

# Hashimoto's thyroiditis and papillary thyroid carcinoma. Are cytokeratin 19 and P63 proteins of any diagnostic value?

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## Abstract

**Objective:** The relationship between Hashimoto's thyroiditis (HT) and papillary thyroid carcinoma (PTC) remains controversial. We aimed to study the coexistence of PTC and HT and the diagnostic utility of cytokeratin19 and P63 proteins expression in all positive for PTC cases of HT. **Subjects and Methods:** We analyzed data from 343 patients who underwent fine needle aspiration cytology followed by thyroid surgery over a six years period. Thyroid scans and blood measurements for anti-thyroid peroxidase antibodies (TPOAb), thyroid stimulating hormone (TSH), anti-thyroglobulin antibodies (TgAb) and free thyroid hormones were performed. We assessed the expression of monoclonal antibodies against cytokeratin 19 (CK-19) and factor P63 in all positive for PTC patients. **Results:** Hashimoto's thyroiditis was diagnosed by histology in 93 patients. They were: 90 female and 3 male patients. Both HT and PTC were present in 6 female patients (6, 7%). All their thyroid scans showed heterogeneous distribution of the tracer with a cold nodule. Laboratory examination showed high levels of TSH as well as of TPOAb in all PTC patients. Cytokeratin 19 showed positive expression in all PTC patients, whereas P63 showed focal positivity in 4/6 cases. We did not estimate the duration of HT in our study. **Conclusion:** This study showed that 6,7% of female patients with HT also had PTC and all had elevated serum TSH, which may be a risk factor for PTC. The age of PTC patients was between 19-42 years. Immunological and genetical factors may be of diagnostic importance.

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## Introduction

**Keywords:** -Hashimoto's thyroiditis  
-Papillary carcinoma -CK19-P63

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**H**ashimoto's thyroiditis is an autoimmune thyroid disease which can affect all age groups and is more common in female than in male. Hashimoto's thyroiditis was the first disease recognized as of autoimmune origin [1] and the most common cause of primary hypothyroidism.

Among risk factors for HT are: a) genes HLA-DR5 HLA-DR3 and CTLA-4 (Cytotoxic T-Lymphocyte Antigen-4) [2]. Type 1 diabetes is also associated with polymorphisms affecting CTLA-4 [3]. b) High iodine intake c) Selenium deficiency d) Sex hormones e) Radiation exposure f) There is evidence supporting an association between human herpes virus-6 and HT [4]. HHV-6 is a T-cell lymphotropic virus with high affinity for CD4 lymphocytes.

Papillary carcinoma is the most common malignant tumor of the thyroid and the most common form of thyroid cancer. This cancer is associated with exposure of head and neck to radiation [5]. The follicular type of thyroid cancer accounts for 15% of cases and tends to affect older adults. Medullary carcinoma accounts only for 5%-8% of thyroid malignancies. Anaplastic thyroid carcinoma is the rarest, most aggressive type and affects older patients.

The association between HT and PTC remains controversial [6-12]. Although PTC can be basically diagnosed by cytology, it has many variants who are diagnosed by specific antibodies or other specific diagnostic procedures like immunohistochemistry [13].

Our aim was to study the relation of PTC and HT and the diagnostic utility of cytokeratin19 (CK19) and P63 proteins expression in PTC.

## Subjects and Methods

In a retrospective study we analyzed data on 343 patients who underwent fine needle

aspiration cytology followed by thyroid surgery over a six years period, from 2010 to 2015. Thyroid aspirates were prepared by conventional and liquid based cytology. Formalin-fixed paraffin-embedded tissues from the same cases were examined histologically. There were 93 patients diagnosed with HT cytologically in accordance with the Bethesda system for reporting thyroid cytology and by tissue pathology. Ninety (96,7%) HT patients were female and 3 (3,2%) male. The ages of the patients ranged from 16 to 72 (mean age 43,9 years).

