Prognostic Role of β-catenin/PTEN Expressions and Braf/Kras Mutations in Papillary Thyroid Carcinoma: Correlation with Response to Radioactive Iodine Therapy

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Abstract:

Background: Papillary thyroid carcinoma (PTC) is the most common endocrine cancer; nevertheless, despite a favorable prognosis, some PTCs demonstrate aggressive behavior and are refractory to radioactive iodine therapy (RAIT). The study aims to evaluate the association between PTEN/β-catenin expression as well as BRAF/KRAS mutations on the pathological features and responsiveness to RAIT.

Methods: A retrospective study included 52 cases of PTC. β-catenin and PTEN expressions were evaluated by immunohistochemistry. BRAFV600 and KRAS mutations were evaluated using PCR technique. All patients underwent thyroidectomy followed by RAIT, and at least 12 months of follow-up following initial therapy.

Results: The study included 52 patients (38 females and 14 males, mean age: 42.07±14.17 years). Lost β-catenin membranous expression was significantly associated with nodal metastasis (P=0.011), lymphovascular invasion (P=0.041), and higher cumulative doses of RAI (P=0.0.034). Positive β-catenin cytoplasmic expression was significantly linked to persistent/recurrent structural disease (P=0.007). Negative PTEN cytoplasmic expression was significantly associated with advanced TNM staging (P=0.022), thyroid capsular infiltration, and extrathyroidal extension (P=0.005 and 0.008, respectively). There was no significant relationship found between PTCs harboring $BRAF^{V600E}$ mutation and pathological characteristics or responsiveness to RAI (P=0.521).

Conclusions: Our results demonstrate that PTCs lacking membranous and expressing cytoplasmic β-catenin are significantly linked to more aggressive pathology, greater RAI dosages, and disease recurrence/persistence. Negative PTEN expression is substantially associated with advanced TNM staging and pathological characteristics. Furthermore, our data suggest that a positive BRAF mutation has no significant impact on RAIT efficacy in PTC patients without known distant metastases.

Keywords: Papillary thyroid carcinoma, Radioiodine therapy, PTEN, β-catenin, $BRAF^{V600E}$, and KRAS mutation

Background:

Papillary thyroid carcinoma (PTC) constitutes about 90% of all thyroid carcinoma (TCs) [1]. PTC is generally a highly curable cancer with a quite favorable prognosis following appropriate treatment, which includes surgical treatment, consecutive radioactive

iodine (RAI) therapy (RAIT), and thyroid-stimulating hormone (TSH) suppression therapy [2]. Nevertheless, up to 30% of PTC patients are at risk of recurrence or persistent disease [3]. Two-thirds of these patients had neoplastic lesions that lost iodine-131 (131I) uptake at first or over time, which is known as RAI refractoriness [4]. Therefore, predicting the prognosis of PTC and tailoring treatment strategies have become clinically relevant issues.

PTC occurrence and progression are often preceded by molecular changes in the sequence composition of biological molecules including DNA, RNA, and proteins [5].

β-catenin is a multifunctional protein that is crucial in the Wnt/β-catenin signaling transduction pathway [6].

The Wnt/β-catenin signaling transduction pathway plays a vital role in regulating cellular growth and proliferation, and its immanent activation is frequently observed in human cancers [7]. In TCs, upstream of Wnt/β-catenin signaling pathway by activating mutations in exon 3 of CTNNB1 (a proto-oncogene encodes β-catenin) renders β-catenin resistant to degradation, resulting in its constitutive accumulation in the cytoplasm, then translocation into the nucleus and transcription of different tumor-promoting genes including cyclin D1. It is particularly found in poorly differentiated TC (PDTC) and anaplastic TC (ATC) [8]. Previous literature has shown that decreased membranous levels of β-catenin and its aberrant nuclear localization are strongly associated with loss of tumor differentiation and poor prognosis [9].

Mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling pathways mediate a wide range of imperative cellular processes such as differentiation, cell survival, reproduction, and apoptosis [10].

Phosphatase and tensin homolog gene (PTEN) is a tumor suppressor gene that plays a pivotal role in programmed cell death, cell cycle, and motility. It has been linked to the pathogenesis of TCs via the termination of PI3K/AKT/mTOR signaling [11], therefore PTEN silencing mutation resulting in radioresistance and metastasis development in TCs [12].

The gateway to MAPK is the activation of receptor tyrosine kinase (RTK) by binding cytokines or growth factors to the receptor's extracellular domain, resulting in an intracellular cascade of phosphorylation events. The Rat sarcoma viral oncogene homolog (RAS) kinase is the first target of the cascade to be activated, which in turn triggers phosphorylation of the downstream serine/threonine-protein (RAF) kinase, MAP/extracellular signal-regulated kinase (MEK), and extracellular signal-regulated kinases (ERK). Activated ERK then translocates into the nucleus, where it regulates numerous transcription factors and hormone receptors involved in the aforementioned cell cycle processes [13].

An activating missense point mutation within axon 15 causes replacement of valine by glutamic amino acid at codon 600 of the BRAF protein, resulting in the $BRAF^{V600E}$ mutation [14]. $BRAF^{V600E}$ mutation is the most prevalent and specific genetic aberration in PTC (60-74%) [15]. It promotes tumorigenesis of PTC by constitutive activation of the MAPK pathway [16]. Positive correlations were found between $BRAF^{V600E}$ mutations and PTC recurrence, metastasis, and dedifferentiation [17]. RAS oncoproteins are GTP-binding proteins on the inner surface of cell membranes that act via both MAPK and PI3K/AKT/mTOR signaling pathways (dual activator) and transmit extracellular signals that promote cell proliferation, differentiation, and longevity [18]. Point mutations at codons 12, 13, and 61 hinder RAS in a constitutively active state, which plays a critical role in human tumorigenesis (mutant RAS gene is found in nearly all human cancers), including TCs [19]. RAS mutations are found in 30–45% of follicular thyroid carcinoma (FTC), 30–45% of PTC; nearly all are follicular variant (FVPTC); 20–40% of PDTC; and 10–20% of ATC [19].

To date, no previous studies have evaluated the potential importance of PTEN and β-catenin simultaneously as prognostic and predictive biomarkers in PTC.

The current study aimed to explore the prognostic role of PTEN/ß-catenin expression, as well as BRAF/KRAS mutations in PTCs and to determine the impact of these biomarkers on the response to RAIT.

Methods:

Samples and patient population

This retrospective study received approval from the Institutional Review Board (IRB. 04-2023-300280) with a waiver of consent.

Patients ≥18 years old at initial surgery, who underwent one/two-stage total thyroidectomy ± lateral/central lymph node dissection, with histopathologically confirmed PTC, available histological tumor blocks at the time of study, and complete follow-up data (retrieved from patients' medical files in our nuclear medicine unit) were enrolled.

