patients.
Holler and Anderson (36)	OB	59	Age: 19–73 years. Goiter: NA	MSIS	MSIS: Swallowing complaints: 43% (at least some of the time) and 27% (often or always).
Tae et al. (22)	TH	111	Age: mean 48 years. Goiter: nodule < 5 cm	SIS	Swallowing symptom score: Increase 1 day, 1 week, 1 month, and 3 months after surgery. At 6 months after surgery values returned to presurgical values. No difference between robotic- and conventional thyroidectomy
Lombardi et al. (37)	TH	127	Age: 43 ± 11 years. Goiter: 26 ± 9 g	SIS	Mean SIS. Baseline: 0.5 points. After 1 week: deterioration to 10.3 points. After 1 month: 6.0 points. After 3 months: 2.8 points
Lombardi et al. (15)	TH	53	Age: NA Goiter: <30 mL	SIS	VAT had a significantly lower and improved SIS score at 1 week after surgery compared to conventional thyroidectomy. No change at 1 or 3 months after surgery
Grover et al. (38)	RE	202	Age: 55 ± 16 years. Goiter: mean 78 g	SIS	One year after total thyroidectomy or completion thyroidectomy: 41% have normal SIS score (<10 points), 28% have moderately affected score (28%) and 31% have severely affected score (>16 points). Scores did not change beyond 1 year.
Burns and Timon (39)	TH	58	Age: mean 40 years. Goiter: 62 g	VAS (0–10 points)	Globus sensation: Baseline 58% (mean 5.2 points), significant improvement 3–6 months after surgery 6% (mean 1.1 point)
Pereira et al. (40)	TH	26	Age: 46 ± 4 years. Goiter: 2.2 ± 1.4 cm	Interview	80% had at least 1 swallowing-related symptom 2 weeks after surgery, 42% at 6 weeks, and 17% at 6 months
Kahrilas et al. (41)	RE	120	Age: 58 ± 3 years. Goiter: NA	Chart review	Dysphagia in 2 of 60 patients with goiter before thyroidectomy and in 9 of these patients 4 years after surgery.

RE, Retrospective; OB, Observational; TH, Thyroidectomy; SWAL-QOL, Swallowing quality of life questionnaire; GETS, Glascow-Edinburgh Throat Scale; NA, Not available; VAT, Video-Assisted Thyroidectomy; SIS, Swallowing Impairment Score; VAS, Visual Analog Score; MSIS, Modified Swallowing Impairment Score.

symptoms beyond 6 months after surgery (11, 12, 17, 22, 38), although a subgroup of patients have persistent complaints more than 1 year after surgery (38). More generally, the Goiter Symptom Score, extracted from the ThyPRO instrument, improved after surgery, with significantly reduced globus sensation and swallowing difficulties (25, 30). However, symptom assessment obtained by questionnaires cannot be performed with blinding of the patients, which may have influenced the outcome in all studies of this kind.

Further studies are needed to examine the relation between goiter and the esophagus, and the effect of treatment. In particular, it is of interest to compare surgical and non-surgical techniques–such as radioiodine therapy (43) and ultrasound-guided thermal procedures in the form of e.g., laser or radiofrequency ablation (44). These non-surgical ablation techniques are less prone to side-effects such as voice changes and postoperative hypothyroidism (45–48), while the potential effects on the esophagus remain unknown. Awaiting such studies, and comparison with the long-established surgical approaches, leaves a gap in our ability to provide individual-tailored therapy for goiter patients.

STUDY LIMITATIONS

Although inclusion of solely English language publications theoretically might have caused bias, this does not seem to be the case (49). Women constitute the majority of patients, de facto and in the cited literature. This means that some of the conclusions may not be as valid for male as for female goiter patients. The majority of the identified studies contained a limited number of patients, and many of the intervention studies are hampered by loss to follow-up in 20% of patients (15, 17, 33), or higher (11, 37, 38), especially in studies of esophagus motility due to patient discomfort associated with these procedures (9).

Comparing studies of mixed patient populations regarding age, setting of recruitment, diagnosis i.e., thyroid malignancy, hyper- and hypothyroidism, and benign nodular goiter, complicates interpretation of the results. The fact that patients were included from highly selected populations and from both primary, secondary and tertiary centers, as well as from surgical and medical departments constitute additional limitations. Needless to say, it is unknown to which degree these influence our conclusions and generalizability of the manuscript.

TABLE 4 | Recommendations based on strength of evidence (50).

Theme	Physiological effect	Level of evidence	Sources (references)
Esophageal anatomy	Patients with goiter can have esophageal deviation and/or compression. The prevalence increases if the goiters increase in size or becomes substernal	Moderate (several studies with some limitations)	(8, 16, 24, 25)
	After thyroidectomy some esophageal deviation might persist as normal physiology, but esophageal compression is relieved	Moderate (several studies with some limitations)	
Esophageal physiology	Patients with goiter may show increased esophageal transit time, correlating with the goiter size. This seems not related to esophageal motility disturbances	Low (one or more studies with severe limitations)	(13, 27–30)
	Some esophageal motility disturbance may persist in the weeks after surgery, but not for longer than 6 months after surgery	Low (one or more studies with severe limitations)	
Patient reported outcome	Patients with goiter have a high prevalence of swallowing symptoms.	Moderate (several studies with some limitations)	(11–13, 15, 17, 18, 22, 29, 34, 36–38)
	Swallowing symptoms might deteriorate in the weeks and months after thyroidectomy, but from 6 months swallowing symptom are reduced to a lower level than preoperatively. A subgroup of patients might have persistent swallowing complaints	Low (one or more studies with severe limitations)	

Moderate level of evidence: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate. Low level of evidence: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Implications for the Future

Future studies should compare ^{131}I therapy and ultrasound-guided thermal ablation techniques with various surgical techniques for goiter ablation, as for the effect on the esophagus. Such studies should be performed using validated swallowing and quality-of-life-related questionnaires, with measurements of the esophageal anatomy and function as the potential primary endpoints. Follow-up should be a minimum of 6 months, since improvement of swallowing dysfunction at an earlier time point cannot be expected, at least not after surgical goiter ablation.

CONCLUSIONS

Patients with goiter benefit substantially from thyroidectomy, with profound improvements in dysphagia and increase in the cross-sectional area of the esophagus. However, in most patients the improvements do not occur before 6 months after thyroidectomy. In fact, deterioration in dysphagia may be seen in the early period after surgery. Studies evaluating the long-term status of these parameters, and comparing surgical and non-surgical goiter treatment techniques, are warranted.

AUTHOR CONTRIBUTIONS

All authors (JS, CG, SB, and LH) contributed in the conception of design, interpretation, revising the manuscript critically, and approval of the final manuscript. JS contributed with acquisition and analysis of data, and drafting the work.

REFERENCES

1. Carle A, Krejbjerg A, Laurberg P. Epidemiology of nodular goitre. Influence of iodine intake. *Best Pract Res Clin Endocrinol Metab.* (2014) 28:465–79. doi: 10.1016/j.beem.2014.01.001
2. Gharib H, Papini E, Paschke R, Duick DS, Valcavi R, Hegedüs L, et al. American Association of Clinical Endocrinologists, Americal College of Endocrinology, and Associazione Medici Endocrinologi medical guidelines for clinical practice for the diagnosis and management of thyroid nodules—2016 update. *Endocr Pract.* (2016) 22:622–39. doi: 10.4158/EP161208.GL
3. Brix TH, Kyvik KO, Hegedüs L. Major role of genes in the etiology of simple goiter in females: a population-based twin study. *J Clin Endocrinol Metab.* (1999) 84:3071–5. doi: 10.1210/jcem.84.9.5958
4. Hegedüs L, Bonnema SJ, Bennedbaek FN. Management of simple nodular goiter: current status and future perspectives. *Endocr Rev.* (2003) 24:102–32. doi: 10.1210/er.2002-0016
5. Abdul-Sater L, Henry M, Majdan A, Mijovic T, Franklin JH, Brandt MG, et al. What are thyroidectomy patients really concerned about? *Otolaryngol Head Neck Surg.* (2011) 144:685–90. doi: 10.1177/0194599811399556
6. Stang MT, Armstrong MJ, Ogilvie JB, Yip L, McCoy KL, Faber CN, et al. Positional dyspnea and tracheal compression as indications for goiter resection. *Arch Surg.* (2012) 147:621–6. doi: 10.1001/archsurg.2012.96
7. Watt T, Hegedüs L, Rasmussen AK, Groenvold M, Bonnema SJ, Bjorner JB, et al. Which domains of thyroid-related quality of life are most relevant? Patients and clinicians provide complementary perspectives. *Thyroid* (2007) 17:647–54. doi: 10.1089/thy.2007.0069
8. Netterville JL, Coleman SC, Smith JC, Smith MM, Day TA, Burkey BB. Management of substernal goiter. *Laryngoscope* (1998) 108:1611–7. doi: 10.1097/00005537-199811000-00005
9. Sorensen JR. The impact of surgery on quality of life, esophageal motility, and tracheal anatomy and airflow in patients with benign nodular goiter. *Dan Med J.* (2018) 65:B5472.

10. Sorensen JR, Lauridsen JK, Døssing H, Nguyen N, Hegedüs L, Bonnema SJ, et al. Thyroidectomy improves tracheal anatomy and airflow in patients with nodular goiter: a prospective cohort study. *Eur Thyroid J.* (2017) 6:307–14. doi: 10.1159/000480348
11. Lombardi CP, Raffaelli M, De Crea C, D'Alatri L, Maccora D, Marchese MR, et al. Long-term outcome of functional post-thyroidectomy voice and swallowing symptoms. *Surgery* (2009) 146:1174–81. doi: 10.1016/j.surg.2009.09.010
12. Sabaretnam M, Mishra A, Chand G, Agarwal G, Agarwal A, Verma AK, et al. Assessment of swallowing function impairment in patients with benign goiters and impact of thyroidectomy: a case control study. *World J Surg.* (2012) 36:1293–9. doi: 10.1007/s00268-012-1562-8
13. Scerrino G, Inviati A, Di Giovanni S, Paladino NC, Di Paola V, Lo Re G, et al. Esophageal motility changes after thyroidectomy; possible associations with postoperative voice and swallowing disorders: preliminary results. *Otolaryngol Head Neck Surg.* (2013) 148:926–32. doi: 10.1177/0194599813482299
14. Sorensen JR, Hegedüs L, Kruse-Andersen S, Godballe C, Bonnema SJ. The impact of goitre and its treatment on the trachea, airflow, oesophagus and swallowing function. A systematic review. *Best Pract Res Clin Endocrinol Metab.* (2014) 28:481–94. doi: 10.1016/j.beem.2014.03.002
15. Lombardi CP, Raffaelli M, D'Alatri L, De Crea C, Marchese MR, Maccora D, et al. Video-assisted thyroidectomy significantly reduces the risk of early postthyroidectomy voice and swallowing symptoms. *World J Surg.* (2008) 32:693–700. doi: 10.1007/s00268-007-9443-2
16. Shin JJ, Grillo HC, Mathisen D, Katlic MR, Zurakowski D, Kamani D, et al. The surgical management of goiter: part I. Preoperative evaluation. *Laryngoscope* (2011) 121:60–7. doi: 10.1002/lary.21084
17. Greenblatt DY, Sippel R, Leverson G, Frydman J, Schaefer S, Chen H. Thyroid resection improves perception of swallowing function in patients with thyroid disease. *World J Surg.* (2009) 33:255–60. doi: 10.1007/s00268-008-9837-9
18. Maung KH, Hayworth D, Nix PA, Atkin SL, England RJ. Thyroidectomy does not cause globus pattern symptoms. *J Laryngol Otol.* (2005) 119:973–5. doi: 10.1258/002221505775010760
19. Sorensen JR, Watt T, Cramon P, Dossing H, Hegedüs L, Bonnema SJ, et al. Quality of life after thyroidectomy in patients with nontoxic nodular goiter: a prospective cohort study. *Head Neck* (2017) 39:2232–40. doi: 10.1002/hed.24886
20. Cramon P, Bonnema SJ, Bjorner JB, Ekholm O, Feldt-Rasmussen U, Frendl DM, et al. Quality of life in patients with benign nontoxic goiter: impact of disease and treatment response, and comparison with the general population. *Thyroid* (2015) 25:284–91. doi: 10.1089/thy.2014.0433
21. Bukvic BR, Zivaljevic VR, Sipetic SB, Diklic AD, Tausanovic KM, Paunovic IR. Improvement of quality of life in patients with benign goiter after surgical treatment. *Langenbecks Arch Surg.* (2014) 399:755–64. doi: 10.1007/s00423-014-1221-7
22. Tae K, Kim KY, Yun BR, Ji YB, Park CW, Kim DS, et al. Functional voice and swallowing outcomes after robotic thyroidectomy by a gasless unilateral axillo-breast approach: comparison with open thyroidectomy. *Surg Endosc.* (2012) 26:1871–7. doi: 10.1007/s00464-011-2116-0
23. Higgins JPT, Green S. *Cochrane Handbook for Systematic Revieews of Interventions.* Version 5.1.0. (2011). Available online at: http://handbook.cochrane.org
24. Mackle T, Meaney J, Timon C. Tracheoesophageal compression associated with substernal goitre. Correlation of symptoms with cross-sectional imaging findings. *J Laryngol Otol.* (2007) 121:358–61. doi: 10.1017/s0022215106004142
25. Brinch A, Døssing H, Nguyen N, Bonnema S, Hegedüs L, Godballe C, et al. The impact of esophageal compression on goiter symptoms before and after thyroid surgery. *Eur Thyroid J.* (2018) 6:307–14. doi: 10.1159/000493542
26. Nam IC, Choi H, Kim ES, Mo EY, Park YH, Sun DI. Characteristics of thyroid nodules causing globus symptoms. *Eur Arch Otorhinolaryngol.* (2015) 272:1181–8. doi: 10.1007/s00405-015-3525-9
27. Jorgensen F, Hesse B, Gronbaek P, Fogh J, Haunso S. Abnormal oesophageal function in patients with non-toxic goiter or enlarged left atrium, demonstrated by radionuclide transit measurements. *Scand J Gastroenterol.* (1989) 24:1186–92. doi: 10.3109/00365528909090785
28. Glinoer D, Verelst J, Ham HR. Abnormalities of esophageal transit in patients with sporadic nontoxic goitre. *Eur J Nucl Med.* (1987) 13:239–43. doi: 10.1007/BF00252600
29. Scerrino G, Inviati A, Di Giovanni S, Paladino NC, Di Giovanni S, Paladino NC, et al. Long-term esophageal motility changes after thyroidectomy: associations with aerodigestive disorders. *G Chir.* (2017) 37:193–9. doi: 10.11138/gchir/2016.37.5.193
30. Sorensen JR, Markoew S, Dossing H, Hegedüs L, Bonnema SJ, Godballe C. Changes in swallowing symptoms and esophageal motility after thyroid surgery: a prospective cohort study. *World J Surg.* (2017). 42:998–1004. doi: 10.1007/s00268-017-4247-5
31. Arakawa-Sugueno L, Ferraz AR, Morandi J, Capobianco DM, Cernea CR, Sampaio MA, et al. Videoendoscopic evaluation of swallowing after thyroidectomy: 7 and 60 days. *Dysphagia* (2015) 30:496–505. doi: 10.1007/s00455-015-9628-z
32. Gohrbandt AE, Aschoff A, Gohrbandt B, Keilmann A, Lang H, Musholt TJ. Changes of laryngeal mobility and symptoms following thyroid surgery: 6-month follow-up. *World J Surg.* (2016) 40:636–43. doi: 10.1007/s00268-015-3323-y
33. Fiorentino E, Cipolla C, Graceffa G, Cusimano A, Cupido F, Lo Re G, et al. Local neck symptoms before and after thyroidectomy: a possible correlation with reflux laryngopharyngitis. *Eur Arch Otorhinolaryngol.* (2011) 268:715–20. doi: 10.1007/s00405-010-1394-9
34. Hyun K, Byon W, Park HJ, Park Y, Park C, Yun JS. Comparison of swallowing disorder following gasless transaxillary endoscopic thyroidectomy versus conventional open thyroidectomy. *Surg Endosc.* (2014) 28:1914–20. doi: 10.1007/s00464-013-3413-6
35. Rodrigues MG, Araujo VJFF, Matos LL, Hojaij FC, Simoes CA, Araujo VJFN, et al. Substernal goiter and laryngopharyngeal reflux. *Arch Endocrinol Metab.* (2017) 61:348–53. doi: 10.1590/2359-3997000000266
36. Holler T, Anderson J. Prevalence of voice & swallowing complaints in pre-operative thyroidectomy patients: a prospective cohort study. *J Otolaryngol Head Neck Surg.* (2014) 43:28. doi: 10.1186/PREACCEPT-8455644671045748
37. Lombardi CP, Raffaelli M, D'Alatri L, Marchese MR, Rigante M, Paludetti G, et al. Voice and swallowing changes after thyroidectomy in patients without inferior laryngeal nerve injuries. *Surgery* (2006) 140:1026–32; discussion 32–4. doi: 10.1016/j.surg.2006.08.008
38. Grover G, Sadler GP, Mihai R. Morbidity after thyroid surgery: patient perspective. *Laryngoscope* (2013) 123:2319–23. doi: 10.1002/lary.23850
39. Burns P, Timon C. Thyroid pathology and the globus symptom: are they related? A two year prospective trial. *J Laryngol Otol.* (2007) 121:242–5. doi: 10.1017/S0022215106002465
40. Krekeler BN, Wendt E, Macdonald C, Orne J, Francis DO, Sippel R, et al. Patient-reported dysphagia after thyroidectomy: a qualitative study. *JAMA Otolaryngol Head Neck Surg.* (2018) 144:342–8. doi: 10.1001/jamaoto.2017.3378
41. Pereira JA, Girvent M, Sancho JJ, Parada C, Sitges-Serra A. Prevalence of long-term upper aerodigestive symptoms after uncomplicated bilateral thyroidectomy. *Surgery* (2003) 133:318–22. doi: 10.1067/msy.2003.58
42. Kahrilas PJ, Bredenoord AJ, Fox M, Gyawali CP, Roman S, Smout AJ, et al. The Chicago classification of esophageal motility disorders, v3.0. *Neurogastroenterol Motil.* (2015) 27:160–74. doi: 10.1111/nmo.12477
43. Bonnema SJ, Hegedüs L. Radioiodine therapy in benign thyroid diseases: effects, side effects, and factors affecting therapeutic outcome. *Endocr Rev.* (2012) 33:920–80. doi: 10.1210/er.2012-1030
44. Ha EJ, Baek JH, Kim KW, Pyo J, Lee JH, Baek SH, et al. Comparative efficacy of radiofrequency and laser ablation for the treatment of benign thyroid nodules: systematic review including traditional pooling and bayesian network meta-analysis. *J Clin Endocrinol Metab.* (2015) 100:1903–11. doi: 10.1210/jc.2014-4077
45. Chung SR, Suh CH, Baek JH, Park HS, Choi YJ, Lee JH. Safety of radiofrequency ablation of benign thyroid nodules and recurrent thyroid cancers: a systematic review and meta-analysis. *Int J Hyperthermia* (2017) 33:920–30. doi: 10.1080/02656736.2017.1337936
46. Gharib H, Hegedüs L, Pacella CM, Baek JH, Papini E. Clinical review: nonsurgical, image-guided, minimally invasive therapy for thyroid nodules. *J Clin Endocrinol Metab.* (2013) 98:3949–57. doi: 10.1210/jc.2013-1806
47. Dossing H, Bennedbaek FN, Hegedüs L. Interstitial laser photocoagulation (ILP) of benign cystic thyroid nodules–a prospective randomized trial.

J Clin Endocrinol Metab. (2013) 98:E1213–7. doi: 10.1210/jc.2013-1503

48. Smith TJ, Hegedüs L. Graves' disease. *NEJM* (2016) 375:1552–65. doi: 10.1056/NEJMra1510030
49. Moher D, Pham B, Lawson ML, Klassen TP. The inclusion of reports of randomised trials published in languages other than english in systematic reviews. *Health Technol Assess.* (2003) 7:1–90. doi:10.3310/hta7410
50. Guyatt G, Oxman AD, Akl EA, Kunz R, Vist G, Brozek J, et al. GRADE guidelines: 1. introduction-GRADE evidence profiles and summary of findings tables. *J Clin Epidemiol.* (2011) 64:383–94. doi: 10.1016/j.jclinepi.2010.04.026

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

CASE REPORT
published: 01 November 2018
doi: 10.3389/fendo.2018.00641

Intrathyroidal Parathyroid Carcinoma: An Atypical Thyroid Lesion

Noran Alharbi[1,2], *Sylvia L. Asa*[3], *Marta Szybowska*[4], *Raymond H. Kim*[2,4] and *Shereen Ezzat*[2]*

[1] Department of Internal Medicine and Endocrinology, Prince Mohammed bin Abdulaziz Hospital, Riyadh, Saudi Arabia, [2] Department of Medicine, University Health Network, University of Toronto, Toronto, ON, Canada, [3] Department of Pathology, University Health Network, University of Toronto, Toronto, ON, Canada, [4] Fred A Litwin Family Centre in Genetic Medicine, University Health Network, University of Toronto, Toronto, ON, Canada

Parathyroid carcinoma is a rare endocrine malignancy that is typically difficult to diagnose at presentation. Here, we report a 63 year-old man who had symptomatic hypercalcemia. Investigations revealed a thyroid nodule and a lateral neck mass that was biopsied and diagnosed as "suspicious for a neuroendocrine neoplasm." He underwent total thyroidectomy with central and left neck node dissection. Histology and immunohistochemistry revealed an intrathyroidal angioinvasive parathyroid carcinoma with lymph node metastases. The tumor showed loss of parafibromin expression; germline testing revealed no pathogenic germline variants of *CDC73*, suggesting either a cryptic germline variant or a sporadic malignancy. Multiple pulmonary nodules consistent with metastatic disease explained persistent hypercalcemia and the patient was treated with denosumab as well as Sorafenib resulting in early regression of the lung nodules. This case illustrates an unusual parathyroid carcinoma with respect to anatomic presentation and the importance of complete pathological workup in securing the diagnosis. The management of these rare malignancies is discussed.

Keywords: thyroid nodule, parathyroid carcinoma, hypercalcemia, pathology, sorafenib

OPEN ACCESS

Edited by:
Giampaolo Papi,
Azienda Unità Sanitaria Locale di Modena, Italy

Reviewed by:
Jean-Yves Scoazec,
Institut Gustave Roussy, France
Rosa Maria Paragliola,
Università Cattolica del Sacro Cuore, Italy

***Correspondence:**
Shereen Ezzat
shereen.ezzat@utoronto.ca

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *20 July 2018*
Accepted: *09 October 2018*
Published: *01 November 2018*

Citation:
Alharbi N, Asa SL, Szybowska M, Kim RH and Ezzat S (2018) Intrathyroidal Parathyroid Carcinoma: An Atypical Thyroid Lesion. Front. Endocrinol. 9:641. doi: 10.3389/fendo.2018.00641

BACKGROUND

Parathyroid carcinoma is a rare disease that must be distinguished from other types of parathyroid pathologies (1). It is a particularly difficult diagnosis to establish when the disease is detected outside of its typical anatomical sites (1). Of these, the intrathyroidal occurrence of parathyroid carcinoma can be problematic. Here, we present a case of intrathyroidal parathyroid carcinoma that demonstrates the challenges in diagnosis as well as in endocrine and oncologic control of this disease. The patient provided written informed consent for publication of this case report.

CASE PRESENTATION

A 63 year-old gentleman with known hypertension presented in October 2016 with diffuse bony aches, polyuria, polydipsia, constipation, fatigue, loss of appetite, and weight loss of 25 pounds over two months. Biochemical testing revealed normal thyroid function tests but an elevated serum calcium of 4.17 mmol/L (Normal range 2.2–2.5), phosphate 1.4 mmol/L (0.74–1.52), parathyroid hormone (PTH) 168.2 pmol/L (1.6–9.4), serum creatinine 237 umol/L (64–111), glomerular

filtration rate (GFR) 24 (>60), 24 h urinary calcium 13.89 mmol/day (2.5–7.5). His past medical history was remarkable for a kidney stone 12 years earlier although he denied bony fractures of the spine and hips or renal dysfunction. Family history was non-contributory with no known history of parathyroid disease or endocrine neoplasia.

His treating physicians noted a left thyroid nodule and a left neck node which was biopsied; cytologic examination revealed features suspicious for a neuroendocrine neoplasm. He underwent a total thyroidectomy with central and left neck node dissection. Review of the pathology in the thyroidectomy specimen revealed that the index thyroid mass was indeed an infiltrative intrathyroidal neuroendocrine tumor (**Figure 1a**) that measured 2.7 cm and had multiple foci of vascular invasion characterized by intravascular tumor cells admixed with thrombus (**Figure 1b**). There was single cell tumor necrosis. Mitoses, including atypical mitoses, were conspicuous and a phospho-histone 3 (pHH3)-assisted mitotic count identified 26 mitotic figures per 50 high power fields. The tumor was positive for keratins using the CAM5.2 and CK7 antibodies, CD56, chromogranin, PTH (**Figure 1c**), and GATA-3 (**Figure 1d**), confirming that it was indeed a parathyroid neoplasm; it was negative for CK20, S100, CD5, Pax-8, TTF-1, thyroglobulin, CEA, and calcitonin. The case was then evaluated to confirm biomarkers of malignancy in parathyroid tumors. It was positive for Galectin-3 (**Figure 1e**); The Ki67 labeling index was 19.6% in an automated count of 1158 cells (Leica Biosystems; **Figure 1f**), there was reduced expression of BCL-2 and focal upregulation of cyclin D1 and p53. There was no loss of RB but marked reduction of p27. Staining for menin was technically unsatisfactory. There was loss of nuclear parafibromin reactivity (**Figure 1g**) and PGP 9.5 was positive (**Figure 1h**).

The painted margin of resection of the thyroidectomy was negative for malignancy, however, metastatic parathyroid carcinoma was identified in one left perithyroidal lymph node included in the total thyroidectomy and central neck dissection specimen and in one of 38 lymph nodes from the left neck dissection specimen. A left inferior parathyroid gland was biopsied and had normal morphology.

Elsewhere in the thyroid there was an incidental 0.05 cm classical variant papillary microcarcinoma.

Post-operatively, he noted a marked improvement in his symptoms with reduction of corrected serum calcium to 2.58 mmol/L and PTH to 12.9 pmol/L. However a few months later, his PTH level remained elevated precipitating a referral to our institution. Our investigations yielded the following findings: serum PTH of 27.7 pmol/L (1.3–7.6), corrected calcium 2.84 mmol/L (2.32–2.62), phosphate 0.55 mmol/L (0.8–1.4), 25(OH) vitamin D3 84 nmol/L (25–200), ALP 43 U/L (40–150), creatinine 107 umol/L (64–110), and calculated GFR 64 ml/min/1.73 m^2 (> 60). CT imaging identified no visible disease in the neck but multiple pulmonary nodules consistent with metastatic disease were noted (**Figure 2a**). An abdominal ultrasound showed multiple renal cysts. Nuclear octreotide scintigraphy was completely negative.

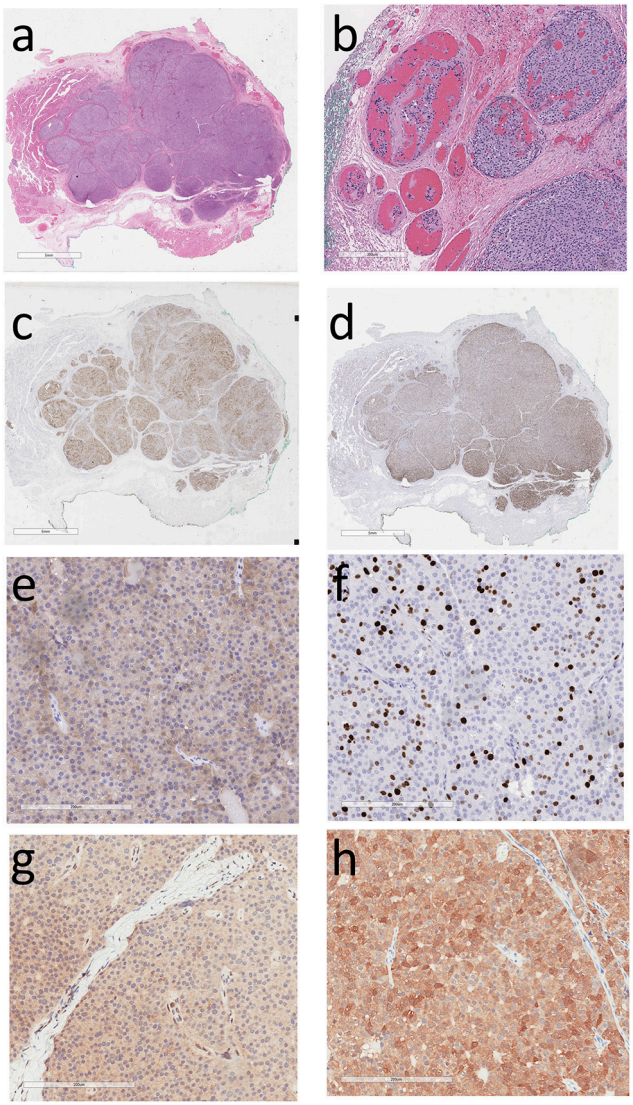


FIGURE 1 | Histology and Immunoprofile of Intrathyroidal Parathyroid Carcinoma. **(a)** The tumor is infiltrative within the thyroid. **(b)** There is vascular invasion characterized by intravascular tumor cells admixed with thrombus. **(c)** The tumor cells exhibit cytoplasmic reactivity for parathyroid hormone and **(d)** nuclear staining for GATA-3, confirming parathyroid differentiation. **(e)** Cytoplasmic reactivity for Galectin-3 is a feature suggesting parathyroid carcinoma. **(f)** The Ki67 labeling index is approximately 20%. **(g)** The tumor cells show loss of nuclear parafibromin reactivity and **(h)** there is diffuse staining for PGP 9.5.

Based on the clinical findings genetic testing was performed with sequencing and deletion/duplication analysis of *CDC73*. No pathogenic variants were detected. He was started on denosumab 120 mg subcutaneously monthly to help control his hypercalcemia. To control his structural metastatic disease, targeted therapy with sorafenib 400 mg orally twice daily was started. Three months later, CT imaging showed early regression of the lung nodules (**Figure 2b**).

DISCUSSION

Parathyroid glands can be located in various ectopic locations in the neck (1). The superior parathyroids are usually located close to the posterior thyroid near the cricothyroid junction; they may be in perithyroidal fascia or completely intrathyroidal. They may also be retropharyngeal or retroesophageal. The inferior glands may be associated with the thymus anywhere from near the inferior pole of the thyroid to lower in the mediastinum or higher, even associated with the hyoid bone (2). We report a parathyroid carcinoma that was completely intrathyroidal, consistent with neoplasia arising in an intrathyroidal superior parathyroid gland. The patient's ipsilateral inferior parathyroid gland was identified and biopsied and proved to be normal on histology.

Parathyroid carcinoma comprises ∼1% of all cases of primary hyperparathyroidism (PHPT) (1). Risk factors include long-standing secondary hyperparathyroidism or previous history of head and neck irradiation. Parathyroid cancer has been associated with a rare autosomal dominant inherited disorder known as hyperparathyroidism–jaw tumor syndrome (HPT-JT) due to germline mutations in the *CDC73* gene that encodes parafibromin (1). Less frequently, it is associated with familial isolated hyperparathyroidism and multiple endocrine neoplasia type 1 and type 2A (1).

The classic symptoms of PHPT include many complications such as kidney stones, bony loss, abdominal pain, and neuropsychiatric complaints. As opposed to the more benign form of the disease, patients with parathyroid cancer are usually severely symptomatic at the time of presentation with severe hypercalcemia. Indeed, severe nephrolithiasis, nephrocalcinosis, and impaired renal function with bone loss is noted in nearly 80% of affected patients (3). The latter may include osteitis fibrosa cystica, diffuse osteopenia, or even pathologic fractures. Other constitutional symptoms associated with PHPT such as fatigue, loss of concentration, bony pain, polydipsia, polyuria, peptic ulcerations, pancreatitis, and depression may also be noted. Other manifestations of severe hypercalcemia including acute pancreatitis, shortened QT interval, drowsiness, and diminished level of consciousness, which if left untreated, can be fatal.

In addition to the stigmata of hyperparathyroidism, a palpable neck mass, such as in our case, can be present in 30–75% of patients with parathyroid carcinoma (3). It should be emphasized that this latter finding is quite rare in benign forms of parathyroid disease. More recent series, however, indicate that a palpable mass may be less common. Kleinpeter et al noted a palpable mass in only 22% of their patients with parathyroid carcinomas (4). Hoarseness, resulting from recurrent laryngeal nerve palsy, and palpably enlarged lymph nodes can also provide a clue to the presence of a parathyroid carcinoma.

The occurrence of an intrathyroidal parathyroid carcinoma is rare but not unknown (5–17). The complexity of the diagnosis in the face of a thyroid nodule rests on the clinical suspicion of severe hyperparathyroidism with an infiltrative thyroid mass. However, the more common scenario is a parathyroid lesion associated with an unrelated thyroid mass. In this case, the lack of resolution of biochemical abnormalities after surgery prompted pathology review to ensure that the culprit parathyroid was indeed resected, since it was possible that the thyroid lesion was unrelated and the abnormal parathyroid might have been left behind in a different ectopic location.

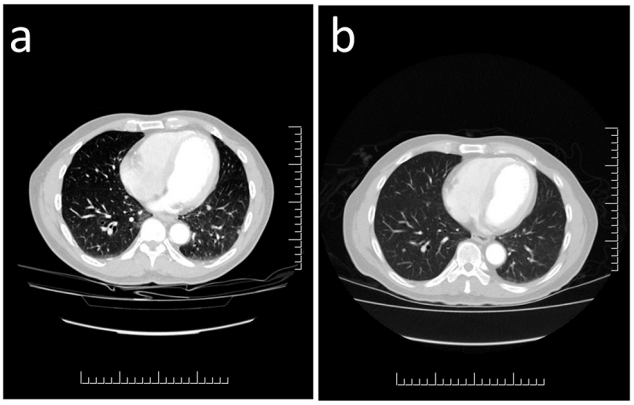


FIGURE 2 | Computed Tomography Imaging of Metastatic Parathyroid Carcinoma. **(a)** Multiple scattered lesions throughout both lung fields are consistent with metastatic parathyroid carcinoma. **(b)** Repeat imaging 3 months following treatment with the tyrosine kinase inhibitor sorafenib demonstrates reduction in the size of several of the pulmonary nodules which are now less prominent.

The diagnosis of malignancy in a parathyroid neoplasm should be suspected in patients with PHPT if at the time of neck exploration, the mass is large, white or gray, and adherent to adjacent structures. This is in contradistinction to benign parathyroid neoplasms, which tend to be soft, flat, and more red-brown in color and easy to dissect surgically. Surgical pathology remains the cornerstone of diagnosis. Carcinomas are typically larger, tend to be more firm, irregularly-shaped, and have a whitish-gray color; they are often adherent or invasive into surrounding neck structures. However, benign parathyroid lesions can exhibit worrisome features such as fibrosis and degeneration if they have been subjected to pre-operative biopsy (18, 19). This is a common finding especially in patients who present with a thyroid mass, as in our case. The diagnosis of malignancy therefore requires identification of unequivocal angioinvasion and can be assisted by the assessment of biomarkers that are features of parathyroid malignancy (20). Our case showed loss of BCL-2, overexpression of galectin-3, reduction of p27, and a high Ki67 labeling index that all support the diagnosis of malignancy. Negative staining for parafibromin and positive staining for PGP9.5 is an abnormal pattern which is very suggestive of *CDC73* mutation/inactivation.

Currently there are no formal clinical diagnostic criteria for HPT-JT, and the diagnosis relies on the detection of a pathogenic variant in *CDC73*. Sequencing (21) and copy number analysis (22) of *CDC73* is highly sensitive for HPT-JT, but not all variants in *CDC73* have been characterized (22). Our patient did not have an identifiable germline pathogenic variant in the *CDC73* gene, suggesting that an undetectable variant germline variant

may be present or the tumor may have had a somatically acquired mutation or epigenetic silencing of this gene. Renal manifestations including renal cysts can be a finding in HPT-JT (23). Despite our patient's negative genetic testing results, the findings of absent parafibromin expression in his tumor and the presence of renal cysts were highly suggestive of HPT-JT, and family members were also recommended to have HPT-JT surveillance.