This study included 6 patients diagnosed with HT and co-existing PTC. All patients were female, aged from 17 to 46 (mean age 27,5 years). Thyroid scans and blood measurements for antithyroidperoxidase antibodies (TPOAb), thyroid stimulating hormone (TSH), antithyroglobulin antibodies (TgAb) and free thyroid hormones (fT<sub>3</sub>, fT<sub>4</sub>), were performed (Table 1). Immunocytochemical staining was also performed on all positive for malignancy cases using a Bond Max immunostainer (Vision Biosystems Limited, Melbourne, Australia). The following monoclonal antibodies were studied: CK19, Clone RCK108 (Biogenex, Fremont California, USA) and P63, Clone 4A4 (Biogenex, Fremont California, USA).

All Hashimoto's thyroiditis PTC were evaluated for the expression of the above mentioned markers.

## Results

Thyroid scintiscans of 65 (69,8%) HT patients showed enlarged thyroid with diffusely increased radiotracer uptake, whereas 28 (30,1%) of the patients had heterogeneous distribution of the tracer with one cold nodule of  $\geq 1\text{cm}$  and other smaller. All patients with PTC were in a separate group.

Elevated levels of antithyroidperoxidase antibodies (TPOAb) and serum TSH were detected in almost all of the 93 patients (Table 1). Two patients with HT were diagnosed by fine needle aspiration cytology.

Free T<sub>3</sub> levels were decreased in 91 (97,8%) patients' serum. Free T<sub>4</sub> levels were also decreased in 90 (96,7%) HT patient's serum.

Of the 93 patients with HT 6 (6,45%) had PTC (Figures 1 and 2) and also high levels of TSH and of (TPOAb) (Table 1). The mean age of the 6 patients with coexistence of PTC and HT was 39,6 years lower as compared to the entire HT group.

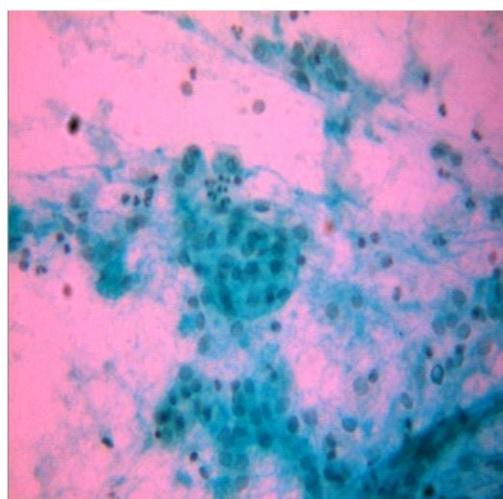
Ck19 showed positive expression in all PTC cases with strong diffuse cytoplasmic reactivity (Figures 3 and 4).

Within the subgroup of the 6 patients with HT and PTC, there was a case with Hürthle cells, lymphocytes and some oncocytes demonstrating intranuclear cytoplasmic invaginations and foci of papillary clusters. Serum CK19 and P63 were positive. A total thyroidectomy was performed revealing HT coexistent and an oncocytic variant of PTC. Serum P63 showed focal positivity in 4 (66,6%) malignant cases (Figures 5, 6). A thyroid HT scan with a cold node is in figure 7.

