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Original Contribution

Role of P53, E-cadherin and BRAF as predictors of regional nodal recurrence for papillary thyroid cancer

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ABSTRACT

Background: Regional nodal recurrence (RNR) in patients diagnosed with papillary thyroid carcinoma (PTC) has increased. Variable immunohistochemical (IHC) markers have been studied for predicting the likelihood of PTC for recurrence. We aimed to clarify the IHC expression of p53, Ecadherin and BRAF as potential markers of RNR in PTC.

Method: 145 (73 study group and 72 control group) patients with PTC were analyzed retrospectively between January 2010 and June 2017. Further classification to a specific histological variant was done, and IHC expression of p53, Ecadherin and BRAF was analyzed both in the primary tumor and in nodal recurrence.

Results: Regarding the risk of RNR, we found certain clinicopathologic features as elder age ≥55 years, tumor size > 1 cm, presence of microscopic extrathyroid extension, presence of lymphovascular emboli, and conventional papillary subtype. Furthermore, IHC results for negative E-cadherin, and positive P53 and BRAF are significant risk factors, while radioactive iodine (RAI) adjuvant therapy decrease recurrence risk.

Conclusion: We found several risk factors for RNR in PTC diagnosed patients, all of which are easily achievable in clinical settings. In this regard, we suggested that patients with specific clinicopathologic and immunohistochemical features have strict follow up for early detection of RNR as it has a great impact on their survival.

1. Introduction

Papillary thyroid carcinoma (PTC) is a well-differentiated thyroid carcinoma that originates from the thyroid follicular cells and accounts for 70–80% of all thyroid cancer [1]. The diagnosis of PTC is made mainly by the presence of certain nuclear features as nuclear grooves, clearing, overlapping, and pseudo-inclusions in addition to papillary architectural, but the latter is not necessary for the diagnosis [2].

Despite that PTC is of excellent prognosis with a 10-year survival rate of over 90%, its clinical behaviors are variable and complex. PTC spreads through lymphatics easily, that leads to tumor recurrence, distant metastases, and even death [3].

Recurrent PTC is variably interpreted either as primary tumor recurrence, lymph node metastases, invasion of the surrounding structures as esophagus and trachea, or distant metastases [4]. Many factors as age, histologic subtype, staging, presence of extrathyroid invasion, and lymph node metastases, in addition to the primary surgery approach are related to PTC recurrence, but final confirmation has not yet been done [5].

Many immunohistochemical (IHC) markers have been used for predicting prognosis in PTC. E-cadherin is an intercellular adhesion molecule that is completely expressed on the surface of the normal follicular cell's surface [6]. P53 is a well known tumor suppressor gene that controls different steps in cell cycle such as DNA repair, cell cycle arrest, differentiation, and apoptosis. P53 mutations could be seen in about 50% of the human cancers [7] in general and in 40–62% of undifferentiated thyroid carcinomas but in well-differentiated thyroid carcinomas, the mutations are only found in 0–25% [8].

BRAF is a member of the mitogen-activated protein kinase (MAPK) pathway that is associated with cell proliferation, cell differentiation,

Abbreviations: FV PTC, follicular variant papillary thyroid cancer; PMC, papillary microcarcinoma; PTC, papillary thyroid cancer; RAI, radioactive iodine; RNR, regional nodal recurrence

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Table 1
Immunohistochemical markers used in the study.

Antibody	Code	Clone	Antigen retrieval	Dilution	Source	Positive control
Anti-p53	60-0050-7	BP53-12	Sodium citrate buffer, pH 6	Ready to use	GENEMED	Normal colon
E-cadherin	IR059	NCH-38	EDTA, pH 8	Ready to use	DAKO	Normal breast
BRAF V600E	NB100-92145	VE1	EDTA, pH 8	1/100	NovusBio	Normal colon

and apoptosis [9]. The V600E mutation of the BRAF gene is a well known genetic event in PTC and thought to have a role in the development and progression of various malignancies. Various methods have been reported for BRAF mutation analysis with different accuracies, including polymerase chain reaction (PCR) and gene sequencing. However, these methods are somewhat complex and expensive. Furthermore; inadequate DNA preservation in formalin-fixed and paraffin-embedded tissues and the presence of non tumoral thyroid tissue may complicate the process of genetic mutation detection [10].