Overall, 52 formalin-fixed paraffin-embedded (FFPE) tissue blocks from 52 patients with PTC (obtained between November 2016 and April 2021) were molecularly profiled at the Oncologic Pathology Department. All H&E slides were re-examined to validate the diagnosis and reevaluate histopathologic characteristics, including PTC variant, tumor size, focality and laterality, lymphovascular invasion (LVI), lymph node metastasis (LNM), thyroid capsular invasion, and extrathyroidal extension (ETE). Ten FFPE blocks of normal thyroid tissue were selected for patients referred for thyroidectomy for reasons other than cancer to serve as controls.

The eighth edition of the American Joint Committee on Cancer was used to figure out the TNM staging [20]. Risk stratification was categorized as low, intermediate, or high according to the 2015 American Thyroid Association (ATA) guidelines [21].

Surgical procedure

Preoperative preparation including laboratory investigations, neck ultrasound (NU) and ENT examination for vocal cord mobility. For all included patients, traditional neck collar incision and full evaluation of thyroid swelling as well as proper identification of recurrent and external laryngeal nerves, and parathyroid glands were done. Then, total thyroidectomy ± ipsilateral/bilateral neck dissection was carried out according to clinical, pathological or radiological nodal involvement.

Treatment and follow-up

Following surgery, all patients were given RAI (30–200 mCi) under stimulated endogenous TSH (≥ 30 μIU/ml) followed by TSH suppressive therapy. Patients were then followed up for a minimum of twelve months from the surgery date or until a documented event (persistent/recurrent disease) was recorded.

The first dose of RAI for each patient was based on how high their risk was. For patients who needed more than one dose due to persistent disease, the next doses were based on the response to the previous ones.

Cases with no evidence of persistent disease were followed up twice a year for the first year, then yearly, while those with persistent disease were followed up every six months. Clinical evaluation, measuring stimulated serum thyroglobulin (Tg) and anti-Tg antibodies (TgAbs), and NU were all parts of the evaluation.

131I-diagnostic whole-body scintigraphy (131I-Dx-WBS) was routinely carried out in the initial follow-up visit, and then repeated as needed for certain patients. 18F-fluorodeoxyglucose-computed tomography was decided for cases with elevated Tg and negative iodine scintigraphy.

We assessed the response to the initial therapy (surgery + RAIT) over one year. Recurrence was described as the reappearance of (clinical, histopathological, biochemical, or radiological) evidence of disease after a disease-free status of ≥12 months following initial therapy. Persistent disease, on the other hand, was considered if there was evidence of disease during the first year following initial therapy [22].

The therapeutic response was assessed in accordance with the ATA classification as an excellent response [no evidence of disease (NED)], a structural incomplete response, or a biochemical incomplete response.

The following conditions were met for disease-free status: TSH-stimulated Tg<1 ng/ml in the lack of TgAbs, negative 131I Dx-WBS, and free NU (no thyroid residue or cervical lymphadenopathy).

In contrast, patients were categorized as having persistent/recurrent structural disease if a disease event was identified in functional (131I Dx-WBS or 18F-FDG PET/CT) or anatomical (NU, CT, or magnetic resonance imaging) imaging modalities.

Biochemical disease was considered when Tg is detectable (in the absence of TgAbs interference) or persistent/increasing TgAb titre in the absence of structural evidence of disease. Patients with NED were thought to be disease-free, while those with biochemical or structural disease were thought to have persistent/recurrent disease.

Immunohistochemistry

FFPE blocks were cut into 4μ thickness and mounted on positively charged slides. Sections were de-paraffinized and rehydrated in xylene and descending alcohol manner. Antigen retrieval was done by heat by putting the slides in high PH Tris EDTA solution at 97 for 20 minutes. Hydrogen peroxide block was applied for 5 min, then washed with PBS. As illustrated in the table below, two primary antibodies were applied to two separate tissue sections. Universal staining kit "PolyQ stain 2 step detection system goat Anti-mouse/rabbit HRP, peroxidase quench, DAP-kit (Ready-To-Use)" (Protaqs® Quartett, catalog # DK-211-015, BIOCYC Gesellschaft für Biotechnologie, Kosmetik und Recyclingverfahren mbH & Co. Entwicklung KG Am Mühlenberg 11, 14476 Postdam Germany) was applied following the manufacturer's instructions. First: the post-blocking/ primary antibody enhancer was applied for 15 min, then washed with PBS. Second: the Ms/Rb polymer was applied for 30 min, then washed with PBS. Diaminobenzidine (DAB) buffer substrate was applied to the slides for 5 min. A counterstain for the tissue sections was performed ng Mayer's hematoxylin. Positive membranous and cytoplasmic staining of adjacent thyroid follicular cells in the same slide was used as an internal positive control for both β-catenin and PTEN expressions, respectively.

Antibody (AB)	Company	Catalog No.	Concen-tration	Time applied
β-catenin rabbit monoclonal AB	Abclonal inc, China	A19657	1/150	60 min
PTEN mouse monoclonal AB	Abbkine, inc, China	ABM40314	1/100	30 min

Evaluation of ß-catenin and PTEN expression

β-catenin positivity was evaluated as either membranous, cytoplasmic, or nuclear brown staining. Membranous staining was evaluated as retained (positive) or lost (negative) staining. For cytoplasmic staining, any cytoplasmic expression regardless of the intensity considered positive. Nuclear β-catenin staining was either negative or positive staining [23]. PTEN immunohistochemistry was scored according to the intensity of cytoplasmic staining from 0-2, which corresponds to absent, weak and strong staining, respectively; (scores 0 was considered as a negative expression, while scores 1 and 2 considered positive) [24]. The nuclear staining was lost earlier than the cytoplasmic staining. Membranous staining of β-catenin and either nuclear and/or cytoplasmic staining of PTEN of normal follicular epithelial cells of thyroid gland were used as internal positive controls.

PCR technique

Five-µm-thick sections were cut from FFPE and pooled into a 1.5-mL tube. The sections were dewaxed, followed by extraction in 100% xylene and washing with 100% ethanol. Genomic DNA was derived from FFPE using Qiagen's QIAamp FFPE Mini Kit, according to the manufacturer's instructions. The extracted DNA was quantified using a fluorometric method based on binding of double-stranded DNA (dsDNA)-selective fluorescent dyes (dsDNA) (Qubit 3.0 Fluorometer/Life Technologies, Invitrogen). The DNA integrity was assessed using 2% agarose gel electrophoresis and DNA concentration was adjusted to 1-10 ng/µl for PCR reaction. Detection of KRAS-BRAF mutation was conducted by using KRAS-BRAF Strip Assay; Vienna Lab Diagnostic GmbH; based on polymerase chain reaction (PCR) and reverse hybridization. PCR amplification was done in a 25 µL reaction using KRAS & BRAF specific biotinylated primers and following the manufacturer's instructions. Biotinylated amplification products were then hybridized to nitrocellulose test strips containing allele specific oligonucleotide probes immobilized as an array of parallel lines. Bound sequences were visualized using a streptavidin-alkaline phosphatase conjugate and color substrates.