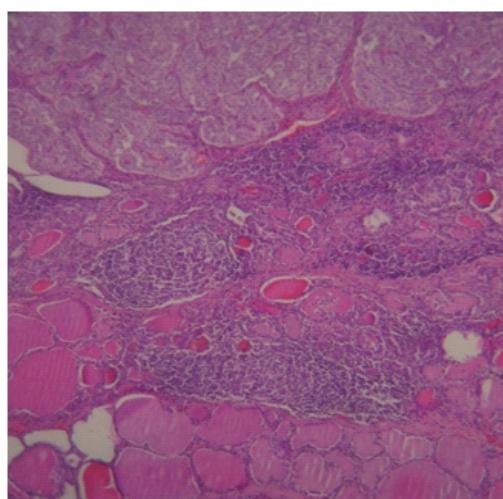
## Discussion

**Table 1.** Patients with Hashimoto's thyroiditis. Laboratory tests

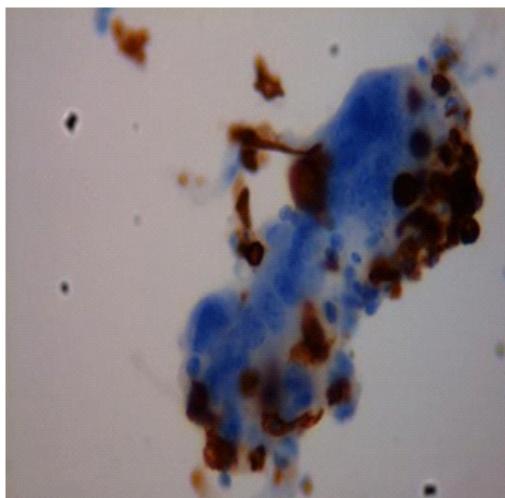
	Patients with HT	Hashimoto's thyroiditis + PTC
No of patients	87	6
Age	16-72	19-42
Male	3	0
Female	90	6
TSH	2,82-107,3 $\mu\text{IU}/\text{mL}$	17,6-107,33 $\mu\text{IU}/\text{mL}$
TPO	6-4500U/mL	200-2600U/mL
Free T <sub>3</sub>	2,6-4,3pmol/L	3,2-3,9pmol/L
Free T <sub>4</sub>	8,4-9pmol/L	8,1-9pmol/L



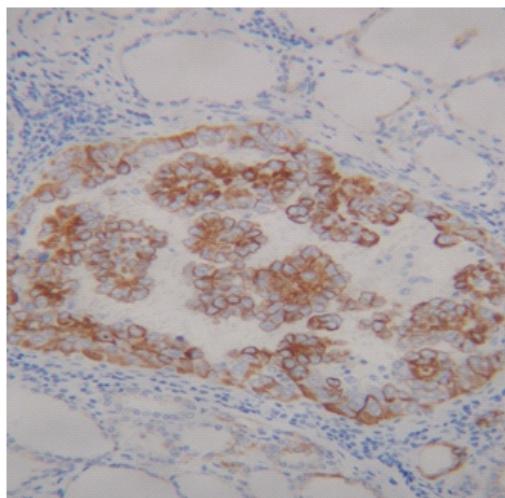
**Figure 1.** Coexistence of papillary carcinoma and Hashimoto's thyroiditis (Papanicolaou X400).



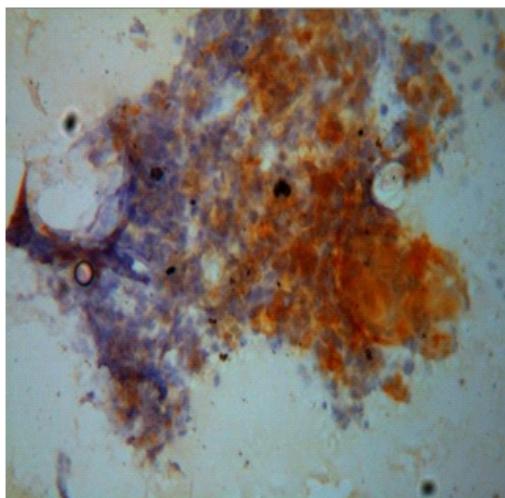
**Figure 2.** Coexistence of papillary carcinoma and Hashimoto's thyroiditis (Papanicolaou X400).



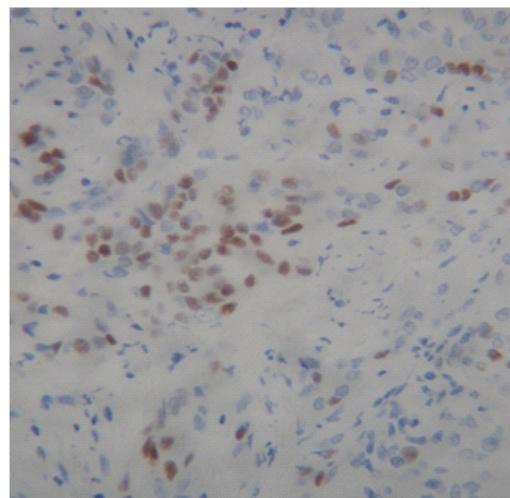
**Figure 3.** FNA Cytokeratin 19 (CK 19) shows strong diffuse cytoplasmic staining X400).



