Zagazig University Medical Journal
www.zumj.journals.ekb.eg
https://doi.org/10.21608/zumj.2025.321083.3602 Volume 31, Issue 6, June 2025
**Manuscript ID:** ZUMJ-2409-3602
**DOI:10.21608/zumj.2025.321083.3602**

**Original article**

# Prognostic significance of APOE and FOXP3 immunohistochemical expression in papillary thyroid carcinoma and concomitant Hashimoto's thyroiditis

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Submit Date: 16-12-2024
Accept Date: 18-04-2025

**ABSTRACT**

**Background:** APOE is involved in immunological control mediated by lymphocytes. The biological processes of several malignant tumors were accelerated by its overexpression. APOE reduces antigen-activated lymphocytes, which has an impact on immunity. Regulatory T cells (Treg) are a subset of T helper (CD4+) cells, which are essential to the immune system because they counteract the actions of T cells to decrease autoimmune reactions. For this regulation, the transcription factor forkhead box (FOXP3) is crucial. Due to their involvement in the onset and progression of PTC, APOE and FOXP3 may be utilized in molecularly targeted treatments. Immunocheck-point inhibitors are essential to support novel immunotherapy approaches in treatment and increase the prognosis of PTC linked with HT since both indicators have significant immunoregulatory processes.

**Our aim of this study** is to explore the effect of APOE and FOXP3 on PTC prognosis and its clinical outcome, which could help in novel treatments development against PTC.

**Methods:** 46 tissue blocks were obtained retrospectively spanning the period between May 2018 to May 2023. These sections are then exposed to all steps of IHC technique for staining of APOE and FOXP3.

SPSS 23.0 was used to computerize and statistically evaluate the data .Chi-sq test ($\chi 2$) and Fisher exact were used to calculate the difference between qualitative variables. Overall and Progression-free survival were estimated by using Kaplan and Meier's method . A P-value of less than 0.05 shows significance.

**Results:** The analysis showed there was a significant association between the expression of APOE and FOXP3 and various prognostic factors such as tumor size, grade, stage, lymphovascular invasion, recurrence, and mortality.

**Conclusions:** Increased tissue expression of novel immunomarkers APOE and FOXP3 are associated with poor clinical outcome which predicted the possibility of using these markers as targeted therapy in combination with the ordinary used therapies for improving the prognosis of PTC with associated HT.

**Keywords**: APOE; FOXP3; PTC; Prognosis; IHC

## INTRODUCTION

The most common subtype of thyroid carcinoma is papillary thyroid carcinoma (PTC). Its mortality rate has significantly grown recently, with its frequency hovering around 80% **(1).** Early-stage PTC is predisposed to lymph node metastases. At the time of diagnosis, between 20% - 30% of PTC patients already had involvement of malignant lymph nodes

**(2).** High tumor recurrence, distant metastasis, and elevated mortality rates are associated with predominance of lymph node metastases **(3).**

Hashimoto's thyroiditis (HT) is the most frequent autoimmune disease of the thyroid gland. It is characterized by existence of lymphocytic infiltration, with the thyroid acini destruction**.** It is more prevalent in females **(4) (5).**

Thyroid cancer is predisposed by Chronic inflammation via allowing an appropriate environment that is an essential factor in most of cancers. Moreover, chronic inflammation associated with incidence of high mutation rate **(6).** Its mediators may lead to tumor progression and DNA damage **(7).**

PTC commonly exhibit lymphocytic infiltration, indicating the involvement of immune systems in PTC development as stated by previous study that cleared this nearby clinical association that is present between PTC and HT by the same pluripotent stem cell origins, and may have an oncogenic role **(8).** So, it is an essential to search for novel prognostic markers that might enable early diagnosis and prognosis of PTC with accompanying HT as APOE and FOXP3 IHC.

Previous studies have cleared that high plasma cholesterol levels are linked with high tumor burden and tumor development and progression (**9).** Apolipoprotein E (APOE) is one of the key proteins that is important in cholesterol metabolism and regulation of various diseases such as atherosclerosis, Alzheimer's disease and diabetic nephropathies (**10).**

APOE can regulate tumor development via re controlling lipid metabolism via TGFβ, EMT, ER signaling pathways **(11).** APOE effects on synthesis of DNA, cell proliferation, and angiogenesis leading to tumorigenesis and progression thus enhancing tumor growth and metastasis in many types of cancers (**12).**

The transcription factor FOXP3 is distinguished by its forkhead domain (FKH), which binds to DNA and plays a function in the regulation of Treg cells **(13).** Regulatory T cells (Treg) are a subset of T helper (CD4+) cells that work against T cells to prevent autoimmune reactions. Appropriate follow-up is required since FOXP3 up-regulation may facilitate the migration and invasion of malignant cells through a series of autoimmune inflammatory processes that culminate in PTC formation **(14).**

Our aim of this work is evaluation of the clinical, pathological and the prognostic importance of IHC staining of APOE and FOXP3 in PTC and concomitant HT.