In contrast to current sequencing methods for molecular testing, IHC for BRAF is cheaper and has a rapid turn-around time. It is not dependent upon DNA quality or percentage of tumor cells in a sample and allows assessment of the mutant protein only in the tumor cells giving more confidence in results obtained. Furthermore, it is routinely available in most pathology laboratories [11].

In this study, we evaluated the immunohistochemical expression of E-cadherin, p53, and BRAF as prognostic indicators for regional nodal recurrence (RNR) of PTC both in the primary tumor and in nodal recurrence.

2. Materials & methods

2.1. Patients

We retrospectively reviewed medical records, archival slides and paraffin blocks of 338 patients referred to the oncology surgery ward of Oncology Centre Mansoura University (OCMU), Egypt, between January 2010 and June 2017, who underwent total thyroidectomy for PTC. The study fulfills ethics of Good Clinical Practice (GCP) guidelines, approved by Intuitional Research Board (IRB), Faculty of Medicine, Mansoura University.

Patients included in this study are those diagnosed with PTC, underwent total thyroidectomy at OCMU and continued for postoperative follow up for at least three years. Patients who were managed primarily in other institutions, as well as their pathological specimens, but were referred to our center for second expert opinion or lost follow up in the postoperative 3 year period were excluded. Only 145 PTC patients who met the inclusion criteria were classified into two groups; those initially neck negative and remained without evidence of recurrent disease for at least 3 years postoperative (control group, 72 cases) and those having nodal recurrence (study group, 73 cases).

2.2. Histopathological analysis

The formalin fixed paraffin blocks and H&E slides of 145 selected patients were retrieved from pathology archive and revised by two pathologists (Kh. A., D. A.) for histopathological criteria and divided according to the 2017 World Health Organization (WHO) classification into follicular variant (FV), conventional variant, tall cell variant and microcarcinoma variant [12]. All these variants were diagnosed when the specific components represented > 75% of the tumor, except for the follicular variant, that required exclusive follicular growth for diagnosis.

All patients were categorized based on TNM classification [13]. Extrathyroid extension is defined by the invasiveness of the primary tumor beyond the thyroid tissue into adjacent tissues and is classified into either macroscopic extrathyroid extension that is confirmed by the surgeon during thyroidectomy, or microscopic extrathyroid extension being ascertained through microscopic examination of biopsied tissue by the pathologist [14].

2.3. Immunohistochemistry and interpretation

Tissue microarrays (TMA) were constructed after selection of proper foci of tumors (TMA, Beecher Instruments, Sun Prairie, WI, USA). Every tumor was represented by three cores; distributed in blocks; 4 μm-thick sections were prepared for H&E. Other sections were prepared for immunohistochemistry.

Table 1 illustrates the primary antibodies used in the present study. Negative control for markers was prepared without addition of primary antibody. IHC staining for P53, E-cadherin and BRAF was done on specimens of the primary tumor and further on nodal recurrence specimens. P53 was scored as positive only if > 10% of tumor cells positively expressed moderate to strong nuclear reaction [15]. E-cadherin was considered positive if ≥10% of the tumor cells showed membranous staining either complete or incomplete [16]; and negative when none or < 10% of the tumor cells had membrane staining. BRAF V600E IHC using VE1 antibody was scored positive only when there was evident diffuse cytoplasmic staining in > 85% of tumor cells [11]. Focal or weak staining of single scattered cells was scored negative.

2.4. Statistical analysis

The data of these patients were analyzed and statistical values were obtained using SPSS version 22 (Inc., Chicago, IL). Continuous variables are presented as mean when symmetrical or median and range when asymmetrical. Categorical variables are presented as proportions. Bivariate analysis was done using Chi-Square test and Fisher's exact test (if cell count < 5) for nominal independents, while Mann-Whitney test and student *t*-test, one way Annova was used for continuous independents. Multivariate analysis was done using binary logistic regression. P value < 0.05 was considered significant.

3. Results

3.1. Clinicopathologic features

As regard WHO classification of PTC; this study included 106 cases of conventional PTC, 21 of FVPTC, 16 of microcarcinoma and 2 cases of tall cell variant (total = 145 cases). 145 PTC patients were divided in two groups; study group with RNR (73 patients) and control group (72 patients). The clinicopathological data is given in Table 2 with a mean follow up period 56 months.