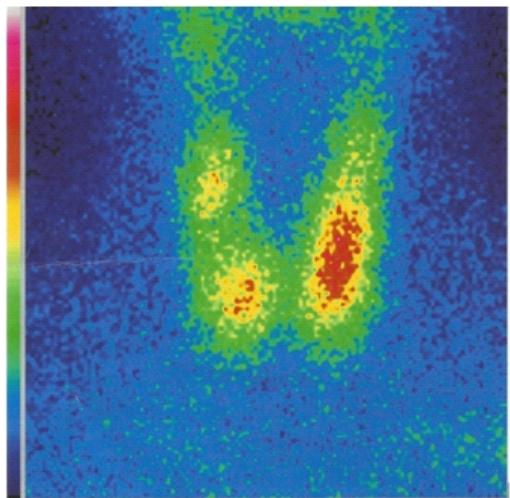
**Figure 4.** Histology: CK19. A case of papillary thyroid carcinoma exhibits diffuse cytoplasmic immunoreactivity X400.



**Figure 5.** P63 showed focal positivity in a case of papillary thyroid carcinoma X400.



**Figure 6.** Histology from the same case of figure X400 shows P63.



**Figure 7.** A case of HT with a cold nodule

The association between HT and PTC has been reported before and the frequency varies between 0,5% to 23,7% [14-16], while other authors failed to show any correlation between these diseases [17-19].

Akhtar and Scognamiglio (2007) [20] suggested that HT and PTC may originate from the same pluripotent stem cells whereas retrospective studies suggested that their association is antibody specific and TgAb may have a tumorigenic effect [21]. Elevated TSH levels may also be a risk factors for cancer [22].

Sheils et al. (2000) [23] detected (explain acronym) RET/PTC1 rearrangements in 95% of the investigated patients with HT. The oncogenic RET/PTC1 and RET/PTC3 sequences are also present in PTC.

This study indicated that women with HT are more likely to have PTC. Additionally HT is about seven times more common in women than in men. It has to be noted that any nodule in the thyroid scan in a patient with HT has to be investigated cytologically in order to exclude malignancy.

Hashimoto's thyroiditis-associated PTC may frequently display prominent stromal desmoplasia and pseudovascular pattern, which can present diagnostic difficulties [24] especially in the cytologic material.

An oncocytic variant of PTC associated with HT can also be a differential diagnostic problem. Follicular as well as the tall-cell variant of PTC often poses a diagnostic challenge. Hürtle cells in HT cases can show nuclear characteristics such as nuclear membrane irregularities, nuclear clearing and grooves, mimicking PTC.

Immunocytochemistry may be helpful in questionable diagnostic cases although these cases represent a minority. [25, 26]. We evaluated the proteins' expression of CK19 and P63. Cytokeratin 19 (CK19) is a type 1 intermediate filament protein and regulation of its expression is different from other keratin-encoding genes [27]. P63 is a P53-homologue nuclear transcription factor that encodes six different isoforms, which harbor either trans-activating or negative dominant effects on P53 reporter genes [28]. In our study CK19 showed positive expression in all PTC, whereas P63 showed focal positivity in 66,6% of the cases. P63 is a specific but less sensitive marker for PTC than CK19 and has been shown to be a sensitive marker for PTC with diffuse cytoplasmic positivity.

In conclusion, we found, although in a small number of HT patients, the coexistence of PTC and HT by using immunological and genetic factors which may be of diagnostic and treatment importance.

The authors declare that they have no conflicts of interest

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