## METHODS

This retrospective, cross-sectional study was conducted at the Zagazig University Faculty of Medicine's departments of nuclear medicine, clinical oncology, and pathology as well as the Ismailia Teaching Oncology Hospital. Fourty six thyroid biopsies that were paraffin embedded and preserved with formalin were chosen at random from the pathology department's archive between May 2018 and May 2023. Informed written consent were obtained from all study participants before study enrollment. Patients who had systemic follow-ups, received no adjuvant therapy before tumor recurrence, and were diagnosed with PTC were selected. The histopathologic subtypes of PTCs in the current study are conventional PTC and PTC follicular variant. Other variants are not evaluated due to their rare incidence

Each of our patients' clinical and pathological characteristics were obtained from their medical records in Nuclear Medicine and Clinical Oncology. According

to the patients' files, the patients underwent surgical resection (total or near-total thyroidectomy) and then referred to the Ismailia teaching oncology hospital to receive RAI131 therapy according to their risk stratification group based on American Thyroid Association Guidelines (ATA) **(15)**

The Ismailia Teaching Oncology Hospital patient records contained the data regarding radioiodine treatment and patient outcomes (16). The WHO Classification of Tumors **(17)** criteria were used for PTC grading, and the TNM staging approach **(18)** was used for staging. The Faculty of Medicine at Zagazig University's Institutional Review Board gave its approval to this study (IRB, No. 1010).

Overall survival was defined as the duration from the first diagnosis to death or censored at the last known alive-data. Disease-free survival was measured in months from the date of initial diagnosis to the date of recurrence.

**Immunohistochemical staining.**

The Streptavidin-biotin technique was employed, utilizing a primary mouse monoclonal antibody targeting APOE (Santa Cruz Biotechnology used at a 1:100 dilution), a mouse monoclonal primary anti-FOXP3 antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA, used at a dilution of 1:200) .The sections (4–5 μm) obtained from the paraffin blocks were dewaxed, rehydrated, and placed in 0.5% hydrogen peroxide in methanol for 10 min for blockage endogenous peroxidase activity. Positive and negative control slides were included. Positive controls were sections from liver tissue and normal human tonsil for APOE ,FOXP3 respectively, the negative controls were done by using the non-immune serum instead of primary antibodies

**Interpretation of the immunohistochemistry:**

Any cytoplasmic or membranous staining is regarded as positive APOE immunostaining. On the other hand, where cytoplasmic or nuclear staining was present, FOXP3 was considered positive.

The staining of APOE was scored as follows: 0 (no staining); 1-2 (weak); >2 (strong). The positive cell percentages of APOE were graded as follows: 0 (0%); 1 (1-25%); 2 (26-50%); 3 (51-75%); 4 (76-100%). Total immunohistochemistry score = staining intensity × percentage. APOE protein expressions were classified as follows: < 2 low expressions, ≥ 2 high expressions **(19)**

For FOXP3, the distribution of positive cells was estimated according to the following: 0=no stained cells; 1=up to 10% stained cells; 2=10–30% stained cells; and 3≥30% stained cells. Simply, the slides with zero scores were considered negative, and the slides with total scores from one up to three were considered positive (20).

**Statistics.**

- SPSS 23.0 was used to computerize and statistically evaluate the data that were collected.
- Chi-square test ($\chi 2$) and Fisher exact were used to calculate the difference between qualitative variables.
- Overall and Progression-free survival were estimated by using Kaplan and Meier's method
- A p-value of less than 0.05 shows significance.

## RESULTS

**Patients' characteristics**

**Comparison of clinicopathological factors in PTC patients with and without HT (Table 1)** Forty-six thyroid biopsies from PTC patients were included in the current study; 63.1% of these were older than 45, 65.3% were female, 78.3% involved PTC without HT, and 21.7% involved PTC with HT. The 46 PTC patients were divided into 10 follicular subtype PTC cases and 36 classic PTC cases. On comparing clinicopathological factors between the two groups, PTC with HT have a lower percentage of extrathyroidal extension, advanced stage, lymph node and distant metastasis (good prognostic variables). Follow up of both groups showed that patients PTC with HT had 100% no local recurrence, deaths, and progressions.

Regarding the LN and distant metastasis which were present in 28.3% and 2.8% of patients, respectively, radioactive I-131 was given to 27.8% of PTC patients in one dose, 33.3% of patients in multiple doses, and 38.9% of patients did not receive I-131 doses because they were low risk and under follow-up. The clinico-pathological characteristics of the Forty-six cases with PTC are summarized in Table (1).