Surgery is currently the only effective form of treatment for parathyroid carcinoma (1). Complete resection avoiding capsular disruption is recommended (1). Outcome studies have shown that complete resection with free margins is a feature of tumors that do well (24). Although radiotherapy has been reported to have beneficial effects (24, 25), it is not recommended as a routine ancillary tool, rather it is reserved for palliation (1). Conventional chemotherapy does not effectively correct hypercalcemia nor influence overall outcomes from the disease (26). A large number of agents including cyclophosphamide, 5-fluorouracil and decarbazine have been tested as single or combination therapies without appreciable effects. Partial responses with a marked drop in serum calcium lasting from weeks to several months have been observed in some individual cases; however, in general the results are largely disappointing. Denosumab, a monoclonal antibody against RANKL, inhibits osteoclast maturation, function, and survival and is useful to treat hypercalcemia of malignancy, including in patients with parathyroid carcinoma (27).

Recurrence of tumors can be monitored by serum calcium and parathyroid hormone levels. However, in view of the potential for loss of differentiation with cancer progression, serum PTH alone may not be fully revealing and long-term imaging surveillance is often required for patients with parathyroid carcinoma. Most recurrences occur within 2–3 years from the time of initial presentation, but prolonged disease-free interval of as long as 23 years has also been reported in some cases (28).

AUTHOR CONTRIBUTIONS

NA, SA, RK, and SE: case review; NA, SA, and SE: writing; NA and SA: image preparation; RK, MS, and SE: review of manuscript.

ACKNOWLEDGMENTS

We acknowledge the assistance of Dr. Anthony Gill, Royal North Shore Hospital, Sydney, Australia, for technical assistance with parafibromin and PGP 9.5 staining. We are grateful for the patient's consent to allow publication of this report.

REFERENCES

1. Wilhelm SM, Wang TS, Ruan DT, , Lee JA, Asa SL, Duh QY, et al. The American association of endocrine surgeons guidelines for definitive management of primary hyperparathyroidism. *JAMA Surg.* (2016) 151:959–68. doi: 10.1001/jamasurg.2016.2310
2. Asa SL, Mete O. Parathyroids. In: Mills SE, editor. *Histology for Pathologists*, Philadelphia, PA: Wolters Kluwer (2018).
3. Fang SH, Lal G. Parathyroid cancer. *Endocr Pract* (2011) 17(Suppl. 1):36–43. doi: 10.4158/EP10310.RA
4. Kleinpeter KP, Lovato JF, Clark PB, Wooldridge T, Norman ES, Bergman S, et al. Is parathyroid carcinoma indeed a lethal disease? *Ann Surg Oncol.* (2005) 12:260–6. doi: 10.1245/ASO.2005.03.036
5. Crescenzo DG, Shabahang M, Garvin D, Evans SR. Intrathyroidal parathyroid cancer presenting as a left neck mass. *Thyroid* (1998) 8:597–9. doi: 10.1089/thy.1998.8.597
6. Kirstein LJ, Ghosh BC. Intrathyroid parathyroid carcinoma. *J Surg Oncol.* (2001) 77:136–8. doi: 10.1002/jso.1084
7. Schmidt JL, Perry RC, Philippsen LP, Wu HH. Intrathyroidal parathyroid carcinoma presenting with only hypercalcemia. *Otolaryngol Head Neck Surg.* (2002) 127:352–3. doi: 10.1067/mhn.2002.128553
8. Hussein WI, El-Maghraby TA, Al-Sanea O. Hyperfunctioning intrathyroidal parathyroid carcinoma. *Saudi Med J.* (2006) 27:1226–9.
9. Foppiani L, Del MP, Sartini G, Arlandini A, Quilici P, Bandelloni R, et al. Intrathyroidal parathyroid carcinoma as cause of hypercalcemia and pitfall of localization techniques: clinical and biologic features. *Endocr Pract.* (2007) 13:176–81. doi: 10.4158/EP.13.2.176
10. Temmim L, Sinowatz F, Hussein WI, Al-Sanea O, El-Khodary H. Intrathyroidal parathyroid carcinoma: a case report with clinical and histological findings. *Diagn Pathol.* (2008) 3:46. doi: 10.1186/1746-1596-3-46
11. Herrera-Hernandez AA, Aranda-Valderrama P, Diaz-Perez JA, Herrera LP. Intrathyroidal parathyroid carcinoma in a pediatric patient. *Pediatr Surg Int.* (2011) 27:1361–5. doi: 10.1007/s00383-011-2904-6
12. Quartey B, Shriver C, Russell D. Intrathyroidal parathyroid carcinoma presenting as asymptomatic high normal serum calcium and slightly elevated intact parathyroid hormone: a case report and review of literature. *World J Oncol.* (2011) 2:138–42. doi: 10.4021/wjon311w
13. Kruljac I, Pavic I, Matesa N, Mirosevic G, Maric A, Becejac B, et al. Intrathyroid parathyroid carcinoma with intrathyroidal metastasis to the contralateral lobe: source of diagnostic and treatment pitfalls. *Jpn J Clin Oncol.* (2011) 41:1142–6. doi: 10.1093/jjco/hyr094
14. Vila DL, Winter WE, Vaysberg M, Moran CA, Al-Quran SZ. Intrathyroidal parathyroid carcinoma: report of an unusual case and review of the literature. *Case Rep Pathol.* (2013) 2013:198643. doi: 10.1155/2013/198643
15. Lee KM, Kim EJ, Choi WS, Park WS, Kim SW. Intrathyroidal parathyroid carcinoma mimicking a thyroid nodule in a MEN type 1 patient. *J Clin Ultrasound* (2014) 42:212–4. doi: 10.1002/jcu.22090
16. Tejera Hernandez AA, Gutierrez Giner MI, Vega B, V, Fernandez San MD, Hernandez Hernandez JR. Intrathyroidal parathyroid carcinoma. a case report and review of literature. *Endocrinol Nutr.* (2016) 63:46–8. doi: 10.1016/j.endonu.2015.09.004
17. Wong YP, Sharifah NA, Tan GC, Gill AJ, Ali SZ. Intrathyroidal oxyphilic parathyroid carcinoma: a potential diagnostic caveat in cytology? *Diagn Cytopathol.* (2016) 44:688–92. doi: 10.1002/dc.23493
18. Alwaheeb S, Rambaldini G, Boerner S, Coire C, Fiser J, Asa SL. Worrisome histologic alterations following fine-needle aspiration of the parathyroid. *J Clin Pathol.* (2006) 59:1094–6. doi: 10.1136/jcp.2005.029017
19. Kim J, Horowitz G, Hong M, Orsini M, Asa SL, Higgins K. The dangers of parathyroid biopsy. *J Otolaryngol Head Neck Surg.* (2017) 46:4. doi: 10.1186/s40463-016-0178-7
20. Erovic BM, Harris L, Jamali M, Goldstein DP, Irish JC, Asa SL, et al. Biomarkers of parathyroid carcinoma. *Endocr Pathol.* (2012) 23:221–31. doi: 10.1007/s12022-012-9222-y
21. van der Tuin K, Tops CMJ, Adank MA, Cobben JM, Hamdy NAT, Jongmans MC, et al. CDC73-related disorders: clinical manifestations and case detection in primary hyperparathyroidism. *J Clin Endocrinol Metab.* (2017) 102:4534–40. doi: 10.1210/jc.2017-01249
22. Bricaire L, Odou MF, Cardot-Bauters C, Delemer B, North MO, Salenave S, et al. Frequent large germline HRPT2 deletions in a French National cohort of patients with primary hyperparathyroidism. *J Clin Endocrinol Metab.* (2013) 98:E403–8. doi: 10.1210/jc.2012-2789

23. Tan MH, Teh BT. Renal neoplasia in the hyperparathyroidism-jaw tumor syndrome. *Curr Mol Med.* (2004) 4:895–7. doi: 10.2174/1566524043359719
24. Erovic BM, Goldstein DP, Kim D, Mete O, Brierley J, Tsang R, et al. Parathyroid cancer: outcome analysis of 16 patients treated at the princess margaret hospital. *Head Neck* (2012) 35:35–9. doi: 10.1002/hed.22908
25. Rasmuson T, Kristoffersson A, Boquist L. Positive effect of radiotherapy and surgery on hormonally active pulmonary metastases of primary parathyroid carcinoma. *Eur J Endocrinol.* (2000) 143:749–54. doi: 10.1530/eje.0.1430749
26. Kassahun WT, Jonas S. Focus on parathyroid carcinoma. *Int J Surg.* (2011) 9:13–9. doi: 10.1016/j.ijsu.2010.09.003
27. Thosani S, Hu MI. Denosumab: a new agent in the management of hypercalcemia of malignancy. *Future Oncol.* (2015) 11:2865–71. doi: 10.2217/fon.15.232
28. Al-Fadhli M, Doi SA, Muttikkal T, Al-Sumait B. Severe hyperparathyroidism versus parathyroid carcinoma: a clinical dilemma. *Sultan Qaboos Univ Med J.* (2010) 10:94–100.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 09 October 2018
doi: 10.3389/fendo.2018.00565

Hashimoto's Disease and Thyroid Cancer in Children: Are They Associated?

Laura Penta[1], *Marta Cofini*[1], *Lucia Lanciotti*[1], *Alberto Leonardi*[1], *Nicola Principi*[2] *and Susanna Esposito*[1]*

[1] *Pediatric Clinic, Department of Surgical and Biomedical Sciences, Università Degli Studi di Perugia, Perugia, Italy,*
[2] *Università degli Studi di Milano, Milan, Italy*

Hashimoto's thyroiditis (HT) is the most common cause of thyroid disease in children and adolescents. Along with significant modifications of thyroid function, HT in pediatric age can be accompanied by relevant thyroid structural alterations. Over time, benign thyroid nodules, carcinoma and, rarely, primary non-Hodgkin lymphoma can develop. However, the relationships between HT and neoplasms are poorly defined. The main aim of this paper is to discuss what is presently known regarding the coexistence of HT and thyroid tumors. Moreover, we attempt to define the pathogenesis of cancer development in children with HT. Literature analysis showed that despite its rarity and relatively promising prognosis, thyroid cancer is associated with HT. Although not all reasons for the coexistence of these diseases are clearly defined, children with HT should be considered at higher risk for thyroid cancer development. Strict correlations between high levels of serum TSH and anti-thyroid antibodies with cancer must be remembered. The same is true for the presence of nodules, especially if multiple nodules are present and ultrasonography and thyroid fine needle aspiration cytology should be promptly used in uncertain cases.

Keywords: anti-thyroid antibodies, Hashinoto's disease, thyroid cancer, thyroid nodules, TSH

OPEN ACCESS

Edited by:
Giampaolo Papi,
Azienda Unità Sanitaria Locale di Modena, Italy

Reviewed by:
Roberto Vita,
Università degli Studi di Messina, Italy
Misa Imaizumi,
Radiation Effects Research Foundation, Japan

***Correspondence:**
Susanna Esposito
susanna.esposito@unimi.it

Specialty section:
This article was submitted to Thyroid Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *27 July 2018*
Accepted: *05 September 2018*
Published: *09 October 2018*

Citation:
Penta L, Cofini M, Lanciotti L, Leonardi A, Principi N and Esposito S (2018) Hashimoto's Disease and Thyroid Cancer in Children: Are They Associated?. Front. Endocrinol. 9:565. doi: 10.3389/fendo.2018.00565

INTRODUCTION

Hashimoto's thyroiditis (HT), also termed chronic lymphocytic thyroiditis, is the most common cause of thyroid disease in children and adolescents. It is an autoimmune disease with an estimated prevalence in pediatrics of 1–2%, with variations according to genetic susceptibility, age and gender, ethnicity, iodine status, the presence of other autoimmune diseases or genetic syndromes and the criteria used for diagnosis (1). HT is more common in children aged 6 to 16 years, in females, in Caucasians, and in countries with iodine deficiency. Moreover, it is more frequently diagnosed in children who suffer from type 1 diabetes, coeliac disease, Addison's disease, autoimmune hypoparathyroidism, Down syndrome, Noonan syndrome, and Turner syndrome, as well as when antibody assays and thyroid fine needle aspiration cytology (TFNAC) are available (1).

At the time of diagnosis, most children with HT show few to no symptoms. A small goiter or the presence of mild clinical symptoms of hypothyroidism are observed in ∼70% of the causes of hospital referrals (2). Other reasons include findings upon work-up for an unrelated problem or for one of the diseases mentioned above that pose the child at higher risk of developing HT. Thyroid function, as evidenced by blood thyroid hormone levels, is normal in up to 80% of cases (2).

Only a minority has low hormone concentrations, suggesting overt hypothyroidism. In rare cases, hyperthyroidism can be demonstrated. Long-term outcomes of HT can significantly vary and are not predictable in single cases. However, both children who are initially euthyroid and those with subclinical hypothyroidism can develop overt hypothyroidism within a few years from diagnosis. Although this is more common in subclinically hypothyroid patients (3–6), conversion to Graves' disease cannot be excluded (7). The presence of goiter and elevated serum concentrations of anti-thyroglobulin antibody (TG-Ab) and anti-thyroid peroxidase antibody (TPO-Ab) at diagnosis or the progressive increase in serum TSH levels suggests an increased risk of hypothyroidism (5, 8). Finally, patients with hyperthyroidism generally become euthyroid and only occasionally develop hypothyroidism (4).

Along with significant modifications of thyroid function, HT in children can be accompanied by relevant thyroid structural alterations. Over time, benign thyroid nodules, carcinoma and, rarely, primary non-Hodgkin lymphoma can develop (9–11). However, the relationships between HT and neoplasms are poorly defined. In particular, it is not known whether HT is a predisposing factor to the development of thyroid neoplasms and whether other clinical manifestations or laboratory biomarkers can permit the early identification of HT children that are at higher risk of tumor development. The main aim of this paper is to discuss what is presently known regarding the coexistence of HT and thyroid tumors. Moreover, we attempt to define the pathogenesis of cancer development in children with HT.

EPIDEMIOLOGY OF PEDIATRIC THYROID CANCER

Although the incidence rates of pediatric thyroid cancer have progressively increased over the past thirty years (9–11), this disease remains rare in children and adolescents compared to adults. Only 2% of the ∼60,000 cases annually diagnosed in the USA regard subjects younger than 19 years of age (12). However, thyroid cancer plays a relevant role in pediatric oncology, as, in the USA, it is the eighth most common cancer diagnosed in patients aged 15–19 years and is the second most common cancer among adolescent girls (13, 14).

Along with differences in frequency compared to adult cases, pediatric thyroid cancer has several other differences regarding both histology and clinical characteristics. From a histological point of view, differentiated thyroid cancer comprises 90–95% of all childhood thyroid cancers, with papillary thyroid carcinoma (PTC) accounting for the majority of cases. In contrast, medullary thyroid cancer and undifferentiated and anaplastic forms that can be diagnosed in adults are exceptionally rare in children (15). Thyroid nodules are significantly less common in children (16, 17). However, pediatric thyroid nodules have a higher likelihood of malignancy compared to adults (16–20).

Several factors were found to be potential predictors of thyroid nodule malignancy. Microcalcifications, hypoechoic pattern, intranodular vascularization, lymph node alterations, and TSH concentration were identified by Mussa et al. (21). Papendieck et al. (22) added multinodular goiter, solid nodules, irregular margins and TSH values >2.5 mIU/L.

Finally, in contrast to adults, children with cancer at presentation have more extensive disease, with positive cervical lymph nodes and evidence of local or distant metastasis and have a higher risk of recurrence. In contrast, pediatric PTC has an excellent long-term prognosis, with 30-year survival rates of 90–99% (20, 23–26). All these findings, that are summarized in **Table 1**, have raised the supposition that pediatric thyroid cancer might be distinct from that of adults (27).

COEXISTENCE OF THYROID CANCER AND HASHIMOTO'S THYROIDITIS (HT) IN CHILDREN

Most of the studies regarding the potential association between HT and thyroid cancer development have been carried out in adults. With few exceptions (28), most have clearly demonstrated that the coexistence of HT and thyroid tumors, mainly PTC, is common and that the risk of development of thyroid cancer in patients with HT is significantly higher than that in patients without HT. Moreover, HT seemed to have a certain protective effect on the short- and long-term prognosis of cancer. In a meta-analysis of 38 articles published before September 2011, including 10,648 PTC cases (29), the frequency of HT in PTC cases was ∼23%, ranging from 5 to 85%. Different diagnostic criteria for HT, various surgical procedures, and heterogeneity of enrolled patient characteristics may explain the differences. HT was more frequently observed in PTC than in benign thyroid diseases and other carcinomas (odds ratio [OR] = 2.8 and 2.4, respectively; $P < 0.001$). The association was more common in females (OR = 2.7; $P < 0.001$) and was found to have more favorable clinical and histological characteristics than PTC without HT (29). In patients with HT-associated PTC, carcinoma had no extrathyroidal extension (OR = 1.3; $P = 0.002$), no lymph node metastasis (OR = 1.3; $P = 0.041$), and a long recurrence-free survival (hazard ratio [HR] = 0.6; $P = 0.001$). Similar results were reported in a more recent meta-analysis (30). In this case, 27 studies published until December 2015, enrolling 76,281 patients and including 12,476 cases of thyroid cancer, were analyzed. The mean rate of PTC among patients with HT ranged from 1.1 to 40.1%, with variations strictly related to the methods used to diagnose thyroid cancer, being higher when diagnosis

TABLE 1 | Main characteristics of pediatric thyroid cancer.

Characteristic	Comparison with adults
Incidence rate	Significantly lower
Histology	Mainly represented by papillary thyroid cancer
	Lower frequency of thyroid nodules that have a higher likelihood of malignancy
Clinical findings	More extensive disease with positive cervical lymph nodes and evidence of local or distant metastasis
Outcome	Higher risk of recurrence
	Excellent long-term survival rate

is performed with more effective radiological and laboratory methods (30). The overall pooled OR of the PTC risk for HT (HT vs. non-HT) was 2.12 (95% confidence interval [CI] = 1.78–2.52).

In children, the few available studies had even more severe limitations than those enrolling adults. They were retrospective, frequently included small numbers of children and used different criteria for the diagnosis of both HT and thyroid cancer. This precludes pooling and comparison. However, for children and adolescents, the association between HT and thyroid cancer seems relatively common. The frequency of PTC in children and adolescents with HT was found variable from 0.67 to ∼3% (31–33). In patients with PTC, the prevalence of coexisting HT varied from 6.3% to more than 40% of the cases. In a recent study, in which modern histological and laboratory methods, including thyroid fine needle aspiration cytology (TFNAC), were used to diagnose HT and thyroid cancer, HT was detected in 28.7% of the 108 thyroid cancer cases (34).

However, the impact of pediatric HT on the short- and long-term prognosis of cancer was not clearly defined. In the study by Iliadou et al. (34), HT was associated with a more severe clinical manifestation of the neoplasm. Histological examination revealed that infiltration of the thyroid parenchyma, revealing invasive characteristics of the cancer, was more frequent in children with HT (74.2 vs. 48.1%; $P = 0.024$), but the final prognosis was not influenced by HT. The clinical condition of patients with or without HT was strikingly similar after 5 or 10 years of follow-up. In contrast, in the study by Ren et al. (35), in which a frequent association between HT and cancer was shown (HT in 44.2% of differentiated thyroid cancer and 41.3% of thyroid nodules), no significant differences were observed in the clinical characteristics of the thyroid tumor among children with or without HT. No differences in tumor multifocality ($P = 0.7$), tumor size ($P = 0.09$), extrathyroidal infiltration ($P = 0.6$), or metastasis ($P = 0.34$) were shown (35). However, as evidenced by Iliadou et al. (34), no effect of HT on short-term disease-free survival was shown.

SUPPOSED REASONS FOR THE COEXISTENCE OF THYROID CANCER AND HASHIMOTO'S THYROIDITIS

Several hypotheses have been proposed to explain the potential relationship between HT and thyroid cancer (**Table 2**). One of them regards the role of TSH. It has been shown that the risk of thyroid malignancy is strictly related to the serum level of TSH, even with serum TSH levels within the normal range (36). In a recent prospective study, it was found that the risk of malignancy was ∼3-fold higher in patients with TSH levels ≥2.26 μIU/mL than in patients with lower TSH levels ($P = 0.001$) (37). Thyroid autoimmunity that leads to higher serum TSH concentrations may partially explain the association between HT and PTC. However, it remains unclear whether TSH simply promotes the growth of a pre-existing cancer or truly causes cancer development.

A second hypothesis considers the role of chronic inflammation due to autoimmunity. HT is associated with chronic inflammation of thyroid tissue. Inflammation can increase the risk of cancer by providing bioactive molecules from cells infiltrating the tumor microenvironment (38). Cytokines, chemokines, and growth factors favor sustained proliferation, a significant reduction of apoptosis, and angiogenesis. Moreover, the production of extracellular matrix-modifying enzymes, such as metalloproteinases, is induced. This production promotes epithelial-mesenchymal transition and facilitates other carcinogenesis programmes, such as genome instability, immune evasion, and modifications of energy metabolism (39, 40). On the other hand, a complete concordance of all the markers of thyroid autoimmunity with thyroid cancer development has been reported. Boi et al. (39) carried out a retrospective analysis on 2,053 patients with single/prevalent thyroid nodules submitted to TFNAC and found that a higher prevalence of suspicious/malignant or indeterminate cytological findings was detected in patients with positive TG-Ab and thyroid microsomal antibody (TM-Ab) than in those with benign cytology. Increased independent OR for malignancy was conferred by any anti-thyroid antibody (OR 2.21; 95% CI = 1.49–3.29, $P < 0.0001$), TPO-Ab (OR 2.15; CI = 1.42–3.25, $P < 0.0001$) and TG-Ab (OR 1.67; CI = 1.05–2.67, $P < 0.05$).

TABLE 2 | Hypotheses proposed to explain the potential relationship between Hashimoto's thyroiditis (HT) and thyroid cancer.

Mechanism	Action
High serum TSH level	Growth of a pre-existing cancer or cancer development induced by TSH
Chronic inflammation due to autoimmunity	Proliferation, reduction of apoptosis and angiogenesis sustained by cytokines, chemokines, and growth factors
	Facilitation of carcinogenesis programmes by extracellular matrix-modifying enzymes
Gene expression	Proinflammatory proteins produced by gene rearrangements and point mutations in proto-oncogenes increase proliferation and invasiveness of tumor cells, stimulation of angiogenesis, and reduction of anti-tumoural immune responses

TSH, thyroid-stimulating hormone.

A third hypothesis considers genetics. Molecular genetic studies have shown an association of HT with gene rearrangements and point mutations in the proto-oncogenes implicated in PTC, suggesting a potential interrelationship between the two diseases (18). Proinflammatory proteins (several cytokines and chemokines) induced by these mutated genes are relevant for the mobility, proliferation, survival, and invasiveness of tumor cells; stimulation of angiogenesis; and reduction of anti-tumoural immune responses. The best example in this regard is given by the chromosomal rearrangement involving the *RET* receptor tyrosine kinase gene. The rearrangement, named RET/PTC, fuses the 3′ terminal portion of *RET* coding for the tyrosine kinase domain with the 5′ terminal sequence of different unrelated genes, leading to constitutive activation of the *RET* tyrosine kinase. It is frequently identified in patients with PTC, although with significant differences according to several factors, including methodological and ethnic differences. However, one of the

most important differences is age: RET/PTC rearrangements are much more frequent in younger patients with PTC, especially in children. These rearrangements constitute 40–70% of sporadic papillary carcinomas diagnosed in children and young adults (18). Regarding association with HT, RET/PTC rearrangement was more frequently observed in PTC associated with HT than in PTC without HT (31 vs. 13%, $P = 0.02$) (38). It was found that RET/PTC rearrangements were correlated with high TSH levels ($P = 0.037$) (41). Moreover, thyroid cell lines expressing RET/PTC may induce genes encoding molecules involved in the immune response (42), including *CXCL10*, which plays an important role in the first steps of HT lymphocytic infiltration (43, 44). Finally, it was reported that RET/PTC could be found both in areas of PTC and in areas with classic histological thyroid modifications typical of HT (45). Other genes have been theoretically implicated in the association between HT and thyroid cancer. *Human 8-oxoguanine glycosylase* is one of these genes. Mutations of this gene have commonly been found in both PTC (94%) and HT (73%), but not in other thyroid diseases (8%) (46). However, also in this case, children are different from adults because rearrangements appear more common in children, whereas mutations are more frequently detected in adults (46).

Independently of mutations in proto-oncogenes implicated in PTC, it seems likely that other genetic alterations may play a role in favoring the association between HT and thyroid cancer. This is suggested by the description of some clinical reports regarding children with thyroid cancer and HT associated with other autoimmune diseases, such as type 1 diabetes (47) and the autoimmune polyglandular syndrome type II (48).

CONCLUSION

Despite its rarity and relatively promising prognosis, thyroid cancer remains a significant clinical problem in pediatrics. Its association with HT, despite being based on a significantly lower number of reliable studies than in adults, seems likely. However, although not all reasons for the coexistence of these diseases are clearly defined, children with HT should be considered at highest risk of cancer development. Strict correlations between high levels of serum TSH and anti-thyroid antibodies must be remembered. The same is true for the presence of nodules, especially if multiple nodules are present, Ultrasonography and TFNAC can favor an early identification of patients with malignant changes and should be promptly used in uncertain cases.

AUTHOR CONTRIBUTIONS

LP conceptualized the work and wrote the first draft of the manuscript. MC, LL, and AL performed the literature analysis. NP gave a significant contribution of the event. SE supervised the work and gave a substantial scientific contribution. All the authors approved the final report.

REFERENCES

1. Diaz A, Lipman Diaz EG. Hypothyroidism. *Pediatr Rev.* (2014) 35:336–47. doi: 10.1542/pir.35-8-336
2. de Vries L, Bulvik S, Phillip M. Chronic autoimmune thyroiditis in children and adolescents: at presentation and during long-term follow-up. *Arch Dis Child.* (2009) 94:33–7. doi: 10.1136/adc.2007.134841
3. Aversa T, Corrias A, Salerno M, Tessaris D, Di Mase R, Valenzise M, et al. Five-year prospective evaluation of thyroid function test evolution in children with Hashimoto's thyroiditis presenting with either euthyroidism or subclinical hypothyroidism. *Thyroid* (2016) 26:1450–6. doi: 10.1089/thy.2016.0080
4. Crisafulli G, Gallizzi R, Aversa T, Salzano G, Valenzise M, Wasniewska M, et al. Thyroid function test evolution in children with Hashimoto's thyroiditis is closely conditioned by the biochemical picture at diagnosis. *Ital J Pediatr.* (2018) 44:22. doi: 10.1186/s13052-018-0461-5
5. Radetti G, Gottardi E, Bona G, Corrias A, Salardi S, Loche S. The natural history of euthyroid Hashimoto's thyroiditis in children. *J Pediatr.* (2006) 149:827–32. doi: 10.1016/j.jpeds.2006.08.045
6. Rallison ML, Dobyns BM, Keating FR, Rall JE, Tyler FH. Occurrence and natural history of chronic lymphocytic thyroiditis in childhood. *J Pediatr.* (1975) 86:675–82. doi: 10.1016/S0022-3476(75)80350-7
7. Ohye H, Nishihara E, Sasaki I, Kubota S, Fukata S, Amino N, et al. Four cases of Graves' disease which developed after painful Hashimoto's thyroiditis. *Intern Med.* (2006) 45:385–9. doi: 10.2169/internalmedicine.45.1506
8. Champion B, Gopinath B, Ma G, El-Kaissi S, Wall JR. Conversion to Graves' hyperthyroidism in a patient with hypothyroidism due to Hashimoto's thyroiditis documented by real-time thyroid ultrasonography. *Thyroid* (2008) 18:1135–7. doi: 10.1089/thy.2008.0142
9. Bergamini LB, Frazier AL, Abrantes FL, Ribeiro KB, Rodriguez-Galindo C. Increase in the incidence of differentiated thyroid carcinoma in children, adolescents, and young adults: a population-based study. *J Pediatr.* (2014) 164:1481–5. doi: 10.1016/j.jpeds.2014.01.059
10. Holmes L, Hossain J, Opara F. Paediatric thyroid carcinoma incidence and temporal trends in the USA (1973–2007): race or shifting diagnostic paradigm? *ISRN Oncol.* (2012) 2012:1–10. doi: 10.5402/2012/906197
11. Ieni A, Vita R, Magliolo E, Santarpia M, Di Bari F, Benvenga S, et al. One-third of an archivial series of papillary thyroid cancer (years 2007–2015) has coexistent chronic lymphocytic thyroiditis, which is associated with a more favorable tumor-node-metastasis staging. *Front Endocrinol.* (2017) 8:337. doi: 10.3389/fendo.2017.00337
12. Hogan AR, Zhuge Y, Perez EA, Koniaris LG, Lew JI, Sola JE. Paediatric thyroid carcinoma: incidence and outcomes in 1753 patients. *J Surg Res.* (2009) 156:167–72. doi: 10.1016/j.jss.2009.03.098
13. Siegel DA, King J, Tai E, Buchanan N, Ajani UA, Li J. Cancer incidence rates and trends among children and adolescents in the United States, 2001–2009. *Paediatrics* (2014) 134:e945–55. doi: 10.1542/peds.2013-3926
14. Ward E, DeSantis C, Robbins A, Kohler B, Jemal A. Childhood and adolescent cancer statistics, 2014. *CA Cancer J Clin.* (2014) 64:83–103. doi: 10.3322/caac.21219
15. Demidchik YE, Demidchik EP, Reiners C, Biko J, Mine M, Saenko VA, et al. Comprehensive clinical assessment of 740 cases of surgically treated thyroid cancer in children of Belarus. *Ann Surg.* (2006) 243:525. doi: 10.1097/01.sla.0000205977.74806.0b
16. Niedziela M. Thyroid nodules. *Best Pract Res Clin Endocrinol Metab.* (2014) 28:245–77. doi: 10.1016/j.beem.2013.08.007
17. Dean DS, Gharib H. Epidemiology of thyroid nodules. *Best Pract Res Clin Endocrinol Metab.* (2008) 22:901–11. doi: 10.1016/j.beem.2008.09.019
18. Yamashita S, Saenko V. Mechanisms of disease: molecular genetics of childhood thyroid cancers. *Nat Clin Pract Endocrinol Metab.* (2007) 3:22–9. doi: 10.1038/ncpendmet0499
19. Wiersinga WM. Management of thyroid nodules in children and adolescents. *Hormones (Athens)* (2007) 6:194–9.
20. Jarzab B, Handkiewicz-Junak D. Differentiated thyroid cancer in children and adults: same or distinct disease? *Hormones (Athens)* (2007) 6:200–9.

21. Mussa A, De Andrea M, Motta M, Mormile A, Palestini N, Corrias A. Predictors of malignancy in children with thyroid nodules. *J Pediatr.* (2015) 167:886–92. doi: 10.1016/j.jpeds.2015.06.026
22. Papendieck P, Gruñeiro-Papendieck L, Venara M, Acha O, Cozzani H, Mateos F, et al. Differentiated thyroid cancer in children: prevalence and predictors in a large cohort with thyroid nodules followed prospectively. *J Pediatr.* (2015) 167:199–201. doi: 10.1016/j.jpeds.2015.04.041
23. Gigsby PW, Gal-or A, Michalski JM, Doherty GM. Childhood and adolescent thyroid carcinoma. *Cancer* (2002) 95:724–9. doi: 10.1002/cncr.10725
24. Hay ID, Gonzalez-Losada T, Reinalda MS, Honetschlager JA, Richards ML, Thompson GB. Long term outcome in 215 children and adolescents with papillary thyroid cancer treated during 1940 through 2008. *World J Surg.* (2010) 34:1192–202. doi: 10.1007/s00268-009-0364-0
25. La Quaglia MP, Black T, Holcomb TB, Sklar CA, Azizkhan RG, et al. Differentiated thyroid cancer; clinical characteristics, treatment, and outcome in patients under 21 years of age who present with distant metastases. A report from the Surgical Discipline Committee of the Children's Cancer Group. *J Pediatr Surg.* (2000) 35:955–9. doi: 10.1053/jpsu.2000.6935
26. Rivkees SA, Mazzaferri EL, Verburg FA, Reiners C, Luster M, Breuer CK, et al. The treatment of differentiated thyroid cancer in children; emphasis on surgical approach and radioactive iodine therapy. *Endocr Rev.* (2011) 32:798–826. doi: 10.1210/er.2011-0011
27. Gupta A, Ly S, Castroneves LA, Frates MC, Benson CB, Feldman HA, et al. A standardized assessment of thyroid nodules in children confirms higher cancer prevalence than in adults. *J Clin Endocrinol Metab.* (2013) 98:3238–45. doi: 10.1210/jc.2013-1796
28. Jankovic B, Le KT, Hershman JM. Clinical review: Hashimoto's thyroiditis and papillary thyroid carcinoma: is there a correlation? *J Clin Endocrinol Metab.* (2013) 98:474–82. doi: 10.1210/jc.2012-2978
29. Lee JH, Kim Y, Choi JW, Kim YS. The association between papillary thyroid carcinoma and histologically proven Hashimoto's thyroiditis: a meta-analysis. *Eur J Endocrinol.* (2013) 168:343–9. doi: 10.1530/EJE-12-0903
30. Lai X, Xia Y, Zhang B, Li J, Jiang Y. A meta-analysis of Hashimoto's thyroiditis and papillary thyroid carcinoma risk. *Oncotarget* (2017) 8:62414–24. doi: 10.18632/oncotarget.18620
31. Keskin M, Savas-Erdeve S, Aycan Z. Co-existence of thyroid nodule and thyroid cancer in children and adolescents with Hashimoto thyroiditis: a single-center study. *Horm Res Paediatr.* (2016) 85:181–7. doi: 10.1159/000443143
32. Skarpa V, Kousta E, Tertipi A, Anyfandakis K, Vakaki M, Dolianiti M, et al. Epidemiological characteristics of children with autoimmune thyroid disease. *Hormones (Athens)* (2011) 10:207–14. doi: 10.14310/horm.2002.1310
33. Corrias A, Cassio A, Weber G, Mussa A, Wasniewska M, Rapa A, et al. Thyroid nodules and cancer in children and adolescents affected by autoimmune thyroiditis. *Arch Pediatr Adolesc Med.* (2008) 162:526–31. doi: 10.1001/archpedi.162.6.526
34. Iliadou PK, Effraimidis G, Konstantinos M, Grigorios P, Mitsakis P, Patakiouta F, et al. Chronic lymphocytic thyroiditis is associated with invasive characteristics of differentiated thyroid carcinoma in children and adolescents. *Eur J Endocrinol.* (2015) 173:827–33. doi: 10.1530/EJE-14-1046
35. Ren PY, Liu J, Xue S, Chen G. Paediatric differentiated thyroid carcinoma: the clinicopahological features and the coexistence of Hashimoto's thyroiditis. *Asian J Surg.* (2017). doi: 10.1016/j.asjsur.2017.10.006. [Epub ahead of print].
36. McLeod DS, Watters KF, Carpenter AD, Ladenson PW, Cooper DS, Ding EL. Thyrotropin and thyroid cancer diagnosis: a systematic review and dose-response meta-analysis. *J Clin Endocrinol Metab.* (2012) 97:2682–92. doi: 10.1210/jc.2012-1083
37. Golbert L, de Cristo AP, Faccin CS, Farenzena M, Folgierini H, Graudenz MS, et al. Serum TSH levels as a predictor of malignancy in thyroid nodules: a prospective study. *PLoS ONE* (2017) 12:e0188123. doi: 10.1371/journal.pone.0188123
38. Landskron G, De la Fuente M, Thuwajit P, Thuwajit C, Hermoso MA. Chronic inflammation and cytokines in the tumour microenvironment. *J Immunol Res.* (2014) 2014:149185. doi: 10.1155/2014/149185
39. Boi F, Minerba L, Lai ML, Marziani B, Figus B, Spanu F, et al. Both thyroid autoimmunity and increased serum TSH are independent risk factors for malignancy in patients with thyroid nodules. *J Endocrinol Invest.* (2013) 36:313–20. doi: 10.3275/8579
40. Muzza M, Degl'Innocenti D, Colombo C, Perrino M, Ravasi E, Rossi S, et al. The tight relationship between papillary thyroid cancer, autoimmunity and inflammation: clinical and molecular studies. *Clin Endocrinol (Oxf)* (2010) 72:702–8. doi: 10.1111/j.1365-2265.2009.03699.x
41. Su X, He C, Ma J, Tang T, Zhang X, Ye Z, et al. RET/PTC Rearrangements are associated with elevated postoperative TSH levels and multifocal lesions in papillary thyroid cancer without concomitant thyroid benign disease. *PLoS ONE* (2016) 11:e0165596. doi: 10.1371/journal.pone.0165596
42. Borrello MG, Alberti L, Fischer A, Degl'innocenti D, Ferrario C, Gariboldi M, et al. Induction of a proinflammatory program in normal human thyrocytes by the RET/PTC1 oncogene. *Proc Natl Acad Sci USA.* (2005) 102:14825–30. doi: 10.1073/pnas.0503039102
43. Antonelli A, Rotondi M, Fallahi P, Grosso M, Boni G, Ferrari SM, et al. Iodine-131 given for therapeutic purposes modulates differently interferon-gamma-inducible alpha-chemokine CXCL10 serum levels in patients with active Graves' disease or toxic nodular goiter. *J Clin Endocrinol Metab.* (2007) 92:1485–90. doi: 10.1210/jc.2006-1571
44. Rotondi M, Chiovato L, Romagnani S, Serio M, Romagnani P. Role of chemokines in endocrine autoimmune diseases. *Endocr Rev.* (2007) 28:492–520. doi: 10.1210/er.2006-0044
45. Mechler C, Bounacer A, Suarez H, Saint Frison M, Magois C, Aillet G, et al. Papillary thyroid carcinoma: 6 cases from 2 families with associated lymphocytic thyroiditis harbouring RET/PTC rearrangements. *Br J Cancer* (2001) 85:1831–7. doi: 10.1054/bjoc.2001.2187
46. Royer MC, Zhang H, Fan CY, Kokoska MS. Genetic alterations in papillary thyroid carcinoma and Hashimoto thyroiditis: an analysis of hOGG1 loss of heterozygosity. *Arch Otolaryngol Head Neck Surg.* (2010) 136:240–2. doi: 10.1001/archoto.2010.20
47. Karavanaki K, Karayianni C, Vassiliou I, Tzanela M, Sdogou T, Kakleas K, et al. Multiple autoimmunity, type 1 diabetes (T1DM), autoimmune thyroiditis and thyroid cancer: is there an association? A case report and literature review. *J Pediatr Endocrinol Metab.* (2014) 27:1011–6. doi: 10.1515/jpem-2013-0370
48. Mussa A, Matarazzo P, Corrias A. Papillary thyroid cancer and autoimmune polyglandular syndrome. *J Pediatr Endocrinol Metab.* (2015) 28:793–5. doi: 10.1515/jpem-2014-0268

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

CASE REPORT
published: 09 October 2018
doi: 10.3389/fendo.2018.00590

Lung Recurrence of Papillary Thyroid Cancer Diagnosed With Antithyroglobulin Antibodies After 10 Years From Initial Treatment

David Viola[1], Laura Agate[1], Eleonora Molinaro[1], Valeria Bottici[1], Loredana Lorusso[1], Francesco Latrofa[1], Liborio Torregrossa[2], Laura Boldrini[2], Teresa Ramone[1], Paolo Vitti[1] and Rossella Elisei[1]**

[1] Unit of Endocrinology, Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy, [2] Unit of Pathological Anatomy, Department of Surgical, Medical, and Molecular Pathology and Critical Care Medicine, University of Pisa, Pisa, Italy

OPEN ACCESS

Edited by:
Giampaolo Papi,
Azienda Unità Sanitaria Locale di Modena, Italy

Reviewed by:
Zubair Wahid Baloch,
University of Pennsylvania, United States
Pasqualino Malandrino,
Università degli Studi di Catania, Italy

***Correspondence:**
David Viola
david.viola@for.unipi.it
Rossella Elisei
rossella.elisei@med.unipi.it

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: 18 July 2018
Accepted: 18 September 2018
Published: 09 October 2018

Citation:
Viola D, Agate L, Molinaro E, Bottici V, Lorusso L, Latrofa F, Torregrossa L, Boldrini L, Ramone T, Vitti P and Elisei R (2018) Lung Recurrence of Papillary Thyroid Cancer Diagnosed With Antithyroglobulin Antibodies After 10 Years From Initial Treatment. Front. Endocrinol. 9:590. doi: 10.3389/fendo.2018.00590

Introduction: Papillary thyroid cancer (PTC) is the most common endocrine malignancy. More than 98% of patients achieve an excellent response with no evidence of clinical, biochemical, or structural disease after initial treatment. In these patients structural recurrence is rare, more frequently diagnosed in the first 5 years from initial treatment and almost invariably localized in neck lymph nodes.