Interpretation of results

For RH, oligonucleotides were synthesized as probes targeting 10 mutations in codons 12 and 13 of the KRAS gene, namely Val12 (GGT→GTT), Asp12 (GGT→GAT), Leu12 (GGT→CTT), Ser12 (GGT→AGT), Ala12 (GGT→GCT), Ile12 (GGT→ATT), Cys12 (GGT→TGT), Arg12 (GGT→ CGT), Cys13 (GGC→TGC), and Asp13 (GGC→GAC). $BRAF^{V600E}$ (c.1799T>A).

Additionally, an oligonucleotide probe specific for the human HLA-DRA locus and a 5′-biotinylated oligonucleotide were used to control PCR performance and detection reagents, respectively. The genotype of a sample is determined using the enclosed Collector TM sheet.

- Upper control line: should permanently stain positive.
- KRAS (line1-10): one or more positive indicates Respective KRAS mutation present.
- BRAF (line11): positive indicates $BRAF^{V600E}$ mutation detection.
- (KRAS & BRAF) PCR negative control (line 12 & 13 respectively): should be negative as they indicate complete suppression of wild-type KRAS and BRAF amplification. If a PCR Negative Control is positive (e.g., due to an excess of DNA template used for PCR), the assay's sensitivity may be impaired.
- PCR positive control (line 14): should be positive, it indicates the presence and adequate quality of PCR components and DNA template. If it stains negative, repeat the analysis starting from DNA preparation. Fig. 3.

Statistical analysis

Statistical analysis was performed using SPSS version 22 (Chicago, IL, USA). Quantitative data were statistically reported as mean±SD and median (range). Qualitative data were statistically reported as frequencies (numbers) and relative frequencies (percentages) as appropriate. Qualitative variables were compared using chi square test, while Mann Whitney U test was applied for comparison of continuous variables. Kaplan-Meier's method using log rank test was employed for survival analysis. DFS was determined as the time between surgical therapy and the date of last follow-up or verified event (recurrent/persistent disease). Spearman rho test was used to determine the correlation between different variables. P-value is always 2 tailed set significant at 0.05 level.

Results:

Clinicopathological characteristics

This study included 52 patients with PTCs (38 females and 14 males) with a mean age of 42.07±14.17 years (range: 18-75). In total, 35 patients (67.3%) had a classic variant of PTC (CVPTC), 6 (11.5%) had FVPTC, 10 had papillary microcarcinoma (19.2%), and a solid variant was detected in 1 patient (2%). Eight patients (15.4%) had family history of PCT. According to ATA risk stratification, 13, 31, and 8 patients were categorized as low, intermediate, and high risk, respectively, Table 1. The mean duration of follow up was 29.87±15.96 months.

Association of β-catenin membranous expression with clinicopathological characteristics, ATA response category, and cumulative dose of RAI

A significant statistical association was noted between lost β-catenin membranous expression and N1b stage (P=0.011), LVI (P=0.041), and increased cumulative dose of RAI (P=0.034). Recurrent/persistent structural disease was more frequent among cases with PTCs not expressing membranous β-catenin, yet without statistical significance (P=0.085), Table 2.

Association of β-catenin cytoplasmic expression with clinicopathological characteristics, ATA response category, and cumulative dose of RAI

Positive β-catenin cytoplasmic expression was significantly associated with advanced pT stage, increased cumulative RAI dose, and persistent/recurrent structural disease (P=0.017, 0.045, and 0.007, respectively), Table 3.

Association of PTEN cytoplasmic expression with clinicopathological characteristics, ATA response category, cumulative dose of RAI, and ATA risk stratification

Positive PTEN cytoplasmic expression was significantly associated with favorable pathologic parameters in the form of the early pT stage (P=0.026) and absence of capsular infiltration (p=0.005). Alternatively, negative PTEN cytoplasmic expression was significantly associated with ETE (P=0.008),

distant metastasis (M1) (P=0.013) and advanced TNM staging (p=0.022). In addition, positive PTEN expression was highly associated with the low and intermediated ATA risk groups, while negative expression was highly linked to the high risk one with marginal statistical significance (p=0.054). There was no statistically significant difference in cumulative RAI doses between the negative-PTEN and positive-PTEN groups (p=0.933), Table 4.

DFS analysis for ATA risk stratification regarding to β-catenin and PTEN expression:

Among patients with retained membranous β-catenin expression, positive cytoplasmic β-catenin expression and negative PTEN expression; the high-risk group was significantly associated with poor DFS (P= 0.011, P=0.020, and P=0.007, respectively), Fig. 4.

$BRAF^{V600E}$ /KRAS mutational analysis

Due to financial constraints, only 20 (38.5%) of the original 52 patients underwent BRAF/KRAS mutational analysis. In 18 of them, the $BRAF^{V600E}$ mutation was identified. $BRAF^{V600E}$ expression had no significant relationship with any of the clinicopathological characteristics, ATA response type, or cumulative RAI dosage. On the other hand, none of these patients had a positive KRAS mutation. Table 5.