**Table1.** The clinical and pathological differences between PTC and PTC with HT (N=46)

| **Age (years)** | **PTC N=36** | | **PTC with HT N=10** | | **P value** |
|---|---|---|---|---|---|
| **≤45** | **13** | **36.1%** | **4** | **40%** | |
| **>45** | **23** | **63.9%** | **6** | **60%** | **0.8216** |
| **Pathological subtypes** | | | | | |
| **Follicular variant** | **6** | **16.7%** | **4** | **40%** | **0.1893** |
| **Classic variant** | **30** | **83.3%** | **6** | **60%** | |
| **Extrathyroidal extension** | | | | | |
| **No** | **30** | **83.3%** | **9** | **90%** | |
| **Yes** | **6** | **16.7%** | **1** | **10%** | **0.6554** |
| **Stage** | | | | | |
| **I–II** | **22** | **61.1%** | **6** | **60%** | **0.0041** |
| **III–IV** | **14** | **38.9%** | **4** | **40%** | |
| **LN metastasis** | | | | | |
| **No** | **25** | **69.4%** | **8** | **80%** | **0.7003** |
| **Yes** | **11** | **30.6%** | **2** | **20%** | |
| **Tumor size (cm)** | | | | | |
| **≤2** | **20** | **55.6%** | **8** | **80%** | **0.2736** |
| **>2** | **16** | **44.4%** | **2** | **20%** | |
| **Tumor grade** | | | | | |
| **Low grade** | **22** | **61.1%** | **6** | **60%** | **0.004** |
| **High grade** | **14** | **38.9%** | **4** | **40%** | |

| | | | | | |
|---|---|---|---|---|---|
| **Distant metastasis** | | | | | |
| **No** | **34** | **94.4%** | **10** | **100%** | **0.3913** |
| **Yes** | **2** | **5.6%** | **0** | **0.0%** | |
| **FOXP3 immunostaining** | | | | | |
| **Negative** | **20** | **55.6%** | **6** | **60.0%** | |
| **Positive** | **16** | **44.4%** | **4** | **40.0%** | **1.000** |
| **APOE immunostaining** | | | | | |
| **Negative** | **26** | **72.2%** | **6** | **60%** | **0.4646** |
| **Positive** | **10** | **27.8** | **4** | **40%** | |
| **Bilaterality** | | | | | |
| **No** | **29** | **80.6%** | **9** | **90%** | **0.1991** |
| **Yes** | **7** | **19.4%** | **1** | **10%** | |
| **Local recurrence** | | | | | |
| **No** | **33** | **91.7%** | **10** | **100%** | **0.6923** |
| **Yes** | **3** | **8.3%** | **0** | **0.0%** | |
| **Death** | | | | | |
| **No** | **32** | **88.9%** | **10** | **100%** | **0.9574** |
| **YES** | **4** | **11.1%** | **0** | **0.0%** | |
| **Number of I131 doses** | | | | | |
| **No doses** | **14** | **38.9%** | **0** | **0%** | **0.1497** |
| **1** | **10** | **27.8%** | **6** | **60 %** | |
| **>1** | **12** | **33.3%** | **4** | **40%** | |

**Association of APOE and FOXP3 expression with clinicopathological parameters:**

Results of immunohistochemical expression of FOXP3 are presented in Table 2. The neoplastic cells showed nuclear and or cytoplasmic FOXP3 immunoreactivity. All positive samples showed weak to moderate FOXP3 expression. In PTC, the positive FOXP3 expression was more in female more than 45 years. FOXP3 expression was related to a series of poor clinicopathological features like large tumor size, higher tumor grade, stage, presence of lymph node and distant metastasis, multifocality, and extracapsular extension. PTC with associated HT showed FOXP3+ lymphocytes in the lymphocytic infiltrate of HT indicating the presence of regulatory T lymphocytes (Treg) (Fig. 1). Regarding PTC patients, APOE immunostaining is positive in cytoplasmic or membranous staining and correlated with higher tumor grade and stage (Fig. 2).

**Table 2**. Association of clinical and pathological features with marker expression in the studied PTC (N=46).

| | **FOXP 3** | | | | **P value** | **APOE** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Negative N=26** | | **Positive N=20** | | | **Negative N=32** | | **Positive N=14** | | **P value** |
| | **N** | **%** | **N** | **%** | | **N** | **%** | **N** | **%** | |
| **Age (years)** | | | | | | | | | | |
| **≤45** | **10** | **38.5** | **7** | **35** | **0.8094** | **11** | **34.4** | **6** | **42.8** | **0.5834** |
| **>45** | **16** | **61.5** | **13** | **65** | | **21** | **65.6** | **8** | **57.2** | |