Seventy three RNR patients were investigated as study group. Median time between diagnosis and nodal recurrence is 1 year, earliest 4 months and latest 9 years. Median number of resected LN is 12 (range 1–43) with median 4 positive nodes (range 1 to 34), and extranodal extension in 23 case (31.5%). Postoperative RNR was detected mainly in the first two years (54 patients, 74%) which decreased with increasing postoperative period from (> 2 − ≤5), and > 5 years (16 patients, 21.9% and 3 patients, 4.1%; respectively). Most of the patients (68.5%, 50/73) were in stage I, while distant metastasis (M1) was present in 3/145 cases (2%) (two classic type and one FV).

Histologically, the conventional type showed larger tumor size;

Table 2
Demographic, clinicopathological and immunohistochemical characteristics of control and study group.

Parameter	Study (n = 73)	Control (n = 72)	P-value
Age (mean and SD[a])	49.3 (SD = 17.4)	44.1 (SD = 12.8)	0.041
< 55 years	41	55	0.008
≥55 years	32	17	
Gender (male/female)	29/44	23/49	0.211
Tumor size (largest dimension in cm)			
≤1 cm	2	14	0.005
1–2 cm	47	41	
> 2 cm	24	17	
Bilaterality (absent/present)	43/30	49/23	0.166
Tumor type			0
Micropapillary/conventional/follicular/tall cell variant	2/64/5/2	14/42/16/0	0.00
Necrosis (absent/present)	48/25	57/15	0.052
Microscopic extrathyroid extension (absent/present)	48/25	67/5	0.00
Lymphovascular emboli (absent/present)	51/22	69/3	0.00
Adjacent thyroid (goitre/lymphocytic thyroiditis/Hashimoto's thyroiditis)	67/5/1	56/14/2	0.062
T stage (T1/T2/T3)	49/21/3	55/16/1	0.36
Metastasis (absent/present)	69/4	72/0	0.062
Stage (I/II/IVB)	50/20/3	71/1/0	0.00
Immunohistochemistry (on primary tumor)			
P53 (negative/positive)	22/51	33/39	0.038
E-cadherin (negative/incomplete positive/complete positive)	19/15/39	0/5/67	0.00
BRAF (negative/positive)	33/40	63/9	0.00
Adjuvant therapy (no/RAI/radiotherapy)	46/26/1	29/43/0	0.011
Combined BRAF & P53 positive (no/yes)	36/37	63/9	0.00
Triple print of BRAF & P53 positive and E-cadherin negative (no/yes)	55/18	72/0	0.00

[a] SD = standard deviation, n = number.

Table 3
Clinicopathologic and immunohistochemical characteristics of histological variants.

Parameter	Conventional 73.1% (n = 106)	FV 14.5% (n = 21)	PMC 11% (n = 16)	P value
Age				
< 55 years	69	13	13	0.4
≥55 years	37	8	3	
Sex (F:M)	65:41	15:6	12:4	0.43
Tumor size				
≤1 cm	0	0	16	0.00
1–2 cm	72	15	0	
> 2 cm	34	6	0	
Microscopic extrathyroid extension	29	0	0	0.002
LN metastasis	64	5	2	0.00
Adjacent thyroid				0.5
Goitre	92	17	12	
Lymphocytic thyroiditis	12	3	4	
Hashimoto thyroiditis	2	1	0	
Distant metastasis	2	1	0	0.58
P53 expression				0.001
Positive	72	13	3	
Negative	34	8	13	
E-cadherin				0.083
Complete	73	17	16	
Incomplete	17	3	0	
Negative	16	1	0	
BRAF				
Positive	42	5	0	0.005
Negative	64	16	16	

especially ≥2 cm (P = 0.00) and was the only one to show microscopic extrathyroidal extension (P = 0.002) and more frequent RNR (P = 0.00, Table 3).

3.2. Expression of immunohistochemical markers

Each of P53, and BRAF positive expression showed significant correlation both between study and control group (P = 0.038, P = 0.00 respectively, Table 2) and in between different histologic variants (P = 0.001, P = 0.005 respectively, Table 3). In addition, double print of combined positive P53 and BRAF had the same significant correlation between the study and control group (P = 0.00, Table 2).

E-cadherin positive reaction was found in 88% of cases either complete (106/143) or incomplete (20/143). None of the control group showed negative reaction for E-cadherin with 93% of them having complete positive reaction in contrast to only 54.9% of the study group. Despite the significant correlation between the study and control group (P = 0.00, Table 2), E-cadherin failed to show any significance between different histologic subtypes (P = 0.83, Table 3). Furthermore; the triple print of combined P53 and BRAF positivity in addition to E-cadherin negativity showed significant correlation between the study and control group (P = 0.00, Table 2).