Patient: We report the case of a woman affected by PTC who presented with rapidly rising anti-thyroglobulin antibodies (TgAb) level after 10 years from clinical, morphological and biochemical remission.

Diagnosis and Treatment: In 2003, a 56 year old patient was treated with total thyroidectomy and radioiodine remnant ablation (RRA) for a PTC (2 cm) with minimal extrathyroidal extension (T3N1aM0 according to the 6th AJCC TNM staging system) associated with diffuse lymphocytic thyroiditis. In 2004 the patient was free of disease defined as undetectable Tg after recombinant human TSH administration in the absence of TgAb and structural disease. Since February 2012 the appearance and progressive increase of TgAb titer was observed and in 2014 a ^{18}FDG-PET scan documented three hypermetabolic lesions suggestive of lung micrometastases. The lung lesions were cytologically confirmed as PTC metastases. Both the primary tissue and the lung metastasis were positive for BRAF *V600E* mutation. The patient was treated with 131-radioiodine that showed radioiodine avid lung lesions that lose the ability to take up iodine at the following treatment. The patient is still alive and the lung lesions are growing slowly.

Conclusions: Structural recurrence in patients that demonstrated an excellent response after initial treatment for PTC is extremely rare, and distant metastases exceptional but possible. This case is peculiar because recurrence was early identified after 10 years from initial treatment for the presence of detectable TgAb in a patient that had an histological diagnosis of lymphocytic thyroiditis but with an atypical clinical presentation

(normal thyroid at neck ultrasound and undetectable TgAb and anti-thyroid peroxidase antibodies). For this reason TgAb should be tested with Tg in patients with a history of lymphocytic thyroiditis, either histological or humoral, also when TgAb is in the normal range and not suggestive of autoimmune thyroiditis.

Keywords: thyroid cancer (TC), TgAb, recurrence, BRAF mutation, lymphocytic thyroiditis

BACKGROUND

Thyroid carcinoma (TC) is the most common endocrine malignancy accounting for about 4% of all human tumors (1). The most frequent histotype is PTC accounting for 85–90% of all TC, followed by follicular thyroid cancer (FTC), medullary thyroid cancer (MTC), poorly differentiated thyroid cancer (PDTC), and anaplastic thyroid cancer (ATC) representing 5–10, 3–5, 2–3, and 1% of all TC, respectively (2). Well-differentiated thyroid carcinoma (DTC), both PTC and FTC, originate from follicular cells and maintain the ability to concentrate iodine and producing Tg. These features of differentiation have important clinical and prognostic implications. In fact, the slow growth rate of differentiated PTC and the possibility to treat it with radioactive iodine make it one of the most curable human cancers (3). Despite an overall survival of 98.1% after 5 years from diagnosis PTC recurrence rate is rather high reaching in some series 30% (4, 5). In the vast majority of cases, structural recurrent disease is represented by neck lymph nodes and can be suspected on the basis of detectable levels of serum Tg and easily diagnosed with neck ultrasound followed by fine needle aspiration biopsy. Although more frequent in the first 5 years after initial treatment, recurrent disease could manifest also after 10–20 years (4).

Although Tg is a quite well-recognized and reliable tumor marker, in subjects with detectable/high titer of anti-thyroglobulin antibodies (TgAb), that can interfere in its measurement, Tg is no longer trustable as tumoral marker (6, 7). In these cases TgAb titer can be used as a surrogate marker since its disappearance is correlated with the clinical remission of the disease while its persistence or increase can suggest the persistence or recurrence of the disease (8–10). In fact, after the initial treatment (i.e., total thyroidectomy and RRA) thyroid autoantibodies, specifically TgAb, decrease over the following years disappearing in a mean of 3 years when the disease is cured (11). At variance, the persistence or increase of TgAb titer should induce the suspicious of a persistent or recurrent disease and further diagnostic tests should be performed (12). On this regard, several studies have shown that changes in the TgAb levels can predict the risk of persistence/recurrence in TgAb-positive PTC patients while patients who obtain undetectable TgAb titer have an excellent prognosis (13). More recently, the evidence that an increase in TgAb titer should not be undervalued was described also in subjects that were not treated with radioiodine and could indicate the presence of either normal or tumoral thyroid tissue (14). However, while the previous reported series were focused on patients with PTC and positive TgAb from the beginning, the present case is peculiar because the recurrence was discovered for the appearance, followed by a progressive increase, of TgAb titer that was negative at the time of PTC diagnosis. To our knowledge no similar cases have been reported so far.

CASE PRESENTATION

On January 2003 a 56-year-old woman underwent total thyroidectomy for a multinodular goiter with a thyroid nodule that was suspicious for malignancy at cytology. No evidences of biochemical and/or ultrasonographic features of autoimmune thyroiditis were present before surgery (15). The histological diagnosis was papillary thyroid carcinoma (PTC), classical variant (**Figure 1A1**) but with focal areas of tall cells, perithyroid soft tissue invasion, and multifocality. Histology showed also the presence of a diffuse lymphocytic infiltration (**Figure 1A2**).A few central compartment lymph node metastases were also present (**Figure 1A3**) (pT3mN1aMx according to the 6th AJCC-TNM staging system) (16).

On May 2003 the patient was referred to the Endocrine Oncology Unit of the Department of Clinical and Experimental Medicine of the University Hospital of Pisa to perform radioiodine remnant ablation (RRA) with 30 mCi of 131-I, after levothyroxine (L-T4) withdrawal. Post-therapeutic whole body scan (pWBS) showed an exclusive uptake in the central neck that was suggestive for thyroid remnant, serum thyroglobulin (Tg) was 1.2 ng/ml with undetectable levels of TgAb. On May 2004 the patient had undetectable Tg (i.e., <0.5 ng/ml) serum (Immulite 2000 Thyroglobulin; DPC, Los Angeles, CA) after the administration of recombinant human thyroid stimulating hormone (rhTSH; Thyrogen; Sanofi Genzyme, Cambridge, Massachusetts), negative TgAb (AIA-Pack 2000, Tosoh Corporation, Tokyo, Japan) and negative neck ultrasound (US). Considering the excellent response to the initial treatment the patient, accordingly to the American Thyroid Association guidelines (17), was considered in clinical remission and then followed with clinical and biochemical (i.e., Tg and TgAb) controls and neck US every 12–24 months. The clinical evaluations, neck US and both Tg and TgAb were negative and/or undetectable for the following 5 years.

In 2012 an unexpected positive serum TgAb titer was noted still in the absence of detectable serum Tg. A small (8 mm) indeterminate lymph node was newly detected at neck US. The titer of TgAb slightly increased over the years and for this reason on August 2015 the patient was subjected to a computerized tomography (CT) scan that showed three small lesions (maximum diameter 12 mm) in the lung. A ^{18}Fluorodeoxyglucose-Positron Emission Tomography (^{18}FDG-PET) scan confirmed the presence of these lesions that were hypermetabolic (**Figure 2A**). On November 2015 the largest

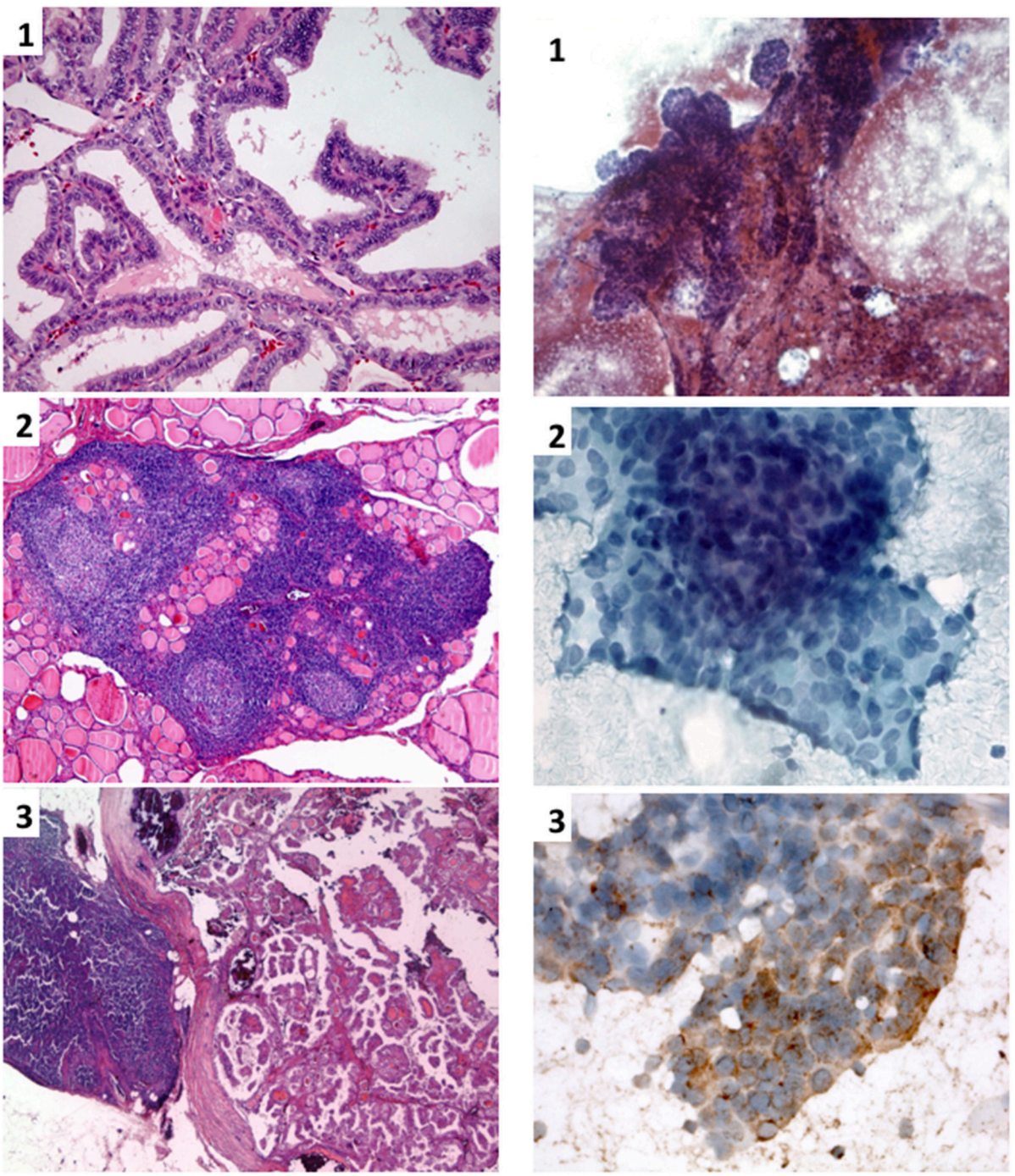


FIGURE 1 | (A) Histological slides of primary PTC and lymph node metastasis. **(A1)** Primary PTC with well-formed papillary structures (20X, hematoxilyn/eosin); **(A2)** collateral diffuse lymphocytic thyroiditis with lymphoid follicles and germinal centers(5X, hematoxilyn/eosin); **(A3)** lymph node metastatic lesion of PTC (2.5X, hematoxilyn/eosin). **(B)** Fine-needle aspiration cytological smears of lung metastastic lesion. **(B1)** low power magnification showing papillary-like structures with smooth contours and palisading of nuclei (10X, papanicolau stain); **(B2)** high power magnification showing nuclear characteristics of PTC (finely granular chromatin and one pseudoinclusion) (40X, papanicolau stain); **(B3)** immunocytochemistry showing cytoplasmatic positivity for thyroglobulin, confirming the thyroid origin of the lung lesion (40X).

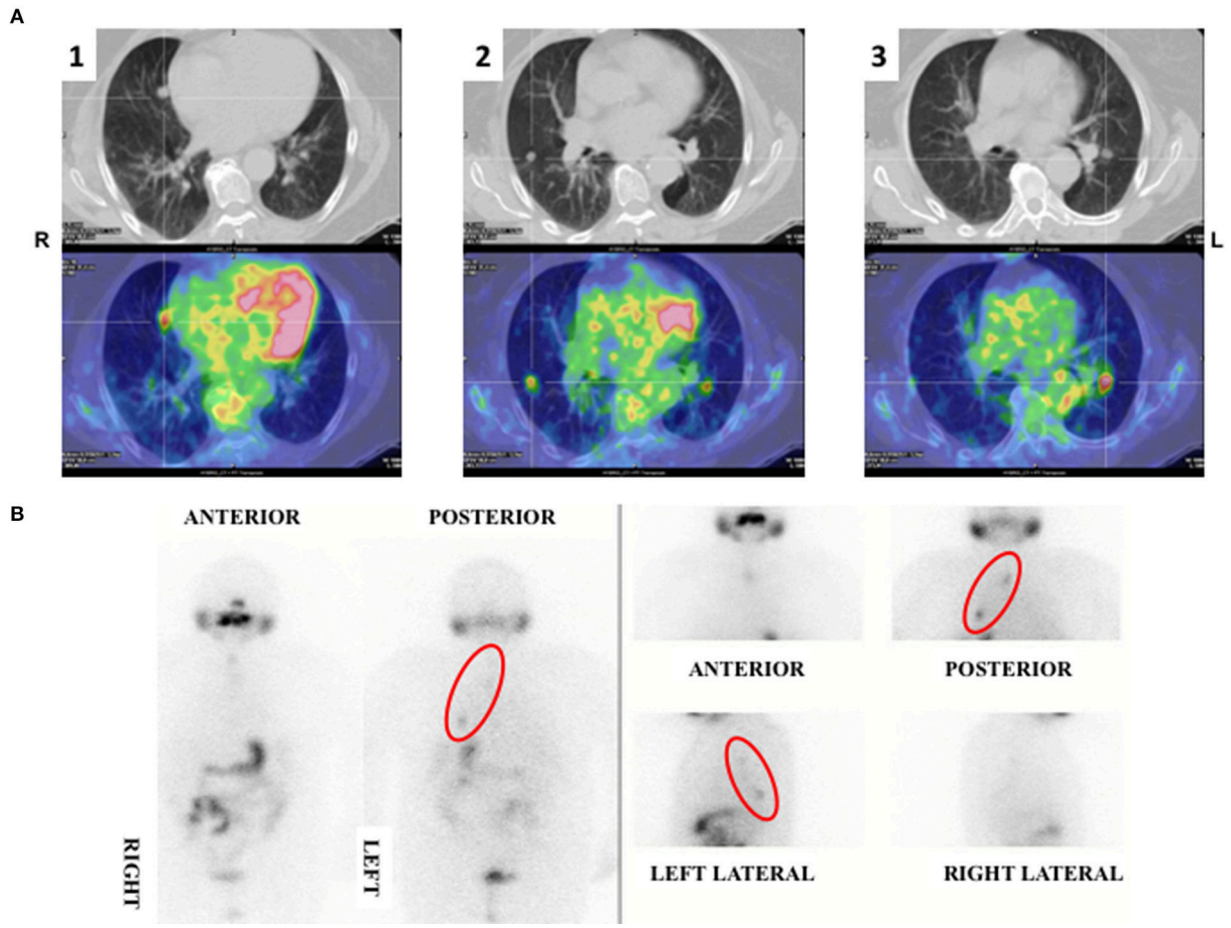


FIGURE 2 | (A) ^{18}FDG-PET scan showing three hypermetabolic nodular lung lesions localized at the medial (1) (SUV max 3.1) and lateral (2) (SUV max 2.7) segment of the right medium lobe and at the apical segment (3) of the left inferior (SUV max 3.9) lobe of the lungs. **(B)** pWBS showing 131-I uptake corresponding to the hypermetabolic lung lesions localized at the medial segment of the right lobe and at the left inferior lobe.

lung nodule, that was located in the inferior left lobe, was subjected to fine needle biopsy and cytology confirmed that cell morphology was suggestive of PTC (**Figures 1B1**,**2**). Moreover, the immunohistochemistry was positive for TTF-1 and focally for Tg (**Figure 1B3**) and the measurement of Tg in the wash out of the needle used for the lung cytology was 1780 ng/ml, confirming the thyroid origin of the lesion. On December 2015 the serum Tg became slightly detectable and the patient was treated with 150 mCi of 131-I. The pWBS showed two areas of uptake in the lung that were suggestive of iodine avid lung metastases and likely corresponding to two of the lesions found at the CT and PET scan (**Figure 2B**).On May 2016 serum biomarkers, namely TgAb, continued to increase and the patient was subjected to a CT scan that showed a slight increase of the lung lesions (maximum diameter 14 mm). Taking into account the slow but continue increase of serum biomarkers on December 2016 the patient was treated with additional 150 mCi of 131-I. At that time the pWBS didn't show any radioiodine uptake. On February 2018 the CT scan showed a further increase in one lung lesion that reached a maximum diameter of 22 mm. A detailed history of serum biomarkers (i.e., Tg and TgAb) variation is summarized and shown in **Table 1** and **Figure 3**.

TABLE 1 | Patient's serum Tg values and TgAb titer between 2004 and 2018.

Date	Tg (ng/mL)	TgAb (IU/mL)
2004–2011	0	0
Feb-2012*	0	5.7
Nov-2014	0	49
Dec-2015	2.31	154
May-2016	0.18	587
Nov-2016	1.15	568
Oct-2017	0	1,369
Feb-2018	0	2,000

**Since 2012 Tg measurement was performed with an ultrasensitive method (Beckman Coulter, Inc., Fullerton CA) with a functional sensitivity of 0.1 ng/mL while TgAb assay used was the same during all the follow-up period.*

Paraffin embedded slices of the primary tumor tissue and cytological smears of the metastatic lung lesion were used for

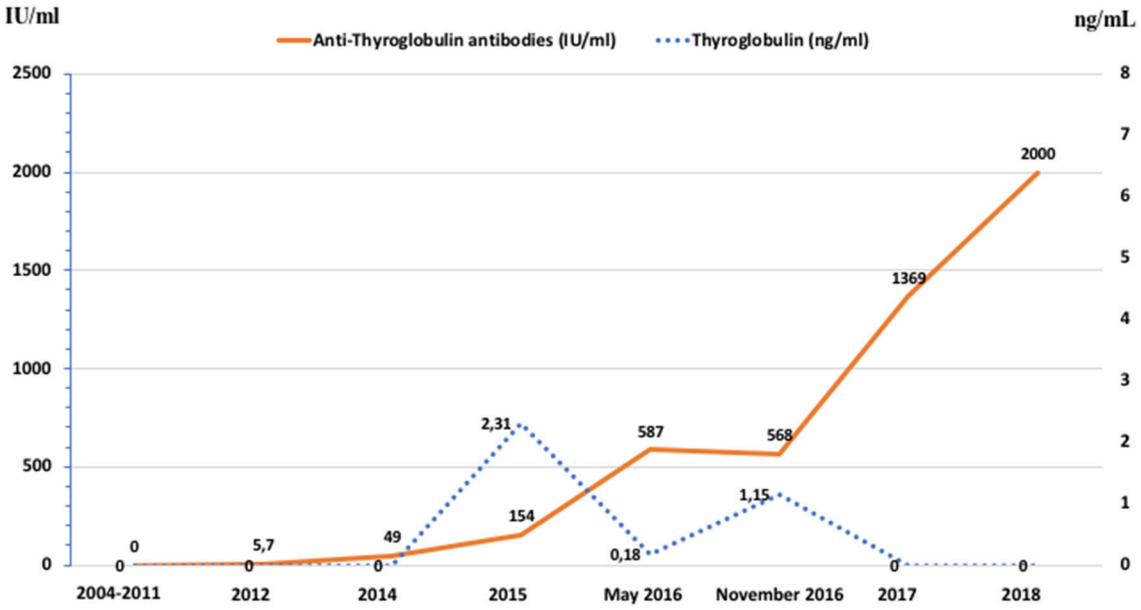


FIGURE 3 | Graphic representation of the patient's serum Tg values and TgAb titer between 2004 and 2018.

the DNA extraction that was targeted sequenced with a next generation sequencing system (Ion S5 deep sequencer, Ion Torrent, Applied Biosystem) by using a custom panel designed to analyze all thyroid related oncogene mutations. The analysis showed the presence of BRAF *V600E* mutation with an allelic frequency of 18 and 27% in the lung and primary tumor tissue, respectively. No other alterations were found with this analysis.

According to the Hospital rules, the patient signed an informed consent for the use of her clinical data and biological specimens for research purposes and publication of this case report; the study was approved by the Internal Review Board.

DISCUSSION

Papillary thyroid carcinoma is a lymphotropic tumor, especially in young patients, which shows a high lymphatic spread at diagnosis while distant metastases are quite rare and account for <5% of all TC patients, including other histotype (5). The most frequent sites of PTC metastatization, excluding lymph nodes and in order of frequency, are lungs, bone, and liver. Although the presence of a persistent disease after initial treatment, either biochemical and/or structural, has been reported up to 20–30% in different series of DTC, structural recurrent disease (i.e., the reappearance of disease after a period of documented cure) is not so frequent and it is present in about 1–2% of cases (18, 19). Disease recurrence rate depends mainly on tumor histology, gender, patient's age, and stage of the disease at diagnosis. This risk is very well-predicted by the risk of recurrence categories (i.e., low, intermediate and high) described in the American Thyroid Association guidelines (17). The periodical monitoring of serum Tg allows to perform an early diagnosis and the cure of these rare cases. Although the first 5–10 years after the initial treatment are those with the highest rate of recurrence, a lifelong follow-up is suggested because of the late onset recurrences (4).

In about 20–25% of PTC an autoimmune thyroiditis with circulating TgAb and/or TPOAb, is associated to the tumor (20). In these cases, the follow-up requires the concurrent measurement of Tg and TgAb because of the interference of the latter on the Tg assays and in many cases TgAb acquire the role of "Tg surrogate" marker (21, 22). However, although cases of PTC with a lymphocytic infiltration in the absence of concomitant serum autoimmunity are also described (21), no studies on the need to measure TgAb also during the follow-up of these patients have been reported so far and our case, although peculiar, testifies this need.

About 60% of PTC are positive for BRAF *V600E* mutation and its prognostic role for recurrence and/or mortality is still under debate (19, 23–26). Nevertheless, there are evidences that BRAF *V600E* positive cases have a lower degree of differentiation and, as a consequence, a lower ability to take up iodine (23, 27, 28). It is worth to note that this case was harboring a BRAF *V600E* mutation with a rather high allelic prevalence both in the primary and in the lung metastases. This finding can at least partially support the hypothesis that this mutation could be the driver of the recurrence and the cause of low uptake and response to radioiodine (19).

In conclusion, the description of this case is relevant not only because the recurrence with distant metastases in a PTC patient who achieved excellent response is exceptional but also because the way in which recurrent disease was diagnosed is unusual. In particular, this case underlines the importance to always measure serum TgAb along with Tg in

TC patients not only for the interference that the antibodies can determine on Tg measurement but also for their role as Tg surrogate marker. The case demonstrates that this is particularly relevant when histological lymphocytic infiltration is present even if serum TgAb are negative. In fact the appearance of the positivity of TgAb allowed the early diagnosis of disease recurrence and the appropriate treatment and follow-up the patient.

ETHICS STATEMENT

The study was approved by the patient and Internal Review Board.

AUTHOR CONTRIBUTIONS

RE obtained funding, DV and RE study concept, design and drafting of the manuscript, FL critical revision of the manuscript. DV, RE, LA, EM, VB, LL, FL, PV, and RE acquisition and interpretation of data, LT, LB, and TR technical and/or material support.

ACKNOWLEDGMENTS

This study was partially supported by the Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR, Investigator Grant 2015, PRIN 2015HPMLFY).

REFERENCES

1. Hayat MJ, Howlader N, Reichman ME, Edwards BK. Cancer statistics, trends, and multiple primary cancer analyses from the Surveillance, Epidemiology, and End Results (SEER) Program. *Oncologist* (2007) 12:20–37. doi: 10.1634/theoncologist.12-1-20
2. Elisei R, Pinchera A. Advances in the follow-up of differentiated or medullary thyroid cancer. *Nat Rev Endocrinol.* (2012) 8:466–75. doi: 10.1038/nrendo.2012.38
3. Elisei R, Molinaro E, Agate L, Bottici V, Masserini L, Ceccarelli C, et al. Are the clinical and pathological features of differentiated thyroid carcinoma really changed over the last 35 years? Study on 4187 patients from a single Italian institution to answer this question. *J Clin Endocrinol Metab.* (2010) 95:1516–27. doi: 10.1210/jc.2009-1536
4. Mazzaferri EL, Jhiang SM. Long-term impact of initial surgical and medical therapy on papillary and follicular thyroid cancer. *Am J Med.* (1994) 97:418–28. doi: 10.1016/0002-9343(94)90321-2
5. *SEER Cancer Statistics Review NCI*. Available online at: https://seer.cancer.gov/statfacts/html/thyro.html (2018).
6. 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) 83:1121–7.
7. Pacini F, Pinchera A. Serum and tissue thyroglobulin measurement: clinical applications in thyroid disease. *Biochimie* (1999) 81:463–7. doi: 10.1016/S0300-9084(99)80096-0
8. Spencer CA. Clinical review: clinical utility of thyroglobulin antibody (TgAb) measurements for patients with differentiated thyroid cancers (DTC). *J Clin Endocrinol Metab.* (2011) 96:3615–27. doi: 10.1210/jc.2011-1740
9. Spencer C, LoPresti J, Fatemi S. How sensitive (second-generation) thyroglobulin measurement is changing paradigms for monitoring patients with differentiated thyroid cancer, in the absence or presence of thyroglobulin autoantibodies. *Curr Opin Endocrinol Diabetes Obes.* (2014) 21:394–404. doi: 10.1097/MED.0000000000000092
10. Kim WG, Yoon JH, Kim WB, Kim TY, Kim EY, Kim JM, et al. Change of serum antithyroglobulin antibody levels is useful for prediction of clinical recurrence in thyroglobulin-negative patients with differentiated thyroid carcinoma. *J Clin Endocrinol Metab.* (2008) 93:4683–9. doi: 10.1210/jc.2008-0962
11. Chiovato L, Latrofa F, Braverman LE, Pacini F, Capezzone M, Masserini L, et al. Disappearance of humoral thyroid autoimmunity after complete removal of thyroid antigens. *Ann Inter Med.* (2003) 139(5 Pt 1):346–51. doi: 10.7326/0003-4819-139-5_Part_1-200309020-00010
12. Ozkan E, Soydal C, Araz M, Aras G, Ibis E. The additive clinical value of 18F-FDG PET/CT in defining the recurrence of disease in patients with differentiated thyroid cancer who have isolated increased antithyroglobulin antibody levels. *Clin Nucl Med.* (2012) 37:755–8. doi: 10.1097/RLU.0b013e31825ae77b
13. Ernaga-Lorea A, Hernandez-Morhain MC, Anda-Apinaniz E, Pineda-Arribas JJ, Migueliz-Bermejo I, Eguilaz-Esparza N, et al. Prognostic value of change in anti-thyroglobulin antibodies after thyroidectomy in patients with papillary thyroid carcinoma. *Clin Transl Oncol.* (2018) 20:740–4. doi: 10.1007/s12094-017-1782-3
14. Matrone A, Latrofa F, Torregrossa L, Piaggi P, Gambale C, Faranda A, et al. Changing trend of thyroglobulin antibodies in not 131I ablated differentiated thyroid cancer patients. *Thyroid* (2018) 28:871–9. doi: 10.1089/thy.2018.0080
15. Marcocci C, Vitti P, Cetani F, Catalano F, Concetti R, Pinchera A. Thyroid ultrasonography helps to identify patients with diffuse lymphocytic thyroiditis who are prone to develop hypothyroidism. *J Clin Endocrinol Metab.* (1991) 72:209–13. doi: 10.1210/jcem-72-1-209
16. Greene FL PD, Fleming ID, Fritz AG, Balch CM, Haller DG, Morrow M. *AJCC cancer staging handbook.* New York, NY: Springer (2002).
17. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid* (2016) 26:1–133. doi: 10.1089/thy.2015.0020
18. Grant CS. Recurrence of papillary thyroid cancer after optimized surgery. *Gland Surg.* (2015) 4:52–62. doi: 10.3978/j.issn.2227-684X.2014.12.06
19. Xing M, Alzahrani AS, Carson KA, Shong YK, Kim TY, Viola D, et al. Association between BRAF V600E mutation and recurrence of papillary thyroid cancer. *J Clin Oncol.* (2015) 33:42–50. doi: 10.1200/JCO.2014.56.8253
20. Pacini F, Mariotti S, Formica N, Elisei R, Anelli S, Capotorti E, et al. Thyroid autoantibodies in thyroid cancer: incidence and relationship with tumour outcome. *Acta Endocrinol.* (1988) 119:373–80. doi: 10.1530/acta.0.1190373
21. Latrofa F, Ricci D, Montanelli L, Rocchi R, Piaggi P, Sisti E, et al. Lymphocytic thyroiditis on histology correlates with serum thyroglobulin autoantibodies in patients with papillary thyroid carcinoma: impact on detection of serum thyroglobulin. *J Clin Endocrinol Metab.* (2012) 97:2380–7. doi: 10.1210/jc.2011-2812
22. Latrofa F, Ricci D, Sisti E, Piaggi P, Nencetti C, Marino M, et al. Significance of low levels of thyroglobulin autoantibodies associated with undetectable thyroglobulin after thyroidectomy for differentiated thyroid carcinoma. *Thyroid* (2016) 26:798–806. doi: 10.1089/thy.2015.0621
23. Cancer Genome Atlas Research N. Integrated genomic characterization of papillary thyroid carcinoma. *Cell* (2014) 159:676–90. doi: 10.1016/j.cell.2014.09.050
24. Elisei R, Ugolini C, Viola D, Lupi C, Biagini A, Giannini R, et al. BRAF(V600E) mutation and outcome of patients with papillary thyroid carcinoma: a 15-year median follow-up study. *J Clin Endocrinol Metab.* (2008) 93:3943–9. doi: 10.1210/jc.2008-0607
25. Xing M, Alzahrani AS, Carson KA, Viola D, Elisei R, Bendlova B, et al. Association between BRAF V600E mutation and mortality in patients with papillary thyroid cancer. *JAMA* (2013) 309:1493–501. doi: 10.1001/jama.2013.3190

26. Trovisco V, Soares P, Preto A, de Castro IV, Lima J, Castro P, et al. Type and prevalence of BRAF mutations are closely associated with papillary thyroid carcinoma histotype and patients' age but not with tumour aggressiveness. *Virchows Arch.* (2005) 446:589–95. doi: 10.1007/s00428-005-1236-0
27. Riesco-Eizaguirre G, Gutierrez-Martinez P, Garcia-Cabezas MA, Nistal M, Santisteban P. The oncogene BRAF V600E is associated with a high risk of recurrence and less differentiated papillary thyroid carcinoma due to the impairment of Na+/I- targeting to the membrane. *Endocr Relat Cancer* (2006) 13:257–69. doi: 10.1677/erc.1.01119
28. Romei C, Ciampi R, Faviana P, Agate L, Molinaro E, Bottici V, et al. BRAFV600E mutation, but not RET/PTC rearrangements, is correlated with a lower expression of both thyroperoxidase and sodium iodide symporter genes in papillary thyroid cancer. *Endocr Relat Cancer* (2008) 15:511–20. doi: 10.1677/ERC-07-0130

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 09 October 2018
doi: 10.3389/fendo.2018.00591

Elevated Interleukin-36α And $CD4^+IL\text{-}36\alpha^+$T Cells Are Involved in the Pathogenesis of Graves' Disease

*Qiu-ming Yao[1], Ling Li[1], Zhen-Yu Song[2], Bin Wang[1], Qiu Qin[1], Xiao-fei An[3] and Jin-an Zhang[1]**

[1] Department of Endocrinology, Jinshan Hospital of Fudan University, Shanghai, China, [2] Department of Urology, Jinshan Hospital of Fudan University, Shanghai, China, [3] Department of Endocrinology, The Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, China

Background: IL-36α is involved in the pathogenesis of a variety of autoimmune diseases, but the relationship between IL-36α and Graves' disease (GD) has rarely investigated. In the present study, we aimed to explore the expression of IL-36α and elucidate the potential role of IL-36α in GD.

Methods: The expression of IL-36α mRNA in peripheral blood mononuclear cells (PBMCs) from 32 newly diagnosed GD patients, 15 refractory GD patients and 30 normal controls (NC) was examined using quantitative real-time polymerase chain reaction (qRT-PCR). The level of IL-36α in serum from 46 newly diagnosed GD patients, 10 refractory GD patients and 24 NC was measured using enzyme linked immunosorbent assay (ELISA). The percentage of $CD4^+IL\text{-}36\alpha^+$T cells was detected by flow cytometry. PBMCs from newly diagnosed GD patients and NC group were cultured in the presence or absence of recombinant human IL-36α, and the expression levels of IFN-γ, TNF-α, IL-6, and IL-17A in culture supernatant were detected by cytokine array.