| | FOXP 3 | | | | P value | APOE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Negative N=26 | | Positive N=20 | | | Negative N=32 | | Positive N=14 | | P value |
| | N | % | N | % | | N | % | N | % | |
| **Sex** | | | | | | | | | | |
| **M** | 3 | 11.5 | 10 | 50 | 0.0040 | 7 | 21.9 | 5 | 35.7 | 0 .3253 |
| **F** | 23 | 88.5 | 10 | 50 | | 25 | 78.1 | 9 | 64.3 | |
| **Pathology** | | | | | | | | | | |
| **PTC** | 21 | 80.8 | 15 | 75 | 0.6381 | 31 | 96.9 | 5 | 35.7 | < 0.00001 |
| **PTC with HT** | 5 | 19.2 | 5 | 25 | | 1 | 3.1 | 9 | 64.3 | |
| **Path subtype** | | | | | | | | | | |
| **Follicular** | 5 | 19.2 | 1 | 5 | 0.2900 | 5 | 15.6 | 1 | 7.1 | 0.7322 |
| **Classic** | 16 | 61.5 | 14 | 70 | | 21 | 65.6 | 9 | 64.3 | |
| **Follicular with HT** | 1 | 3.8 | 3 | 15 | | 2 | 6.3 | 2 | 14.3 | |
| **Classic with HT** | 4 | 15.5 | 2 | 10 | | 4 | 12.5 | 2 | 14.3 | |
| **Extra thyroid Ext** | | | | | | | | | | |
| **No** | 24 | 92.3 | 15 | 75 | 0.1052 | 29 | 90.6 | 10 | 71.4 | 0.0953 |
| **Yes** | 2 | 7.7 | 5 | 25 | | 3 | 9.4 | 4 | 28.6 | |
| **Stage** | | | | | | | | | | |
| **I–II** | 21 | 80.8 | 7 | 35 | 0.0016 | 23 | 71.9 | 5 | 35.7 | 0.002 |
| **III–IV** | 5 | 19.2 | 13 | 65 | | 9 | 28.1 | 9 | 64.3 | |
| **LN. met** | | | | | | | | | | |
| **No** | 20 | 76.9 | 13 | 65 | 0.3733 | 25 | 78.1 | 8 | 57.2 | 0.1458 |
| **N1** | 6 | 23.1 | 7 | 35 | | 7 | 21.9 | 6 | 42.8 | |
| **Distant met** | | | | | | | | | | |
| **M0** | 26 | 100 | 18 | 90 | 0.4020 | 32 | 100 | 13 | 92.9 | 0.5386 |
| **M1** | 0 | 00 | 2 | 10 | | 0 | 00 | 1 | 7.1 | |
| **Tumor grade** | | | | | | | | | | |
| **Low grade** | 21 | 80.8 | 7 | 35 | 0.001 | 23 | 71.9 | 5 | 35.7 | 0.002 |
| **High grade** | 5 | 19.2 | 13 | 65 | | 9 | 28.1 | 9 | 64.3 | |
| **T** | | | | | | | | | | |
| **T1–T2** | 18 | 69.2 | 10 | 50 | 0.1852 | 20 | 62.5 | 8 | 57.2 | 0.7319 |
| **T3- T4** | 8 | 30.8 | 10 | 50 | | 12 | 37.5 | 6 | 42.8 | |
| **Surgery** | | | | | | | | | | |
| **Lobectomy** | 2 | 7.7 | 1 | 95 | 0.7139 | 3 | 9.4 | 0 | 00 | 0.8047 |
| **thyroidectomy** | 24 | 92.3 | 19 | 5 | | 29 | 90.6 | 14 | 100 | |
| **Number of I131 doses** | | | | | | | | | | |
| **No doses** | 6 | 23.1 | 8 | 40 | 0.3609 | 6 | 18.8 | 8 | 57.2 | 0.0337 |
| **1** | 11 | 42.3 | 5 | 25 | | 13 | 40.6 | 3 | 21.4 | |
| **>1** | 9 | 34,6 | 7 | 35 | | 13 | 40.6 | 3 | 21.4 | |
| **Cumulative radioiodine dose (mCI)** | | | | | | | | | | |
| **<200** | 13 | 50 | 10 | 50 | 0.8898 | 15 | 46.9 | 8 | 57.2 | 0.5487 |
| **≥200–400** | 5 | 19.2 | 3 | 15 | | 7 | 21.9 | 1 | 7.1 | |
| **>400–600** | 3 | 11.5 | 3 | 15 | | 3 | 9.4 | 3 | 21.5 | |
| **>600–800** | 4 | 15.5 | 2 | 10 | | 5 | 15.6 | 1 | 7.1 | |

| | FOXP 3 | | | | P value | APOE | | | | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| | Negative N=26 | | Positive N=20 | | | Negative N=32 | | Positive N=14 | | |
| | N | % | N | % | | N | % | N | % | |
| 0>800–1000 | 1 | 3.8 | 2 | 10 | | 2 | 6.3 | 1 | 7.1 | |

**Association between APOE and FOXP3 expression and survival**

Analysis of overall survival (OS) and disease-free survival (DFS) of PTC patients using the Kaplan-Meier method (Figure 2) clearly revealed that shorter OS & DFS is associated with positive expressions of APOE and FOXP3**(Fig. 3).**

**Association between APOE and FOXP3 expression with both tumor relapse and mortality.**

Concerning recurrence, relapse and mortality rates, a significant correlation between APOE and FOXP3 positive IHC staining was observed, which denotes that APOE and FOXP3 can be considered poor prognostic indicators (Table 3). There is a significant correlation between APOE and FOXP3 positive IHC staining (p= 0.000132**).** (Table 4).