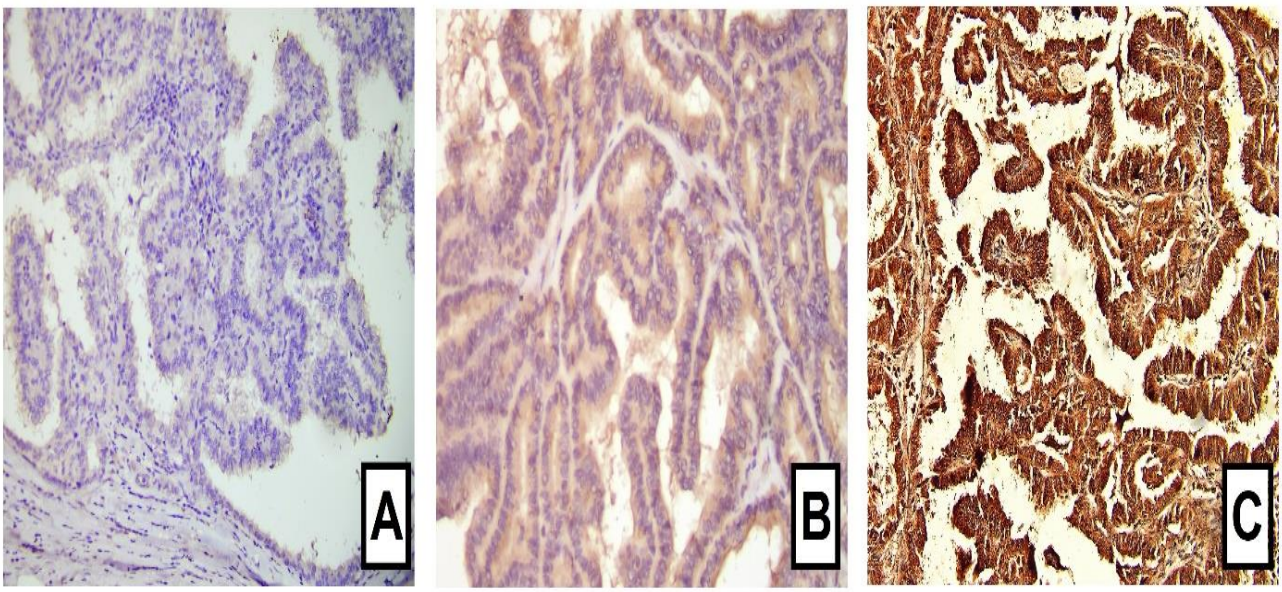

Fig. 1 PTEN expression in PTC

(A) CVPTC showed lost PTEN expression. (B) CVPTC showed weak positive cytoplasmic PTEN expression. (C) CVPTC showed strong positive cytoplasmic and retained nuclear PTEN expression.

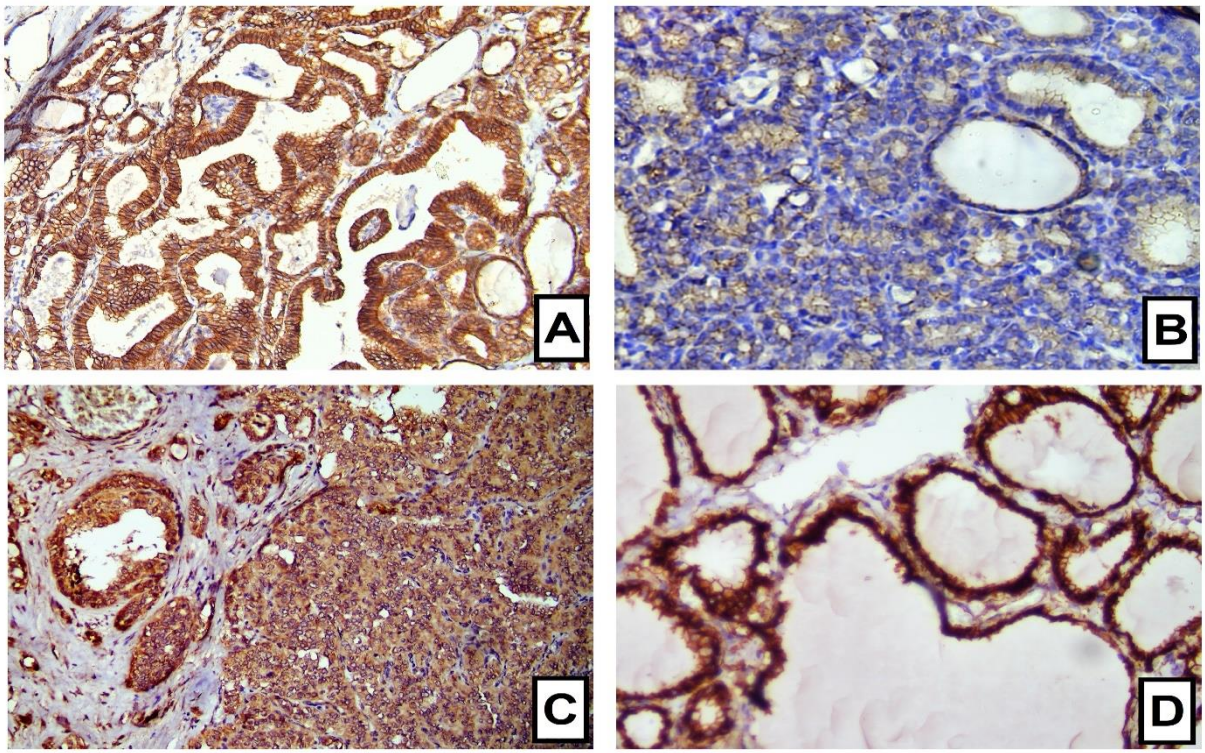

Fig. 2 β-catenin expression in PTC

(A) CVPTC showed retained β-catenin membranous expression. (B) FVPTC showed lost β-catenin membranous expression. (C) Solid variant of PTC showed lost β-catenin membranous expression and positive β-catenin cytoplasmic expression. (D) FVPTC showed positive cytoplasmic and aberrant nuclear β-catenin expression.