Results: The expression of IL-36α mRNA in newly diagnosed GD patients was significantly higher than that in NC group ($P = 0.019$). IL-36α mRNA expression was positively associated with thyrotropin receptor antibody (TRAb) ($P = 0.004$, $r = 0.498$) in newly diagnosed GD patients. The level of IL-36α in serum from newly diagnosed GD patients was significantly higher than that in refractory GD patients and NC group ($P = 0.01$; $P = 0.007$). The percentage of $CD4^+IL\text{-}36\alpha^+$T cells in newly diagnosed GD patients was significantly higher than that in NC group ($P = 0.030$). In GD group, recombinant human IL-36α stimulation resulted in the increase of INF-γ, TNF-α, IL-6 and IL-17A ($P = 0.015$; $P = 0.016$; $P = 0.039$; $P = 0.017$).

Conclusion: IL-36α and $CD4^+IL\text{-}36\alpha^+$T cells may be involved in the pathogenesis of GD by promoting the production of Th1, Th2, and Th17 cytokines.

Keywords: graves' disease, interleukin-36α, $CD4^+IL\text{-}36\alpha^+$T cells, thyroid, cytokines

OPEN ACCESS

Edited by:
Giampaolo Papi, Azienda Unità Sanitaria Locale di Modena, Italy

Reviewed by:
Takao Ando, Nagasaki University Hospital, Japan
Rosa Maria Paragliola, Università Cattolica del Sacro Cuore, Italy

****Correspondence:***
Jin-an Zhang
zhangjinan@hotmail.com

Specialty section:
This article was submitted to Thyroid Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *10 July 2018*
Accepted: *18 September 2018*
Published: *09 October 2018*

Citation:
Yao Q, Li L, Song Z-Y, Wang B, Qin Q, An X and Zhang J (2018) Elevated Interleukin-36α And $CD4^+IL\text{-}36\alpha^+$T Cells Are Involved in the Pathogenesis of Graves' Disease. Front. Endocrinol. 9:591. doi: 10.3389/fendo.2018.00591

INTRODUCTION

Graves' disease (GD), also known as the diffuse toxic goiter, is a common subtype of autoimmune thyroid disease (AITD). As an organ-specific autoimmune disease, GD is characterized by hyperthyroidism and a large amount of thyrotropin receptor antibody (TRAb) in serum. GD is the most common cause of hyperthyroidism, and its annual incidence is approximately 20–50/100,000 (1). In addition to hyperthyroidism syndrome, GD may also accompany some clinical manifestations outside thyroid, mainly including ophthalmopathy, pretibial myxedema and clubbing. GD can be observed in any age, but more frequently encountered in women at the productive age. The risk ratio of GD between male and female is about 1: 6 (2). Previous studies from our and other research teams have documented that the interruption of classical Th1/Th2 cell balance, and newly defined Th17/Treg cell balance, as well as newly discovered lymphocytes such as Th22 cells and follicular helper T (Tfh) cells are involved in the development of GD (3–8), but so far, the specific pathogenesis of GD is still unclear.

IL-36α is a new member of the IL-1 family (9), also known as IL-36A or IL-1F6. IL-36α, IL-36β (IL-1F8) and IL-36γ (IL-1F9) are collectively referred to as IL-36 due to the same biological function (10). IL-36α can execute pro-inflammatory and immunomodulatory functions through binding its heterodimeric receptors consisting of IL-36R and IL-1 receptor accessory protein (IL-1R/AcP), and activating mitogen-activated protein kinase (MAPK) and transcription factor NF-kB signaling cascades (11). As a kind of pro-inflammatory factor, IL-36 is expressed in many tissues, with the most notably expression in skin, esophagus, tonsils, lungs, bowel, and brain; in addition, IL-36 can also be expressed in immune cells, including monocytes, macrophages and T cells (12). Because IL-36R expressed in human monocytes, IL-36 can stimulate human monocyte-derived dendritic cells (MDC) to produce a variety of pro-inflammatory cytokines, including IL-1, IL-6 IL-12, IL-12, and IL-18, and can also enhance the expression of CD83 and MHC-II on the cell surface, thus promoting MDC maturation; vice versa, under the stimulation of IL-36, MDC can promote T cell proliferation and IFN-γ production (13, 14). It is found that IL-36α and IL-36β can up-regulate the production of pro-inflammatory cytokines such as TNF-α and IL-17; in turn, TNF-α, IL-17 and IL-22 can also stimulate the expression of IL-36 (15). IL-36 may not only act solely on naive $CD4^+$ T cells and promote its proliferation and IL-2 expression, but also may execute a synergistic effect on Th1 polarization when combined with IL-12 (16).

Previous studies have confirmed that IL-36α is involved in the pathogenesis of a variety of autoimmune diseases such as rheumatoid arthritis, Sjogren's syndrome, inflammatory bowel disease, and so on, but the relationship between IL-36α and GD has rarely been investigated. Therefore, in the present study, the possible role of IL-36α and underlying mechanism in the pathogenesis of GD were explored.

MATERIALS AND METHODS

Subjects

In the present study, all GD samples were collected from the Department of Endocrinology, Jinshan Hospital of Fudan University and used as the case group, the normal controls with matched sex and age of the case group were selected from the Physical Examination Center of the same hospital. All subjects included in this study signed informed consent. In this study, 134 GD patients and 73 normal controls (NC) were included. As shown in **Supplementary Table 1**, among them, 32 newly diagnosed GD patients (7 males and 25 females, 37.6 ± 13.7 years old), 15 refractory GD patients (3 males and 12 females, 33.6 ± 11.1 years old), and 30 normal controls (8 males and 22 females, 35.2 ± 12.3 years old) were collected for quantitative real-time polymerase chain reaction (qRT-PCR); 46 newly diagnosed GD patients (15 males and 31 females, 43.8 ± 12.4 years old), 10 refractory GD patients (5 males and 5 females, 41.3 ± 9.4 years old) and 24 normal controls (8 males and 16 females, 40.7 ± 9.8 years old) were collected for ELISA; 19 newly diagnosed GD patients (3 males and 16 females, 41.0 ± 9.2 years old) and 10 normal controls (2 males and 8 females, 38.2 ± 6.0 years old) were collected for flow cytometry. Twelve patients with newly diagnosed GD (4 males and 8 females, 36.7 ± 11.6 years old) and 9 normal controls (2 males and 7 females, 35.3 ± 9.0 years old) were collected for cell stimulation. Newly diagnosed GD also known as newly onset GD patients, were the first to be diagnosed as GD without drug therapy; refractory GD patients were treated with anti-thyroid drugs for at least 4 years and still positive for thyrotropin receptor antibody (TRAb) (17). Thyroid function test and TRAb levels of all subjects were summarized in **Supplementary Table 1**. This project was approved of by the Ethics Committee of Jinshan Hospital of Fudan University.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Peripheral blood monocytes (PBMCs) were separated from blood using Lymphoprep density gradient centrifugation (TianGen Biotech, China). Total RNA was extracted from PBMCs using Trizol reagent (Invitrogen, USA) according to the manufacturer's protocol. Then, the concentration of RNA was determined and 1 μg of RNA was used to synthesize cDNA by reverse transcription kit (TaKaRa, Japan). The qRT-PCR was performed in ABI PRISM 7300 Fast Real-Time PCR system (BIO-RAD) using SYBR Premix Ex TaqTM II (Perfect Real Time) (TaKaRa, Japan). The primer sequences were ATC AAT CAT CGG GTG TGG as the forward primer and AAG GCA ATA GTG ACT GGA GAC as the reversed primer for IL-36α; CAT TGC CGA CAG GAT GCA G as the forward primer and CTC GTC ATA CTC CTG CTT GCT G as the reversed primer for β-actin.

Serum IL-36α Assay

Serum samples were collected from 2 mL of EDTA-containing whole blood. After centrifuged at 4,000 rpm for 10 min, supernatants were obtained and then centrifuged at 13,000 rpm for 2 min. Serum samples were obtained and

stored at −80°C. The concentration of IL-36α in serum was determined using commercial sandwich ELISA kits (CUSABIO, Wuhan, China) according to the manufacturer's instructions.

Flow Cytometry

PBMCs were stimulated with 2 μL mixture (BD Bioscience, USA) containing phorbol myristate acetate, ionomycin and monensin at 37°C and 5% CO_2 for 4 h. Then, the cells were stained with anti-human CD4-APC at 4°C under light-free environment for 30 min. Fixation and permeabilization were conducted with a Cytofix/Cytoperm kit (BD Biosciences, USA). Then, the cells were incubated with primary antibody anti-human IL-36α (Lifespan Bioscience, USA) at 4°C for 30 min under light-free environment. The cells were sequentially washed with 1 mL BD Perm/Wash™ buffer (1×) (BD Bioscience, USA), and stained with secondary antibody goat anti-rabbit IgG-FITC (Santa, USA) at room temperature for 30 min. Finally, FACScalibur Flow cytometer (Beckman coulter) was used to analyze the stained cells ($CD4^+IL\text{-}36\alpha^+T$ cells) immediately.

Cell Culture

Like the previously published researches (7, 18), the separated PBMCs from GD patients or controls were divided into two equal parts (1∼2× 10^6 /ml). Both of them were seeded into culture dishes with a diameter of 35 mm. One was incubated with recombinant human IL-2 (50 ng/ml) (PeproTech, USA) and 100 U/ml penicillin/streptomycin (Hyclone, USA) in the presence of recombinant human IL-36α (100 ng/ml) (R&D system, USA) at a 37°C incubator supplemented with 5% CO2. Another was incubated only with recombinant human IL-2 (50 ng/ml) (PeproTech, USA) and served as the negative control. After 24 h, we harvested the cultured supernatants of the both and stored them at −80°C. The levels of T-cell-derived cytokines including IFN-γ, TNF-α, IL-6 and IL-17A in supernatants were determined by Human High Sensitivity T Cell Magnetic Bead Panel (Merck Millipore, Germany) according to the manufacturer's instructions.

Statistical Analysis

All data are reported as mean ± standard deviation (M ± SD). SPSS 17.0 was used for statistical analysis. The comparison of multiple groups was conducted through the one-way ANOVA. Comparison between two groups was performed by using two-independent-sample T test, or nonparametric test. For the correlation of two variables, the non-parametric spearman's test was conducted. Comparison of cytokine levels in supernatant of PBMCs between stimulation with recombinant IL-36α combined with IL-2 and IL-2 only was analyzed using paired T test. The $2^{-\Delta\Delta CT}$ method was used to analyze the data of RT-PCR. Data of the levels of IFN-γ, IL-6 and TNF-α were log10 transformed. $P < 0.05$ was considered to be statistically significant difference.

RESULTS

The Expression of IL-36α mRNA in PBMCs

As shown in **Figure 1A**, the expression of IL-36α mRNA in newly onset GD patients was significant higher than that in NC group ($P = 0.019$). There was no significant difference between refractory GD and newly onset GD as well as NC ($P > 0.05$). Correlation analysis showed that the expression of IL-36α mRNA was positively correlated with TRAb ($P = 0.004$, $r = 0.498$, **Figure 1B**) in newly onset GD patients. In refractory GD patients, the expression of IL-36α mRNA was not significantly correlated with free triiodothyronine (FT3), free thyroxine (FT4) and TRAb ($P > 0.05$) (data not shown).

Serum Levels of IL-36α

As shown in **Figure 2**, the concentration of IL-36α in newly onset GD patients was significantly higher than that of refractory GD patients and NC group ($P = 0.010$; $P = 0.007$). There was no significant difference in serum IL-36α concentration between refractory GD patients and NC group ($P = 0.406$). In newly diagnosed GD group and refractory GD group, there was no significant correlation between IL-36α concentration and FT3, FT4 and TRAb ($P > 0.05$).

Frequency of $CD4^+IL\text{-}36\alpha^+T$ Cells in PBMCs

Flow cytometric analysis revealed that the percentage of $CD4^+IL\text{-}36\alpha^+T$ cells in GD group was significantly higher than that in NC group ($P = 0.030$, **Figure 3**), but the percentage of

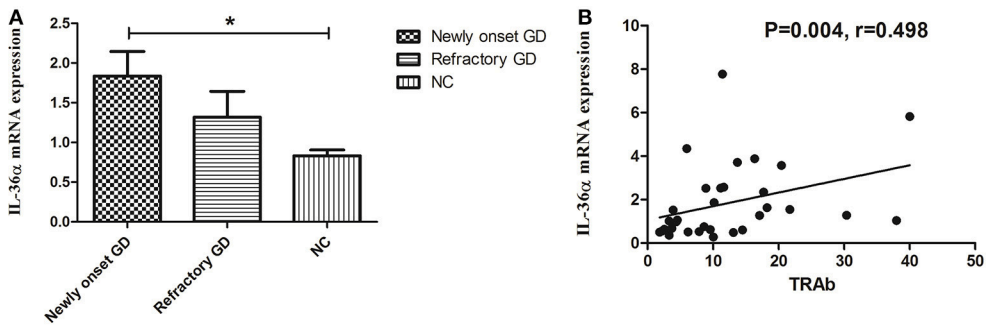


FIGURE 1 | The expression of IL-36α mRNAs in GD patients **(A)**. IL-36α mRNA expression in newly onset GD patients was significantly higher than that in NC group; **(B)**. Correlation analysis of IL-36α mRNA expression and TRAb. IL-36α mRNA expression in newly onset GD patients was positively correlated TRAb. *$P < 0.05$.

CD4+IL-36α+T cells was not correlated with FT3, FT4 and TRAb ($P > 0.05$).

The Expression of Cytokines in Cultured PBMCs After Recombinant Human IL-36α Stimulation

As shown in **Figure 4**, in supernatant of PBMCs from newly onset GD patients, recombinant human IL-36α stimulation resulted in the increase of INF-γ, IL-6, IL-17A, and TNF-α (2.4 ± 1.2 pg/mL vs. 2.7 ±1.1 pg/mL, $P = 0.015$; 3.3 ± 0.5 pg/mL vs. 3.5 ± 0.3 pg/mL, $P = 0.039$; 24.2 ± 11.0 pg/mL vs. 28.2 ± 11.0 pg/mL, $P = 0.017$; 2.6 ± 0.9 pg/mL vs. 2.9 ± 0.7 pg/mL, $P = 0.016$, respectively). While the levels of INF-γ, IL-6, IL-17A, and TNF-α in PBMCs from NC group were unaffected by the stimulation of recombinant human IL-36α ($P > 0.05$).

DISCUSSION

The dysfunction of immune cells is a key factor in the development of GD. In recent years, the studies on immune cells and their corresponding cytokines have become a hotspot in the field of GD pathogenesis. Previous reports have demonstrated that Th1 and Th17 cells and their cytokines are abnormally expressed in autoimmune thyroid disease (19). Compared with the normal control group, the expression of Th2 cell-related cytokines such as IL-4 and IL-10 is significantly increased in GD patients (3). Similarly, protein concentrations of Th1-related cytokines such as IL-12 and IL-18 in GD patients are significantly higher than that in the normal controls (8). The percentage of Th17 cells in peripheral blood of patients with intractable GD is significantly higher than that in patients at the remission state (4). It is also confirmed that the proportion of $CD4^+CD25^+Foxp3^+$Treg cells in the untreated GD group is significantly lower than that in the normal group and negatively correlated with the autoantibody concentration against thyroid-stimulating hormone receptor (TSHR) (5). The proportion of follicular helper T (Tfh) cells in the peripheral blood of GD patients is significantly increased, and the number of Tfh cells is positively correlated with FT3 and FT4. The proportion of circulating Tfh cells is decreased after anti-thyroid drug treatment in follow-up duration (6). Moreover, the expression of IL-22 mRNA, the concentration of serum IL-22 and the percentage of Th22 cells in PBMCs from GD patients are significantly higher than those in the normal control group (7). All those data suggest that various immune cells and cytokines may be involved in the development of GD.

IL-36α is a new member of IL-1 family (IL-1F) located on IL-1F gene cluster of human chromosome 2 (9). The IL-1 family includes seven agonists or pro-inflammatory factors (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, and IL-36r) and four inhibitors or anti-inflammatory factors (IL-1Ra, IL-36Ra, IL-37 and IL-38) (20, 21). IL-36α and IL-IRa have 24% homology (22). IL-36α can induce the production of pro-inflammatory cytokines, chemokines and costimulatory molecules, and then accomplish the recruitment of neutrophils, the activation of dendritic cells and the polarization of Th1 cells (16, 23).

The abnormal expression of IL-36α has been found in a series of autoimmune inflammatory diseases such as psoriasis, rheumatoid arthritis, and Crohn's disease (24). IL-36RN is a gene encoding the native inhibitor for IL-36, and is deficient in severe pustular psoriasis (25). IL-36α can up-regulate the expression of IL-17A, IL-23, and tumor necrosis factor (TNF-α) in psoriasis arthritis (PsA) (26). The expression of IL-36α in the lesion skin is significantly higher than that in the normal

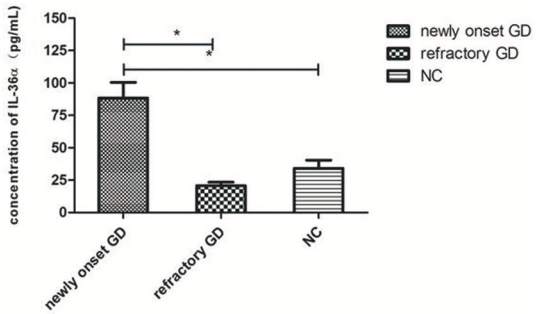


FIGURE 2 | Serum IL-36α levels of GD patients (including newly onset GD, refractory patients) and healthy controls. *$P < 0.05$.

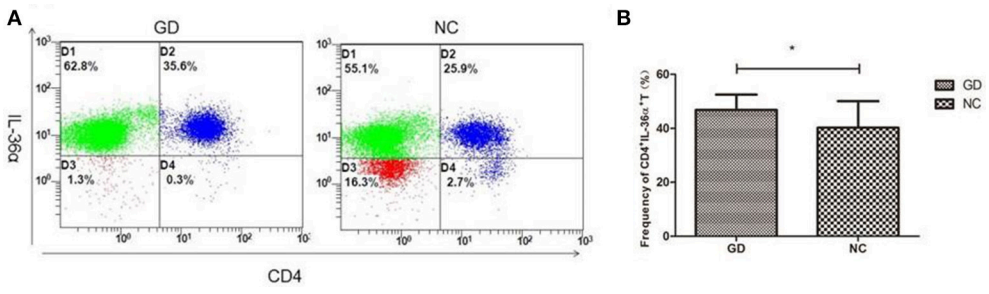


FIGURE 3 | Flow cytometry analysis detected $CD4^+$IL-$36\alpha^+$ T cells. **(A)** Representative flow cytometry data showing the expression of $CD4^+$IL-$36\alpha^+$ T cells in newly onset GD patients and NC group. **(B)** The frequency of $CD4^+$IL-$36\alpha^+$ T cells in newly onset GD patients and controls. *$P < 0.05$.

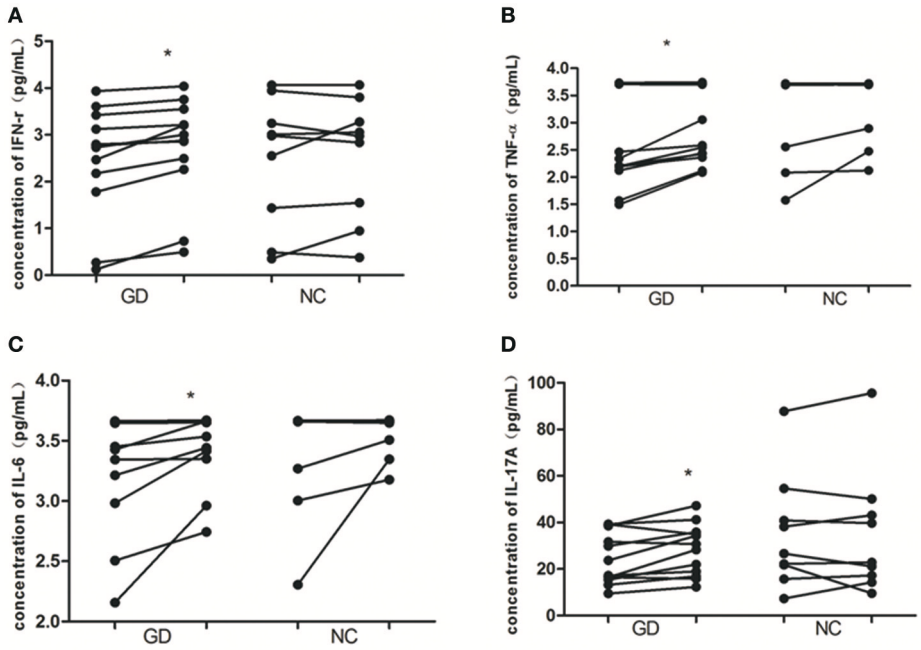


FIGURE 4 | IL-36α promotes the expression of inflammatory cytokines in PBMCs of patients with GD. In GD patients, the levels of IFN-γ **(A)**, TNF-α **(B)**, IL-6 **(C)**, and IL-17A **(D)** were increased in supernatant of PBMCs stimulated with human recombinant IL-36α combined with IL-2 than that of supernatant of PBMCs stimulated with IL-2 only ($P = 0.015$, $P = 0.016$, $P = 0.039$, $P = 0.017$, respectively). For these cytokines in supernatant of PBMCs from NC, no significant difference was found between stimulation with recombinant human IL-36α combined with IL-2 and IL-2 only. Each symbol represents an individual patient or healthy control. In GD patients or NC group, the points on the left column represent the levels of cytokines after stimulation with IL-2 only; the points on the right column represent the levels of cytokines after stimulation with IL36 in combination with IL-2. $^*P < 0.05$.

skin of psoriasis (27). IL-36 can also induce the secretion of IL-6 and IL-8 in the culture of synovial cells (28). The increased level of IL-36α in serum from patients with rheumatoid arthritis is positively correlated with C-reactive protein (CRP) (29). The elevated expression of IL-36α in patients with Sjogren's syndrome is positively associated with the expression of IL-22, IL-17 and IL-23p19 in the lip gland tissues (30). The higher level of IL-36α in serum of patients with systemic lupus erythematosus (SLE) is also positively correlated with disease procession (31). Upon the stimulation with recombinant IL-36α and IL-36γ, the concentrations of IL-6 and CXCL8 in the culture of PBMCs are significantly increased when compared with NC (31).

In the present study, the expression of IL-36α in PBMCs and serum of newly diagnosed GD patients was significantly higher than that of NC group. This is consistent with the results of previous studies on the association of IL-36 with Sjogren's syndrome (30) and SLE (31). Correlation analysis showed that the expression of IL-36α mRNA in newly diagnosed GD patients was positively correlated with TRAb, suggesting that IL-36α was involved in the development of GD and correlated with the severity of the disease. There was no significant difference in IL-36α mRNA in PBMCs and level of IL-36α in serum between refractory GD patients and NC group. The reason may be that refractory GD patients take drugs for a long time, thereby the expression of IL-36α was inhibited to some extent. Flow cytometric assay showed that the percentage of $CD4^+IL\text{-}36\alpha^+$ T cells in PBMCs from newly onset GD patients was higher than that in normal controls, implicating that $CD4^+$ T cells are one of the major cells for the production of IL-36α in GD. The up-regulation of IL-36α in serum from newly diagnosed GD patients may be associated with an increase in the percentage of $CD4^+$T cells secreting IL-36α in PBMCs.

The PBMCs stimulation test showed that the expression of IFN-γ, TNF-α, IL-6, and IL-17A in the supernatant of PBMCs from GD patients was significantly increased after stimulation with human recombinant IL-36α, while the concentrations of above cytokines in the culture supernatant did not reveal a significant change in NC group. These results suggest that IL-36α may participate in the process of immune disorder in GD by acting on Th1, Th2, and Th17 cells and inducing the secretion of inflammatory cytokines. However, our results were not consistent with the results from a previous study about IL-36α in SLE with the only elevated level of IL-6, instead of IFN-γ and IL-17A (31), which may be related to IL-36α concentration, detection kit and cell population because the target cells and the intensity of IL-36α are different in different autoimmune inflammatory diseases.

In summary, the pro-inflammatory factor IL-36α is involved in GD pathogenesis, which is beneficial to elucidate the pathogenic mechanisms of GD and to develop a new immune-specific therapy.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of human participants, Ethics Committee of the Jinshan Hospital of Fudan University with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Committee of the Jinshan Hospital of Fudan University committee.

AUTHOR CONTRIBUTIONS

QY carried out the work, conducted the data analysis and wrote the manuscript. LL, Z-YS, BW, QQ, XA helped with the collection of specimens. JZ revised the manuscript.

FUNDING

This study was supported by the National Natural Science Foundation of China (81471004 and 81670722) and the Key Discipline Development of Shanghai Jinshan District (No. JSZK2015A02).

ACKNOWLEDGMENTS

The authors would like to thank all participants in the study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2018.00591/full#supplementary-material

REFERENCES

1. Zimmermann MB, Boelaert K. Iodine deficiency and thyroid disorders. *Lancet Diabetes Endocrinol*. (2015) 3:286–295. doi: 10.1016/S2213-8587(14)70225-6
2. Smith TJ, Hegedus L. Graves' disease. *N Engl J Med*. (2016) 375:1552–65. doi: 10.1056/NEJMra1510030
3. Kocjan T, Wraber B, Repnik U, Hojker S. Changes in Th1/Th2 cytokine balance in Graves' disease. *Pflugers Arch*. (2000) 440(5 Suppl):R94–5.
4. Nanba T, Watanabe M, Inoue N, Iwatani Y. Increases of the Th1/Th2 cell ratio in severe Hashimoto's disease and in the proportion of Th17 cells in intractable Graves' disease. *Thyroid* (2009) 19:495–501. doi: 10.1089/thy.2008.0423
5. Mao C, Wang S, Xiao Y, Xu J, Jiang Q, Jin M, et al. Impairment of regulatory capacity of CD4+CD25+ regulatory T cells mediated by dendritic cell polarization and hyperthyroidism in Graves' disease. *J Immunol*. (2011) 186:4734–43. doi: 10.4049/jimmunol.0904135
6. Zhu C, Ma J, Liu Y, Tong J, Tian J, Chen J, et al. Increased frequency of follicular helper T cells in patients with autoimmune thyroid disease. *J Clin Endocrinol Metab*. (2012) 97:943–50. doi: 10.1210/jc.2011-2003
7. Song RH, Yu ZY, Qin Q, Wang X, Muhali FS, Shi LF, et al. Different levels of circulating Th22 cell and its related molecules in Graves' disease and Hashimoto's thyroiditis. *Int J Clin Exp Pathol*. (2014) 7:4024–31.
8. Miyauchi S, Matsuura B, Onji M. Increased levels of serum interleukin-18 in Graves' disease. *Thyroid* (2000) 10:815–9. doi: 10.1089/thy.2000.10.815
9. Dunn E, Sims JE, Nicklin MJ, O'Neill LA. Annotating genes with potential roles in the immune system: six new members of the IL-1 family. *Trends Immunol*. (2001) 22:533–6. doi: 10.1016/S1471-4906(01)02034-8
10. Dinarello C, Arend W, Sims J, Smith D, Blumberg H, O'Neill L, et al. IL-1 family nomenclature. *Nat Immunol*. (2010) 11:973. doi: 10.1038/ni1110-973
11. Towne JE, Garka KE, Renshaw BR, Virca GD, Sims JE. Interleukin (IL)-1F6, .IL-1F8, and IL-1F9 signal through IL-1Rrp2 and IL-1RAcP to activate the pathway leading to NF-kappaB and MAPKs. *J Biol Chem*. (2004) 279:13677–88. doi: 10.1074/jbc.M400117200
12. Gresnigt MS, Rosler B, Jacobs CW, Becker KL, Joosten LA, van der Meer JW, et al. The IL-36 receptor pathway regulates *Aspergillus fumigatus*-induced Th1 and Th17 responses. *Eur J Immunol*. (2013) 43:416–26. doi: 10.1002/eji.201242711
13. Mutamba S, Allison A, Mahida Y, Barrow P, Foster N. Expression of IL-1Rrp2 by human myelomonocytic cells is unique to DCs and facilitates DC maturation by IL-1F8 and IL-1F9. *Eur J Immunol*. (2012) 42:607–17. doi: 10.1002/eji.201142035
14. Foster AM, Baliwag J, Chen CS, Guzman AM, Stoll SW, Gudjonsson JE, et al. IL-36 promotes myeloid cell infiltration, activation, and inflammatory activity in skin. *J Immunol*. (2014) 192:6053–61. doi: 10.4049/jimmunol.1301481
15. Carrier Y, Ma HL, Ramon HE, Napierata L, Small C, O'Toole M, et al. Inter-regulation of Th17 cytokines and the IL-36 cytokines *in vitro* and *in vivo*: implications in psoriasis pathogenesis. *J Invest Dermatol*. (2011) 131:2428–37. doi: 10.1038/jid.2011.234
16. Vigne S, Palmer G, Martin P, Lamacchia C, Strebel D, Rodriguez E,et al. IL-36 signaling amplifies Th1 responses by enhancing proliferation and Th1 polarization of naive CD4+ T cells. *Blood* (2012) 120:3478–87. doi: 10.1182/blood-2012-06-439026
17. Yasuda T, Okamoto Y, Hamada N, Miyashita K, Takahara M, Sakamoto F, et al. Serum vitamin D levels are decreased in patients without remission of Graves' disease. *Endocrine* (2013) 43:230–2. doi: 10.1007/s12020-012-9789-6
18. Guan LJ, Wang X, Meng S, Shi LF, Jiang WJ, Xiao L, et al. Increased IL-21/IL-21R expression and its proinflammatory effects in autoimmune thyroid disease. *Cytokine* (2015) 72:160–5. doi: 10.1016/j.cyto.2014.11.005
19. Bossowski A, Moniuszko M, Idzkowska E, Dabrowska M, Jeznach M, Sawicka B, et al. Evaluation of CD4+CD161+CD196+ and CD4+IL-17+ Th17 cells in the peripheral blood of young patients with Hashimoto's thyroiditis and Graves' disease. *Pediatr Endocrinol Diabetes Metab*. (2012) 18:89–95.
20. Gabay C, Lamacchia C, Palmer G. IL-1 pathways in inflammation and human diseases. *Nat Rev Rheumatol*. (2010) 6:232–41. doi: 10.1038/nrrheum.2010.4
21. Garlanda C, Dinarello CA, Mantovani A. The interleukin-1 family: back to the future. *Immunity* (2013) 39:1003–18. doi: 10.1016/j.immuni.2013.11.010
22. Gresnigt MS, van de Veerdonk FL. Biology of IL-36 cytokines and their role in disease. *Semin Immunol*. (2013) 25:458–65. doi: 10.1016/j.smim.2013.11.003
23. Gabay C, Towne JE. Regulation and function of interleukin-36 cytokines in homeostasis and pathological conditions. *J Leukoc Biol*. (2015) 97:645–52. doi: 10.1189/jlb.3RI1014-495R
24. Boutet MA, Bart G, Penhoat M, Amiaud J, Brulin B, Charrier C, et al. Distinct expression of interleukin (IL)-36alpha, beta and gamma, their antagonist IL-36Ra and IL-38 in psoriasis, rheumatoid arthritis, and Crohn's disease. *Clin Exp Immunol*. (2016) 184:159–73. doi: 10.1111/cei.12761
25. Marrakchi S, Guigue P, Renshaw BR, Puel A, Pei XY, Fraitag S, et al. Interleukin-36-receptor antagonist deficiency and generalized pustular psoriasis. *N Engl J Med*. (2011) 365:620–8. doi: 10.1056/NEJMoa1013068
26. Blumberg H, Dinh H, Dean CJ, Trueblood ES, Bailey K, Shows D, et al. IL-1RL2 and its ligands contribute to the cytokine network in psoriasis. *J Immunol*. (2010) 185:4354–62. doi: 10.4049/jimmunol.1000313
27. Keermann M, Koks S, Reimann E, Abram K, Erm T, Silm H, et al. Expression of IL-36 family cytokines and IL-37 but not IL-38 is altered in psoriatic skin. *J Dermatol Sci*. (2015) 80:150–2. doi: 10.1016/j.jdermsci.2015.08.002
28. Frey S, Derer A, Messbacher ME, Baeten DL, Bugatti S, Montecucco C, et al. The novel cytokine interleukin-36alpha is expressed in psoriatic and rheumatoid arthritis synovium. *Ann Rheum Dis*. (2013) 72:1569–74. doi: 10.1136/annrheumdis-2012-202264

29. Wang M, Wang B, Ma Z, Sun X, Tang Y, Li X, et al. Detection of the novel IL-1 family cytokines by QAH-IL1F-1 assay in rheumatoid arthritis. *Cell Mol Biol.* (2016) 62:31–4.
30. Ciccia F, Accardo-Palumbo A, Alessandro R, Alessandri C, Priori R, Guggino G, et al. Interleukin-36alpha axis is modulated in patients with primary Sjogren's syndrome. *Clin Exp Immunol.* (2015) 181:230–8. doi: 10.1111/cei.12644
31. Chu M, Wong CK, Cai Z, Dong J, Jiao D, Kam NW, et al. Elevated expression and Pro-Inflammatory activity of IL-36 in patients with systemic lupus erythematosus. *Molecules* (2015) 20:19588–604. doi: 10.3390/molecules201019588

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

CASE REPORT
published: 01 October 2018
doi: 10.3389/fendo.2018.00577

Primary Epithelioid Angiosarcoma of the Thyroid in a Patient Occupationally Exposed to Radiations

*Michela Marina[1], Luigi Corcione[2], Maria Francesca Serra[1], Teore Ferri[3], Enrico Maria Silini[2] and Graziano Ceresini[1]**

[1] Endocrinology of Aging Unit, Department of Medicine and Surgery, University of Parma, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy, [2] Pathology Unit, Department of Medicine and Surgery, University of Parma, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy, [3] Otolaryngology Unit, Department of Medicine and Surgery, University of Parma, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy

Background: Angiosarcoma (AS) of the thyroid is a rare and aggressive tumor. Its incidence is higher in iodine-deficient areas but cases unrelated to endemic goiter have been reported.

Case Presentation: We describe a case of a 63-year-old Italian man living in a non-iodine-deficient area, with no previous diagnosis of thyroid disease with a history of radiation exposure. The patient—an interventional cardiologist who had worked for 15 years in an angiographic room- came to the clinical observation because of the rapid onset of dyspnea and dysphonia. Computed tomography (CT) showed a 13-cm inhomogeneous neck mass, originating from the left thyroid lobe which caused displacement and stenosis of the trachea. The patient underwent diagnostic fine-needle aspiration that was followed by total thyroidectomy and lymphadenectomy of central and left lateral cervical nodes. The final pathological diagnosis was epithelioid angiosarcoma (EAS), high grade. The preoperative staging by CT of the head, neck, abdomen, chest and pelvis was negative. At pathological staging, the tumor was angionvasive but it was limited to the thyroid; no lymphnode metastases were detected. Chemotherapy with Epirubicin and Ifosfamide was administered for 4 cycles and, then, it was discontinued due to significant bone marrow toxicity.

Conclusion: One year after diagnosis, the CT of neck, abdomen, chest, and pelvis were negative. At 2 years after diagnosis, the FDG-PET was negative with no evidence of the disease at CT Due to the known association between the occurrence of angiosarcoma after radiation therapy it is tempting to speculate that in this patient the presence of thyroid EAS may be linked to radiation exposure.The patient is still alive at 62 months after diagnosis. He is on a follow-up program by a 6-month /1-year neck, chest, abdomen, and pelvis CT evaluation with no signs of metastases.

Keywords: epithelioid, angiosarcoma, thyroid, radiation, survival

OPEN ACCESS

Edited by:
Giampaolo Papi, Azienda Unità Sanitaria Locale di Modena, Italy

Reviewed by:
Maria Chiara Zatelli, University of Ferrara, Italy
Salvatore Maria Corsello, Università Cattolica del Sacro Cuore, Italy

***Correspondence:**
Graziano Ceresini
graziano.ceresini@unipr.it

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *01 August 2018*
Accepted: *11 September 2018*
Published: *01 October 2018*

Citation:
Marina M, Corcione L, Serra MF, Ferri T, Silini EM and Ceresini G (2018) Primary Epithelioid Angiosarcoma of the Thyroid in a Patient Occupationally Exposed to Radiations. Front. Endocrinol. 9:577. doi: 10.3389/fendo.2018.00577

BACKROUND

Thyroid angiosarcoma (AS) is a rare, aggressive, mesenchymal tumor of the thyroid gland with vascular differentiation (1). It mainly occurs in adult females; the highest incidence is reported in the seventh decade (2–4). Its prognosis is considered very poor with early metastases occurring at lymphnodes, lung, skin, bones, soft tissues, and with a mean overall survival of 6 months (2, 3, 5–7). Thyroid AS usually presents as a large and hemorrhagic thyroid mass that extends to local tissues, lymphnode, and distant sites. The non-neoplastic gland frequently shows multinodular goiter.