There is statistically significant association between OS and expression of both APOE and FOXP3. Patients with negative APOE and FOXP3 had significantly higher OS.

**Table 3.** Correlation between APOE and FOXP3 immunostaining with clinicopathologic parameters, mortality and relapse.

| | FOXP3 | | | | P value | APOE | | | | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| | Negative N=26 | | Positive N=20 | | | Negative N=32 | | Positive N=14 | | |
| | N | % | N | % | | N | % | N | % | |
| **Metastasis** | | | | | | | | | | |
| No<br>Yes | 26<br>0 | 100<br>00 | 19<br>1 | 95<br>5 | 0.8491 | 32<br>0 | 100<br>0 | 13<br>1 | 92.9<br>7.1 | 0.5386 |
| **Local recurrence** | | | | | | | | | | |
| No | 22 | 84.6 | 17 | 85 | 0 .9712 | 29 | 90.6 | 10 | 71.4 | 0 .0953 |
| Yes | 4 | 15.4 | 3 | 15 | | 3 | 9.4 | 4 | 28.6 | |
| **Death** | | | | | | | | | | |
| No | 24 | 92.3 | 18 | 90 | 0.7830 | 31 | 96.9 | 11 | 78.6 | 0.0426 |
| Yes | 2 | 7.7 | 2 | 10 | | 1 | 3.1 | 3 | 21.4 | |

**Table 4.** Correlation between FOX 3 and APOE expression.

| | | Fox 3 | | P value |
|---|---|---|---|---|
| | | Negative N=26 | Positive N=20 | |
| APOE | Negative N=32 | 24 | 8 | **0.000132** |
| | Positive N=14 | 2 | 12 | |