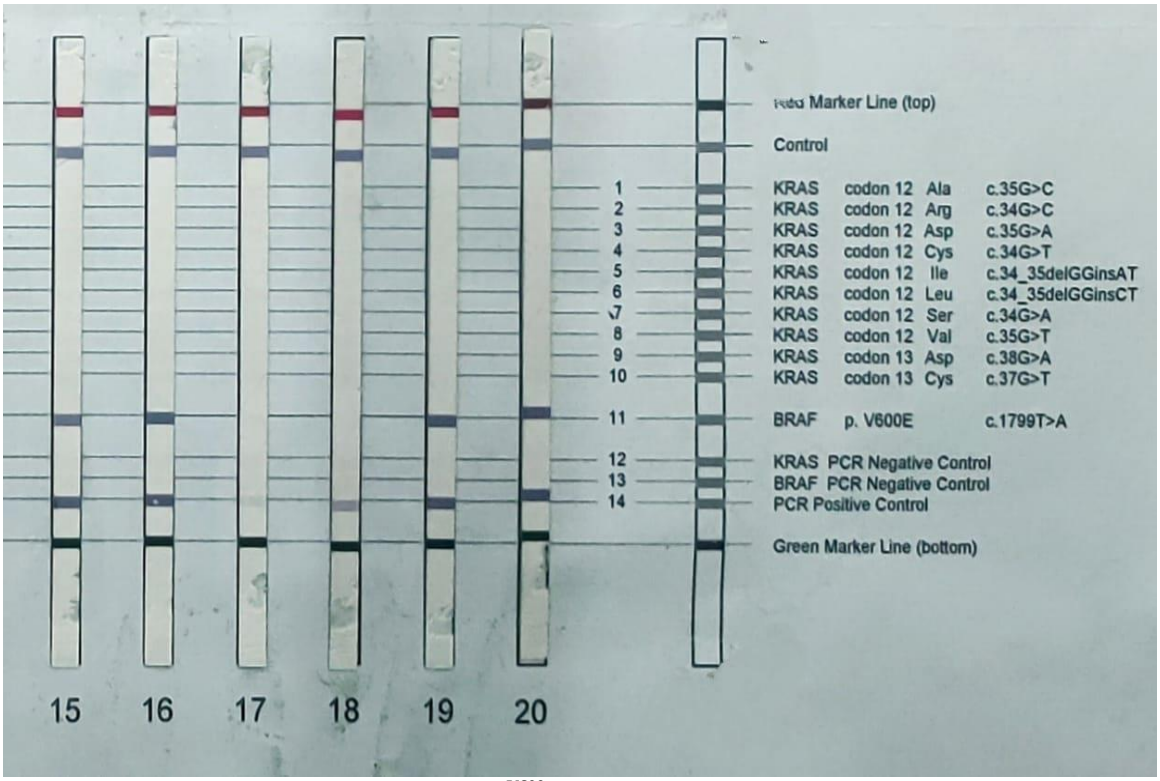

Fig. 3 BRAF^{V600} and KRAS mutations

Collector sheet for test strip of KRAS-BRAF strip assay shows to the right a demonstrative strip; top red marker line, control line for validation of the test result, line 1 to 10 for detection of KRAS codon 12 & 13 mutation, line 11 for BRAF^{V600} mutation, line 12 for KRAS negative control, line 13 for BRAF negative control and line 14 for PCR positive control for validation of the test result and bottom green marker line. First 2 Strips to the left (case 15& 16) are positive for BRAF^{V600E} (c.1799T>A), (case 17 & 18) are negative for both KRAS & BRAF mutations, (case 19 & 20) are positive for BRAF^{V600E} (c.1799T>A)

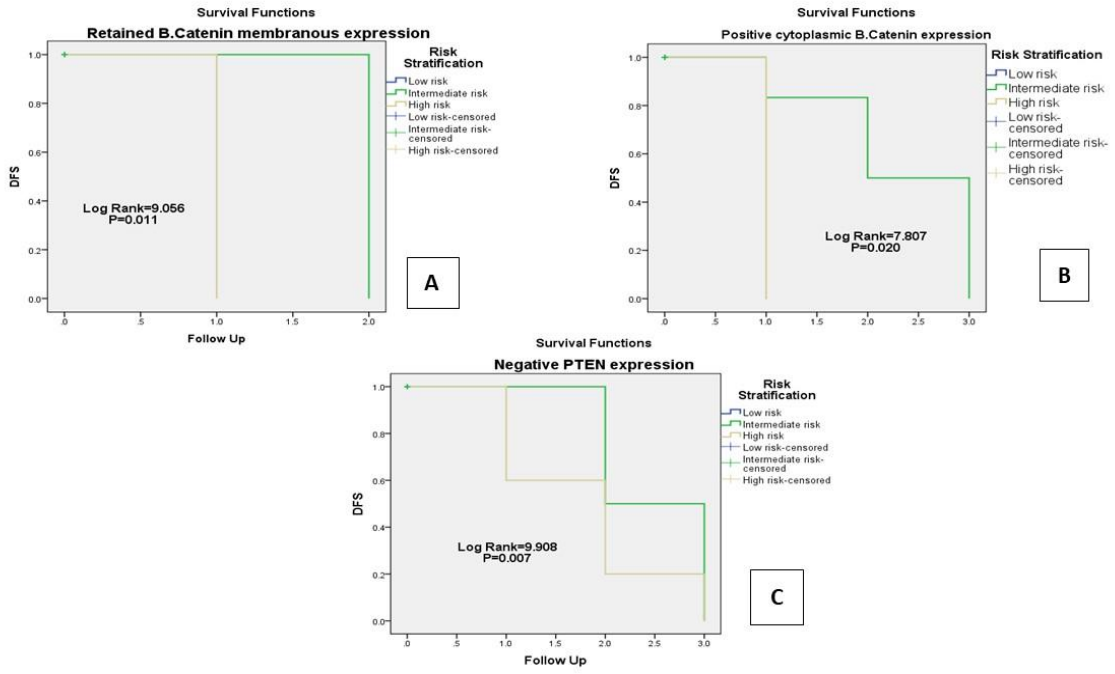

Fig. 4 DFS analysis for ATA risk stratification regarding β-catenin and PTEN expression

Table 1 Clinicopathological characteristics of studied patients

Clinicopathological characteristics	N (%)
Age (years)	
• Mean ± SD (range)	42.07 ± 14.17(18 – 75)
• < 55 years	43 (82.7)
• ≥ 55 years	9 (17.3)
Gender	
• Male	14 (26.9)
• Female	38 (73.1)
Familial/ sporadic	
• Familial	8 (15.4)
• Sporadic	44 (84.6)
Primary tumor size (cm)	
• ≤ 1 cm	11 (21.2)
• > 1 cm	41 (78.8)
Variant	
• Classic	35 (67.3)
• Follicular	6 (11.5)
• Solid	1 (1.9)
• Microcarcinoma	10 (19.2)
Laterality	
• Unilateral	36 (69.2)
• Bilateral	16 (30.8)
Focality	
• Unifocal	27 (51.9)
• Multifocal	25 (48.1)
T stage	
• pT1a	10 (19.2)
• pT1b	12 (23.1)
• pT2	17 (32.7)
• PT3a	8 (15.4)
• PT3b	2 (3.8)
• PT4a	3 (5.8)
N stage	
• Nx	10 (19.2)
• N0	6 (11.5)
• N1a	7 (13.5)
• N1b	29 (55.8)
M stage	
• Mx	6 (11.5)
• M0	44 (84.6)
• M1	2 (3.8)
Thyroid Capsule	
• Intact	36 (69.2)
• Infiltrated	16 (30.8)
Extra-thyroid Extension	
• Absent	43 (82.7)
• Present	9 (17.3)
Lymphovascular Invasion	
• Absent	14 (26.9)
• Present	38 (73.1)
TNM staging	
• Stage I	44 (84.7)
• Stage II	4 (7.7)
• Stage III	2 (3.8)
• stage IVB	2 (3.8)
ATA risk stratification	
• Low risk	13 (25.0)
• Intermediate risk	31 (59.6)
• High risk	8 (15.4)
RAI cumulative dose: Median (Range)	100 (30 – 800)
ATA response	
• No evidence of disease	30 (57.7)
• Persistent/ recurrent structural disease	14 (62.9)
• Persistent /recurrent biochemical disease	8 (15.4)

SD: Standard deviation, ATA: American Thyroid Association, RAI: Radioactive iodine