Thyroid AS was originally described in iodine-deficient areas of the Alps and other mountain regions in association with endemic goiter. It accounts for up to 4.3% of all malignant thyroid tumors in Switzerland (8) and its presence is documented in other mountain regions such as Austria and Northern Italy (9). Although there have been case reports of AS in patients without goiter, many patients may not be aware of an underlying thyroid disease until a tumor is detected. Several cases have also been reported in non-alpine areas although with unknown incidence (9–14). The coexistence of AS with Hashimoto's thyroiditis (15) or differentiated thyroid cancer (13, 16) has been reported.

Here, we report a case of primary epitelioid angiosarcoma of the thyroid diagnosed in a physician professionally exposed to radiation who lived in a non-Alpine region and had no personal history of goiter or thyroid disease.

CASE PRESENTATION

A 63-year-old man came to the clinical observation because of a rapid onset of dyspnea and dysphonia along with the development of a bulky node in the left side of the neck. He had been working as an interventional cardiologist in an angiographic room for 15 years at the local Hospital. Family history was negative with regard to malignancies and thyroid disease.

The relevant medical history included hypertension treated with valsartan and hydrochlorothiazide and non-insulin-dependent diabetes mellitus treated with metformin. There was no previous history of thyroid disease. Two years before the admission, he was treated with warfarin because of a deep venous thrombosis of the left leg occurred after a short bed rest for prostatitis. He was a heavy smoker.

The iodine status of the patient was not known; however, he was from a non-Alpine region and he was still living in the same area which is considered as a mildly iodine insufficient (17).

On physical examination, the patient had a 8 × 10-cm firm left-sided neck mass with a right-sided shift of the larynx. On ultrasound examination, a nodule of the left thyroid lobe was found measuring 5 and 6-cm in its antero-posterior (AP) and transverse (T) diameters, respectively. The nodule was hypoechoic but inhomogeneous, with no vascularization; at the strain elastography, the nodule ranged from a medium elasticity to a hard pattern. The volume of the right thyroid lobe was reduced with a small hypoechoic nodule. No enlarged lymphnodes were found at the neck ultrasound. Computed tomography (CT) of the neck confirmed a 7 × 5 × 13-cm (T × AP × Long diameters) large, inhomogeneous neck mass originating from the left lobe that caused displacement of the trachea, the left common carotid artery and the left internal jugular vein. No evidence of primary malignancies or suspicious for secondary lesions was found at the CT of the head, abdomen, and pelvis. The chest CT showed a 6-mm round-shaped nodule not suspicious for malignancy close to the parietal pleura at the lower lobe of the right lung.

A fine needle aspiration cytology (FNAC) of the mass was performed which yielded hemorrhagic smears with few groups of large, epithelioid cells, with vesicular, severely atypical nuclei and eosinophilic dense cytoplasms. A diagnosis of malignancy was given (Category 6 according to Bethesda 2010) with a suggestion for an anaplastic carcinoma (**Figure 1A**).

The patient underwent a total thyroidectomy and lymphadenectomy of central and left lateral cervical nodes. At the gross pathology examination, the tumor measured 6 × 6 × 12 cm (T × AP × Long diameters) and was partially circumscribed by a fibrous pseudocapsule. The mass had a gray, tan and red cut surface, with areas of hemorrhagic necrosis. Histology showed a vasoformative high grade neoplasia characterized by large epithelioid cells growing in sheets and lining abnormal vascular spaces; some cells showed intracytoplasmic lumina. There were areas of spontaneous necrosis and hemorrhage and a brisk mitotic activity; angioinvasion was noted. The tumor immunostained for vascular markers (CD31, ERG, CD34, factor VIII and vimentin), whereas epithelial differentiation markers were negative (cytokeratins, thyroid transcription factor 1, thyroglobulin, and EMA). The final histologic diagnosis was primary epithelioid angiosarcoma of the thyroid, grade 3 according to FFCCS (**Figures 1B–D**). This diagnosis was confirmed at a second opinion from a different institution. The tumor was restricted to the thyroid with free surgical margins. The mass had substernal extension and displaced the surrounding structures but it did not infiltrate the thyroid capsule, the strap muscles, or other neck tissues. The remaining thyroid tissue had nodular colloid goiter. No lymphnode metastases were detected.

Fifteen days after the thyroidectomy, the patient was operated to prevent rupture of an aneurysm of the abdominal aorta. One month after thyroidectomy, the chest CT showed multiple pulmonary nodular lesions some of them with a solid pattern surrounded by a ground-glass halo, 12 mm in maximum diameter. There was no consensus as to the oncologic relevance of these lesions, therefore, no biopsy was performed. A bone scintigraphy yielded negative results.

Chemotherapy with Epirubicin, Ifosfamide, and Mesna was administered but it was discontinued after 4 cycles because of pancytopenia and infection by *Klebsiella Pneumoniae,* treated with piperacillin/tazobactam, and by *Clostridium difficile,* treated with vancomycin. The patient recovered from the infections and, at a 6-month follow-up, the chest CT showed a reduction of the number and volume of the lung lesions with only three of them remaining in the medial lobe of the right lung.

At a further 18-month control, the chest CT was unchanged. The 6 mm round-shaped nodule close to the parietal pleura at the lower lobe of the right lung was also found to be stable. One

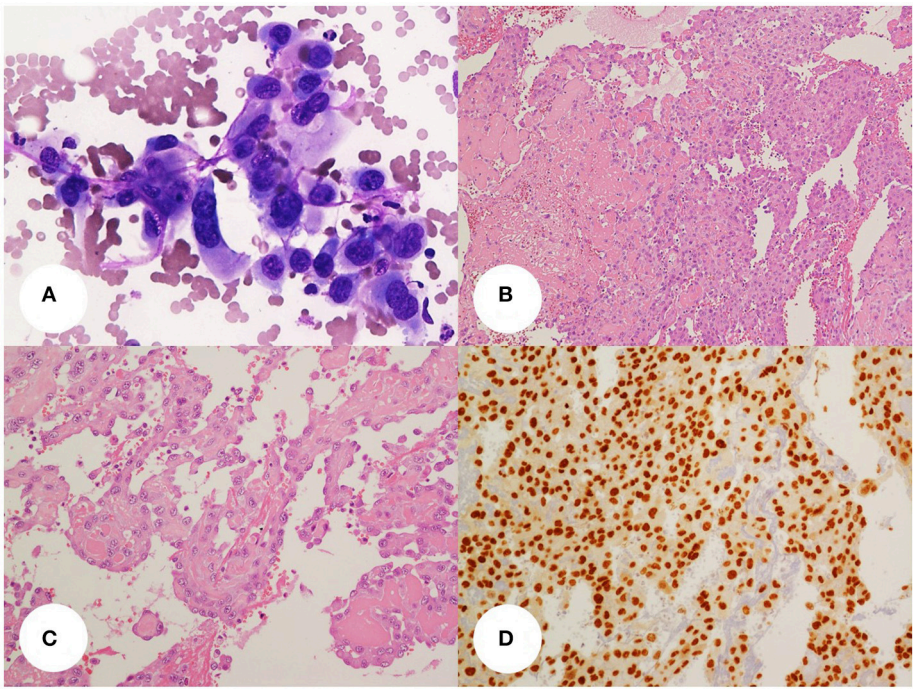


FIGURE 1 | (A) thyroid fine-needle aspiration smear(400x, Giemsa stain) showing a small dishesive group of large, atypical, epithelioid cells with well-defined cell borders, pleomorphic and vesicular, central nuclei and eosinophilic cytoplasms. **(B,C)** Histology slides (**B**, 100x; **C**, 200x; H&E stain) show the above cells organized in sheets and papillae with fibrin cores or lining irregular vascular. Areas of recent and remote hemorrhage with siderophages can be seen. The tumor cell delimiting the vascular spaces are dishesive and pluristratified. Some cells show small intracytoplasmic lumina. **(D)** the tumor cells display diffuse nuclear stain for the vascular transcription factor Erg-1 (200x, DAB stain).

year later, the patient developed pneumonitis and recovered after antibiotic therapy. At that time, he was investigated by neck, chest, abdomen and pelvis CT as well as with FDG-PET without any evidence of disease recurrence.

Afterward, a 6-month CT follow-up program was started which is still ongoing. At present, the patient is alive with no evidence of disease after 62 months from initial diagnosis.

DISCUSSION

A recent systematic review (18) indicates that the majority of thyroid AS cases are still diagnosed in people living in alpine regions of Europe, especially Switzerland, Northern Italy and Austria and they are associated with endemic nodular goiter (1, 8, 19, 20). The association between AS and nodular goiter, however, is not absolute and up to 50% of thyroid AS may arise in different contexts (3, 12, 14, 21).

In the liver, AS has been observed in subjects exposed to vinyl chloride, arsenic, and thorium dioxide (Thorotrast) (1). The occurrence of AS after radiation therapy is also well documented (22, 23), although mainly for superficial rather than visceral sites (1, 22, 24). It has been hypothesized that this association between radiation therapy and the development of AS can be due to stasis within lymphatic channels due to the development of fibrosis or a radiation-induced sarcoma (25–27). Potential risk factors, apart from long-standing goiter, have been rarely recognized and reported in thyroid AS cases. In 2016, Collini et al. in a series of six cases of thyroid AS from a non-iodine-deficinent area found a male patient with occupational exposure to vinyl chloride and a female patient with a history of radiation therapy for malignant timoma (22). No known risk factor was recognized for the other patients. The patient herein reported had worked for 15 years in an angiographic room as an interventional cardiologist. Although no data are available as to the radiation dose to the thyroid, it is tempting to speculated a link between this professional exposure and the development of AS that should alert for the occurrence of similar cases.

Histologically, the morphology of thyroid AS may range from well-differentiated forms, to poorly differentiated tumors with solid growth of spindle and/or epithelioid cells. The main differential diagnosis is with anaplastic thyroid carcinoma or other high-grade sarcomas. The diagnosis is now made easy by the availability of several antigenic markers; this does not necessarily apply to old literature data.

The patient had a R0 thyroidectomy with no extra-thyroidal invasion or lymphnode disease and, despite angionvasion and an incomplete chemotherapy treatment due to infectious complications, he remains free of disease at 62 months from diagnosis. The reported prognosis of patients with thyroid AS is poor and survival rates are limited to few months after the initial diagnosis (2, 3). However, single cases with longer survival (in one case, up to 82 months) have been reported (22).

Angionvasion has been suggested as a possible risk factor for progression on a limited series of cases and review of the literature (28). In larger series, tumors confined to the thyroid and without distant metastases at diagnosis seem to have a better prognosis (18, 22, 29).

There is no established therapeutic strategy for post-surgical management of thyroid AS. Some authors reported promising results with adjuvant radiotherapy (9, 30–32), although its role still remains unclear. Also the role of chemotherapy remains to be elucidated. Several drugs have been used, such as epirubucine, adriamicine, taxanes, and ifosfamide, either alone or in combination. Chemotherapy has been used either in adjuvant or neo-adjuvant protocols as well as in combination with radiotherapy (18) but the overall outcome is poor. New treatment strategies are under investigation, including drugs targeting the vascular endothelial growth factor (VEGF) and its receptor (VEGFR) pathway (i.e., bevacizumab) as well as tyrosine kinase inhibitors with activity against VEGFR (i.e., sunitinib and pazopanib) (33, 34). Also, inhibition of phosphatidylinositol 3-kinase pathway has been hypothesized to virtually represent a further therapeutic tool, based on the findings of the association between this pathway and AS, although of non-thyroidal origin (35, 36). However, further studies are needed in order to verify and confirm the effects of these novel therapeutic approaches on thyroid AS.

Our patient was treated with epirubicin in combination with ifosfamide. The therapy was discontinued after four cycles of the 6-cycles planned treatment because of severe bone marrow toxicity. Nevertheless, no evidence of disease is still recorded at the follow-up. Notably, Maiorana et al. reported a thyroid AS with 66-month disease-free survival in a patient with no treatments after surgery (11). Undoubtedly, the critical issue in discussing the clinical course is the limited clinical experience available for this tumor and the related difficulty to properly design therapeutic protocols.

No evidence-based explanation can be provided for the favorable clinical course so far observed in our patient. The expansile pattern of growth of the tumor that was limited to the thyroid and lacked distant metastases are pathological variables that suggest a less aggressive behavior (37). Angioinvasion is the norm in vaso-formative tumors and seems of little consequence in this setting. Conversely, it is tempting to speculate that AS arising in different settings than goiter may have a lower biological potential than tumors complicating endemic iodine deficiency which still make most of the current literature on this subject. More attention should be given to this issue in future studies.

ETHICS STATEMENT

The patient gave written informed consent for the publication of this report.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Weiss A, Antonescu CR, Deyrup AT. Angiosarcoma of soft tissue. In: Fletcher Christopher DM, Bridge Julia A, Hogendoorn Pancras CW, Fredrik M, editors. *World Health Organization Classification of Tumors. Pathology and Genetics of Tumors of Soft Tissue and Bone.* Lyon: IARC Press (2013). p. 156–8.
2. Eusebi V. Angiosarcoma. In: De Lellis Ronal A, Lloyd Ricardo V, Heitz Philipp U, Charis E, editors. *World Health Organization Classification of Tumors. Pathology and Genetics of Tumors of Endocrine Organs.* Lyon: IARC Press (2004). p. 113–4.
3. Kaur A, Didolkar MS, Thomas A. Angiosarcoma of the thyroid: a case report with review of the literature. *Endocr Pathol.* (2013) 24:156–61. doi: 10.1007/s12022-013-9253-z
4. Rosai J, Carcangiu ML, DeLellis RA. Sarcomas. In: *Atlas of Tumor Pathology. Tumors of the Thyroid Gland, 3rd series.* Washington, DC: Armed Forces Institute of Pathology (1992).
5. Papotti M, Arrondini M, Tavaglione V, Veltri A, Volante M. Diagnostic controversies in vascular proliferations of the thyroid gland. *Endocr Pathol.* (2008)19:175–83. doi: 10.1007/s12022-008-9039-x
6. Wiedermann JP, Sadeghi N, Baird-Howell M. Rapidly growing vascular mass within the thyroid. *Angiosarcoma. JAMA Otolaryngol Head Neck Surg* (2016) 142:397–8. doi: 10.1001/jamaoto.2015.3282
7. Prather J, Mehrotra S. Thyroid nodule. Primary thyroid angiosarcoma. *JAMA Otolaryngol Head Neck Surg.* (2014)140:469–70 doi: 10.1001/jamaoto.2014.95
8. Hedinger C. Geographic pathology of thyroid diseases. *Pathol Res Pract.* (1981) 171:285–92 doi: 10.1016/S0344-0338(81)80101-X
9. Ryska A, Ludvikova M, Szepe P, Boor A. Epithelioid haemangiosarcoma of the thyroid gland. Report of six cases from a non-Alpine region. *Histopathology* (2004) 44:40–6 doi: 10.1111/j.1365-2559.2004.01772.x
10. Goh SGN, Chuah KL, Goh HKC, Chen YYC. Two cases of epithelioid angiosarcoma involving the thyroid and a brief review of non-Alpine epithelioid angiosarcoma of the thyroid. *Arch Pathol Lab Med.* (2003) 127:e70–3. doi: 10.1043/0003-9985(2003)127<e70:TCOEAI>2.0.CO;2
11. Maiorana A, Collina G, Cesinaro AM, Fano RA, Eusebi V. Epithelioid angiosarcoma of the thyroid. Clinicopathologic analysis of seven cases from non-Alpine areas. *Virchows Arch.* (1996) 429:131–7.
12. Rotellini M, Vezzosi V, Bianchi S. Epithelioid angiosarcoma of the thyroid: report of a case from an italian non-alpine area and review of the literature. *Endocr Pathol.* (2015) 26:152–6. doi: 10.1007/s12022-015-9372-9
13. Del Rio P, Cataldo S, Sommaruga L, Corcione L, Guazzi A, Sianesi M. A rare case of thyroid haemangiosarcoma. *Chir Ital.* (2007) 59:747–9.
14. Bayir Ö, Yilmazer D, Ersoy R, Akca Y, Saylam G, Han Ü et al. An extremely rare case of thyroid malignancy from the non-Alpine region. *Angiosarcoma. Int J Surg Case Rep.* (2016) 19:92–6. doi: 10.1016/j.ijscr.2015.12.028
15. Innaro N, Succurro E, Tomaino G, Arturi F. Nonalpine thyroid angiosarcoma in a patient with Hashimoto thyroiditis. *Case Rep Oncol Med.* (2013) 2013:901246. doi: 10.1155/2013/901246
16. Kefeli M, Mete O. An unusual malignant thyroid nodule: coexistence of epithelioid angiosarcoma and follicular variant papillary thyroid carcinoma. *Endocr Pathol.* (2014) 25:350–2. doi: 10.1007/s12022-013-9243-1
17. Ceresini G, Corcione L, Michiara M, Sgargi P, Teresi G, Gilli A, et al. Thyroid cancer incidence by histological type and related variants in a mildly iodine-deficient area of Northern Italy, 1998 to 2009. *Cancer* (2012) 118:5473–80. doi: 10.1002/cncr.27591
18. De Felice F, Moscatelli E, Orelli S, Bulzonetti N, Musio D, Tombolini V. Primary thyroid angiosarcoma: A systematic review. *Oral Oncology* (2018) 82:48–52. doi: 10.1016/j.oraloncology.2018.05.004
19. Eckert F, Schmid U, Gloor F, Hedinger C. Evidence of vascular differentiation in anaplastic tumours of the thyroid- an immunohistological study. *Virchows Archiv.* (1986) 410:203–15. doi: 10.1007/BF00710826

20. Totsch M, Dobler G, Feichtinger H, Sandbichler P, Ladurner D, Schmid KW. Malignant hemangioendothelioma of the thyroid. Its immunohistochemical discrimination from undifferentiated thyroid carcinoma. *Am J Surg Pathol.* (1990) 14:69–74. doi: 10.1097/00000478-199001000-00007
21. Gouveia P, Silva C, Magalhães F, Santos C, Guerreiro E, Santos F, et al. Non-Alpine thyroid angiosarcoma. *Int J Surg Case Rep.* (2013) 4:524–7. doi: 10.1016/j.ijscr.2013.02.005
22. Collini P, Barisella M, Renne SL, Pizzi N, Mattavelli D, Stacchiotti S, et al. Epithelioid angiosarcoma of the thyroid gland without distant metastases at diagnosis: report of six cases with a long follow-up. *Virchows Arch.* (2016) 469:223–32. doi: 10.1007/s00428-016-1964-3
23. Young RJ, Brown NJ, Reed MW, Hughes D, Woll PJ. Angiosarcoma. *Lancet Oncol.* (2010) 11:983–91. doi: 10.1016/S1470-2045(10)70023-1
24. Cabibi D, Pipitone G, Porcasi R, Ingrao S, Benza I, Porrello C, et al. Pleural epithelioid angiosarcoma with lymphatic differentiation arisen after radiometabolic therapy for thyroid carcinoma: immunohistochemical findings and review of the literature. *Diagn Pathol.* (2017) 12:60. doi: 10.1186/s13000-017-0652-1
25. Davies JD, Rees GJG, Mera SL. Angiosarcoma in irradiated postmastectomy chest wall. *Histopathology* (1983) 7:947–56. doi: 10.1111/j.1365-2559.1983.tb02309.x
26. Nanus DM, Kelsen D, Clark DGC. Radiation-induced angiosarcoma. *Cancer* (1987) 60:777–9. doi: 10.1002/1097-0142(19870815)60:4<777::AID-CNCR2820600412>3.0.CO;2-T
27. Meis-Kindblom JM1, Kindblom LG. Angiosarcoma of soft tissue: a study of 80 cases. *Am J Surg Pathol.* (1998) 22:683–97. doi: 10.1097/00000478-199806000-00005
28. Nechifor-Boilă A, Decaussin-Petrucci M, Varga-Ilyés A, Chinezu L, Carașca C, Borda A. Angioinvasion as a factor for predicting aggressive outcome in primary thyroid angiosarcoma: three case reports and literature review. *Pol J Pathol.* (2018) 69:53–61. doi: 10.5114/pjp.2018.75337.
29. Wick MR, Eusebi V, Lamovec J, Ryska A. Angiosarcoma. In: Lloyd RV, Osamura RY, Kloppel G, Rosai J, editors. *WHO Classification of Tumors of Endocrine Organs*, *4th edn.* Lyon:WHO Press (2017). p 129–32.
30. Couto J, Martins RG, Santos AP, Matos J, Torres I. Invasive thyroid angiosarcoma with a favorable outcome. *Int J Endocrinol Metab.* (2014) 12:e15806. doi: 10.5812/ijem.15806
31. Rhomberg W, Boehler F, Eiter H, Fritzsche H, Breitfellner G. Treatment options for malignant hemangioendotheliomas of the thyroid. *Int J Radiat Oncol Biol Phys.* (2004) 60:401–5. doi: 10.1016/j.ijrobp.2004.03.023
32. Yoon Moon S, Su Park H, Young Woo J, Kyun Choi J, Oh H, Up Kim K, et al. Primary thyroid angiosarcoma with tracheal invasion. *Int Med.* (2016) 55:1165–9. doi: 10.2169/internalmedicine.55.5447
33. Tokuyama W, Mikami T, Masuzawa M, Okayasu I. Autocrine and paracrine roles of VEGF/VEGFR-2 and VEGF-C/VEGFR-3 signaling in angiosarcomas of the scalp and face. *Hum Pathol.* (2010) 41:407–14. doi: 10.1016/j.humpath.2009.08.021
34. Park MS, Ravi V, Araujo DM. Inhibiting the VEGF-VEGFR pathway in angiosarcoma, epithelioid hemangioendothelioma, and hemangiopericytoma/solitary fibrous tumor. *Curr Opin Oncol.* (2010) 22:351–5. doi: 10.1097/CCO.0b013e32833aaad4
35. Italiano A, Chen CL, Thomas R, Breen M, Bonnet F, Sevenet N, et al. Alterations of the p53 and PIK3CA/AKT/mTOR pathways in angiosarcomas: a pattern distinct from other sarcomas with complex genomics. *Cancer* (2012) 118:5878–87. doi: 10.1002/cncr.27614
36. De Felice F, Guerrero Urbano T. New drug development in head and neck squamous cell carcinoma: the PI3-K inhibitors. *Oral Oncol.* (2017) 67:119–23. doi: 10.1016/j.oraloncology.2017.02.020
37. Kalitova P, Plzak, Kodet, Astl J. Angiosarcoma of the thyroid. *Eur Arch Otorhinolaryngol.* (2009) 266:903–5. doi: 10.1007/s00405-008-0820-8

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

CASE REPORT
published: 25 September 2018
doi: 10.3389/fendo.2018.00555

An Unusual Salivary Gland Tumor Mimicking Papillary Thyroid Carcinoma: Mammary Analog Secretory Carcinoma

Sylvia L. Asa [1,2]* *and Ozgur Mete* [1,2]

[1] *Department of Pathology, University Health Network, Toronto, ON, Canada,* [2] *Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada*

Thyroid tumors usually present as masses in the thyroid gland. While the majority of these tumors represent neoplasms of thyroid tissues, mainly of follicular epithelial cell differentiation, the differential diagnosis includes other lesions, such as C cell neoplasms (medullary thyroid carcinoma), intrathyroidal parathyroid, or thymic tumors, soft tissue tumors, and hematologic neoplasms as well as metastatic malignancies. Rare tumors are of salivary gland types. This case illustrates an unusual tumor of salivary gland type, an intrathyroidal mammary analog secretory carcinoma (MASC). The pathogenesis, diagnostic pitfalls, and therapeutic implications of this unusual tumor are discussed.

Keywords: thyroid, salivary gland, mammary analog secretory carcinoma, papillary thyroid cancer, immunohistochemistry

OPEN ACCESS

Edited by:
Giampaolo Papi,
Azienda Unità Sanitaria Locale di Modena, Italy

Reviewed by:
Agnese Barnabei,
Istituto Nazionale del Cancro Regina Elena, Italy
Dario Giuffrida,
Istituto Oncologico del Mediterraneo, Italy

***Correspondence:**
Sylvia L. Asa
sylvia.asa@utoronto.ca

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *05 July 2018*
Accepted: *30 August 2018*
Published: *25 September 2018*

Citation:
Asa SL and Mete O (2018) An Unusual Salivary Gland Tumor Mimicking Papillary Thyroid Carcinoma: Mammary Analog Secretory Carcinoma. Front. Endocrinol. 9:555. doi: 10.3389/fendo.2018.00555

BACKGROUND

Thyroid tumors are common and the vast majority represent tumors derived from follicular epithelial cells (1). Most are differentiated thyroid neoplasms, including follicular adenomas, well differentiated follicular tumors with low metastatic potential (including NIFT-P, encapsulated follicular variant papillary thyroid carcinoma with minimal capsular invasion and minimally invasive follicular carcinomas), and classical papillary carcinomas. Rare aggressive follicular carcinomas or follicular variant papillary carcinomas show widespread capsular invasion or angioinvasion and some tumors progress with loss of differentiation, including poorly differentiated carcinomas and anaplastic carcinomas.

Other thyroid tumors include medullary carcinomas of C cell differentiation and tumors that are composed of cells that are not unique to the thyroid (1). These include intrathyroidal parathyroid lesions and thymic tumors that are explained by their embryologic origin and locations near and occasionally within the thyroid. Soft tissue tumors can arise from soft tissue components of the thyroid region. Primary thyroid lymphomas and other hematologic neoplasms can present as thyroid masses and some thyroid nodules represent metastatic malignancies. Other unusual thyroid tumors can be of salivary gland types, and these are explained by the occasional finding of salivary gland tissue within the thyroid (**Figure 1a**).

This report describes a case of a salivary gland type of tumor that can be confused with a papillary thyroid carcinoma. The clinical and therapeutic implications of the diagnosis are discussed.

CASE REPORT

A 72 year old woman was found to have a 2.5 cm nodule in the left thyroid. Thyroid function tests were within the normal range. She had no family history of thyroid or other endocrine disease. Her medical history was unremarkable. A fine needle biopsy of the lesion was diagnosed as "suspicious for neoplasm." She underwent left hemithyroidectomy.

The tumor was diagnosed as papillary thyroid carcinoma by the pathologist at the originating institution. There was extrathyroidal extension. A consultation from a thyroid expert confirmed the diagnosis. The patient was referred to our institution for completion thyroidectomy and radioactive iodine therapy. Pathology review was requested.

The patient was evaluated for metastatic disease and none was identified. She is alive and well with no evidence of recurrence 18 months later. The patient provided informed signed consent for publication of her data.

Pathology Findings

The thyroid contained an infiltrative tumor that had areas of follicular and papillary architecture but the overall morphology and cytologic features were atypical for a tumor of thyroid follicular differentiation. The surrounding thyroid exhibited

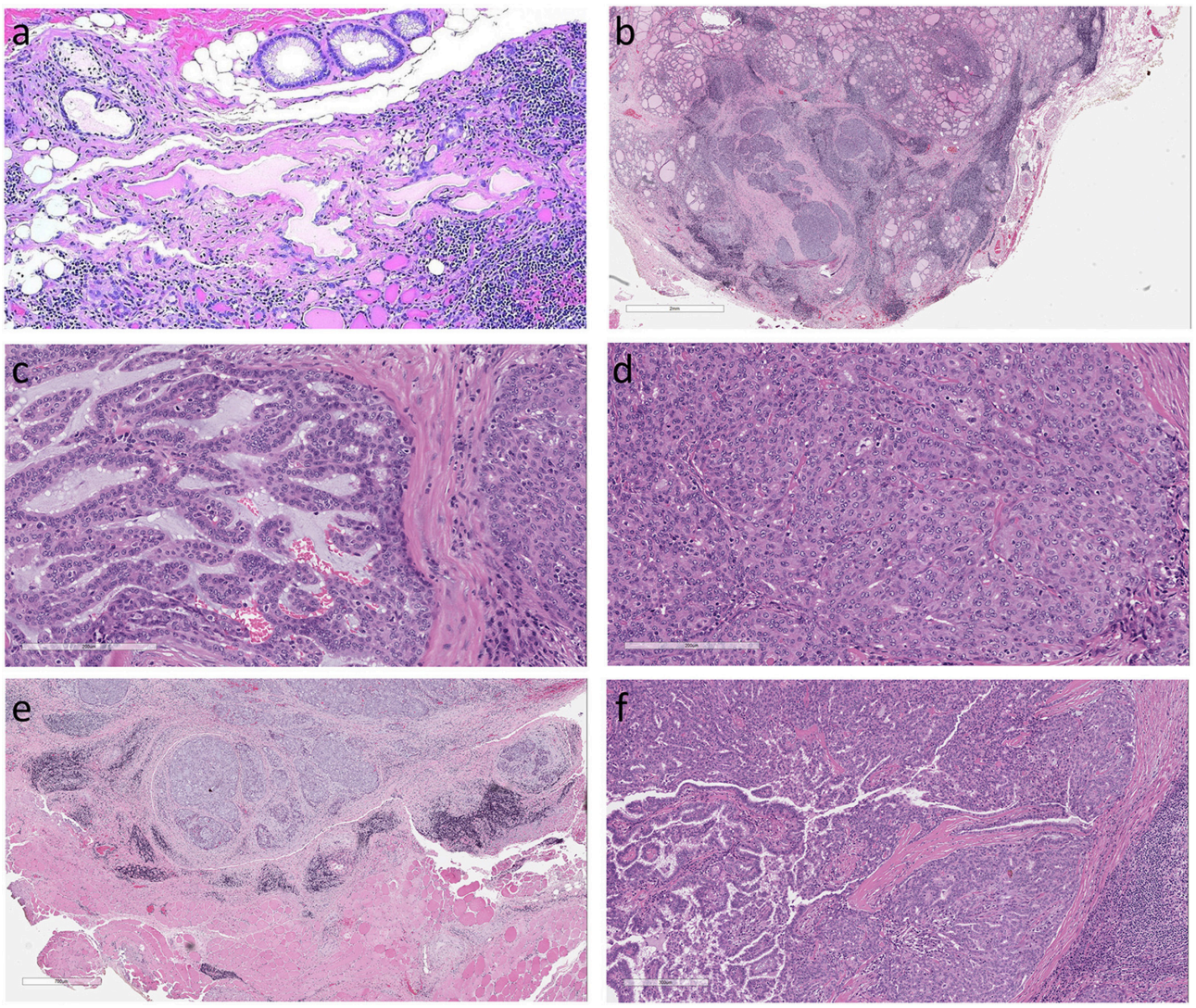


FIGURE 1 | Histologic features of Mammary Analog Secretory Carcinoma of Thyroid **(a)**. The origin of this unusual tumor is unknown but may be from incidental intrathyroidal salivary gland rests as seen in this normal thyroid (not from the patient reported) **(b)**. The thyroid tumor in the case described is an infiltrative tumor composed of solid sheets and nests of epithelial cells in a fibrous stroma. The surrounding thyroid exhibits chronic lymphocytic thyroiditis **(c)**. The solid sheets were punctuated by small cribriform areas and microcysts with pseudopapillae and a few true papillae with fibrovascular cores **(d)**. The homogeneous tumor cells had abundant cytoplasm and monotonous round nuclei with clear nucleoplasm and conspicuous large nucleoli but no indentations or inclusions **(e)**. There was extrathyroidal extension into surrounding skeletal muscle **(f)**. In one area of the tumor there was a small 0.2 cm focus of classical papillary microcarcinoma.

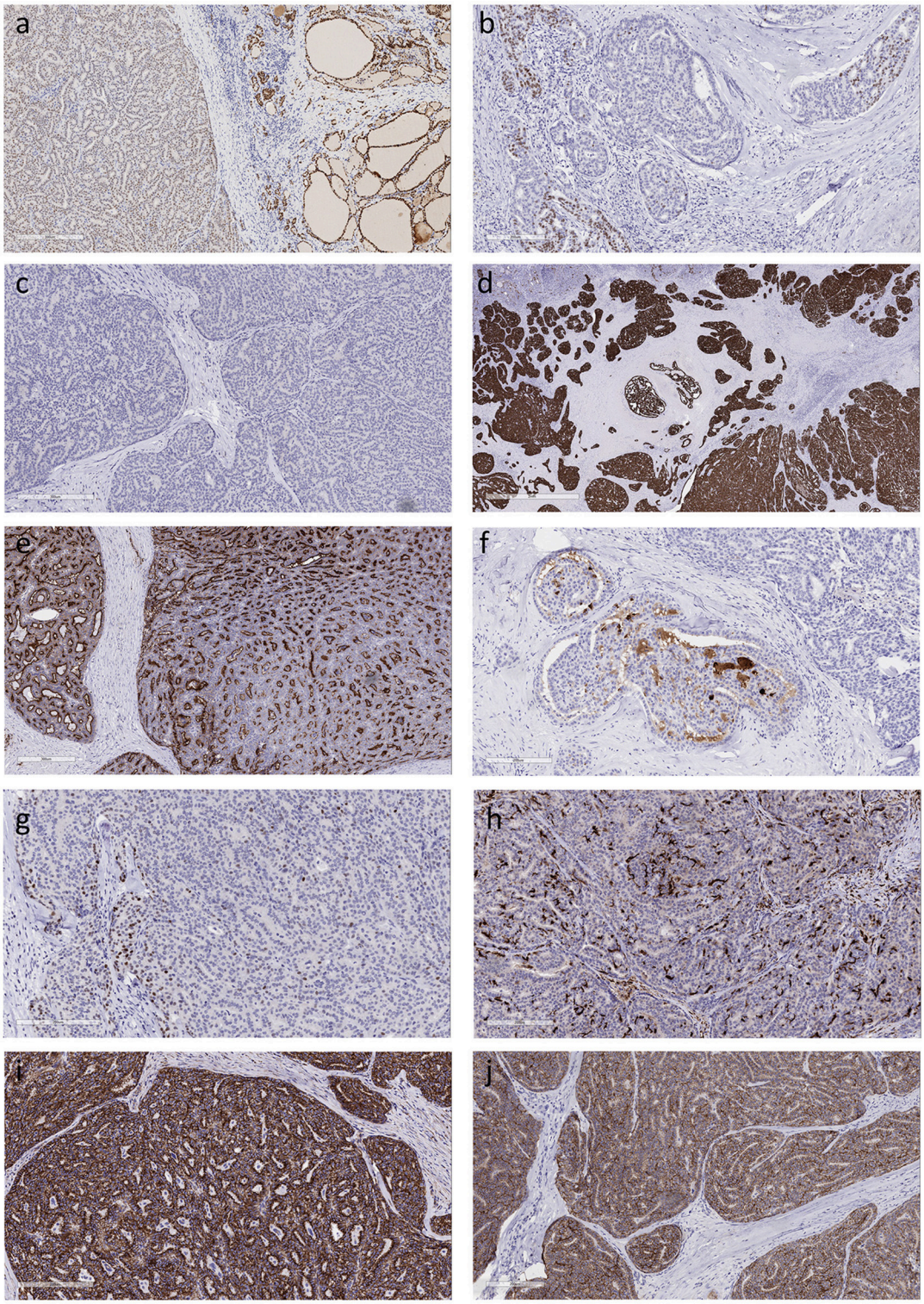


FIGURE 2 | Immunohistochemical features of Mammary Analog Secretory Carcinoma of Thyroid **(a)**. The tumor cells exhibit diffuse positivity for monoclonal PAX-8 that is much weaker than in the surrounding thyroid **(b)**. There is very focal positivity for TTF-1 (clone: SPT24); some of the stained cells might be entrapped follicular epithelial cells **(c)**. The tumor cells are completely negative for thyroglobulin **(d)**. The tumor exhibits strong diffuse positivity for cytokeratin 19 **(e)**. Staining for CEA with a polyclonal antiserum yields diffuse reactivity, however a monoclonal CEA antibody resulted in a completely negative stain **(f)**. Tumor cells are positive for gross cystic disease fluid protein-15 **(g)**. Scattered tumor cells express p63 **(h)**. Dendritic type cells that are strongly positive for S100 protein are distributed throughout the tumor **(i)**. Beta-catenin staining is intact at the cell membrane and there is no nuclear translocation **(j)**. Positivity for E-cadherin is retained at the cell borders.

chronic lymphocytic thyroiditis. The tumor was composed of solid sheets and nests in a fibrovascular stroma (**Figure 1b**) with cribriform areas, microcysts, cleft-like structures, and focal pseudopapillae with a few true papillae (**Figure 1c**). The tumor cells were relatively homogeneous with abundant eosinophilic cytoplasm and monotonous round nuclei with clear nucleoplasm and conspicuous large nucleoli but no indentations or inclusions (**Figure 1d**). There was frank extrathyroidal extension into surrounding skeletal muscle (**Figure 1e**). In one area of the tumor there was a small 0.2 cm focus of classical papillary microcarcinoma with the distinctive features of that entity that were clearly different from the rest of the lesion (**Figure 1f**).