## FIGURES

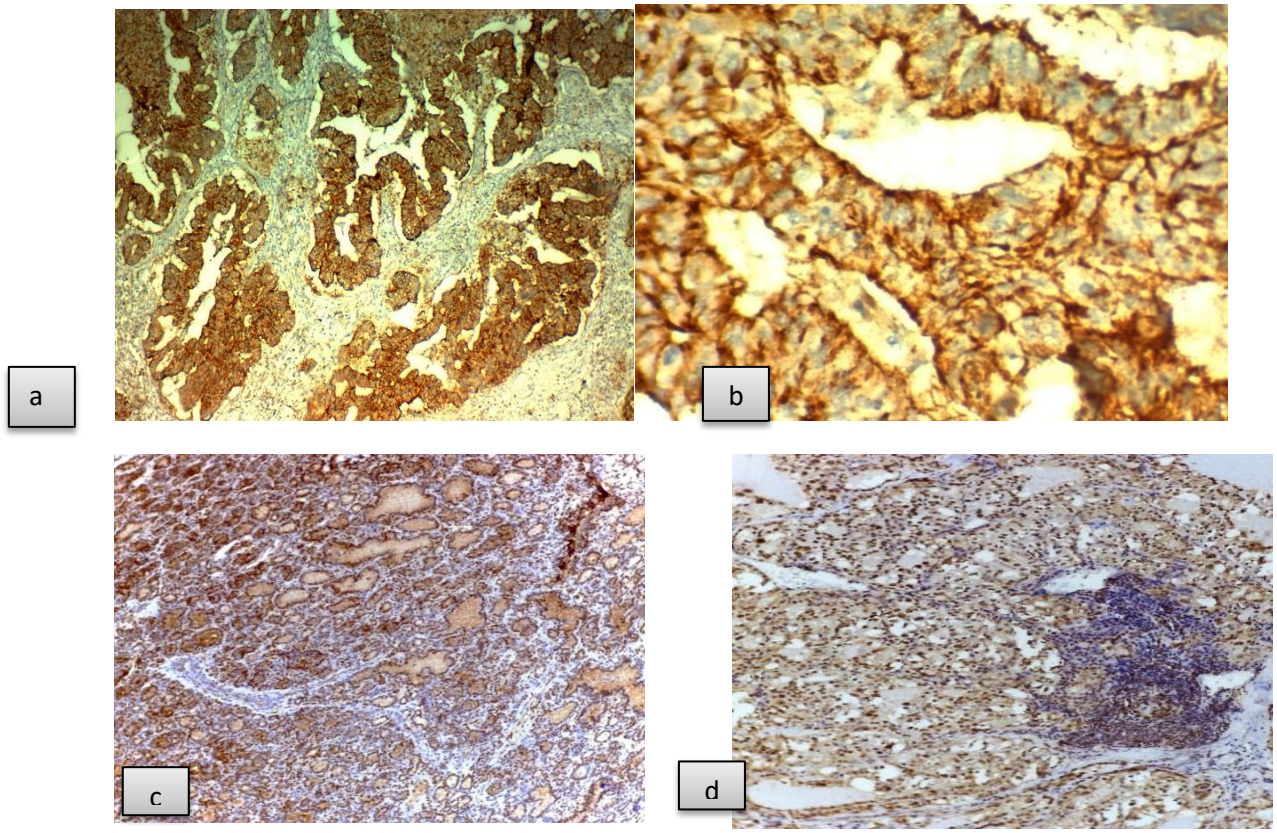


**Fig. 1**, Foxp3 cytoplasmic expression in classic variant of papillary thyroid carcinoma immunostaining ((a)×200) and ((b)×400). FOXP3 nuclear and cytoplasmic immunostaining in follicular variant of PTC((C)×200) and infiltrating lymphocyte in HT ((d)×200).

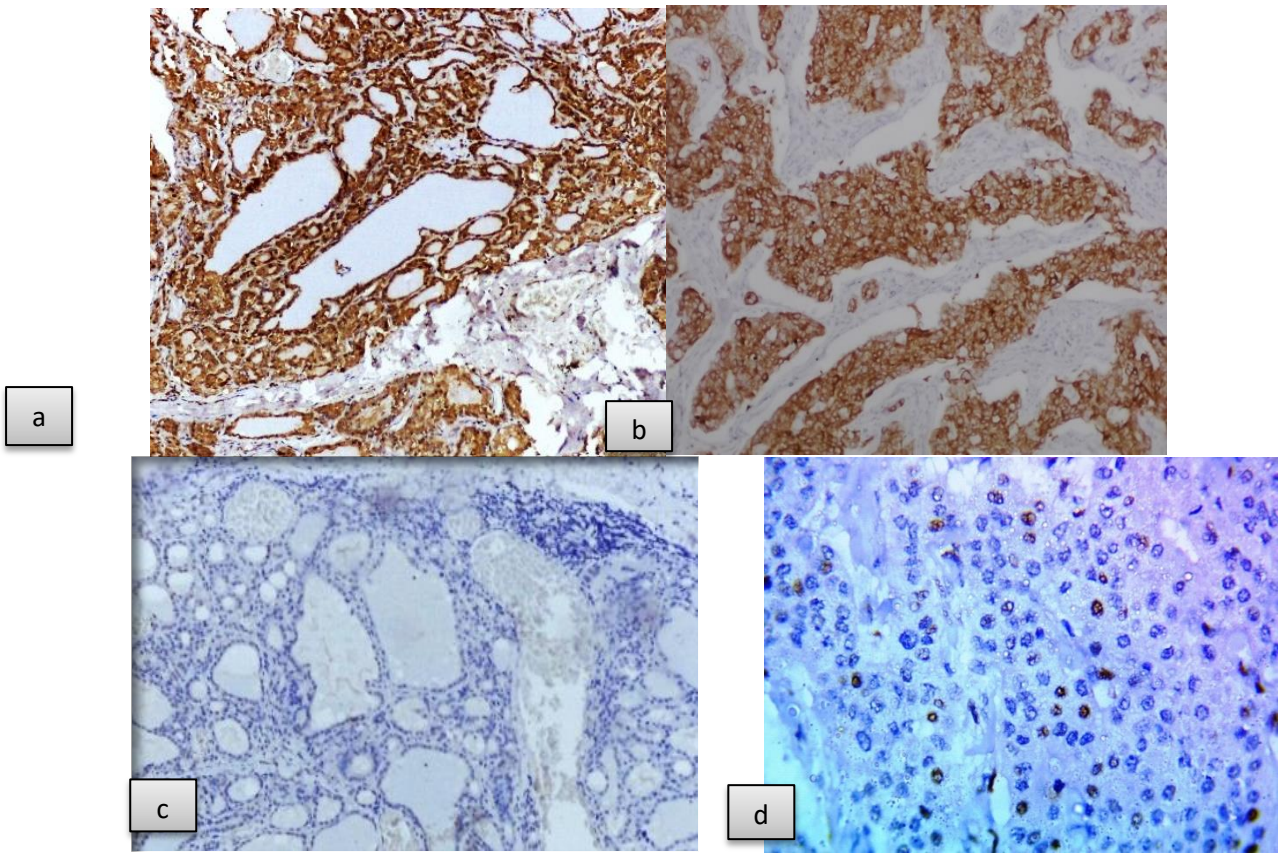


**Fig. 2**, Positive APOE immunostaining in papillary thyroid carcinoma ((a)×200) and ((b)×400). Negative APOE immunostaining in papillary thyroid carcinoma with HT ((c)×200) and ((d) ×400)