Table 2 Association of β-catenin membranous expression with clinicopathological characteristics, ATA response category, and cumulative dose of RAI

	β-Catenin membranous expression				
Clinicopathological characteristics	**Retained (n=15)**		**Lost (n=37)**		**P value**
Age (years)					0.335
• Mean ± SD	38.93 ± 10.12		42.84 ± 14.84		
• < 55 years	13	(30.2)	30	(69.8)	1
• ≥ 55 years	2	(22.2)	7	(77.8)	
Gender					1
• Male	4	(28.6)	10	(71.4)	
• Female	11	(28.9)	27	(71.1)	
Familial/ sporadic					**0.005***
• Familial	6	(75)	2	(25)	
• Sporadic	9	(20.5)	35	(79.5)	
Primary tumor size (cm)					0.477
• ≤ 1 cm	2	(18.2)	9	(81.8)	
• > 1 cm	13	(31.7)	28	(68.3)	
Variant					0.132
• Classic	12	(34.3)	23	(65.7)	
• Follicular	0	(0.0)	6	(100.0)	
• Solid	1	(100.0)	0	(0.0)	
• Microcarcinoma	2	(20.0)	8	(80.0)	
Focality					0.273
• Unifocal	6	(22.2)	21	(77.8)	
• Multifocal	9	(36.0)	16	(64.0)	
T stage					0.826
• pT1a	2	(20.0)	8	(80.0)	
• pT1b	4	(33.3)	8	(66.7)	
• pT2	6	(35.3)	11	(64.7)	
• PT3a	3	(37.5)	5	(62.5)	
• PT3b	0	(0.0)	2	(100.0)	
• PT4a	0	(0.0)	3	(100.0)	
N stage					**0.011***
• Nx	0	(0.0)	10	(100.0)	
• N0	2	(33.3)	4	(66.7)	
• N1a	5	(71.4)	2	(28.6)	
• N1b	8	(27.6)	21	(72.4)	
M stage					0.826
• Mx	1	(16.7)	5	(83.3)	
• M0	14	(31.8)	30	(68.2)	
• M1	0	(0.0)	2	(100.0)	
Thyroid capsule					1
• Intact	10	(27.8)	26	(72.2)	
• Infiltrated	5	(31.2)	11	(68.8)	
Extra-thyroid Extension					0.706
• Absent	12	(27.9)	31	(72.1)	
• Present	3	(33.3)	6	(66.7)	
Lymphovascular invasion					**0.041***
• Absent	1	(7.1)	13	(92.9)	
• Present	14	(36.8)	24	(63.2)	
TNM staging					1
• Stage I	14	(31.8)	30	(68.2)	
• Stage II	1	(25.0)	3	(75.0)	
• Stage III	0	(0.0)	2	(100.0)	
• stage IVB	0	(0.0)	2	(100.0)	
RAI cumulative dose: Median (Range)	30 (30 – 450)		100 (30 – 800)		**0.034***
ATA response category					0.085
• No evidence of disease	12	(40.0)	18	(60.0)	
• Persistent/ recurrent structural disease	1	(7.1)	13	(92.9)	
• Persistent /recurrent biochemical disease	2	(25.0)	6	(75.0)	

SD: Standard deviation, ATA: American Thyroid Association, RAI: Radioactive iodine

Table 3 Association of β-catenin cytoplasmic expression with clinicopathological characteristics, ATA response category, and cumulative dose of RAI

	β-catenin C				
Clinicopathological characteristics	**Negative (n=22)**		**Positive (n=30)**		**P value**
Age (years)					0.084
• Mean ± SD	38.05 ± 9.84		44.04 ± 14.63		
• < 55 years	21	48.8)	22	51.2)	0.062
• ≥ 55 years	1	11.1)	8	88.9)	
Gender					0.559
• Male	5	(35.7)	9	(64.3)	
• Female	17	(44.7)	21	(55.3)	
Familial/ sporadic					**0.014***
• Familial	6	(75.0)	2	(25.0)	
• Sporadic	16	(36.3)	28	(63.6)	
Primary tumor size (cm)					0.169
• ≤ 1 cm	7	(63.6)	4	(36.4)	
• > 1 cm	15	(36.6)	26	(63.4)	
Variant					0.308
• Classic	15	(42.9)	20	(57.1)	
• Follicular	1	(16.7)	5	(83.3)	
• Solid	0	(0.0)	1	(100.0)	
• Microcarcinoma	6	(60.0)	4	(40.0)	
Focality					0.812
• Unifocal	11	(40.7)	16	(59.3)	
• Multifocal	11	(44.0)	14	(56.0)	
T stage					**0.017***
• pT1a	6	(60.0)	4	(40.0)	
• pT1b	9	(75.0)	3	(25.0)	
• pT2	5	(29.4)	12	(70.6)	
• PT3a	1	(12.5)	7	(87.5)	
• PT3b	1	(50.0)	1	(50.0)	
• PT4a	0	(0.0)	3	(100.0)	
N stage					0.719
• Nx	3	(30.0)	7	(70.0)	
• N0	3	(50.0)	3	(50.0)	
• N1a	4	(57.1)	3	(42.9)	
• N1b	12	(41.4)	17	(58.6)	
M stage					0.171
• Mx	1	(16.7)	5	(83.3)	
• M0	21	(47.7)	23	(52.3)	
• M1	0	(0.0)	2	(100.0)	
Thyroid capsule					0.282
• Intact	17	(47.2)	19	(52.8)	
• Infiltrated	5	(31.2)	11	(68.8)	
Extra-thyroid Extension					0.468
• Absent	17	(39.5)	26	(60.5)	
• Present	5	(55.6)	4	(44.4)	
Lymphovascular invasion					0.431
• Absent	7	(50.0)	7	(50.0)	
• Present	15	(39.5)	23	(60.5)	
TNM staging					0.334
• Stage I	21	(47.7)	23	(52.3)	
• Stage II	1	(25.0)	3	(75.0)	
• Stage III	0	(0.0)	2	(100.0)	
• stage IVB	0	(0.0)	2	(100.0)	
RAI cumulative dose: Mean (Range)	100 (30 – 420)		150 (30 – 800)		**0.045***
ATA response category					**0.007***
• No evidence of disease	16	(53.3)	14	(46.7)	
• Persistent/ recurrent structural disease	2	(14.3)	12	(85.7)	
• Persistent /recurrent biochemical disease	6	(75.0)	2	(25.0)	

SD: Standard deviation, ATA: American Thyroid Association, RAI: Radioactive iodine

Table 4 Association of PTEN cytoplasmic expression with clinicopathological characteristics, ATA risk stratification, and cumulative dose of RAI