Overall, PMC cases were completely positive for E-cadherin and negative BRAF while p53 was positive in minority of cases (3/13; 23%).

3.3. IHC discordance in recurrence

On examination of IHC discordance in recurrence (Table 4); 9/73 had discordant P53 (7initially being negative in primary tumor stain positive in nodal recurrence and 2 initially positive changed to

Table 4
Expression of immunohistochemical discordance in nodal recurrence (73 cases).

	Primary tumor	Recurrent node	P-value
P53 positive/negative	51/22	56/17	0.00
E-cadherin complete/incomplete/negative	39/15/19	59/0/14	0.00
BRAF positive/negative	40/33	48/25	0.00

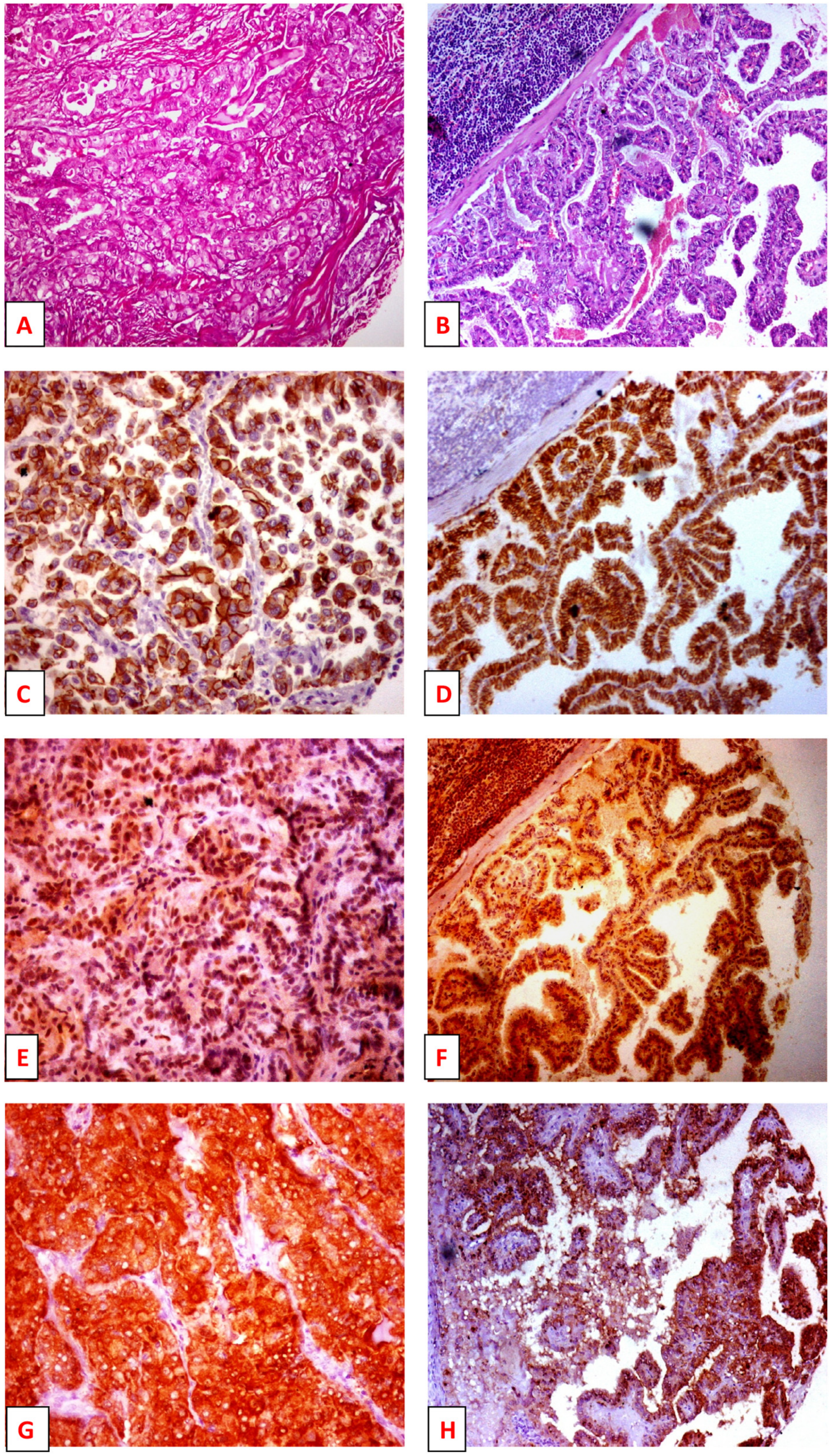

Fig. 1. Representative example of primary (A) and nodal recurrent (B) conventional PTC showing papillary structure with classic PTC nuclear features (H & E stain). IHC expression of E-cadherin (C) showing incomplete membranous expression that became complete membranous in nodal recurrence (D). P53 and, BRAF were positive both in primary and in nodal reoccurrence (from E to H) (all magnification ×10 except C, E, & G are ×20).