Immunohistochemistry of the dominant tumor identified diffuse but weak monoclonal PAX-8 nuclear reactivity (**Figure 2a**) but TTF-1 (clone: SPT24) was only focal and weak (**Figure 2b**) and thyroglobulin staining was completely negative (**Figure 2c**). Stains for Cytokeratin 7 and Cytokeratin 19 (**Figure 2d**) were diffusely positive but Cytokeratin 5 was only focally expressed. Although polyclonal CEA was positive (**Figure 2e**), monoclonal CEA was negative, as was synaptophysin and chromogranin-A. Scattered tumor cells were positive for gross cystic disease fluid protein-15 (GCDFP-15) (**Figure 2f**), some stained for p63 (**Figure 2g**) and stellate cells were identified by localization of S100 protein (**Figure 2h**). CD5 positivity was restricted to infiltrating lymphocytes. Beta-catenin (**Figure 2i**) and E-cadherin (**Figure 2j**) positivity was intact at the tumor cell membrane and there was no nuclear translocation.

The diagnosis was changed to Mammary Analog Secretory Carcinoma (MASC), an unusual tumor of salivary gland, associated with a 0.2 cm papillary microcarcinoma.

DISCUSSION

Mammary analog secretory carcinoma (MASC) has been recently identified as a unique salivary gland tumor that occurs mainly in the parotid but can also occur in minor salivary glands (2). It has also been reported in the sinonasal tract, lip, skin, thyroid gland, and lung (3–5). The thyroid gland is known to be a site of mucoepidermoid carcinoma and other lesions of salivary gland types, so it is not surprising that it has also been found to harbor MASC (4, 6–9).

A characteristic finding in the initial molecular studies of MASC was the presence of *t(12;15)(p13;q25)* resulting in *ETV6-NTRK3* translocation. This was reported to be specific for this tumor type and was not documented in other salivary gland tumors. However, a recent report of 10 cases with typical morphology and immunoprofile identified that some such tumors harbor a novel *ETV6-RET* translocation (10). It is interesting to speculate on the possible cytogenetic and molecular relationships between these tumors and carcinomas of follicular epithelial differentiation that also harbor rearrangements of *RET* and *NTRK* (11).

The diagnosis of MASC in thyroid can be challenging as illustrated in this case. Many features mimic papillary thyroid carcinoma. The cytology can be misdiagnosed (8, 9). The architecture can resemble that of papillary thyroid carcinoma and the diffuse expression for PAX-8 can result in a misdiagnosis, however, the staining for the biomarkers of thyroid differentiation, when present, is weak and focal (7) as in our case. In addition, papillary thyroid carcinomas may exhibit diffuse positivity for cytokeratin 19 (12), again leading to a potential for misdiagnosis. However, differentiated thyroid carcinomas are rarely if ever completely negative for thyroglobulin, and the lack of positivity should raise the possibility that this is not a primary tumor of thyroid follicular cell derivation, especially when the tumor is not diffusely positive for TTF-1 and PAX8. TTF-1 (clone 8G7G3/1) has been reported to be negative in MASC (13) whereas we found very focal TTF-1 reactivity in our case using the SPT24 clone; while some of the stained cells may be entrapped follicular epithelial cells, it is possible that there is clone-dependent focal expression for TTF-1 in these tumors. The identification of basophilic/blue luminal secretory material and numerous S100-positive cells should prompt consideration of the diagnosis of MASC that can then be confirmed by the identification of mammary markers such as GCDFP-15, mammoglobin (2) or GATA-3 (6).

The distinction of MASC from papillary carcinoma can be complicated by the presence of a true papillary carcinoma. Two previous cases have been reported to have a minor component of papillary thyroid carcinoma (13) and similar to our case, the two lesions had distinct morphologic and immunophenotypic features. While the *ETV6-NTRK3* translocation has been described in both tumors, and the two tumor types may indeed be causally related, it remains to be determined whether these represent collision tumors or divergent differentiation (clonal trans-differentiation).

MASC can be a very aggressive tumor; most reported cases have extensive local invasion and lymph node metastases and some have pursued an aggressive clinical course with the development of distant metastases. It is important to ensure the correct diagnosis; several cases have been misdiagnosed as papillary thyroid carcinoma but were not responsive to the usual therapy for that disease, radioactive iodine (6, 13). Our case was referred for radioactive iodine therapy based on a similar misdiagnosis. These tumors require other management approaches, including possible response to entrectinib (a tyrosine kinase inhibitor targeting TrkA, TrkB, TrkC, ROS1, and ALK), however the development of resistance has been reported (14).

CONCLUDING REMARKS

This case illustrates an unusual tumor of salivary gland type, an intrathyroidal MASC. The occurrence of salivary gland tumors in the thyroid is unusual and they can mimic primary thyroid carcinomas, however the distinction of these tumors from neoplasms of thyroid follicular epithelium is important, since the treatment approaches are distinct. The potential association of mammary analog secretory carcinoma with papillary thyroid carcinoma raises important questions about possible common precursor cells. Both tumor types have common gene fusions, pointing to possible similar pathogenetic mechanisms.

AUTHOR CONTRIBUTIONS

SA and OM case review. SA writing and image preparation. OM review and editing of manuscript.

REFERENCES

1. Boerner SL, Asa SL. *Biopsy Interpretation of the Thyroid*. Philadelphia, PA: Wolters Kluwer (2017).
2. Seethala RR, Stenman G. Update from the 4th edition of the World Health Organization classification of head and neck tumours: tumors of the salivary gland. *Head Neck Pathol*. (2017) 11:55–67. doi: 10.1007/s12105-017-0795-0
3. Baneckova M, Agaimy A, Andreasen S, Vanecek T, Steiner P, Slouka D, et al. Mammary analog secretory carcinoma of the nasal cavity: characterization of 2 cases and their distinction from other low-grade sinonasal adenocarcinomas. *Am J Surg Pathol*. (2018) 42:735–43. doi: 10.1097/PAS.0000000000001048
4. Nguyen JK, Bridge JA, Joshi C, McKenney JK. Primary mammary analog secretory carcinoma (MASC) of the Vulva with ETV6-NTRK3 Fusion: a case report. *Int J Gynecol Pathol*. (2018). doi: 10.1097/PGP.0000000000000501. [Epub ahead of print].
5. Huang T, Mchugh JB, Berry GJ, Myers JL. Primary mammary analogue secretory carcinoma of the lung: a case report. *Hum Pathol*. (2018) 74:109–113. doi: 10.1016/j.humpath.2017.10.027
6. Reynolds S, Shaheen M, Olson G, Barry M, Wu J, Bocklage T. A case of primary mammary analog secretory carcinoma (MASC) of the thyroid masquerading as papillary thyroid carcinoma: potentially more than a one off. *Head Neck Pathol*. (2016) 10:405–13. doi: 10.1007/s12105-016-0715-8
7. Dettloff J, Seethala RR, Stevens TM, Brandwein-Gensler M, Centeno BA, Otto K, et al. mammary analog secretory carcinoma (MASC) involving the thyroid gland: a report of the first 3 cases. *Head Neck Pathol*. (2017) 11:124–30. doi: 10.1007/s12105-016-0741-6
8. Rupp AP, Bocklage TJ. Mammary analog secretory carcinoma of thyroid: a case report. *Diagn Cytopathol*. (2017) 45:45–50. doi: 10.1002/dc.23608
9. Rodriguez-Urrego PA, Dogan S, Lin O. Cytologic findings of mammary analogue secretory carcinoma arising in the thyroid. *Diagn Cytopathol*. (2017) 45:552–6. doi: 10.1002/dc.23692
10. Skalova A, Vanecek T, Martinek P, Weinreb I, Stevens TM, Simpson RHW, et al. Molecular profiling of mammary analog secretory carcinoma revealed a subset of tumors harboring a novel ETV6-RET translocation: report of 10 cases. *Am J Surg Pathol*. (2018) 42:234–46. doi: 10.1097/PAS.0000000000000972
11. The cancer genome atlas research network. Integrated genomic characterization of papillary thyroid carcinoma. *Cell* (2014) 159:676–90. doi: 10.1016/j.cell.2014.09.050
12. Fischer S, Asa SL. Application of immunohistochemistry to thyroid neoplasms. *Arch Pathol Lab Med*. (2008) 132:359–72. doi: 10.1043/1543-2165(2008)132[359:AOITTN]2.0.CO;2
13. Dogan S, Wang L, Ptashkin RN, Dawson RR, Shah JP, Sherman EJ, et al. Mammary analog secretory carcinoma of the thyroid gland: a primary thyroid adenocarcinoma harboring ETV6-NTRK3 fusion. *Mod Pathol*. (2016) 29:985–95. doi: 10.1038/modpathol.2016.115
14. Drilon A, Li G, Dogan S, Gounder M, Shen R, Arcila M, et al. What hides behind the MASC: clinical response and acquired resistance to entrectinib after ETV6-NTRK3 identification in a mammary analogue secretory carcinoma (MASC). *Ann Oncol*. 27:920–6. doi: 10.1093/annonc/mdw042

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 21 August 2018
doi: 10.3389/fendo.2018.00471

The Role of the Transcription Factor Nuclear Factor-kappa B in Thyroid Autoimmunity and Cancer

Cesidio Giuliani, Ines Bucci and Giorgio Napolitano*

Unit of Endocrinology, Department of Medicine and Sciences of Aging and Ce.S.I.-Me.T., University of Chieti–Pescara, Chieti, Italy

Nuclear factor-kappa B (NF-κB) is a ubiquitous transcription factor that is involved in inflammatory and immune responses, as well as in regulation of expression of many other genes related to cell survival, proliferation, and differentiation. In mammals, NF-κB comprises five subunits that can bind to promoter regions of target genes as homodimers or heterodimers. The most common dimer is the p50/p65 heterodimer. The several combinations of dimers that can be formed contribute to the heterogeneous regulation of NF-κB target genes, and this heterogeneity is further increased by interactions of the NF-κB dimers with other transcription factors, such as steroid hormone receptors, activator protein-1 (AP-1), and cAMP response element binding protein (CREB). In the thyroid, several studies have demonstrated the involvement of NF-κB in thyroid autoimmunity, thyroid cancer, and thyroid-specific gene regulation. The role of NF-κB in thyroid autoimmunity was hypothesized more than 20 years ago, after the finding that the binding of distinct NF-κB heterodimers to the major histocompatibility complex class I gene is hormonally regulated. Further studies have shown increased activity of NF-κB in thyroid autoimmune diseases and in thyroid orbitopathy. Increased activity of NF-κB has also been observed in thyroid cancer, where it correlates with a more aggressive pattern. Of particular interest, mutation of some oncogenes or tumor suppressor genes involved in thyroid carcinogenesis results in constitutive activation of the NF-κB pathway. More recently, it has been shown that NF-κB also has a role in thyroid physiology, as it is fundamental for the expression of the main thyroid-specific genes, such as sodium iodide symporter, thyroid peroxidase, thyroglobulin, *Pax8*, and *TTF-1* (*NKX2-1*).

Keywords: NF-κB, thyroid autoimmunity, thyroid cancer, transcription factors, gene regulation, major histocompatibility complex, RET/PTC, BRAF^{V600E}

Edited by:
Alessandro Antonelli, Università degli Studi di Pisa, Italy

Reviewed by:
Roberto Vita, Università degli Studi di Messina, Italy
Silvia Martina Ferrari, Università degli Studi di Pisa, Italy

****Correspondence:***
Cesidio Giuliani
cesidio.giuliani@unich.it

Specialty section:
This article was submitted to Thyroid Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *08 June 2018*
Accepted: *31 July 2018*
Published: *21 August 2018*

Citation:
Giuliani C, Bucci I and Napolitano G (2018) The Role of the Transcription Factor Nuclear Factor-kappa B in Thyroid Autoimmunity and Cancer. Front. Endocrinol. 9:471. doi: 10.3389/fendo.2018.00471

INTRODUCTION

Nuclear factor-kappa B (NF-κB) was identified more than 30 years ago as a transcription factor that can stimulate the expression of the immunoglobulin κ light chain in B cells (1, 2). Further studies demonstrated that NF-κB binds to DNA as a dimer that is formed by the combination of several proteins that contain an N-terminal Rel homology domain, which is responsible for NF-κB DNA binding and dimerization. These proteins are members of the NF-κB family, and in mammals they include: subunit p50 and its precursor p105 (encoded by the *NF-κB1* gene); subunit p52 and its precursor p100 (encoded by the *NF-κB2* gene); subunit p65 (also called RelA); c-Rel;

TABLE 1 | The NF-κB family members in mammals.

Protein	Functional domains	Precursor	Gene symbol
p50 subunit	RHD	p105	*NFkB1*
p52 subunit	RHD	p100	*NFkB2*
p65 subunit (RelA)	RHD, TAD	None	*RELA*
c-Rel	RHD, TAD	None	*REL*
RelB	RHD, TAD	None	*RELB*

RHD, Rel homology domain; TAD, transcriptional activation domain
See references (2–4).

TABLE 2 | Main stimuli involved in NF-κB activation.

Pattern recognition receptors ligands (including PAMPs and DAMPs)
Cytokines (such as TNF-α, IL-1, IL-2, IL-17, IFNs)
T-cell receptor signals
B-cell receptor signals
Oxidative stress, hypoxia
Radiation (such as UV radiation, γ-radiation)
Mitogen-activated protein kinase signals

DAMPs, damage-associated molecular patterns; IFN, interferon; IL, interleukin; PAMPs, pathogen-associated molecular patterns; TNF-α, tumor necrosis factor α; UV: ultraviolet.

and RelB **(Table 1)** (2–4). The proteins p65, c-Rel, and RelB contain a C-terminal transcriptional activation domain (TAD) that confers the ability to activate gene expression, whereas p50 and p52 lack the TAD and can only stimulate transcription through formation of heterodimers with transcription factors that have a TAD. Otherwise, p50 and p52 can bind as homodimers and repress gene transcription by preventing binding to the DNA of dimers containing a TAD (5). In this Review, the term NF-κB is used to indicate the NF-κB family of transcription factors as a whole (as given in **Table 1**), whereas the specific proteins are indicated where appropriate.

Although first described in B lymphocytes, NF-κB is almost ubiquitous. It regulates the expression of hundreds of genes, most of which are involved in inflammatory and immune responses. Indeed, NF-κB has a fundamental role in lymphocyte development and activation, and it is essential for innate and adaptive immune responses. In addition to its role in inflammation and immunity, NF-κB regulates other genes involved in cell survival, proliferation, and differentiation (2, 4–8). The list of genes that can be regulated by NF-κB can be found at the following link: http://www.bu.edu/NF-kB/gene-resources/target-genes/.

At its simplest, the mechanism by which NF-κB regulates transcription can be described as follows. In the resting state, NF-κB dimers are located in the cytoplasm in an inactive form, through their binding to inhibitory proteins known as inhibitors of κB (IκB). A wide range of stimuli can activate NF-κB through degradation of IκB **(Table 2)**. This leads to translocation of the dimers into the nucleus, where they bind to a consensus sequence in the promoters of target genes **(Figure 1)**. The first NF-κB dimer that was identified was the p50/p65 heterodimer (9), which is also the most abundant and widespread of the NF-κB dimers. In addition, several combinations have been described, both as homodimers, such as p65/p65, c-Rel/c-Rel, and p50/p50, and as heterodimers, such as p52/c-Rel, p50/c-Rel, RelB/p50, RelB/p52, p65/c-Rel, and p65/p52 (10).

As indicated above, in unstimulated cells, NF-κB dimers are localized in the cytoplasm through their association with IκB proteins. However, it has been reported that the complex constituted by IκBα and the p50/p65 dimer can shuttle between the cytoplasm and the nucleus, although it remains transcriptionally inactive. Indeed, only after degradation of IκB proteins this dimer localizes to the nucleus and binds to DNA (2, 10). The IκB protein family is characterized by the presence of

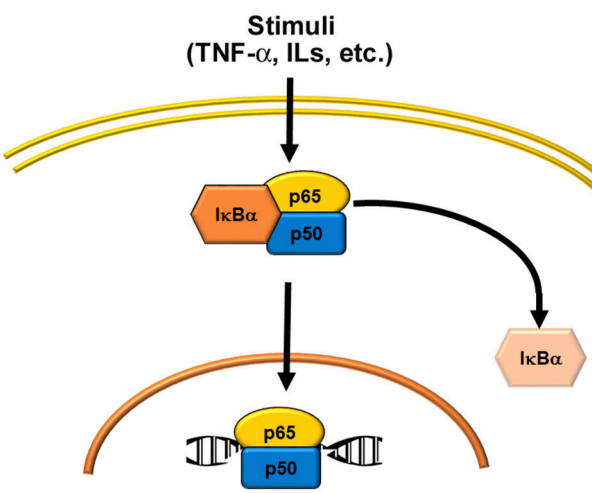


FIGURE 1 | Schematic representations of NF-κB activation. Several stimuli (see **Table 2**) activate the classical or canonical pathway, where the p50/p65 heterodimer is the most common signal.

several ankyrin repeat (ANK) domains (i.e., five to seven) that are responsible for IκB binding to the NF-κB dimers. Several IκB proteins have been identified, including IκBα, IκBβ, IκBε, IκBκ, and Bcl-3. The precursor proteins p100 and p105 have several ANK domains in their C-terminal portions that work as IκB proteins, and therefore they are known as IκBδ and IκBγ, respectively (2, 5). IκBα and IκBβ are the best known members of the IκB family, as they are expressed in almost all tissues; conversely, the expression of IκBε, IκBζ, and Bcl-3 is restricted to hematopoietic cells (5, 10). The primary target of IκBα is the dimer p50/p65, whereas IκBβ is associated mainly with p65/c-Rel dimers. Several stimuli **(Table 2)** can activate NF-κB by triggering a signal cascade that ends in the phosphorylation of the IκB proteins, and their removal from the NF-κB dimer complex.

Two signaling pathways are involved in NF-κB activation: the classical or canonical pathway and the alternative or non-canonical pathway. The canonical pathway is the most common signaling involved in the activation of NF-κB. It is triggered by inflammatory cytokines, toll-like receptors, antigen receptors, and other stimuli, as given in **Table 2**. The canonical pathway activates the most common NF-κB dimers, which are

formed by the p65, p50, c-Rel, and RelB subunits. The non-canonical pathway is involved in the activation of p100/RelB dimers, and it is induced by specific stimuli, such as B-cell activating factor (BAFF), lymphotoxin β, CD40 ligand, receptor activator of NF-κB ligand (RANKL), tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK), and TNF superfamily member 14 (also known as LIGHT) (11). The non-canonical NF-κB pathway is mainly involved in regulation of immune cell function and in bone remodeling (11).

As already mentioned, the precursor proteins p100 and p105 contain ANK domains and can function as IκB proteins. Usually, the precursor p105 is constitutively processed in cells, which produces the p50 subunit that binds to other NF-κB subunits to form dimers. However, dimers between p105 and other NF-κB subunits can also be formed; in this case, the ANK domains of the precursor protein function as an IκB protein, and their phosphorylation and degradation activate p50-containing dimers (12). Instead, precursor p100 is processed only after stimulation of the non-canonical pathway, which generates p52-containing dimers.

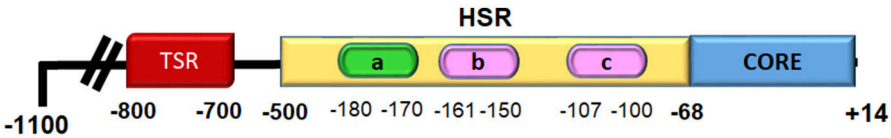
Activated NF-κB dimers bind to specific DNA binding sites (called κB sites) that are located in the promoter regions of the target genes. The consensus κB site has a partial palindromic sequence, 5′-GGGRNWYYCC-3′, where R is any purine (A or G), N is any nucleotide, W is A or T, and Y is any pyrimidine (C or T) (2). The constitution of different NF-κB dimers allows the regulation of distinct sets of genes. The mechanism of transcriptional regulation by NF-κB is complex and remains not fully understood (13). The several combinations of dimers that can be formed are fundamental for selective regulation of the target genes. Indeed, differences in DNA binding affinities have been reported between the different NF-κB subunits and the variant κB sites of target genes. Some dimers, such as c-Rel, p50, and Rel A homodimers, can bind κB sites that contain only half of the site consensus sequence (14). Also, the same NF-κB dimer can have different effects on gene transcription based on small differences in the κB site. An example is seen for the p52/Bcl-3 dimer, which activates transcription in genes with a κB site that contains the nucleotides G/C in the central position, whereas it represses transcription in genes where the nucleotides A/T are located in the central position of the κB site (15). Furthermore, NF-κB dimers can interact with several transcriptional coactivators (e.g., interactions between p65 and the coactivators p300/CBP) and with chromatin complexes. These interactions are particularly important for NF-κB dimers that lack a TAD; i.e., p50 and p52. Indeed, it has been reported that p50 and p52 homodimers can stimulate gene transcription through their interactions with nuclear IkB proteins such as Bcl-3 and IkB ζ, which function as coactivators. Furthermore, p50 and p52 homodimers can repress gene expression through interactions with histone deacetylases. A further mechanism that contributes to heterogeneous regulation of target genes by NF-κB is through its physical interactions with steroid hormone receptors, and it can also form heterodimers with different families of transcription factors, such as activator protein-1 (AP-1) and cAMP response element binding protein (CREB) (16–20).

NF-κB AND THE THYROID

The presence of NF-κB in thyrocytes was reported more than 20 years ago, both in a human thyroid carcinoma cell line (21) and in a non-transformed rat thyroid cell line (17). Thenceforth, several studies have demonstrated the involvement of NF-κB in thyroid autoimmunity, thyroid cancer, and thyroid-specific gene regulation (7, 21–25). It is worth to remark that the role of NF-κB in regulating the expression of the thyroid-specific genes has been demonstrated several years after the discovery of its involvement in thyroid autoimmunity and cancer.

NF-κB and Thyroid Autoimmunity

NF- κB has a fundamental role in both innate and adaptive immune responses. Indeed, NF-κB is one of the main transcription factors that is activated by pattern recognition receptors (PRRs), cytokines receptors, and lymphocyte receptors; therefore, it is not surprising that several studies have demonstrated its involvement in the development of autoimmune diseases (22). Of note, thyroid cells have functional PRRs, such as toll-like receptors (TLRs) and RIG-like receptors, that respond to various pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), to induce the production of several cytokines and chemokines (26). On this basis, it has been hypothesized that several insults to thyrocytes through the production of PAMPs or DAMPs can trigger an innate immune response, and eventually make thyrocytes behave as antigen-presenting cells (APCs), which can recruit and activate lymphocytes, and hence initiate an autoimmune response (26–28). An intriguing observation is that in rat thyroid cells in continuous culture, the FRTL-5 cells, there is a hormonal regulation of NF-κB activation, and of its binding to DNA. Indeed, studies on the major histocompatibility complex (MHC) class I gene in thyrocytes have shown that its expression is regulated by several hormones and growth factors through the regulation of NF-κB binding to the MHC class I promoter. Many studies have demonstrated that the main hormones involved in the regulation of thyroid growth and function decrease MHC class I expression, which include thyroid-stimulating hormone (TSH), insulin/ insulin-like growth factor (IGF)-I and hydrocortisone, (17, 29–31). MHC class I overexpression, as well as MHC class II aberrant expression, on non-immune cells is a feature of autoimmune diseases and thyroid autoimmunity (27, 32–37). The hormonal regulation of MHC molecules in thyroid cells is considered important for the suppression of autoimmunity during hormonally induced changes in cellular growth and function, which results in enhanced expression of potential thyroid autoantigens, such as thyroglobulin, thyroid peroxidase, and the TSH receptor (TSHR). Of note, MHC class I expression is also decreased by iodide, phorbol esters, transforming growth factor (TGF)-β, and methimazole, whereas it is increased by interferon (IFN)-α and IFN-γ, thymosin-α1, and high levels of glucose (17, 38–42). It must be emphasized that the regulation of MHC class I gene expression by these hormones and growth factors involves the binding of NF-κB dimers to the enhancer A region of the MHC class I promoter **(Figure 2)**. The enhancer A region is located in a "hormone-sensitive region"



- **TSR:** Tissue Specific Region with overlapping enhancer and silencer elements
- **HSR:** Hormones/cytokines Sensitive Region including:
  a. **Enhancer A**
  b. **Interferon response element**
  c. **CRE-like element**
- **CORE:** Core promoter containing CAAT and TATAA-like elements

FIGURE 2 | Diagrammatic representation of the MHC class I gene promoter.

of the MHC class I promoter (i.e., −500 to −68 bp), and this region is responsible for the regulation of MHC class I expression by hormones, cytokines, chemokines, and drugs (33, 35, 40). The enhancer A sequence (5′-GGGGAGTCCCC-3′) that spans nucleotides −180 bp to −170 bp is a palindromic variant of the κB consensus site, and it can bind NF-κB dimers (43). Its core sequence, GGGGA, is common to κB sites from other genes, such as that of the immunoglobulin κ light chain (38). Using electrophoretic mobility shift assays, it has been demonstrated that in thyrocytes, the enhancer A sequence can bind several NF-κB dimers. The first dimer identified, named Mod-1, is an unusual heterodimer that comprises the p50 subunit of NF-κB and fra-2, a transcription factor member of the AP-1 family (17). Modulation of Mod-1 binding affects MHC class I expression. Indeed, increased Mod-1 binding to enhancer A results in increased expression of the promoter activity, whereas the opposite is seen when there is decreased Mod-1 binding. Enhancer A also binds the classic NF-κB heterodimer p50/p65, which has an opposite effect compared to Mod-1 (38). In brief, several factors regulate MHC class I expression through modification of the binding of the heterodimers Mod-1 and p50/p65 to enhancer A. As an example, iodide, phorbol esters, and TGF-β decrease MHC class I gene transcription through inhibition of Mod-1 binding, while they allow p50/p65 binding (38, 39). Conversely, factors such as glucose and thymosin-α1, which activate MHC class I gene transcription, act through increasing Mod-1 binding and decreasing p50/p65 binding to enhancer A (41, 42). Further studies have demonstrated that NF-κB also interacts with a dominant regulatory element of the MHC class I promoter that is located between −800 bp and −700 bp, and which regulates tissue-specific transcription through overlapping enhancer and silencer elements. The binding of a complex in this region has been observed, which contains the p65 subunit of NF-κB and c-jun (31, 39). Of note, the two primary regions involved in the regulation of MHC class I transcription in thyroid cells interact with different members of the same family of transcription factors, NF-κB and AP-1, and both these factors are involved in the signaling of PPRs and cytokine receptors.

The data obtained on the regulation of the MHC class I gene are not restricted to this gene, as they can also be applied on the regulation of all of the thyroid genes where transcription is modulated by NF-κB (44–47). In this regard, the intercellular adhesion molecule (ICAM)-1 gene is hormonally regulated in FRTL-5 cells, and this regulation involves NF-κB, similarly to that observed for the MHC class I gene (44).

A conclusion that comes from these data is that different NF-κB dimers can modify the expression of the target genes. Therefore, an observation that is of significant interest is that in thyroid cells, stimulation of TSHR by TSH or stimulating antibodies to TSHR (TSAbs) can modify the composition of the NF-κB dimers activated by TNF-α. Indeed, in the absence of TSH, TNF-α treatment activates only the p50 homodimers, whereas in the presence of TSH, there is also activation of the p50/p65 heterodimers, which results in the modification of target gene expression (48).

Further progress on the understanding of the role of NF-κB in thyroid autoimmunity was derived from studies on CD40 signaling in thyroid cells. CD40 is a member of the TNF family that is expressed in immune cells and some non-immune cells, including thyroid cells. CD40 overexpression on thyroid cells has been associated with the development of autoimmunity (49). A recent study has showed that CD40 activation up-regulates expression of the p65 and p52 subunits of NF-κB in human primary thyroid cell cultures from Graves' patients, which indicates an involvement of both the canonical and noncanonical NF-κB pathways in CD40 signaling in Graves' disease (50).

Involvement of NF-κB activation is also seen in the pathogenesis of Graves' orbitopathy. Graves' orbitopathy is characterized by infiltration of the orbit by fibrocytes, which produce proinflammatory cytokines and induce an inflammatory reaction. Cytokine production by fibrocytes is stimulated by TSH and TSAbs, which interact with a receptor complex that is formed by the TSHR and the IGF-I receptor. Stimulation of this receptor complex results in activation of both the Akt and NF-κB pathways (51). As previously reported for thyroid cells, CD40 signaling can activate NF-κB on these orbit fibrocytes (52). At

present, which NF-κB dimers are activated on orbital fibrocytes is not known.

NF-κB and Thyroid Cancer

A large number of studies on the role of NF-κB in the pathogenesis of cancer followed the observations that NF-κB transcription factors have homology with the avian oncogene *v-REL*, which causes reticuloendotheliosis and lymphoma in poultry (53), and that human *c-REL* can induce transformation of primary chicken spleen cells (54). Abnormal activation of NF-κB has been associated not only with lymphoid malignancies (55), but also with tumors of epithelial origin, including thyroid cancer (23, 56). This is not surprising given the well-recognized connection between inflammation and tumor development (57–59). NF-κB promotes the production of cytokines, chemokines, growth factors, and other molecules that constitute the tumor microenvironment. Furthermore, NF-κB increases the expression of anti-apoptotic genes, such as *BCL2*, and mitogenic genes, such as c-*MYC* and cyclin D1 (23, 56). Therefore, NF-κB activation makes tumor cells resistant to pro-apoptotic stimuli, as observed for TGF-β apoptotic effects in thyroid cancer cells (60). Activation of NF-κB in tumors can arise from both a response to classical inflammatory stimuli, such as infectious and physical or chemical agents **(Figure 3A)**, and the result of oncogene activation **(Figure 3B)** (58). A typical example of the latter is the *RET* oncogene, which is involved in several cancer types, including thyroid cancer (61). Indeed, activating mutations of the *RET* gene are responsible for medullary thyroid carcinomas, and RET/PTC rearrangements are associated with some 5 to 25% of thyroid papillary carcinomas (62). Of interest, activating mutations of the *RET* proto-oncogene cause constitutive activity of NF-κB, and this process is important for *RET*-mediated carcinogenesis (63, 64). Indeed, abnormal expression of the RET/PTC1 oncogene in primary cultures of normal human thyrocytes is sufficient to induce the expression of a large panel of genes that can be activated by NF-κB and are involved in inflammation, among which there are the colony-stimulating factors, interleukin-1β, and cyclooxygenase 2 (65).

For thyroid cancers, constitutive increased DNA-binding activity of NF-κB was reported for the first time about 20 years ago, in a series of seven human thyroid carcinoma cell lines that included papillary, follicular, and anaplastic carcinomas (66). In particular, the increased binding activity was associated with overexpression of the p65 subunit, whereas the p50 subunit was not overexpressed in the anaplastic carcinoma cell lines, and was increased only in cell lines derived from papillary or follicular carcinoma, and to a lesser extent than for p65. The role of the p65 subunit was confirmed by the observation that inhibition of p65 expression using a specific antisense oligonucleotide reduced the growth of cell lines and their colony formation in agar. Successively, several studies showed that activation of NF-κB is a key merging point of distinct transforming signals that are involved in thyroid carcinogenesis, which besides the *RET* oncogene, included others such as $BRAF^{V600E}$ mutation, *PPARγ* insufficiency, and *PTEN* inactivation (67–69). Indeed, induction of a mutated form of the *BRAF* gene (e.g., the $BRAF^{V600E}$ mutation) in models of follicular and papillary thyroid cancer cell lines resulted in NF-κB activation, with involvement of the p65/p50 heterodimer (67). This NF-κB activation resulted in apoptotic resistance and increased invasiveness of these cells, as a consequence of increased expression of anti-apoptotic molecules and matrix metalloproteinases. Furthermore, in a mouse model of thyroid cancer, inactivation of the *PTEN* or *PPARγ* genes gave rise in both cases to a more aggressive form of cancer, which was associated with NF-κB overactivation (68, 69). As well as the experimental data, NF-κB activation has also been shown in human thyroid cancer tissues (23, 70–72). A first study that was performed on 10 specimens of thyroid follicular carcinoma investigated the p65 subunit selectively and showed its constitutive activation and translocation to the nucleus (70). A following study showed increased nuclear expression of the p65 subunit in about 75% of a larger sample of papillary carcinomas. Interestingly, these tumors were associated with a significantly higher frequencies of aggressive features, such as extrathyroidal extension and lymph node metastasis (71). The important role of NF-κB in the pathogenesis of thyroid cancer was also confirmed by the efficacy of several inhibitors of NF-κB activation in the counteracting of its effects on growth and invasiveness in experimental models (23, 73–76).

However, very few studies have evaluated the binding of the distinct NF-κB dimers to DNA in thyroid cancers. Most of the studies performed both *in vitro* and *in vivo* have investigated NF-κB activation using only antibodies against the p65 subunit. A few studies have also investigated the p50 subunit, which showed that the p50/p65 heterodimer is the main complex involved in DNA binding (66, 74, 77). However, no data is available so far concerning the involvement of other NF-κB dimers. There is also no information available on the binding of distinct dimers to the promoters of specific genes involved in thyroid carcinogenesis. Therefore, a working hypothesis would investigate the distinct dimer combinations involved in binding to the promoter region of genes activated in thyroid carcinogenesis, as described above for the MHC class I gene in FRTL-5 cells. Moreover, more data are needed on the interactions between NF-κB and other transcription factors, as some studies have suggested that this is an important step in thyroid carcinogenesis and it can be used as a molecular target for therapy (75, 76). On this point, an experimental study has shown that the efficacy of triptolide in inhibition of the growth and invasiveness of an anaplastic thyroid cancer cell line is due to its blocking of the association between the p65 subunit of NF-κB and CBP/P300 (75). Similarly, the retinoid X receptor agonist bexarotene that has been used to treat metastatic differentiated thyroid cancer in clinical trials, represses NF-κB activation in follicular cancer cells through inhibition of the interaction between p300 and the p65 subunit (76).

NF-κB and Thyroid-Specific Gene Regulation

The findings that NF-κB is involved in the regulation of thyroid-specific genes is of particular interest (45–47). In a study of the effects of lipopolysaccharide on *NIS* gene expression in FRTL-5 cells, Nicola et al. (45) defined a κB binding site in the upstream enhancer region of the *NIS* promoter (NUE). They also observed that this site binds the p65 subunit of NF-κB, and that this subunit acts in synergy with transcription factor Pax8 for the promotion of gene transcription. Indeed, a physical interaction between p65

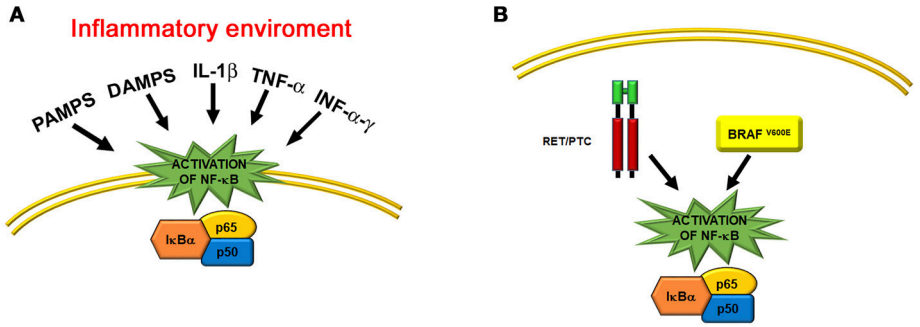


FIGURE 3 | Schematic representations of the two main mechanisms that activate NF-κB in thyroid cells. **(A)** Activation of the NF-κB pathway in an inflammatory environment, such as Hashimoto's thyroiditis. Some of the main factors that act throughout transmembrane and cytosolic receptors are indicated. **(B)** Activation of the NF-κB pathway by thyroid oncogenes, such as the cytosolic RET/PTC rearranged protein and the cytosolic $BRAF^{V600E}$ mutated kinase.

and Pax8 was reported. These data confirm the involvement of heterodimers between NF-κB subunits and other transcription factors in the regulation of gene expression in thyroid cells, as discussed above regarding the expression of the MHC class I gene in FRTL-5 cells. Subsequently, the same group showed that the p65 subunit is also involved in the regulation of *TPO* gene expression by lipopolysaccharide (46). A recent study further highlighted the role of NF-κB in thyroid physiology (25). Indeed, a mouse model bearing a thyroid-specific knock-out of the NF-κB essential modulator (NEMO) gene, which is fundamental for IκB phosphorylation, developed hypothyroidism and thyroid hypoplasia due to massive apoptosis. Of further interest, in this model there was a reduced expression of the thyroid specific genes *NIS, TPO, TG, Pax8*, and *TTF-1* (*NKX2-1*). These data are very important, as they demonstrate that as well as its involvement in thyroid autoimmunity and cancer, NF-κB is also fundamental for the regulation of genes related to normal thyroid function (25, 78).