Clinicopathological characteristics	PTEN C				P value
	Negative (n=14)		Positive(n=38)		
Age (years)					0.287
• Mean ± SD	44.93 ± 15.55		40.53 ± 12.09		
• < 55 years	10	(23.3)	33	(76.7)	0.229
• ≥ 55 years	4	(44.4)	5	(55.6)	
Gender					0.182
• Male	6	(42.9)	8	(57.1)	
• Female	8	(21.1)	30	(78.9)	
Familial/sporadic					**0.025***
• Familial	5	(62.5)	3	(37.5)	
• Sporadic	9	(20.5)	35	(79.5)	
Primary tumor size (cm)					0.251
• ≤ 1 cm	1	(9.1)	10	(90.9)	
• > 1 cm	13	(31.7)	28	(68.3)	
Variant					0.064
• Classic	11	(31.4)	24	(68.6)	
• Follicular	3	(50.0)	3	(50.0)	
• Solid	0	(0.0)	1	(100.0)	
• Microcarcinoma	0	(0.0)	10	(100.0)	
Focality					0.866
• Unifocal	7	(25.9)	20	(74.1)	
• Multifocal	7	(28.0)	18	(72.0)	
T stage					**0.025***
• pT1a	0	(0.0)	10	(100.0)	
• pT1b	3	(25.0)	9	(75.0)	
• pT2	4	(23.5)	13	(76.5)	
• PT3a	3	(37.5)	5	(62.5)	
• PT3b	2	(100.0)	0	(0.0)	
• PT4a	2	(66.7)	1	(33.3)	
N stage					0.081
• Nx	0	(0.0)	10	(100.0)	
• N0	2	(33.3)	4	(66.7)	
• N1a	1	(14.3)	6	(85.7)	
• N1b	11	(37.9)	18	(62.1)	
M stage					**0.013***
• Mx	3	(50.0)	3	(50.0)	
• M0	9	(20.5)	35	(79.5)	
• M1	2	(100.0)	0	(0.0)	
Thyroid capsule					**0.005***
• Intact	5	(13.9)	31	(86.1)	
• Infiltrated	9	(56.3)	7	(43.8)	
Extra-thyroid Extension					**0.008***
• Absent	8	(18.6)	35	(81.4)	
• Present	6	(66.7)	3	(33.3)	
Lymphovascular invasion					0.297
• Absent	2	(14.3)	12	(85.7)	
• Present	12	(31.6)	26	(68.4)	
TNM staging					**0.022***
• Stage I	9	(20.5)	35	(79.5)	
• Stage II	2	(50.0)	2	(50.0)	
• Stage III	1	(50.0)	1	(50.0)	
• stage IVB	2	(100.0)	0	(0.0)	
ATA risk stratification					0.054
• Low risk	2	(15.4)	11	(84.6)	
• Intermediate risk	7	(22.6)	24	(77.4)	
• High risk	5	(62.5)	3	(37.5)	
RAI cumulative dose: Mean (Range)	110 (30 – 800)		100 (30 – 625)		0.933
ATA response category					0.693
• No evidence of disease	7	(23.3)	23	(76.7)	
• Persistent /recurrent structural disease	4	(28.6)	10	(71.4)	
• Persistent /recurrent biochemical disease	3	(37.5)	5	(62.5)	

SD: Standard deviation, ATA: American Thyroid Association, RAI: Radioactive iodine

Table 5 Association of BRAF^{V600E} expression with clinicopathological characteristics, ATA risk stratification, and cumulative dose of RAI

	BRAF^{V600E}				
Clinicopathological characteristics	**Negative (n=2)**		**Positive (n=18)**		**P value**
Age (years)					0.316
• Mean ± SD	32.50 ± 0.71		43.56 ± 14.29		
• < 55 years	2	(12.5)	14	(87.5)	1
• ≥ 55 years	0	0.0)	4	(100.0)	
Gender					0.521
• Male	0	(0.0)	7	(100.0)	
• Female	2	(15.4)	11	(84.6)	
Familial/sporadic					0.521
• Familial	0	(0.0)	7	(100)	
• Sporadic	2	(15.4)	11	(84.6)	
Primary tumor size (cm)					1
• ≤ 1 cm	0	(0.0)	4	(100)	
• > 1 cm	2	(12.5)	14	(87.5)	
Variant					1
• Classic	2	(12.5)	14	(87.5)	
• Follicular	0	(0.0)	1	(100.0)	
• Microcarcinoma	0	(0.0)	3	(100.0)	
Focality					1
• Unifocal	1	(12.5)	7	(87.5)	
• Multifocal	1	(8.3)	11	(91.7)	
T stage					0.368
• pT1a	0	(0.0)	3	(100.0)	
• pT1b	2	(40.0)	3	(60.0)	
• pT2	0	(0.0)	7	(100.0)	
• PT3a	0	(0.0)	2	(100.0)	
• PT3b	0	(0.0)	2	(100.0)	
• PT4a	0	(0.0)	1	(100.0)	
N stage					0.711
• Nx	0	(0.0)	1	(100.0)	
• N0	0	(0.0)	4	(100.0)	
• N1a	1	(25.0)	3	(75.0)	
• N1b	1	(9.1)	10	(90.9)	
M stage					**1**
• Mx	0	(0.0)	3	(100.0)	
• M0	2	(11.8)	15	(88.2)	
• M1	0	(0.0)	0	(0.0)	
Thyroid capsule					0.495
• Intact	2	(16.7)	10	(83.3)	
• Infiltrated	0	(0.0)	8	(100.0)	
Extra-thyroid Extension					1
• Absent	2	(14.3)	12	(85.7)	
• Present	0	(0.0)	6	(100.0)	
Lymphovascular invasion					1
• Absent	0	(0.0)	4	(100.0)	
• Present	2	(12.5)	14	(87.5)	
TNM staging					1
• Stage I	2	(12.5)	14	(87.5)	
• Stage II	0	(0.0)	2	(100.0)	
• Stage III	0	(0.0)	1	(00.0)	
• stage IVB	0	(0.0)	1	(100.0)	
ATA risk stratification					0.521
• Low risk	0	(0.0)	4	(100.0)	
• Intermediate risk	2	(33.3)	11	(66.7)	
• High risk	0	(0.0)	3	(100.0)	
RAI cumulative dose: Median (Range)	150 (150 – 150)		55 (30 – 450)		0.126
ATA response category					0.521
• No evidence of disease	1	(7.1)	13	(92.9)	
• Persistent /recurrent structural disease	1	(50.0)	2	(11.2)	
• Persistent /recurrent biochemical disease	0	(0.0)	3	(16.7)	

SD: Standard deviation, ATA: American Thyroid Association, RAI: Radioactive iodine

Discussion:

Further knowledge of the molecular features of PTC is mandatory to identify prognostic and predictive biomarkers of the therapeutic response to RAI.

We investigated the relationship between PTEN/β-catenin expression, BRAF/KRAS expression and pathological parameters as well as response to RAIT of the 52 cases and a subgroup of patients (n=20) with PTC, respectively.

Reduced membranous expression of β-catenin is associated with a lack of cell adhesion and cohesion, allowing tumor cells to be detached from the primary site and infiltrate surrounding tissue, spread to lymph nodes, or even distant organs [25].

In the current research, we documented that loss of β-catenin membranous expression and its cytoplasmic accumulation was significantly associated with nodal metastasis, LVI, persistent/recurrent disease, and increased cumulative doses of RAI. These finding are in line with those of Ziari et al. [23] and Kordestani et al. [26].

Lan et al., reported that β-catenin nuclear translocation reduced the efficacy of RAIT in TC via impairment of membranous expression of sodium iodine symporter (NIS); a transmembrane glycoprotein responsible for transporting iodide into thyroid follicular cells [27].

According to their explanation, we assume that lost membranous expression of β-catenin and its cytoplasmic overexpression affects NIS subcellular localization, resulting in an inadequate therapeutic response to the initial doses of RAI and thereby increasing its cumulative doses.

Conversely, Ishigaki et al. did not observe any significant correlation between increased cytoplasmic β-catenin expression and various pathologic parameters or recurrence. They found an overexpression of cyclin D1, a cell cycle modulatory gene that acts as a downstream molecule of β-catenin, in the majority of membranous β-catenin expressing PTCs. This denoting that not only triggered Wnt/β-catenin pathway but also stimulated non-Wnt/ β-catenin signaling pathways, such as the Ras-MAPK pathway can precipitate cyclin D1 overexpression, and that cytoplasmic β-catenin accumulation had a lower significant prognostic value than cyclin D1 overexpression in PTCs [28].

The present study revealed loss of nuclear and cytoplasmic PTEN expression in 48 and 14 cases, respectively. This is consistent with a prior study that noticed the reduction in PTEN nuclear staining intensity in TCs typically outweighed the decrease in cytoplasmic staining intensity [29].

In the current work, we found that negative PTEN expression was significantly associated with ETE, M1, and advanced TNM staging. Preserved PTEN expression, on the other hand, was substantially related to the early pT stage and M0. In accordance, Min et al. showed that PTCs with lost PTEN expression are frequently associated with regional LNM and more ETE [24]. Similarly, Zhong and Zhang reported that PTEN expression was considerably downregulated in PTCs with LNM compared to those without nodal metastasis [30].

On the contrary, in a study discussing PTEN expression in FVPTC, demonstrated a significant relationship between lost PTEN expression and lack of ETE [31], the higher frequency of PTEN loss among patients with FVPTC together with the significant association between this variant of PTC and the absence of ETE could be the interpretation for their contradicting findings.

Interestingly, we noted that 62.5% of familial PTCs had negative PTEN expression, indicating that reduction of PTEN expression may play a role in the pathogenesis of familial PTC independent of Cowden disease association. This finding corroborated earlier studies that reported a low prevalence of PTEN-inactivating mutations in sporadic PTC [32, 33].

The present study demonstrated that the mutant $BRAF^{V600E}$ was more prevalent in patients with CVPTC (14/16; 87.5%), whereas the KRAS mutation was absent in all of the studied cases, which could be attributed to the lack of FVPTC in the cases that underwent KRAS mutational analysis. These findings support the results of a previous study, which revealed a predominance of BRAF mutations in patients with CVPTC (70%), whereas 96% of RAS-positive PTCs were of the follicular variant [34].

We found no significant association between the BRAF mutation and aggressive pathological features, which is comparable to the results of a large recent study that investigated the role of $BRAF^{V600E}$ as a predictor of the RAIT effect in PTC patients. However, in contrast to our findings, they hypothesized that a positive BRAF mutation would result in lower RAIT effectiveness [35]. These conflicting results may be explained in part by the small size of the subgroup evaluated with this pathologic marker in our study.

In the other part, considering that all of the patients in our research with positive BRAF did not have distant metastases, we may infer that mutant BRAF had no influence on the clinical outcome of RAIT in PTC patients without known distant metastases, as previously stated in the literature [36].

We noticed that the high- ATA risk group was substantially associated with poor DFS among patients with preserved membranous β-catenin, positive cytoplasmic β-catenin expression, as well as negative PTEN expression. So yet, no published articles have either supported or denied this finding. Given that high-ATA risk PTC is tightly associated with aggressive pathologic criteria, determining the proportion of risk attributable to the expression of the investigated molecular markers versus that attributable to the other clinicopathologic characteristics is difficult.

Conclusion:

In conclusion, our data show that PTCs lacking membranous β-catenin and expressing cytoplasmic β-catenin are substantially associated with aggressive

pathology, greater cumulative doses of RAI, and disease recurrence/persistence. Negative PTEN expression is strongly linked to advanced pathological features. Furthermore, our findings suggest that in PTC patients with no known distant metastases, a positive BRAF mutation has no effect on RAIT effectiveness.

LIST OF ABBREVIATION

TC	Thyroid carcinoma
PTC	Papillary thyroid carcinoma
RAI	Radioactive iodine
RAIT	Radioactive iodine therapy
RAS	Rat sarcoma viral oncogene homolog
TSH	Thyroid-stimulating hormone
PDTC	Poorly differentiated TC
ATC	Anaplastic TC
PTEN	Phosphatase and tensin homolog gene
FTC	Follicular thyroid carcinoma
FVPTC	Follicular variant of PTC
ATA	American Thyroid Association
FFPE	Formalin-fixed paraffin-embedded
NU	Neck ultrasound
Tg	Serum thyroglobulin
TgAbs	Anti-Tg antibodies
WBS	Whole body scintigraphy
NED	No evidence of disease
ETE	Extrathyroidal extension
LVI	Lymphovascular invasion
LNM	Lymph node metastasis
CVPTC	Classic variant of PTC

AUTHOR CONTRIBUTIONS

- Conceptualization: NMM, MTH, HB, KR, NG
- Data curation: NMM, MTH, HB, KR
- Formal analysis: MTH, NMM, BR
- Methodology: MTH, HB, EH, KR, NMM, NG
- Resources: MTH, HB, KR, WA, NMM
- Writing – original draft: NMM, MTH
- Writing – review & editing: NMM, MTH
- Approval of final manuscript: all authors

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This cohort study was approved by the IRB of the faculty of Medicine, Assiut University Ethical Committee (04-2023-300280). All methods were performed in accordance with the relevant guidelines and regulations.

COMPETING INTEREST:

The authors declare that they have no competing interest.

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