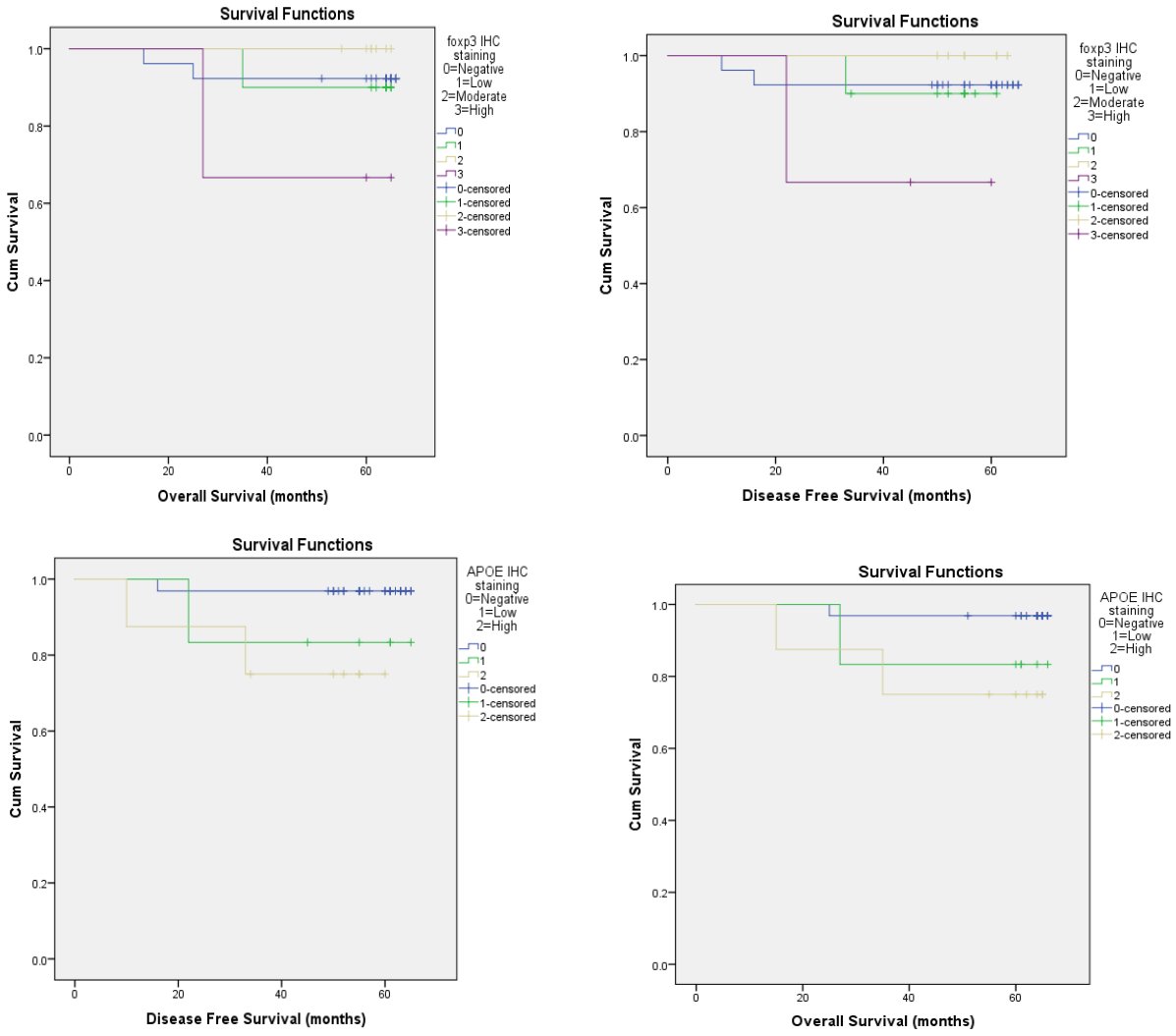


**Fig.3,** Kaplan Meier plot, upper panel: Disease free survival, lower panel: Overall survival, (A&D). All studied patients, (A&B) stratified by FOXP3 and(C&D) stratified by APOE.

## DISCUSSION

Thyroid carcinoma represents about 1% of all malignant tumors, and its commonest form is PTC that constitutes about 80–90% **(21).** To our knowledge, there is no previous study that used combined APOE, FOXP3 in prognosis of PTC with associated HT to predict the disease progression of HT to PTC and so we can avoid or lessen occurrence of malignant transformation.