CONCLUSIONS

Since its discovery more than 30 years ago, NF-κB has become a pillar of cellular biology. A large number of studies have shown its fundamental roles in regulating cellular functions. The importance of this can be perceived by considering the great number of genes that can be regulated by NF-κB. For the thyroid, after the first studies that showed the role of NF-κB in the regulation of MHC genes, several observations underlined its further role as a common target of the distinct PRRs pathways, which confirmed its involvement in the pathogenesis of thyroid autoimmunity. Not surprisingly, given the relationship between thyroid autoimmunity and cancer, NF-κB is also involved in thyroid carcinogenesis, and it is considered a potential pharmacological target for new therapies against the most aggressive types of thyroid cancers. As well as these observations on the role of NF-κB in thyroid pathology, recent studies of great relevance have correlated NF-κB with normal thyroid growth and function. These data indicate how much more we have to learn about the function of NF-κB in both thyroid physiology and physiopathology. As some studies have suggested, an important issue that remains largely unexplored relates to the binding of the distinct NF-κB subunits to DNA, and particularly the different combinations of homodimers and heterodimers involved, including those with other families of transcription factors. Therefore, more studies are needed to understand the physiological role of NF-κB in thyrocytes and its dysfunction in thyroid pathology.

AUTHOR CONTRIBUTIONS

CG: substantial contributions to the conception and design of the work; drafting the work; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. IB: substantial contributions to the design of the work; revising the work critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. GN: substantial contributions to the conception of the work; revising it critically for intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work.

REFERENCES

1. Sen R, Baltimore D. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. *Cell* (1986) 46:705–16. doi: 10.1016/0092-8674(86)90346-6
2. Zhang Q, Lenardo MJ, Baltimore D. Thirty years of NF-κB: a blossoming of relevance to human pathobiology. *Cell* (2017) 168:37–57. doi: 10.1016/j.cell.2016.12.012
3. Napetschnig J, Wu H. Molecular basis of NF-κB signaling. *Annu Rev Biophys.* (2013) 42:443–68. doi: 10.1146/annurev-biophys-083012-130338
4. Hinz M, Scheidereit C. The IκB kinase complex in NF-κB regulation and beyond. *EMBO Rep.* (2013) 15:46–61. doi: 10.1002/embr.201337983
5. Hayden TH, Gosh S. NF-κB, the first quarter-century: remarkable progress and outstanding questions. *Genes Dev.* (2012) 26:203–34. doi: 10.1101/gad.183434.111

6. Gilmore TD, Wolenski FS. NF-κB: where did it come from, and why? *Immunol Rev.* (2012) 246:14–35. doi: 10.1111/j.1600-065X.2012.01096.x
7. Giuliani C, Napolitano G, Bucci I, Montani V, Monaco F. NF-κB transcription factor: role in the pathogenesis of inflammatory autoimmune and neoplastic diseases and therapy implications. *Clin Ter*. (2001) 152:249–53.
8. Santoro MG, Rossi A, Amici C. NF-κB and virus infection: who controls whom? *EMBO J*. (2003) 22:2552–60. doi: 10.1093/emboj/cdg267
9. Baeuerle PA, Baltimore D. A 65-kD subunit of active NF-κB is required for inhibition of NF-κB by IκB. *Genes Dev*. (1989) 3:1689–98. doi: 10.1101/gad.3.11.1689
10. Oeckinghaus A, Gosh S. The NF-κB family of transcription factors and its regulation. *Cold Spring Harb Perspect Biol*. (2009) 1:000034. doi: 10.1101/cshperspect.a000034
11. Cildir C, Low KC, Tergaonkar V. Noncanonical NF-κB signaling in health and disease. *Trends Mol Med*. (2016) 22:414–29. doi: 10.1016/j.molmed.2016.03.002
12. Kravtsova-Ivantsiv Y, Cohen S, Ciechanover A. Modification by single ubiquitin moieties rather than polyubiquitination is sufficient for proteasomal processing of the p105 NF-κB precursor. *Mol Cell* (2009) 33:496–504. doi: 10.1016/j.molcel.2009.01.023
13. Smale ST. Dimer-specific regulatory mechanisms within the NF-κB family of transcription factors. *Immunol Rev*. (2012) 246:193–204. doi: 10.1111/j.1600-065X.2011.01091.x
14. Siggers T, Chang AB, Teixeira A, Wong D, Williams KJ, Ahmed B, et al. Principle of dimer-specific gene regulation revealed by a comprehensive characterization of NF-κB family DNA binding. *Nat Immunol*. (2012) 13:95–102. doi: 10.1038/ni.2151
15. Wang VY-F, Huang W, Asagiri M, Spann N, Hoffmann A, Glass C, et al. The transcriptional specificity of NF-κB dimers is coded within the κB DNA response element. *Cell Rep*. (2012) 2:824–39. doi: 10.1016/j.celrep.2012.08.042
16. Stein B, Baldwin AS, Ballard DW, Greene WC, Angel P, Herrlich P. Cross-coupling of NF-κB p65 and Fos/Jun transcription factors produces potentiated biological function. *EMBO J*. (1993) 12:3879–91.
17. Giuliani C, Saji M, Napolitano G, Palmer LA, Taniguchi S-I, Shong M, et al. Hormonal modulation of major histocompatibility complex class I gene expression involves an enhancer A-binding complex consisting of fra-2 and the p50 subunit of NF-κB. *J Biol Chem.* (1995) 270:11453–62. doi: 10.1074/jbc.270.19.11453
18. Yao C, Purwanti N, Karabasil MR, Azlina A, Javkhlan P, Hasegawa T, et al. Potential down-regulation of salivary gland AQP5 by LPS via cross-coupling of NF-κB and p-c-Jun/c-Fos. *Am J Pathol*. (2010) 177:724–34. doi: 10.2353/ajpath.2010.090282
19. McKay LI, Cidlowski JA. Cross-talk between nuclear factor-κB and the steroid hormone receptors: mechanisms of mutual antagonism. *Mol Endocrinol*. (1998) 12:45–66. doi: 10.1210/mend.12.1.0044
20. Ling J, Kumar R. Cross-talk between NF-κB and glucocorticoid signaling: a potential target of breast cancer therapy. *Cancer Lett*. (2012) 322:119–66. doi: 10.1016/j.canlet.2012.02.033
21. Pang X-P, Ross NS, Park M, Juillard GJF, Stanley TM, Hershman JM. Tumor necrosis factor-α activates nuclear factor κB and induces manganous superoxide dismutase and phosphodiesterase mRNA in human papillary thyroid carcinoma cells. *J Biol Chem.* (1992) 267:12826–30.
22. Kurylowicz A, Nauman J. The role of nuclear factor-κB in the development of autoimmune diseases: a link between genes and environment. *Acta Biochim Pol*. (2008) 55: 629-47.
23. Pacifico F, Leonardi A. Role of NF-κB in thyroid cancer. *Mol Cell Endocrinol*. (2010) 321:29–35. doi: 10.1016/j.mce.2009.10.010
24. Zeligs KP, Neuman MK, Annunziata CM. Molecular pathways: the balance between cancer and the immune system challenges the therapeutic specificity of targeting nuclear factor-κB signaling for cancer treatment. *Clin Cancer Res*. (2016) 22:4302–08. doi: 10.1158/1078-0432.CCR-15-1374
25. Reale C, Iervolino A, Scudiero I, Ferravante A, D'Andrea LE, Mazzone P, et al. NF-κB essential modulator (NEMO) is critical for thyroid function. *J Biol Chem*. (2016) 291:5765–73. doi: 10.1074/jbc.M115.711697
26. Kawashima A, Yamazaki K, Hara T, Akama T, Yoshihara A, Sue M, et al. Demonstration of innate immune responses in the thyroid gland: potential to sense danger and a possible trigger for autoimmune reactions. *Thyroid* (2013) 23:477–87. doi: 10.1089/thy.2011.0480
27. Luo Y, Yoshihara A, Oda K, Ishido Y, Suzuki K. Excessive cytosolic DNA fragments as a potential trigger of Graves' disease: an encrypted message sent by animal models. *Front Endocrinol*. (2016) 7:144. doi: 10.3389/fendo.2016.00144
28. Harii N, Lewis CJ, Vasko V, McCall K, Benavides-Peralta U, Sun X, et al. Thyrocytes express a functional toll-like receptor 3: overexpression can be induced by viral infection and reversed by phenylmethimazole and is associated with Hashimoto's autoimmune thyroiditis. *Mol Endocrinol*. (2005) 19:1231–50. doi: 10.1210/me.2004-0100
29. Saji M, Moriarty J, Ban T, Singer DS, Kohn LD. Major histocompatibility complex class I gene expression in rat thyroid cells is regulated by hormones methimazole and iodide as well as interferon. *J Clin Endocrinol Metab*. (1992) 75:871–8.
30. Saji M, Shong M, Napolitano G, Palmer LA, Taniguchi S-I, Ohmori M, et al. Regulation of major histocompatibility complex class I gene expression in thyroid cells. *J Biol Chem*. (1997) 272:20096–107. doi: 10.1074/jbc.272.32.20096
31. Giuliani C, Saji M, Bucci I, Fiore G, Liberatore M, Singer DS, et al. Transcriptional regulation of major histocompatibility complex class I gene by insulin and IGF-I in FRTL-5 thyroid cells. *J Endocrinol*. (2006) 189:605–15. doi: 10.1677/joe.1.06486
32. Mozes E, Kohn LD, Hakim F, Singer DS. Resistance of MHC class I-deficient mice to experimental systemic lupus erythematosus. *Science* (1993) 261:91–3. doi: 10.1126/science.8316860
33. Singer DS, Mozes E, Kirshner S, Kohn LD. Role of MHC class I molecules in autoimmune disease. *Crit Rev Immunol*. (1997) 17:463–8.
34. Ito T, Meyer KC, Ito N, Paus R. Immune privilege and the skin. *Curr Dir Autoimmun*. (2008) 10:27–52. doi: 10.1159/000131412
35. René C, Lozano C, Eliaou JF. Expression of classical HLA class I molecules: regulation and clinical impacts. *HLA* (2016) 87:338–49. doi: 10.1111/tan.12787
36. Richardson SJ, Rodriguez-Calvo T, Gerling IC, Mathews CE, Kaddis JS, Russell MA, et al. Islet cell hyperexpression of HLA class I antigens: a defining feature in type 1 diabetes. *Diabetologia* (2016) 59:2448–58. doi: 10.1007/s00125-016-4067-4
37. Gianfran C, Pisapia L, Picascia S, Strazzullo M, Del Pozzo G. Expression level of risk genes of MHC class II is a susceptibility factor for autoimmunity: new insights. *J Autoimmun.* (2018) 89:1–10. doi: 10.1016/j.jaut.2017.12.016
38. Taniguchi S-I, Shong M, Giuliani C, Napolitano G, Saji M, Montani V, et al. Iodide suppression of major histocompatibility class I gene expression in thyroid cells involves enhancer A and the transcription faactor NF-κB. *Mol Endocrinol.* (1998) 12:19–33. doi: 10.1210/mend.12.1.0052
39. Napolitano G, Montani V, Giuliani C, Di Vincenzo S, Bucci I, Todisco V, et al. Transforming growth factor-?1 down-regulation of major histocompatibility complex class I in thyrocytes: coordinate regulation of two separate elements by thyroid-specific as well as ubiquitous transcription factors. *Mol Endocrinol.* (2000) 14:486–505. doi: 10.1210/mend.14.4.0454
40. Giuliani C, Bucci I, Montani V, Singer DS, Monaco F, Kohn LD, et al. Regulation of major histocompatibility complex gene expression in thyroid epithelial cells by methimazole and phenylmethimazole. *J Endocrinol*. (2010) 204:57–66. doi: 10.1677/JOE-09-0172
41. Napolitano G, Bucci I, Giuliani C, Massafra C, Di Petta C, Devangelio E, et al. High glucose levels increase major histocompatibility complex class I gene expression in thyroid cells and amplify interferon-γ action. *Endocrinology* (2002) 143:1008–17. doi: 10.1210/endo.143.3.8674
42. Giuliani C, Napolitano G, Mastino A, Di Vincenzo S, D'Agostini C, Grelli S, et al. Thymosin-α1 regulates MHC class I expression in FRTL-5 cells at transcriptional level. *Eur J Immunol.* (2000) 30:778–86. doi: 10.1002/1521-4141(200003)30:3<778::AID-IMMU778>3.0.CO;2-I
43. Baldwin AS, Sharp PA. Two transcription factors NF-κB and H2TF1 interact with a single regulatory sequence in the class I major histocompatibility complex promoter. *Proc Natl Acad Sci USA*. (1988) 85:723–7. doi: 10.1073/pnas.85.3.723
44. Park ES, You SH, Kim H, Kwon OY, Ro HK, Cho BY, et al. Hormone-dependent regulation of intercellular adhesion molecule-1 gene expression: cloning and analysis of 5'-regulatory region of rat intercellular adhesion molecule-1 gene in FRTL-5 rat thyroid cells. *Thyroid* (1999) 9:601–12. doi: 10.1089/thy.1999.9.601

45. Nicola JP, Nazar M, Mascanfroni ID, Pellizas CG, Masini-Repiso AM. NF-κB p65 subunit mediates lipopolysaccharide-induced Na(+)/I(-) symporter gene expression by involving functional interaction with the paired domain transcription factor Pax8. *Mol Endocrinol*. (2010) 24:1846–62. doi: 10.1210/me.2010-0102
46. Nazar M, Nicola JP, Velez ML, Pellizas CG, Masini-Repiso AM. Thyroid peroxidase gene expression is induced by lipopolysaccharide involving nuclear factor (NF)-κB p65 subunit phosphorylation. *Endocrinology* (2012) 153:6114–25. doi: 10.1210/en.2012-1567
47. Nicola JP, Peyret V, Nazar M, Romero JM, Lucero AM, Montesinos Mdel M, et al. S-Nitrosylation of NF-κB p65 inhibits TSH-induced Na(+)/I(-) symporter expression. *Endocrinology* (2015) 156:4741–54. doi: 10.1210/en.2015-1192
48. Kikumori T, Kambe F, Nagaya T, Funahashi H, Seo H. Thyrotropin modifies activation of nuclear factor kB by tumour necrosis factor α in rat thyroid cell line. *Biochem J*. (2001) 354:573–79. doi: 10.1042/bj3540573
49. Kayes T, Fang Y, Yu S, Downey E, Wang S, Braley-Mullen H. Agonistic anti-CD40 induces thyrocyte proliferation and promotes thyroid autoimmunity by increasing CD40 expression on thyroid epithelial cells. *J Immunol.* (2013) 190:3928–38. doi: 10.4049/jimmunol.1202929
50. Lee HJ, Lombardi A, Stefan M, Li CW, Inabnet WBIII, Owen RP, et al. CD40 signaling in Graves' disease is mediated through canonical and noncanonical thyroidal nuclear factor κB activation. *Endocrinology* (2017) 158:410–8. doi: 10.1210/en.2016-1609
51. Chen H, Shan SJC, Mester T, Wei Y-H, Douglas RS. TSH-mediated TNF? production in human fibrocytes is inhibited by teprotumumab an IGF-1R antagonist. *PLoS ONE* (2015) 10:e0130322. doi: 10.1371/journalpone013032.
52. Wu T, Mester T, Gupta S, Sun F, Smith TJ, Douglas RS. Thyrotropin and CD40L stimulate interleukin-12 expression in fibrocytes: implications for pathogenesis of thyroid-associated ophthalmopathy. *Thyroid* (2016) 26:1768–77. doi: 10.1089/thy.2016.0243
53. Gilmore TD. Multiple mutations contribute to the oncogenicity of the retroviral oncoprotein v-Rel. *Oncogene* (1999) 18:6925–37. doi: 10.1038/sj.onc.1203222
54. Gilmore TD, Cormier C, Jean-Jacques J, Gapuzan ME. Malignant transformation of primary chicken spleen cells by human transcription factor c-Rel. *Oncogene* (2001) 20:7098–103. doi: 10.1038/sj.onc.1204898
55. Lim KH, Yang Y, Staudt LM. Pathogenetic importance and therapeutic implications of NF-κB in lymphoid malignancies. *Immunol Rev*. (2012) 246:359–78. doi: 10.1111/j.1600-065X.2012.01105.x
56. Pires BRB, Silva RCMC, Ferreira GM, Abdelhay E. NF-κB: two sides of the same coin. *Genes* (2018) 9:E24. doi: 10.3390/genes9010024
57. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* (2001) 357:539–45. doi: 10.1016/S0140-6736(00)04046-0
58. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* (2008) 454:436–44. doi: 10.1038/nature07205
59. Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S et al. NF-κB functions as a tumour promoter in inflammation-associated cancer. *Nature* (2004) 431:461–6. doi: 10.1038/nature02924
60. Bravo SB, Pampin S, Cameselle-Teijeiro J, Cerneiro C, Dominguez F, Barreiro F, et al. TGF-β-induced apoptosis in human thyrocytes is mediated by p27kip1 reduction and is overridden in neoplastic thyrocytes by NF-κB activation. *Oncogene* (2003) 22:7819–30. doi: 10.1038/sj.onc.1207029
61. De Falco V, Carlomagno F, Li HY, Santoro M. The molecular basis for RET tyrosine-kinase inhibitors in thyroid cancer. *Best Pract Res Clin Endocrinol Metab*. (2017) 31:307–18. doi: 10.1016/j.beem.2017.04.013
62. Acquaviva G, Visani M, Repaci A, Rhoden KJ, de Biase D, Pession A, et al. Molecular pathology of thyroid tumours of follicular cells: a review of genetic alterations and their clinicopathological relevance. *Hystopathology* (2018) 72:6–31. doi: 10.1111/his.13380
63. Ludwig L, Kessler H, Wagner M, Hoang-Vu C, Dralle H, Adler G, et al. Nuclear factor-κB is constitutively active in C-cell carcinoma and required for RET-inducced transformation. *Cancer Res*. (2001) 61:4526–35.
64. Spitschak A, Meier C, Kowtharapu B, Engelmann D, Pützer BM. MiR-182 promotes cancer invasion by linking RET oncogene activated NF-κB to loss of the HES1/Notch1 regulatory circuit. *Mol Cancer* (2017) 16:24. doi: 10.1186/s12943-016-0563-x
65. Borrello MG, Alberti L, Fischer A, Degl'Innocenti D, Ferrario C, Gariboldi M, et al. Induction of a proinflammatory program in normal human thyrocytes by the RET/PTC1 oncogene. *Proc Natl Acad Sci USA* (2005) 102:14825–830. doi: 10.1073/pnas.05030 39102
66. Visconti R, Cerutti J, Battista S, Fedele M, Trapasso F, Zeki K, et al. Expression of the neoplastic phenotype by human thyroid carcinoma cell lines requires NF-κB p65 protein expression. *Oncogene* (1997) 15:1987–94. doi: 10.1038/sj.onc.1201373
67. Palona I, Namba H, Mitsutake N, Starenki D, Podcheko A, Sedliarou I, et al. BRAFV600E promotes invasiveness of thyroid cancer cells through nuclear factor-κB activation. *Endocrinology* (2006) 147:5699–707. doi: 10.1210/en.2006-0400
68. Kato Y, Ying H, Zhao L, Furuya F, Araki O, Willingham MC, et al. PPARγ insufficiency promotes follicular thyroid carcinogenesis via activation of the nuclear factor-κB signaling pathway. *Oncogene* (2006) 25:2736–47. doi: 10.1038/sj.onc.1209299
69. Guigon CJ, Zhao L, Willingham MC, Cheng S-Y. PTEN deficiency accelerates tumour progression in a mouse model of thyroid cancer. *Oncogene* (2009) 28:509–17. doi: 10.1038/onc.2008.407
70. Liu J, Brown RE. Morphoproteomic confirmation of an activated nuclear factor-?B p65 pathway in follicular thyroid carcinoma. *Int J Clin Exp Pathol*. (2012) 5:216–23.
71. Pyo JS, Kang G, Kim DH, Chae SW, Park C, Kim K, et al. Activation of nuclear factor-κB contributes to growth and aggressiveness of papillary thyroid carcinoma. *Pathol Res Pract*. (2013) 209: 228–32. doi: 10.1016/j.prp.2013.02.004
72. Li W, Ming H, Sun D, Li W, Wang D, Zhang G et al. The relationship between BRAFV600E NF-κB and TgAb expression in papillary thyroid carcinoma. *Pathol Res Pract*. (2017) 213:183–8. doi: 10.1016/j.prp.2016.12.022
73. Li X, Abdel-Mageed AB, Mondal D, Kandil E. The nuclear factor-κB signaling pathway as a therapeutic target against thyroid cancers. *Thyroid* (2013) 23: 209–18. doi: 10.1089/thy.2012.0237
74. Starenki D, Namba H, Saenko V, Ohtsuru A, Yamashita S. Inhibition of nuclear factor-κB cascade potentiates the effect of a combination treatment of anaplastic thyroid cancer cells. *J Clin Endocrinol Metab.* (2004) 89:410–8. doi: 10.1210/jc.2003-031216
75. Zhu W, Ou Y, Li Y, Xiao R, Shu M, Zhou Y, et al. A small-molecule triptolide suppresses angiogenesis and invasion of human anaplastic thyroid carcinoma cells via down-regulation of the nuclear factor-κB pathway. *Mol Pharmacol*. (2009) 75:812–9. doi: 10.1124/mol.108.052605
76. Cras A, Politis B, Balitrand N, Darsin-Bettinger D, Boelle PY, Cassinat B, et al. Bexarotene via CBP/p300 induces suppression of NF-κB–dependent cell growth and invasion in thyroid cancer. *Clin Cancer Res*. (2012) 18:442–53. doi: 10.1158/1078-0432.CCR-11-0510
77. Vasudevan KM, Gurumurthy S, Rangnekar VM. Suppression of PTEN expression by NF-κB prevents apoptosis. *Mol Cell Biol*. (2004) 24:1007–21. doi: 10.1128/MCB.24.3.1007-1021.2004
78. Reale C, Zotti T, Scudiero I, Vito P, Stilo R. The NF-κB family of transcription factors and its role in thyroid physiology. *Vitam Horm.* (2018) 106:195–210. doi: 10.1016/bs.vh.2017.05.003

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer SF and handling editor declared their shared affiliation at time of review.

frontiers in Endocrinology

CASE REPORT
published: 19 July 2018
doi: 10.3389/fendo.2018.00398

Medullary Thyroid Carcinoma With Exon 2 p.L56M RET Variant: Clinical Particular Features in Two Patients

*Rosa M. Paragliola[1], Rosa M. Lovicu[1], Giampaolo Papi[1], Ettore Capoluongo[2], Angelo Minucci[2], Giulia Canu[2], Alfredo Pontecorvi[1] and Salvatore M. Corsello[1]**

[1] Unit of Endocrinology, Università Cattolica del Sacro Cuore, Rome, Italy, [2] Unit of Biochemistry and Clinical Biochemistry, F. Policlinico Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy

RET (REarranged during Transfection) proto-oncogene variants are essential for the development of familial and sporadic forms of medullary thyroid carcinoma (MTC). The most frequent variants are usually located in exons 10, 11, and 13 through 16 of the RET gene. We report two cases of apparently sporadic MTC associated with the variant in exon 2 of *RET* gene. Patient 1, a 62-year old man who had undergone adrenalectomy for a 5 cm pheochromocytoma, was screened for type 2 multiple endocrine neoplasia (MEN 2) which showed elevated basal and post-intravenous calcium gluconate calcitonin levels. A fine needle aspiration biopsy (FNAB) confirmed the suspicion of MTC. The patient underwent total thyroidectomy and lymphadenectomy, and the histology showed C-cell hyperplasia with medullary microcarcinoma. Patient 2, a 57 years old woman, underwent total thyroidectomy for toxic multinodular goiter. Pre-operative FNAB had shown benign features, while basal calcitonin levels were only borderline increased. Final histology revealed medullary multifocal microcarcinoma. Genetic testing for RET protoncogene on DNA extracted from peripheral blood was performed in both patients and a missense variant on exon 2 (c.166C>A, p.L56M) was identified. To our knowledge, these are the first time two cases of MTC associated to RET p.L56M variant. Interestingly, one patient had also a pheochromocytoma suggesting a possible pathogenetic role of this variant in the genesis of MEN2A. While the association of this variant with MTC or MEN2A has been never reported, it has been described in association with Hirschsprung's disease.

Keywords: medullary thyroid carcinoma, MEN2, RET, pheochromocytoma, L56M

OPEN ACCESS

Edited by:
Giorgio Stassi,
Università degli Studi di Palermo, Italy

Reviewed by:
Agnese Barnabei,
Istituto Nazionale del Cancro Regina Elena, Italy
Dario Giuffrida,
Istituto Oncologico del Mediterraneo, Italy
Pasqualino Malandrino,
Department of Clinical and Experimental Medicine, Italy

****Correspondence:***
Salvatore M. Corsello
salvatore.corsello@unicatt.it

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *27 April 2018*
Accepted: *27 June 2018*
Published: *19 July 2018*

Citation:
Paragliola RM, Lovicu RM, Papi G, Capoluongo E, Minucci A, Canu G, Pontecorvi A and Corsello SM (2018) Medullary Thyroid Carcinoma With Exon 2 p.L56M RET Variant: Clinical Particular Features in Two Patients. Front. Endocrinol. 9:398. doi: 10.3389/fendo.2018.00398

BACKGROUND

Medullary thyroid carcinoma (MTC), originating from parafollicular C-cells, accounts for 5% of all thyroid cancers and can occur as sporadic (75% of cases) or hereditary (25%) disease (1). Germline *RET* proto-oncogene variants play a crucial pathogenetic role and are found in the majority of hereditary forms (98%). In fact, only few families with hereditary MTC do not show any germline variant (2). On the other hand, somatic *RET* variants are responsible for approximately 40% of cases of sporadic MTC, according to data of COSMIC database published in 2015 (3). The *RET* proto-oncogene, located on chromosome 10, codifies for a member of the tyrosine-kinase family receptors which is expressed on C cells, parathyroid glands, adrenal

medulla and urogenital tract. In the hereditary forms, the causative role of the germline *RET* variants has been clearly demonstrated, and there appears to be a strict correlation between genotype and phenotype, leading to different degree of risk related to the aggressiveness of the tumor and the clinical syndrome [familiar medullary thyroid cancer (FMTC), and type 2A and type 2B multiple endocrine neoplasia (MEN) (4)]. The "classic MEN2A" phenotype is characterized by the concomitant occurrence of pheochromocytoma and primary hyperparathyroidism. In addition to the "classic MEN2A," other forms associated with cutaneous lichen amyloidosis (5) and with Hirschsprung disease have been reported (6). MEN2A is primarily associated with variants in the *RET* gene causing substitution of cysteines at codons 609, 611, 618, and 620 in exon 10, and particularly with the Cys634Arg alteration in exon 11 (3).

On the contrary, MEN2B, where MTC which is associated with pheochromocytoma and other additional clinical features (e.g., mucosal neuromas), is mostly associated with the Met918Thr variant in exon 16.

While genetic testing is mandatory whenever the familial form of MTC is suspected, germline *RET* testing may also be recommended in all patients with newly diagnosed C cell hyperplasia (CCH) or apparently sporadic MTC, since approximately 7% of patients, who would appear to have a "sporadic" form, in truth have an unsuspected germline variant in the *RET* proto-oncogene.

In the past decade, additional *RET* variants, unknown at the time of the International *RET* Consortium (7), have been discovered. These include very rare germline *RET* variants (8) often observed in a single affected member. However, the germline *RET* proto-oncogene variants identified during the past 20 years are localized in specific regions and involve eight exons (exons 5, 8, 10, 11, 13, 14, 15, 16) (9).

We here report the first two cases of MTC associated with the variant of exon 2 of *RET* proto-oncogene causing the substitution of Leucine with Methionine at codon 56 (p.L56M; rs145633958), which, to the best of our knowledge, has not previously been reported in association to MTC. In one case, the diagnosis of MEN2A cannot be excluded, considering the concomitant occurrence of pheochromocytoma.

Interestingly, the above-mentioned variant has been described in association with Hirschsprung disease, which has been excluded in these two patients.

CASE PRESENTATION AND DESCRIPTION OF LABORATORY INVESTIGATIONS AND DIAGNOSTIC TESTS

Patient 1: A 62-year man came to our attention for an incidentally discovered right adrenal mass of 5 cm in diameter, detected during abdominal ultrasound evaluation for suspected nephrolithiasis. An abdominal computer tomography scan showed an adrenal mass suggestive of adrenal adenoma, without the typical radiological characteristics of pheochromocytoma.

The patient had hypertension which was well controlled by lercanidipine 10 mg/day. His family history was negative for endocrine disease.

Blood cortisol, aldosterone, adrenal androgens levels, and 24-h urinary catecholamine and metanephrines levels were normal. However, in consideration of the size of the tumor, the patient was referred for laparoscopic adrenalectomy. Surprisingly, final histology revealed a 55 mm pheochromocytoma. Because of the presence of bilateral thyroid micronodules (7 mm on right lobe and 9 mm on left lobe), serum calcitonin was measured, showing mildly elevated basal levels (20 pg/ml) and post i.v. calcium gluconate levels (296 and 366 pg/ml 2 and 3 min after the infusion, respectively). Fine needle aspiration biopsy (FNAB) performed on the left nodule confirmed the suspicion of MTC. The patient underwent total thyroidectomy and central neck dissection. Final pathology showed diffuse CCH and a 4 mm medullary microcarcinoma on the left nodule which previously had undergone FNAB, and no lymph node involvement.

Patient 2: A 58 years-old woman came to our attention for tachycardia. Laboratory evaluation showed mild hyperthyroidism and thyroid ultrasound revealed a multinodular goiter. Thyroid scintiscan confirmed the diagnosis of a toxic multinodular goiter, with a 2 cm hyperfunctioning left nodule. A FNAB evaluation performed on the most relevant non-hyperfunctioning nodule (15 mm on right lobe) showed was benign features (Thy 2). Serum calcitonin was borderline increased (13.2 pg/ml). Due to the symptoms of hyperthyroidism and dysphagia, the patient underwent total thyroidectomy and the final histology showed struma with CCH and medullary multifocal microcarcinoma (maximum diameter 6 mm on left lobe).

Molecular Analysis of *RET*-Protoncogene

After obtaining informed consent, DNA was isolated from peripheral blood by a manual method (Roche Diagnostics, Basel, Switzerland, http://www.roche.com/index.htm). Molecular analysis of the *RET* onco-gene was performed by sequencing of the coding region and exon-intron boundaries of the exons 2, 5, 8, 10, 11, 13–16, as routinely performed in our laboratory. Primers used and the respective annealing temperatures are reported in **Table 1**. PCR products were sequenced using the Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA, http://www.appliedbiosystems.com/absite/us/en/home.html) in an automated sequencer ABI Prism 3500 Genetic Analyzer (Applied Biosystems).

Sanger sequencing revealed in both patients a missense variant on exon 2 (c.166C>A, p.L56M). This change from cytosine to adenine causes a substitution of Leucine with Methionine at codon 56. Genetic testing was performed also in first degree relatives and the same variant was found in the son of Patient 1, who is being followed with periodic ultrasonography and calcitonin measurements. In order to assess the frequency of this variant within our patient population, we also screened 100 healthy controls (200 alleles) by high-resolution melting analysis; these additional samples were used since the p.L56M was never found within the previous 250 patients referred to our laboratory

TABLE 1 | Primers used for *RET* amplification and sequencing.

Oligo name	Sequence (5′ 3′)	Annealing temperature (Ta) [°C]
RET-2F	GCT TCC CCT GTT TCC TTT TC	
RET-2R	AGT GTC AGC GGC TGT GAT AA	
RET-5F	CGT GCA GCA TTC TAA GGT CTC	
RET-5R	CAT GTG TGT AGG GTG CTG CT	
RET-8F	TGC TCC TGG CAC TGT CTT	
RET-8R	TGG GGA CCA ATC ACT GTA CTC	
RET-1OF	GGA CAC TGC CCT GGA AAT A	
RET-1OR	ACT CGC CTC CCA GCA ATT T	
RET-11F	ATA CGC AGC CTG TAC CCA CT	58
RET-11R	AAA TGG GGG CAG AAC ACA	
RET-13F	CCA TCC TGA CCT GGT ATG GT	
RET-13R	AAA CAG GGC AGG AGC AGT AG	
RET-14F	GTC CAC CCC CTT ACT CAT TG	
RET-14R	GTG GTG AGC CAT AGC ATG G	
RET-15F	CCC CCG GCC CAG GTC TC	
RET-15R	GCT CCA CTA ATC TTC GGT ATC TTT	
RET-16F	TCT CCT TTA CCC CTC CTT CC	
RET-16R	CAG TGA GGG GGT CAT TGC	

with the suspicion of MEN2 or MTC. All screened subjects were from Northern, Central and Southern Italy. This screening resulted mute for the above-mentioned variant, indicating a frequency of <1%.

DISCUSSION

The *RET* proto-oncogene, located on the long arm of chromosome 10 (10q11.2), was first identified in 1985 by transfection of NIH 3T3 cells with human lymphoma DNA (10). The protein encoded by *RET* is a cellular tyrosine kinase transmembrane receptor that is divided into three domains: an N-terminal extracellular domain with cadherin-like regions, a cysteine-rich transmembrane domain, and a cytoplasmic domain with tyrosine kinase activity (11). The activation of *RET* stimulates multiple pathways promoting cell growth, proliferation, differentiation and survival (4).

Once the role of the mutated *RET* in the development of hereditary forms of MTC became clear, *RET* genetic screening was introduced into clinical practice (8), providing an important contribution in the diagnosis of MTC. In fact, before the introduction of the *RET* genetic screening test in clinical practice, the diagnosis of MTC was exclusively relying on FNAB and serum calcitonin measurements (12), which made the identification of making the identification of the familial forms quite challenging. Nowadays, genetic screening is aimed at the early identification of family members who carry the same mutation as the index case and to propose an early treatment considering of the degree of risk associated with the detected variant (13).

Furthermore, the evidence that germline *RET* variants are present in about 6–7% of "apparently sporadic" cases of MTC confirms the need to perform genetic screening in all patients with MTC (8). If a variant is found, the case is then reclassified as hereditary, and genetic screening of the first-degree relatives is strongly recommended (9). Following such recommendation, we performed genetic testing in our two patients following the diagnosis of "sporadic" MTC and, for the first time, a variant in exon 2 of RET was detected in association with MTC. Moreover, in Patient 1 the diagnosis of MEN2A cannot be ruled out, in consideration of the concomitant occurrence of MTC and pheochromocytoma.

As often reported for the "new" variants, the question is if the *RET* germline variant represents a driving force of MTC or if it is an incidental finding due to the increased use of screening (14). In literature, rare variants which cannot be considered to be "polymorphisms" and which have an uncertain role in the pathogenesis on MTC are described as variants of unknown significance (VUS) (15). In our case, the frequency of p.L56M variant in the general population is <1%, and cannot be considered a polymorphisms. Recently, the ClinVar database identified this mutation in the MEN2A. The clinical significance as been defined as "benign/likely benign," because "it is a conservative change, it occurs at a poorly conserved position in the protein, it is predicted to be benign by multiple in silico algorithms, and/or has population frequency not consistent with disease"[1]. On the other hand, further studies are necessary to confirm the possible pathogenic role of this variant in MTC development: in fact, in clinical practice, genetic studies are often limited to exons 5, 8, 10, 11, 13, 14, 15, 16, where the majority of known *RET* pathogenic germline variant is localized. Whether the variant described by our group can be considered "pathogenic" in MTC or even in MEN2A is a hypothesis that needs to be confirmed. However, an interesting consideration is that this very same variant had previously been reported in association with Hirschsprung's disease, the congenital absence of ganglion cells in the submucosal and myenteric plexi of the gut. *RET* is the main gene implicated in this condition which, as mentioned earlier, represents a rare clinical feature of a MEN 2A clinical variant (6). Approximately 50% of familial cases of MTC and 7–35% of non-familial cases of MTC have loss-of-function germline RET variants (16). The concomitant occurrence of Hirschsprung's disease and MEN 2 is a relatively rare event, due to the presence of a "Janus" variant in the *RET* proto-oncogene: these variants can act both as a gain-of-function and a loss-of-function variant. To date, four missense exon 10 *RET* variants have been implicated in this association, most frequently in codon C620 (mostly C620R and occasionally C620S, and rarely C620W), but also in other areas (e.g., C609, C611, and C618) (6). Common variants in the *RET* promoter (rs10900296; rs10900297), at a SOX10 binding site in intron 1 (rs2435357), and in exon 2 (rs1800858; c.135G>A; p.A45A) have also been associated with Hirschsprung's disease, suggesting that common as well as rare variants might influence the occurrence of Hirschsprung's disease (17). It is important to mention that

[1]https://preview.ncbi.nlm.nih.gov/clinvar/variation/36723/evidence/

the presence of Hirschsprung's disease in our two patients was excluded based on clinical evaluation. However, the association between MTC and exon 2 p.L56M variant, which in turn is also related with a disease involved in MEN 2 phenotype, underlines the possibility of a pathogenic role of p.L56M variant in MTC. These findings need further confirmatory results. Nevertheless, it is important to underline the importance to expand the genetic evaluation for *RET* germline variants in MTC or MEN2A patients also to exon 2, especially if genetic testing has excluded the presence of the more common variants located within other exons.