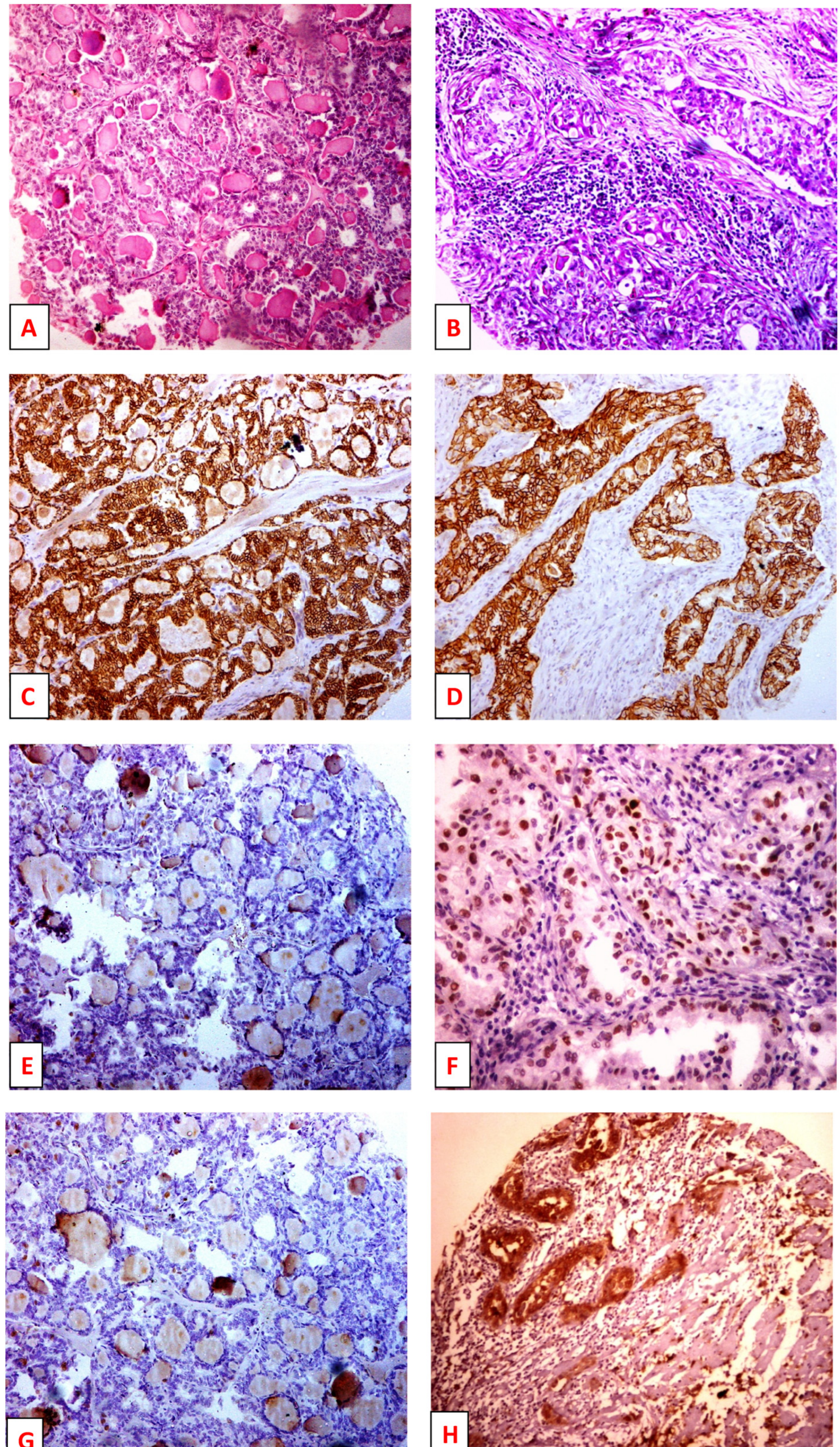

Fig. 2. Representative example of primary (A) and nodal recurrent (B) follicular variant PTC showing predominant follicular growth with PTC nuclear features (H & E stain). IHC expression of E-cadherin showing complete membranous expression in both primary (C) and nodal recurrence (D). P53 and BRAF changed from negative reaction in primary tumor (E and G respectively) to positive expression in nodal recurrence (F and H respectively) (all magnification ×10).

negative) (P = 0.00), 20/73 had discordant E-cadherin (5 initially negative become complete positive and 15 initially incomplete positive changed to complete positive) (P = 0.00), and 10/73 had discordant BRAF (9 initially negative become positive and 1 initially positive become negative) (P = 0.00, Figs. 1 & 2).

Table 5
Multivariate analysis of clinicopathologic and immunohistochemical factors.

Parameter	Hazard ratio (HR)	95% confidence interval (CI)	P-value
Tumor type (conventional)	8.45	1.35–52.79	0.022
Binary logistic regression excluding tumor type due to co-linearity (redundancy)			
Age (≥55)	0.67	0.22–2.06	0.49
Tumor size (> 1 cm)	5.12	1–26.19	0.05
Presence of microscopic extrathyroid extension	0.56	0.07–4.57	0.59
Presence of lymphovascular emboli	1.52	0.29–8.07	0.62
Advanced stage	20.17	1.89–213.5	0.013
Positive P53	0.71	0.27–1.89	0.5
Positive E-cadherin	0.0	0.0	0.45
Positive BRAF	2.31	0.53–10.04	0.26
Adjuvant therapy with RAI	0.35	0.15–0.84	0.018
Binary logistic regression model with double print			
BRAF & P53 positive	2.23	0.59–8.47	0.24
Binary logistic regression model with triple print			
BRAF, P53 positive and E-cadherin negative	α	0.00	0.998

3.4. Bivariate analysis of factors associated with PTC RNR

Bivariate analysis of factors associated with PTC RNR showed that elder age ≥55 years, tumor size > 1 cm, presence of microscopic extrathyroid extension and lymphovascular emboli, conventional PTC subtype and IHC signature of positive P53, negative E-cadherin and positive BRAF are associated with increased risk of recurrence while radioactive iodine (RAI) adjuvant therapy decrease recurrence risk.

3.5. Multivariate analysis of factors associated with PTC RNR

Multivariate analysis showed (Table 5) that conventional PTC (HR = 8.45), advanced stage (HR = 19.52) and tumor size (> 1 cm) (HR = 5.12) are associated with increased risk of RNR while RAI adjuvant therapy is protective (HR = 0.42). It is unfortunate that neither double or triple print (combined positive P53 and BRAF or and P53 and BRAF positive and E-cadherin negative respectively) showed any statistical significance on multivariate analysis.

4. Discussion

Papillary thyroid carcinoma is the most common thyroid malignancy and has an excellent prognosis. However, some studies have demonstrated the increased incidence of recurrent PTC [17]. Many factors can affect PTC recurrence, but final conclusions have not been reached [18]. Our research showed that age ≥55 years, tumor size > 1 cm, presence of microscopic extrathyroid extension, presence lymphovascular emboli, conventional papillary subtype and advanced stage are significant risk factors for RNR. Furthermore, IHC results of positive p53, and BRAF and negative E-cadherin are associated with increased risk of RNR while RAI adjuvant therapy decreases recurrence risk. On the other hand, factors such as gender, unilateral or bilateral lesions, tumor necrosis, and adjacent non tumoral thyroid tissue pathology are not correlated with tumor recurrence.

Although P53 mutation is well established in many human cancers, its prognostic significance in PTC is still controversial. Morita et al. stated a statistical significance in p53 protein expression between the primary tumor and clinicopathologic data such as large tumor size, positive lymph node for tumor metastasis, and the mean number of positive lymph nodes [19]. However, Hamzany et al. could not detect any significant correlation between p53 positivity and various factors as age, tumor size, extrathyroidal extension, vascular invasion, positive lymph node for tumor metastasis, and the mean number of positive lymph nodes [20]. Meanwhile, Balta et al. found in a statistical significance of p53 protein expression in PTC compared with control group (benign lesions) [7].

In our study, E-cadherin showed a statistical significance between study and control group, indicating that E-cadherin can be used as a prognostic marker in PTC. According to some authors [21], reducing the E-cadherin expression is associated with the ability of remote metastases, and local recurrence of thyroid carcinoma. We detected constant and complete membranous reaction of E-cadherin in PMCs. These results are to some extent in accordance with those of previous studies investigating E-cadherin expression in PTC [22].

The loss of E-cadherin expression was one of several steps suggesting epithelial to mesenchymal transition in PTC. It was also observed that E-cadherin was re-expressed in the metastatic cancer cells in the lymph nodes (discordance 19/71). This re-expression of E-cadherin is considered to represent a reversal of epithelial to mesenchymal transition in order to create a suitable environment for cancer cells to survive in the metastatic foci [16].

In the present study we investigated the detection of the BRAF V600E mutation in PTC using IHC with the anti-BRAF V600E (VE1) mouse monoclonal antibody. Similar to our results, Na Ji et al. demonstrated statistically significant correlations between BRAF V600E mutation ascertained by IHC and extrathyroidal extension and stage. They also studied the correlation of the mutation with multifocality, lymph node and distant metastases, PTC subtype, and tumor infiltration by lymphocytes [23].

Although Koperek et al. reported the definite diffuse cytoplasmic expression of the mutated BRAF V600E protein using IHC in 52.8% (76/144 cases) which was confirmed by sequencing analysis [10], Szymonek et al. stated the discordance between BRAF V600E detection by IHC and molecular methods and concluded that IHC cannot replace molecular analyses for identification of this mutation [24].

This study has limitations. Our study was performed retrospectively and not all patients had the same follow-up period. An extended follow-up period may be required for further verification of these selected IHC markers as prognostic predictors of RNR. In addition, the molecular verification of the BRAF mutation and its concordance with IHC was not done and since there is still controversy regarding the confidence in IHC regarding both false-positive and false-negative results; BRAF V600E detection by IHC cannot be used alone to support the utility of BRAF inhibitors, such as Vemurafenib, in the treatment of papillary thyroid carcinoma.

5. Conclusion

We found several risk factors for RNR in PTC diagnosed patients, all of which are easily achievable in clinical settings. In this regard, we suggested that patients with specific clinicopathologic and immunohistochemical features have strict follow up for early detection of RNR as it has a great impact on their survival.

Conflict of interest statement

The authors declare that they had no conflict of interest.

References

[1] DeLellis RA. Pathology and genetics of tumours of endocrine organs. IARC; 2004.
[2] Tan A, Etit D, Bayol U, Altinel D, Tan S. Comparison of proliferating cell nuclear antigen, thyroid transcription factor-1, Ki-67, p63, p53 and high–molecular weight cytokeratin expressions in papillary thyroid carcinoma, follicular carcinoma, and follicular adenoma. Ann Diagn Pathol 2011;15(2):108–16.
[3] Giordano D, Frasoldati A, Kasperbauer JL, Gabrielli E, Pernice C, Zini M, et al. Lateral neck recurrence from papillary thyroid carcinoma: predictive factors and prognostic significance. Laryngoscope 2015;125(9):2226–31.
[4] Teixeira G, Teixeira T, Gubert F, Chikota H, Tufano R. The incidence of central neck micrometastatic disease in patients with papillary thyroid cancer staged

preoperatively and intraoperatively as N0. Surgery 2011;150(6):1161–7.
[5] Zhu J, Wang X, Zhang X, Li P, Hou H. Clinicopathological features of recurrent papillary thyroid cancer. Diagn Pathol 2015;10(1):96.
[6] Radu TG, Mogoantă L, Busuioc CJ, Stănescu C, Grosu F. Histological and immunohistochemical aspects of papillary thyroid cancer. Romanian J Morphol Embryol 2015;56(2 Suppl):789–95.
[7] Balta A, Filiz A, Kurt Y, Sucullu I, Yucel E, Akin M. Prognostic value of oncoprotein expressions in thyroid papillary carcinoma. Med Oncol 2012;29(2):734–41.
[8] Parameswaran R, Brooks S, Sadler GP. Molecular pathogenesis of follicular cell derived thyroid cancers. Int J Surg 2010;8(3):186–93.
[9] Jung JW, Choi JY, Lee KE, Park KW. Immunohistochemical and molecular markers associated with differentiated thyroid carcinoma. J Kor Thyroid Assoc 2015;8(1):50–60.
[10] Koperek O, Kornauth C, Capper D, Berghoff AS, Asari R, Niederle B, et al. Immunohistochemical detection of the BRAF V600E-mutated protein in papillary thyroid carcinoma. Am J Surg Pathol 2012;36(6):844–50.
[11] Dvorak K, Aggeler B, Palting J, McKelvie P, Ruszkiewicz A, Waring P. Immunohistochemistry with the anti-BRAF V600E (VE1) antibody: impact of pre-analytical conditions and concordance with DNA sequencing in colorectal and papillary thyroid carcinoma. Pathology 2014;46(6):509–17.
[12] J. R, J.A. F, S. P, J. AS, K.O. F, M.L. P, et al. Papillary thyroid carcinoma. In: Lloyd R. V., Osamura R.Y., Kloppel G., J R, editors. WHO classification of tumors of endocrine organs (4th edition). Lyon, France: IARC; 2017. p. 81–91.
[13] Gospodarowicz MK, Brierley JD, Wittekind C. TNM classification of malignant tumours. John Wiley & Sons; 2017.
[14] Park JY, Yi JW, Park CH, Lim Y, Lee KH, Lee KE, et al. Role of BRAF and RAS mutations in extrathyroidal extension in papillary thyroid cancer. Cancer Genomics Proteomics 2016;13(2):171–81.
[15] Lee YM, Lee JB. Prognostic value of epidermal growth factor receptor, p53 and galectin-3 expression in papillary thyroid carcinoma. J Int Med Res 2013;41(3):825–34.
[16] Nakamura M, Onoda N, Noda S, Kashiwagi S, Aomatsu N, Kurata K, et al. E-cadherin expression and cell proliferation in the primary tumor and metastatic lymph nodes of papillary thyroid microcarcinoma. Mol Clin Oncol 2014;2(2):226–32.
[17] Lee CW, Roh J-L, Gong G, Cho K-J, Choi S-H, Nam SY, et al. Risk factors for recurrence of papillary thyroid carcinoma with clinically node-positive lateral neck. Ann Surg Oncol 2015;22(1):117–24.
[18] Ardito G, Revelli L, Giustozzi E, Salvatori M, Fadda G, Ardito F, et al. Aggressive papillary thyroid microcarcinoma: prognostic factors and therapeutic strategy. Clin Nucl Med 2013;38(1):25–8.
[19] Morita N, Ikeda Y, Takami H. Clinical significance of p53 protein expression in papillary thyroid carcinoma. World J Surg 2008;32(12):2617.
[20] Hamzany Y, Soudry E, Strenov Y, Lipschitz N, Segal K, Hadar T, et al. Early death from papillary thyroid carcinoma. Am J Otolaryngol 2012;33(1):104–8.
[21] Ceyran AB, Şenol S, Şimşek BÇ, Sağıroğlu J, Aydın A. Role of cd56 and e-cadherin expression in the differential diagnosis of papillary thyroid carcinoma and suspected follicular-patterned lesions of the thyroid: the prognostic importance of e-cadherin. Int J Clin Exp Pathol 2015;8(4):3670.
[22] Batistatou A, Charalabopoulos K, Nakanishi Y, Vagianos C, Hirohashi S, Agnantis NJ, et al. Differential expression of dysadherin in papillary thyroid carcinoma and microcarcinoma: correlation with E-cadherin. Endocr Pathol 2008;19(3):197.
[23] Na J-I, Kim J-H, Kim H-J, Kim H-K, Moon K-S, Lee J-S, et al. VE1 immunohistochemical detection of the BRAF V600E mutation in thyroid carcinoma: a review of its usefulness and limitations. Virchows Arch 2015;467(2):155–68.
[24] Szymonek M, Kowalik A, Kopczyński J, Gąsior-Perczak D, Pałyga I, Walczyk A, et al. Immunohistochemistry cannot replace DNA analysis for evaluation of BRAF V600E mutations in papillary thyroid carcinoma. Oncotarget 2017;8(43):74897.