These patients require new therapeutic modalities besides traditional treatments like combined surgical and radioiodine therapy to reach a definitive cure. In this study, we examined the clinicopathological and prognostic value of immunohistochemical expression of FOXP3 in PTC suggesting a role in the pathogenesis of PTC and if there was a difference in their expression in concomitant HT and their correlation with patients' outcome and survival. The coexistence between PTC and HT has been reported in many studies, but it is

still controversial. In this work, the incidence of PTC over HT in this work was 21.7%. This result almost matches the findings of Ahn et al., who found that 21.6% of PTC had concurrent HT **(22).** However, Girardi et al., found a 35.4% correlation between PTC and HT **(23).** This discrepancy could be brought about by changes in the pathological interpretation of HT, wherein nonspecific lymphocytic infiltration is mistakenly identified as HT.

However, we emphasize that the expression of PTC related marker (FOXP3) by HT infiltrating lymphocyte, point toward a relationship between the development of HT and PTC (the multistep process in autoimmune inflammatory disease). In this study, PTC with coexisting HT was associated with good prognostic variables such as younger age, smaller tumor size, unifocal tumor, a lower percentage of lymph node and distant metastasis in comparison to PTC alone. These results were in line with Ahn et al., who reported that patients with PTC and HT associated with a good prognosis. Previously, FOXP3 was considered to be expressed only in the regulatory T cells, but now it has been found in multiple types of malignant cells(34). Tumors with positive

FOXP3are more liable to invasion and metastasis through induction of the secretion of some cytokines which have immunosuppressive functions such as TGF-β1 and IL-1027. This may represent an example of molecular simulation and could represent a masked method of the escape of malignant cells from the immune system ( 31).

FOXP3 expression was detected in 44.4% of PTC cases and 40 % of PTC with HT, this finding is nearly similar to the finding obtained by Ugolini et al., who reported FOXP3 expression in43% of the studied PTC (32). Other related studies reported a higher incidence of FOXP3 expressions among the studied PTC such as Junior et al., who reported FOXP3 positivity in 72.4% of the studied PTC( 29,30), These differences in the expression of FOXP3 in PTC may be due to the usage of different primary antibody clones, different immunohistochemical techniques, a different method of interpretation of markers positivity, and may be due to different cohort number. Our results showed that FOXP3 tends to be more expressed in PTC cases with large tumor size, advanced tumor stage, presence of lymph node metastasis, and extracapsular extension. Cases of PTC with concomitant Hashimoto thyroiditis showed scattered FOXP3+ lymphocytes in the lymphocytic infiltrate of Hashimoto thyroiditis indicating the presence of regulatory T lymphocytes (Treg). Yang et al. demonstrated that there was a decreased expression of FOXP3and improper Treg function in patients with HT may be due to abnormal acetylation of FOXP3 **(31).** Other results detected the expression of PTC-associated FOXP3 in HT support the hypothesis that epithelial changes of thyroid follicles in HT represent a multistep process of autoimmune inflammatory disease ending by the evolution of PTC, therefore appropriate follow up of these cases are needed **(31) (32) (33).**

According to our findings, PTC with HT had higher levels of APOE expression than PTC without HT. APOE expression was detected in **27.8 %** of PTC without HT and in 40% of PTC with HT. APOE expression rose in the PTC cases in correlation with larger tumor sizes, advanced tumor stages, lymph node metastases, and extracapsular tumor expansion. The identical outcomes aligned with the findings of **Jiang et al., (26).**

APOE can be used in immunotherapy that has become one of the most novel hopeful strategies in treatment of cancer. **Lin et al**., (**28)** found in their study association between APOE level and immune infiltration, so APOE can not only be used as a diagnostic factor for PTC, but also as a prognostic and a possible aim for treatment with immunotherapy. The combination of ICI (pembrolizumab and nivolumab) and targeted drugs (TKIs as Lenvatinib), particularly tyrosine kinase inhibitors, is a crucial direction for future development in thyroid cancer immunotherapy.

**However, Salem et al., (34)** reported significant correlation in both types of PTC**.** This may be due to the link between FOXP3 expression and the underlying unfavorable prognostic factors that have a certain impact on radioiodine sensitivity**.**

**In summary,** both APOE and FOXP3 implicated in PTC initiation and progression so can be used in molecular-targeted therapies. Both markers have important immunoregulatory process, so immune check-point inhibitors are crucial to enhance new immunotherapy strategies in treatment of PTC.

## CONCLUSIONS

Increased tissue expression of novel immunomarkers APOE and FOXP3 are associated with poor outcome So, using these markers is a valuable tool for a recent targeted therapy (TKIs) in conjunction with the currently used treatment regimens to improve PTC with associated HT prognosis.

**Conflict of Interest:** No

**Financial Disclosures:** Self fundamental

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## Citation

**Abdelgeleel, H., Qasem, E., Matar, I., Elnossiry, A., Adel Abdelrahman, E., Elaidy, N. Prognostic significance of APOE and FOXP3 immunohistochemical expression in papillary thyroid carcinoma and concomitant Hashimoto's thyroiditis. *Zagazig University Medical Journal*, 2025; (2302-2315): -. doi: 10.21608/zumj.2025.321083.3602**