AUTHOR CONTRIBUTIONS

RP, RL, GP, EC, AM, GC, AP, and SC have equally made their contribution to the work and approved it for publication.

REFERENCES

1. Wells SA Jr, Pacini F, Robinson BG, Santoro M. Multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma: an update. *J Clin Endocrinol Metab.* (2013) 98:3149–64. doi: 10.1210/jc.2013-1204
2. Romei C, Mariotti S, Fugazzola L, Taccaliti A, Pacini F, Opocher G, et al. Multiple endocrine neoplasia type 2 syndromes (MEN 2): results from the ItaMEN network analysis on the prevalence of different genotypes and phenotypes. *Eur J Endocrinol.* (2010) 163:301–8. doi: 10.1530/EJE-10-0333
3. Romei C, Ciampi R, Elisei R. A comprehensive overview of the role of the RET proto-oncogene in thyroid carcinoma. *Nat Rev Endocrinol.* (2016) 12:192–202. doi: 10.1038/nrendo.2016.11
4. Torino F, Paragliola RM, Barnabei A, Corsello SM. Medullary thyroid cancer: a promising model for targeted therapy. *Curr Mol Med.* (2010) 10:608–25.
5. Scapineli JO, Ceolin L, Punales MK, Dora JM, Maia AL. MEN 2A-related cutaneous lichen amyloidosis: report of three kindred and systematic literature review of clinical, biochemical and molecular characteristics. *Fam Cancer* (2016) 15:625–33. doi: 10.1007/s10689-016-9892-6
6. Coyle D, Friedmacher F, Puri P. The association between Hirschsprung's disease and multiple endocrine neoplasia type 2a: a systematic review. *Pediatr Surg Int.* (2014) 30:751–6. doi: 10.1007/s00383-014-3538-2
7. Eng C, Clayton D, Schuffenecker I, Lenoir G, Cote G, Gagel RF, et al. The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET mutation consortium analysis. *JAMA* (1996) 276:1575–9.
8. Romei C, Tacito A, Molinaro E, Agate L, Bottici V, Viola D, et al. Twenty years of lesson learning: how does the RET genetic screening test impact the clinical management of medullary thyroid cancer? *Clin Endocrinol.* (2015) 82:892–9. doi: 10.1111/cen.12686
9. Elisei R, Alevizaki M, Conte-Devolx B, Frank-Raue K, Leite V, Williams GR. 2012 European thyroid association guidelines for genetic testing and its clinical consequences in medullary thyroid cancer. *Eur Thyroid J.* (2013) 1:216–31. doi: 10.1159/000346174
10. Takahashi M, Ritz J, Cooper GM. Activation of a novel human transforming gene, ret, by DNA rearrangement. *Cell* (1985) 42:581–8.
11. Arighi E, Borrello MG, Sariola H. RET tyrosine kinase signaling in development and cancer. *Cytokine Growth Factor Rev.* (2005) 16:441–67. doi: 10.1016/j.cytogfr.2005.05.010
12. Elisei R, Bottici V, Luchetti F, Di Coscio G, Romei C, Grasso L, et al. Impact of routine measurement of serum calcitonin on the diagnosis and outcome of medullary thyroid cancer: experience in 10,864 patients with nodular thyroid disorders. *J Clin Endocrinol Metab.* (2004) 89:163–8. doi: 10.1210/jc.2003-030550
13. American Thyroid Association Guidelines Task Force, Kloos RT, Eng C, Evans DB, Francis GL, Gagel RF, et al. Medullary thyroid cancer: management guidelines of the American Thyroid Association. *Thyroid* (2009) 19:565–612. doi: 10.1089/thy.2008.0403
14. Orgiana G, Pinna G, Camedda A, De Falco V, Santoro M, Melillo RM, et al. A new germline RET mutation apparently devoid of transforming activity serendipitously discovered in a patient with atrophic autoimmune thyroiditis and primary ovarian failure. *J Clin Endocrinol Metab.* (2004) 89:4810–6. doi: 10.1210/jc.2004-0365
15. Crockett DK, Piccolo SR, Ridge PG, Margraf RL, Lyon E, Williams MS, et al. Predicting phenotypic severity of uncertain gene variants in the RET proto-oncogene. *PLoS ONE* (2011) 6:e18380. doi: 10.1371/journal.pone.0018380
16. Attie T, Pelet A, Edery P, Eng C, Mulligan LM, Amiel J, et al. Diversity of RET proto-oncogene mutations in familial and sporadic Hirschsprung disease. *Hum Mol Genet.* (1995) 4:1381–6.
17. Emison ES, Garcia-Barcelo M, Grice EA, Lantieri F, Amiel J, Burzynski G, et al. Differential contributions of rare and common, coding and noncoding Ret mutations to multifactorial Hirschsprung disease liability. *Am J Hum Genet.* (2010) 87:60–74. doi: 10.1016/j.ajhg.2010.06.007

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

CASE REPORT
published: 18 April 2018
doi: 10.3389/fendo.2018.00150

Increased Requirement of Replacement Doses of Levothyroxine Caused by Liver Cirrhosis

Salvatore Benvenga[1,2,3], Giovanni Capodicasa[1], Sarah Perelli[1], Silvia Martina Ferrari[4], Poupak Fallahi[4] and Alessandro Antonelli[4]*

[1] Department of Clinical and Experimental Medicine, University of Messina, Policlinico Universitario G. Martino, Messina, Italy, [2] Master Program on Childhood, Adolescent and Women's Endocrine Health, University of Messina, Policlinico Universitario G. Martino, Messina, Italy, [3] Interdepartmental Program of Molecular & Clinical Endocrinology, and Women's Endocrine Health, University Hospital, Policlinico Universitario G. Martino, Messina, Italy, [4] Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

Background: Since hypothyroidism is a fairly common dysfunction, levothyroxine (L-T4) is one of the most prescribed medications. Approximately 70% of the administered L-T4 dose is absorbed. The absorption process takes place in the small intestine. Some disorders of the digestive system and some medicines, supplements, and drinks cause L-T4 malabsorption, resulting in failure of serum TSH to be normal. Only rarely liver cirrhosis is mentioned as causing L-T4 malabsorption.

Case report: In this study, we report increased requirement of daily doses of L-thyroxine in two patients with the atrophic variant of Hashimoto's thyroiditis and liver cirrhosis. In one patient, this increased requirement could have been contributed by the increased serum levels of the estrogen-dependent thyroxine-binding globulin (TBG), which is the major plasma carrier of thyroid hormones. In the other patient, we switched from tablet L-T4 to liquid L-T4 at the same daily dose. Normalization of TSH levels was achieved, but TSH increased again when she returned to tablet L-T4.

Conclusion: Liver cirrhosis can cause increased L-T4 requirements. In addition to impaired bile secretion, the mechanism could be increased serum TBG. A similar increased requirement of L-T4 is observed in other situations characterized by elevation of serum TBG. Because of better intestinal absorption, L-T4 oral liquid formulation is able to circumvent the increased need of L-T4 in these patients.

Keywords: liver cirrhosis, undertreated hypothyroidism, thyroxine malabsorption, liquid levothyroxine, thyroxine-binding globulin

OPEN ACCESS

Edited by:
Bernadette Biondi, University of Naples Federico II, Italy

Reviewed by:
Marco Centanni, Sapienza Università di Roma, Italy
Gabriela Brenta, Dr. César Milstein Care Unit, Argentina

****Correspondence:***
Giovanni Capodicasa
capodicasagio@gmail.com

Specialty section:
This article was submitted to Thyroid Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *19 January 2018*
Accepted: *20 March 2018*
Published: *18 April 2018*

Citation:
Benvenga S, Capodicasa G, Perelli S, Ferrari SM, Fallahi P and Antonelli A (2018) Increased Requirement of Replacement Doses of Levothyroxine Caused by Liver Cirrhosis. Front. Endocrinol. 9:150. doi: 10.3389/fendo.2018.00150

INTRODUCTION

Hypothyroidism is a fairly common dysfunction and its incidence grows with age. Therefore levothyroxine (L-T4) is one of the most prescribed medications (1). Approximately 70% of the administered L-T4 dose is absorbed after L-T4 tablet dissolution in the acid intragastric environment. The absorption process takes place in the duodenum, jejunum, and ileum (2–4).

Several gastrointestinal disorders (gastritis, celiac disease, lactose intolerance, intestinal parasitosis, and bariatric surgery) and various medicines, supplements, and drinks reduce the absorption of L-T4 (5–14). Recently, Lobasso et al. (15) reported a woman with systemic sclerosis in whom

dysmotility of distal esophagus caused L-T4 malabsorption. Also, a dysbiosis has been postulated to be involved in pharmacologic homeostasis of thyroxine (16, 17).

Among 210 subjects who were observed by one of us because of undertreated primary hypothyroidism in spite of adequate daily dose of L-T4, 27 (12.9%) had an increased need of L-T4 (5). The gastrointestinal-related causes of increased need of L-T4 were celiac disease ($n = 3$), Crohn's enteritis ($n = 1$), co-ingestion of proton-pump inhibitors (PPIs, $n = 6$) alone or combined with other L-T4 sequestrants (ferrous salts or calcium salts). There are two disorders of the digestive system, liver cirrhosis, and diabetic diarrhea, which are rarely mentioned as the cause of an increase of L-T4 demand (18, 19). Chronic obstructive liver disease and pancreatic insufficiency are mentioned only in less recent reviews (20, 21). Textbooks and literature fail to mention that bile is important to maximize the intestinal absorption of L-T4 (22).

BACKGROUND

Target serum TSH levels have to be used to monitor L-T4 therapy in primary hypothyroidism (1). The target TSH in primary hypothyroidism "*should be the normal range of a third generation TSH assay*" or, if unavailable, should be 0.45–4.2 mU/l. However, according to the reference population of the National Health and Nutrition Examination Survey III, the normal values are 0.45–5.90 mU/l in the 70- to 79-year-old and 0.33–7.50 mU/l in ≥80-year-old patients. By contrast, the 2003 National Academy of Clinical Biochemistry Laboratory and Medicine Practice Guidelines suggest that the upper limit of normal range is 2.5 mU/l (23).

A complete diagnostic work-up for failure of serum TSH to become normal is multidisciplinary, may require hospitalization and be expensive. The work-up includes several steps, whose number and order may vary in the individual patient (24). The initial steps involve confirmation of diagnosis and laboratory data, assessment of patient compliance, and control of the formulations taken. Subsequent steps involve investigation of incorrect ingestion of L-T4, causes of malabsorption, including increased L-T4 turnover or excretion; if necessary, L-thyroxine absorption test is performed. A similar approach has been proposed by one of us elsewhere (5).

Here, we report two cases of undertreated hypothyroidism, causing increased requirement of daily doses of L-thyroxine, in patients with cirrhosis of the liver. In one patient, tablet L-T4 was replaced by oral liquid L-T4, which normalized serum TSH.

Report of the First Case

A 54-year-old man with hepatitis C virus-related liver cirrhosis had been diagnosed autoimmune hypothyroidism (atrophic variant of Hashimoto's thyroiditis) approximately 8 years earlier, which was treated with 100 μg/day L-T4. Serum TSH had been consistently ≤3.4 mU/l, until symptomatology characterized by asthenia, weight loss, and yellowish staining of the sclera appeared. This led to the diagnosis of liver cirrhosis. Serum TSH increased to 9.3 mU/l so that L-T4 was progressively increased to 150 μg/day. However, serum TSH did not fall below 6.6 mU/l. When observed, the patient had scleral jaundice, bilateral gynecomastia and was treated with PPI (lansoprazole), a class of drugs that reduces the absorption of L-T4 by increasing gastric pH (3–8, 25–27). However, lansoprazole was taken discontinuously. Because of gynecomastia, serum testosterone, estradiol (E2), and prolactin (PRL) had been requested before our observation.

Results

Total testosterone was borderline low (300 ng/dl, reference range 290–960), and E2 mildly elevated (53 pg/ml, reference range for men <40), as it was PRL (24 ng/ml, reference range for men <16). Knowing that thyroxine-binding globulin (TBG) is the estrogen-upregulated and androgen-downregulated major thyroid hormone plasma carrier (28–34) and that circulating TBG increases in liver diseases (28, 35–41), we complemented the PRL and sex hormone assays with TBG assay. We hypothesized that, similarly to the increased requirements of L-T4 replacement therapy during gestation (a physiological hyperestrogenic state in which TBG is increased) (42, 43) but unlike the decreased requirements of L-T4 replacement therapy associated with hyperandrogenism (when serum TBG decreases) (34), elevation of serum TBG (and associated increased binding of L-T4 to it) might have contributed to undertreated hypothyroidism in our patient. Indeed, serum TBG was high (60 μg/ml; reference range 12–32), a level comparable to that of pregnant women. The patient was soon lost at follow-up. However, 5 years later, one of his relatives called over the telephone to have a second opinion concerning the combined L-T4 + L-T3 therapy. On that occasion, we learned that in our patient cirrhosis had been managed successfully by liver transplantation and that gynecomastia disappeared. Although he continued to take discontinuously PPI (6 h after L-T4), his latest serum TSH was 2.4 mU/l (reference range 0.27–4.2) under 100 μg/day L-T4.

Report of the Second Case

The second cirrhotic patient is a 64-year-old hypothyroid woman with the atrophic variant of Hashimoto's thyroiditis under treatment with tablet LT-4 over the last 10 years. She was euthyroid upon taking 150 μg/day L-T4, which was taken 30 min before breakfast. Twelve months after the last endocrinological control, she was diagnosed with hepatitis C virus-related cirrhosis. After 7 months, laboratory tests showed subclinical hypothyroidism (TSH = 8.65 mU/l, FT4 = 9.5 pg/ml; **Table 1**), although her body weight had not undergone significant changes.

Results

Since we are aware of a better absorption profile of novel formulations of L-T4 over the classic tablet formulation (see Discussion), we switched tablet L-T4 to oral liquid L-T4, without changing the daily dose. TSH levels became normal after about 60 days (3.0 mU/l), and both FT4 and FT3 levels increased from close to the lower normal limit to close to the mid-range value (12.3 and 3.0 pg/ml) (**Table 1**). Accordingly, she felt better. To confirm that this improvement was not random, L-T4 was switched back to the tablet formulation at the same dosage. One month after this switch, serum TSH bounced back to abnormal levels, in parallel with a decline of both FT4 and FT3 levels (**Table 1**).

TABLE 1 | Thyroid parameters measured when patient no. 2 was receiving oral levothyroxine (L-T4) in the tablet formulation, after 2 months of L-T4 in the liquid formulation (in bold), and then after 1 month of return to the tablet formulation.[a]

L-T4 formulation	TSH, mU/l	FT3, pg/ml	FT4, pg/ml
Tablet	8.65	2.7	9.5
Liquid	**3.03**	**3.0**	**12.3**
Tablet	6.37	2. 9	11. 8

[a]*Because body weight of this patient was stable (67–68 kg) over time, the daily dose of 150 μg/day was also stable when normalized for body weight, namely, 2.23 μg/kg b.w./day.*

Reference ranges are 0.3–3.5 mU/l (TSH), 2.3–4.2 pg/ml (FT3), and 8–17 pg/ml (FT4). The tabulated hormone levels were measured at observation when the patient was under tablet L-T4, after 2 months from switch to oral liquid L-T4 and 1 month after switch back to tablet L-T4.

DISCUSSION

Most patients with hepatitis C virus develop at least one extrahepatic manifestation, including autoimmune thyroid disease (44). Accordingly, hypothyroidism and thyroid autoimmunity are significantly more frequent in HCV patients with respect to controls (44).

The two patients reported here remind us that liver cirrhosis is one of the causes of undertreated hypothyroidism and associated greater L-T4 requirement. The increased L-T4 requirement associated with liver cirrhosis could be due to defects in bile production and excretion. Indeed, bile is important to maximize the intestinal absorption of L-T4 (22). However, one additional mechanism for the increased dosage of L-T4 could be the same as that occurring in pregnancy or other conditions of hyperestrogenism (28, 29, 32, 33), namely, increased binding of L-T4 by the increased estrogen-driven serum levels of TBG, the major thyroid hormone plasma carrier, and failure to compensate for this increased binding. Elevation of serum E2 in cirrhosis and E2 normalization after liver transplantation was shown by other authors (41, 45). Congruent with our data on the second patient, an Italian study found that the increased L-T4 requirement occurring during gestation is significantly less frequent if hypothyroid pregnant women are supplemented with liquid L-T4 compared with tablet L-T4 (1/14 vs 7/17, $P = 0.038$) (46). An increased requirement of L-T4 is more likely to occur in pregnant women with no residual thyroid function (e.g., because of radioablation, thyroidectomy), compared with women with non-atrophic Hashimoto's thyroiditis (42, 43). Non-iatrogenic conditions of absent functional thyroid tissue are thyroid agenesia or, like our patients, thyroid atrophia.

In the first cirrhotic patient described here, co-ingestion of one medication (a PPI) that is known to interfere with L-T4 intestinal absorption (5–8) is unlikely to be the cause of undertreated hypothyroidism. In the Sachmechi et al. study (25), 37 euthyroid patients who had received stable L-T4 replacement for at least 6 months and in whom lansoprazole therapy (the same PPI taken by our patient) was later initiated, serum TSH levels were measured before and at least 2 months after the PPI was started. Of the 37 patients, only 7 (19%) had post-PPI TSH levels >5 mU/l, requiring a mean increase of 20 μg/day L-T4 (+35%). By contrast, short-term (47, 48) and, presumably, discontinuous treatment with PPI (as in our patient) has no impact on L-T4 intestinal absorption.

The second patient permits to add the chronic liver disease setting to the other settings (5, 6, 18, 26, 27, 49–63) in which novel formulations of L-T4, because of their more favorable pharmacokinetics profile, perform better than the classic tablet formulation in achieving target levels of TSH (64). From a gastroenterological perspective, settings of interest are the correction of the impaired tablet L-T4 absorption caused by food and beverages, anti-ulcerants (26, 27), esophageal dysmotility (15), gastritis (58, 59), enteral feeding (63), and bariatric surgery (54, 55).

In sum, liver cirrhosis can cause undertreated primary hypothyroidism and associated increased L-T4 requirement. The L-T4 increased requirement is due to impaired bile secretion and increased serum TBG resulting in increased TBG-T4 binding. This augmented TBG-T4 binding cannot be compensated by increased free T4 levels originating from an increased thyroid gland secretion, if the patient has hyperestrogenism (which would raise serum TBG levels even more) and has no or minimal functioning thyroid tissue. A similar increased requirement of L-T4 is typically observed during gestation (a physiologic condition of elevated serum estrogens and serum TBG), especially in pregnant women with non functioning thyroid tissue, when serum TBG is elevated.

This study suggests that, further to the known situation in hypothyroid pregnant women, elevated serum levels of the major plasma carrier of thyroid hormones (TBG) increase the daily requirement of L-T4 also in hypothyroid men with an atrophied thyroid. Like in iatrogenic conditions of an entirely non-functional thyroid gland (i.e., radioablation and thyroidectomy), the increased binding of L-T4 to circulating TBG cannot be compensated by an increased hormone output by the thyroid.

CONCLUDING REMARKS

Our report highlights live cirrhosis as one cause of increased requirement for L-T4. Hepatic dysfunction may involve the rise in TBG concentrations, like in pregnancy. Our study is also the first showing that the L-T4 oral liquid formulation can solve the problem of the increased need of tablet L-T4 dose in liver cirrhosis because of better intestinal absorption.

ETHICS STATEMENT

The patients gave written informed consent for the publication of this case report.

AUTHOR CONTRIBUTIONS

All the authors contributed equally to recruited patients and wrote the present work.

REFERENCES

1. Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI, et al. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. *Thyroid* (2012) 22:1200–35. doi:10.1089/thy.2012.0205
2. Benvenga S, Bartolone L, Squadrito S, Lo Giudice F, Trimarchi F. Delayed intestinal absorption of levothyroxine. *Thyroid* (1995) 5:249–53. doi:10.1089/thy.1995.5.249
3. Centanni M, Gargano L, Canettieri G, Viceconti N, Franchi A, Delle Fave G, et al. Thyroxine in goiter, *Helicobacter pylori* infection, and chronic gastritis. *N Engl J Med* (2006) 354:1787–95. doi:10.1056/NEJMoa043903
4. Checchi S, Montanaro A, Pasqui L, Ciuoli C, De Palo V, Chiappetta MC, et al. L-thyroxine requirement in patients with autoimmune hypothyroidism and parietal cell antibodies. *J Clin Endocrinol Metab* (2008) 93:465–9. doi:10.1210/jc.2007-1544
5. Benvenga S. When thyroid hormone replacement is ineffective? *Curr Opin Endocrinol Diabetes Obes* (2013) 20:467–77. doi:10.1097/MED.0000000000000003
6. Colucci P, Seng Yue C, Ducharme M, Benvenga S. A review of the pharmacokinetics of levothyroxine for the treatment of hypothyroidism. *Eur Endocrinol* (2013) 9:40–7. doi:10.17925/EE.2013.09.01.40
7. Barbesino G. Drugs affecting thyroid function. *Thyroid* (2010) 20:763–70. doi:10.1089/thy.2010.1635
8. Liwanpo L, Hershman JM. Conditions and drugs interfering with thyroxine absorption. *Best Pract Res Clin Endocrinol Metab* (2009) 23:781–92. doi:10.1016/j.beem.2009.06.006
9. Cellini M, Santaguida MG, Virili C, Capriello S, Brusca N, Gargano L, et al. Hashimoto's thyroiditis and autoimmune gastritis. *Front Endocrinol* (2017) 26(8):92. doi:10.3389/fendo.2017.00092
10. Lahner E, Virili C, Santaguida MG, Annibale B, Centanni M. *Helicobacter pylori* infection and drugs malabsorption. *World J Gastroenterol* (2014) 20:10331–7. doi:10.3748/wjg.v20.i30.10331
11. Virili C, Bassotti G, Santaguida MG, Iuorio R, Del Duca SC, Mercuri V, et al. Atypical celiac disease as cause of increased need for thyroxine: a systematic study. *J Clin Endocrinol Metab* (2012) 97:E419–22. doi:10.1210/jc.2011-1851
12. Cellini M, Santaguida MG, Gatto I, Virili C, Del Duca SC, Brusca N, et al. Systematic appraisal of lactose intolerance as cause of increased need for oral thyroxine. *J Clin Endocrinol Metab* (2014) 99:E1454–8. doi:10.1210/jc.2014-1217
13. Seppel T, Rose F, Schlaghecke R. Chronic intestinal giardiasis with isolated levothyroxine malabsorption as reason for severe hypothyroidism – implications for localization of thyroid hormone absorption in the gut. *Exp Clin Endocrinol Diabetes* (1996) 104:180–2. doi:10.1055/s-0029-1211442
14. Padwal R, Brocks D, Sharma AM. A systematic review of drug absorption following bariatric surgery and its theoretical implications. *Obes Rev* (2009) 11:41–50. doi:10.1111/j.1467-789X.2009.00614.x
15. Lobasso A, Nappi L, Barbieri L, Peirce C, Ippolito S, Arpaia D, et al. Severe hypothyroidism due to the loss of therapeutic efficacy of l-thyroxine in a patient with esophageal complication associated with systemic sclerosis. *Front Endocrinol* (2017) 20(8):241. doi:10.3389/fendo.2017.00241
16. Virili C, Centanni M. Does microbiota composition affect thyroid homeostasis? *Endocrine* (2015) 49(3):583–7. doi:10.1007/s12020-014-0509-2
17. Virili C, Centanni M. "With a little help from my friends" – the role of microbiota in thyroid hormone metabolism and enterohepatic recycling. *Mol Cell Endocrinol* (2017) 15(458):39–43. doi:10.1016/j.mce.2017.01.053
18. Larsen PR, Davies TF. Hypothyroidism and thyroiditis. In: Larsen PR, Kronenberg HM, Melmed S, Polonsky KS, editors. *Williams Textbook of Endocrinology*. 10th ed. Philadelphia: Saunders. (2003). p. 423–55.
19. Hays MT. Absorption of oral thyroxine in man. *J Clin Endocrinol Metab* (1968) 28:749–56. doi:10.1210/jcem-28-6-749
20. Hays MT. Thyroid hormone and the gut. *Endocrine Res* (1988) 14:203–24. doi:10.3109/07435808809032986
21. Watts NB, Blevins LS Jr. Endocrinology. *JAMA* (1994) 271:1666–8.
22. Sinha KN, Van Middlesworth L. Effect of bile on thyroxine absorption in the rat. *Am J Physiol* (1971) 220:253–6.
23. Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF, et al. Laboratory support for the diagnosis and monitoring of thyroid disease. *Thyroid* (2003) 13:3–126. doi:10.1089/105072503321086962
24. Morris JC. How do you approach the problem of TSH elevation in a patient on high-dose thyroid hormone replacement? *Clin Endocrinol (Oxf)* (2009) 70:671–3. doi:10.1111/j.1365-2265.2009.03536.x
25. Sachmechi I, Reich DM, Aninyei M, Wibowo F, Gupta G, Kim PJ. Effect of proton pump inhibitors on serum thyroid-stimulating hormone level in euthyroid patients treated with levothyroxine for hypothyroidism. *Endocr Pract* (2007) 13:345–9. doi:10.4158/EP.13.4.345
26. Vita R, Benvenga S. Tablet levothyroxine (L-T4) malabsorption induced by proton pump inhibitor; a problem that was solved by switching to L-T4 in soft gel capsule. *Endocr Pract* (2014) 20:e38–41. doi:10.4158/EP13316.CR
27. Vita R, Saraceno G, Trimarchi F, Benvenga S. Switching levothyroxine from the tablet to the oral solution formulation corrects the impaired absorption of levothyroxine induced by proton-pump inhibitors. *J Clin Endocrinol Metab* (2014) 99:4481–6. doi:10.1210/jc.2014-2684
28. Benvenga S. Thyroid hormone transport proteins and the physiology of hormone binding. 10th ed. In: Braverman LE, Cooper DS, editors. *Werner and Ingbar's The Thyroid: A Clinical and Fundamental Text*. Philadelphia: Wolters Kluwer, Lippincott Williams & Wilkins (2013). p. 93–103.
29. Skjoldebrand L, Brundin J, Carlstrom A, Pettersson T. Thyroid associated components in serum during normal pregnancy. *Acta Endocrinol* (1982) 100:504–11.
30. Glinoer D, Gershengorn MC, Dubois A, Robbins J. Stimulation of thyroxine-binding globulin synthesis by isolated rhesus monkey hepatocytes after in vivo beta-estradiol administration. *Endocrinology* (1977) 100:807–13. doi:10.1210/endo-100-3-807
31. Ain KB, Mori Y, Refetoff S. Reduced clearance rate of thyroxine binding globulin (TBG) with increased sialylation: a mechanism for estrogen-induced elevation of serum TBG concentration. *J Clin Endocrinol Metab* (1987) 65:686–96. doi:10.1210/jcem-65-4-689
32. Bisschop PH, Toorians AW, Endert E, Wiersinga WM, Gooren LJ, Fliers E. The effects of sex-steroid administration on the pituitary-thyroid axis in transsexuals. *Eur J Endocrinol* (2006) 155:11–6. doi:10.1530/eje.1.02192
33. Arafah BM. Increased need for thyroxine in women with hypothyroidism during estrogen therapy. *N Engl J Med* (2001) 344:1743–9. doi:10.1056/NEJM200106073442302
34. Arafah BM. Decreased levothyroxine requirement in women with hypothyroidism during androgen therapy for breast cancer. *Ann Intern Med* (1994) 121:247–51. doi:10.7326/0003-4819-121-4-199408150-00002
35. Schussler GC, Schaffner F, Korn F. Increased serum thyroid hormone binding and decreased free hormone in chronic active liver disease. *N Engl J Med* (1978) 299:510–5. doi:10.1056/NEJM197809072991003
36. Shigemasa C, Tanaka T, Mitani Y, Ueta Y, Taniguchi S, Urabe K, et al. Are increases in thyroxin-binding globulin in patients with acute hepatitis ascribable to synthesis by regenerating hepatocytes? *Clin Chem* (1988) 34:776–80.
37. Huang MJ, Liaw YF. Thyroxine-binding globulin in patients with chronic hepatitis B virus infection: different implications in hepatitis and hepatocellular carcinoma. *Am J Gastroenterol* (1990) 85:281–4.
38. Danilovic DL, Mendes-Correa MC, Chammas MC, Zambrini H, Barros RK, Marui S. Thyroid disturbance related to chronic hepatitis C infection: role of CXCL10. *Endocr J* (2013) 60:583–90. doi:10.1507/endocrj.EJ12-0321
39. Shimada T, Higashi K, Umeda T, Sato T. Thyroid functions in patients with various chronic liver diseases. *Endocrinol Jpn* (1988) 35:357–69. doi:10.1507/endocrj1954.35.357
40. Nagasue N, Ohmori H, Hashimoto N, Tachibana M, Kubota H, Uchida M, et al. Thyroxine-binding globulin and thyroid hormones after resection of hepatocellular carcinoma. *Am J Gastroenterol* (1997) 92:1187–9.
41. Zacharias BT, Coelho JC, Parolin MB, Matias JE, Freitas AC, Godoy JL. Hypothalamic-pituitary-gonadal function in men with liver cirrhosis before and after liver transplantation. *Rev Col Bras Cir* (2014) 41:421–5. doi:10.1590/0100-69912014006007
42. Alexander EK, Marqusee E, Lawrence J, Jarolim P, Fischer GA, Larsen PR. Timing and magnitude of increases in levothyroxine requirements during pregnancy in women with hypothyroidism. *N Engl J Med* (2004) 351:241–9. doi:10.1056/NEJMoa040079
43. Loh JA, Wartofsky L, Jonklaas J, Burman KD. The magnitude of increased levothyroxine requirements in hypothyroid pregnant women depends upon the etiology of the hypothyroidism. *Thyroid* (2009) 19:269–75. doi:10.1089/thy.2008.0413

44. Fallahi P, Ferrari SM, Vita R, Benvenga S, Antonelli A. The role of human parvovirus B19 and hepatitis C virus in the development of thyroid disorders. *Rev Endocr Metab Disord* (2016) 17:529–35. doi:10.1007/s11154-016-9361-4
45. Aller R, de Luis DA, Moreira V, Boixeda D, Moya JL, Fernandez-Rodriguez CM, et al. The effect of liver transplantation on circulating levels of estradiol and progesterone in male patients: parallelism with hepatopulmonary syndrome and systemic hyperdynamic circulation improvement. *J Endocrinol Invest* (2001) 24:503–9. doi:10.1007/BF03343883
46. Cappelli C, Negro R, Pirola I, Gandossi E, Agosti B, Castellano M. Levothyroxine liquid solution versus tablet form for replacement treatment in pregnant women. *Gynecol Endocrinol* (2016) 32:290–2. doi:10.3109/09513590.2015.1113518
47. Dietrich JW, Gieselbrecht K, Holl RW, Boehm BO. Absorption kinetics of levothyroxine is not altered by proton-pump inhibitor therapy. *Horm Metab Res* (2006) 38:57–9. doi:10.1055/s-2006-924980
48. Ananthakrishnan S, Braverman LE, Levin RM, Magnani B, Pearce EN. The effect of famotidine, esomeprazole, and ezetimibe on levothyroxine absorption. *Thyroid* (2008) 18:493–8. doi:10.1089/thy.2007.0381
49. Benvenga S, Vita R, Di Bari F, Fallahi P, Antonelli A. Do not forget nephrotic syndrome as a cause of increased requirement of levothyroxine replacement therapy. *Eur Thyroid J* (2015) 4:138–42. doi:10.1159/000381310
50. Vita R, Saraceno G, Trimarchi F, Benvenga S. A novel formulation of L-thyroxine (L-T4) reduces the problem of L-T4 malabsorption by coffee observed with traditional tablet formulations. *Endocrine* (2013) 43:154–60. doi:10.1007/s12020-012-9772-2
51. Vita R, Fallahi P, Antonelli A, Benvenga S. The administration of L-thyroxine as soft gel capsule or liquid solution. *Expert Opin Drug Deliv* (2014) 11:1103–11. doi:10.1517/17425247.2014.918101
52. Benvenga S, Di Bari F, Vita R. Undertreated hypothyroidism due to calcium or iron supplementation corrected by oral liquid levothyroxine. *Endocrine* (2017) 56:138–45. doi:10.1007/s12020-017-1244-2
53. Vita R, Di Bari F, Benvenga S. Oral liquid levothyroxine solves the problem of tablet levothyroxine malabsorption due to concomitant intake of multiple drugs. *Expert Opin Drug Deliv* (2017) 14:467–72. doi:10.1080/17425247.2017.1290604
54. Fallahi P, Ferrari SM, Camastra S, Politti U, Ruffilli I, Vita R, et al. TSH normalization in bariatric surgery patients after the switch from L-thyroxine in tablet to an oral liquid formulation. *Obes Surg* (2017) 27:78–82. doi:10.1007/s11695-016-2247-4
55. Pirola I, Formenti AM, Gandossi E, Mittempergher F, Casella C, Agosti B, et al. Oral liquid L-thyroxine (L-t4) may be better absorbed compared to L-T4 tablets following bariatric surgery. *Obes Surg* (2013) 23:1493–6. doi:10.1007/s11695-013-1015-y
56. Fallahi P, Ferrari SM, Antonelli A. In patients with subclinical hypothyroidism while in therapy with tablet L-T4, the liquid L-T4 formulation is more effective in restoring euthyroidism. *Endocr Pract* (2017) 23:170–4. doi:10.4158/EP161545.OR
57. Fallahi P, Ferrari SM, Ruffilli I, Ragusa F, Biricotti M, Materazzi G, et al. Advancements in the treatment of hypothyroidism with L-T4 liquid formulation or soft gel capsule: an update. *Expert Opin Drug Deliv* (2017) 14:647–55. doi:10.1080/17425247.2016.1227782
58. Fallahi P, Ferrari SM, Ruffilli I, Antonelli A. Reversible normalisation of serum TSH levels in patients with autoimmune atrophic gastritis who received L-T4 in tablet form after switching to an oral liquid formulation: a case series. *BMC Gastroenterol* (2016) 16:22. doi:10.1186/s12876-016-0439-y
59. Santaguida MG, Virili C, Del Duca SC, Cellini M, Gatto I, Brusca N, et al. Thyroxine softgel capsule in patients with gastric-related T4 malabsorption. *Endocrine* (2015) 49:51–7. doi:10.1007/s12020-014-0476-7
60. Virili C, Trimboli P, Romanelli F, Centanni M. Liquid and softgel levothyroxine use in clinical practice: state of the art. *Endocrine* (2016) 54:3–14. doi:10.1007/s12020-016-1035-1
61. Brancato D, Scorsone A, Saura G, Ferrara L, Di Noto A, Aiello V, et al. Comparison of TSH Levels with liquid formulation versus tablet formulations of levothyroxine in the treatment of adult hypothyroidism. *Endocr Pract* (2014) 20:657–62. doi:10.4158/EP13418.OR
62. Cappelli C, Pirola I, Gandossi E, Cristiano A, Daffini L, Agosti B, et al. Thyroid hormone profile in patients ingesting soft gel capsule or liquid levothyroxine formulations with breakfast. *Int J Endocrinol* (2016) 2016:9043450. doi:10.1155/2016/9043450
63. Pirola I, Daffini L, Gandossi E, Lombardi D, Formenti A, Castellano M, et al. Comparison between liquid and tablet levothyroxine formulations in patients treated through enteral feeding tube. *J Endocrinol Invest* (2014) 37:583–7. doi:10.1007/s40618-014-0082-9
64. Virili C, Giovannella L, Fallahi P, Antonelli A, Santaguida MG, Centanni M, et al. Levothyroxine therapy: changes of TSH levels by switching patients from tablet to liquid formulation. A systematic review and meta-analysis. *Front Endocrinol* (2018) 9:10. doi:10.3389/fendo.2018.00010

Conflict of Interest Statement: IBSA Farmaceutici Italia s.r.l. and IBSA Institut Biochimique SA (Lugano, Switzerland) provided SB and AA with novel formulations to perform clinical studies. However, IBSA had no role in any phase of writing this paper.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership