Zagazig University Medical Journal
www.zumj.journals.ekb.eg
https://doi.org/10.21608/zumj.2024.342234.3720 Volume 31, Issue 1, January. 2025
Manuscript ID: ZUMJ-2412-3720
DOI: 10.21608/ZUMJ.2024.342234.3720
ORIGINAL ARTICLE

The Predictive Role of Key MAPK Pathway Variants in Differentiated Thyroid Cancer Patients' Response to Radioactive Iodine Therapy

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Submit Date: 06-12-2024
Accept Date: 15-12-2024

ABSTRACT

Background: Radioactive iodine (RAI) therapy is a core part of differentiated thyroid cancer (DTC) treatment. To predict patients' response to RAI is a contributing factor in improving their prognosis and extending their survival. We aimed to investigate the clinical significance of EGFR exon 20, BRAF V600e, and KRAS G13V variants in DTC patients and their predictive role in patients' responses to RAI.

Methods: 117 DTC patients receiving radioactive iodine (I-131) therapy were included; from each, 5 ml of venous blood samples was obtained before RAI treatment, and 3 ml of venous blood samples was obtained after 3 months of treatment. Polymerase chain reaction (PCR) was used to detect the forementioned mutations, and TgAb and TSH were assessed to evaluate DTC patients' response to RAI therapy.

Results: Data showed that EGFR exon 20, BRAF V600e and KRAS G13V mutations were significantly associated with higher TgAbs levels, advanced clinical stage, and positive vascular invasion. EGFR exon 20 and BRAF V600e are associated with larger tumor size. Patients with BRAF V600e and KRAS G13V required higher I-131 doses during RAI and only KRAS was associated with distant metastasis. The co-existence of all three mutations was correlated with higher TgAbs, clinical stage, vascular invasion, larger tumor size, and higher I-131 dose.

Conclusions: EGFR exon 20/BRAF V600e/KRAS G13V mutations are associated with advanced clinical stage and higher radioiodine dose requirements for DTC patients' treatment indicating a significant role in the disease progression of DTC patients and its impact on predicting their response to RAI therapy.

Keywords: Radioactive Iodine Therapy; Thyroid Cancer; BRAF; EGFR; KRAS.

INTRODUCTION

Thyroid cancer (TC) is the most popular endocrine malignancy as well as the most popular head and neck cancer, the seventh in overall cancer incidence rates and the fifth in women [1]. There are four types of thyroid tumors: papillary thyroid carcinoma (PTC), follicular thyroid carcinoma, medullary carcinoma, and undifferentiated thyroid carcinoma. The most common type is papillary thyroid carcinoma, which accounts for 85% to 90% of all occurrences [2]. Due to the relatively inert biological nature of TC,

the general prognosis is positive, with a ten-year survival rate of over 90 percent. However, many cases still have a poor prognosis, and surgical recurrence and metastases sometimes lead to patient death [3]. As a result, clinicians are concerned about predicting a diagnosis for thyroid tumors and developing tailored treatment plans [4]. The etiology of thyroid tumor is still unknown. Genetic and proteomic studies have contributed to the understanding of TC pathogenesis. Surgical removal of the tumor is the primary treatment for PTC, and it is frequently applied in clinical settings. Adjuvant treatment is commonly offered following a thyroidectomy, according to the severity of the condition and the spread of the tumor [5].

Radioactive iodine I-131 (RAI) adjuvant treatment is often offered after a thyroidectomy as thyroid tissue-derived cancer cells typically maintain their ability to absorb iodine. I-131 therapy may effectively remove residual thyroid gland tissue and micro-metastases; hence, this treatment has an essential function in decreasing recurrence rates and elevating survival [6]. Although RAI for residual ablation is effective in large fraction of thyroid tumors, approximately twenty percent of patients develop localized recurrence or distant metastasis, with two-thirds of them develop RAI resistance in less than five years [7] and a ten-year survival rate is sometimes less than ten percent [8]. Additionally, optimal tumor dosage is rarely known and must be moderated to avoid short- and long-term adverse effects on other tissues [9]. Therefore, developing indicators that can predict TC patients' response to RAI is consequential as it can improve treatment outcomes and prolong patients' survival.

Of the numerous molecular pathways that govern the development and progression of thyroid cancer specifically, and other types of cancer in general, is the mitogen-activated protein kinase (MAPK) signaling pathway. Strating with the epidermal growth factor receptor (EGFR) pathway, it has been identified as one of the key factors in cancer proliferation and metastasis [10]. EGFR mutations have been linked to many types of cancers including that of the thyroid, therefore it has been a target of many individualized therapies [11]. Downstream to EGFR, the BRAF proto-oncogene is a member of the RAS-RAF-MEK-ERK-MAP kinase signal transduction pathway [12]. In TC, the BRAF V600e mutation is linked to several undesirable clinicopathological variables, including advanced illness, invasive features, and others [13]. The BRAF V600e mutation may lead to a little downregulation of the sodium iodide symporter (NIS), which might result in an incorrect NIS localization where the NIS is unable to be appropriately localized to the cell membrane [14]. Recent findings suggest that the BRAF V600e mutation could reduce the therapeutic benefit of I-131, which would be detrimental to the patient's prognosis. Rat sarcoma viral (RAS) oncogenes like KRAS, NRAS, and RAS have an important effector function in various signaling cascades that control gene expression, including MAPK. RAS oncogenes play an important function in cell proliferation and differentiation control [15]. Among all RAS mutations, alterations in KRAS codon twelve are the most frequently encountered mutations in cancer [16]. RAS point mutations are common genetic alterations reported in thyroid lesions [17]. The aim of the study was to evaluate the clinical significance of EGFR exon 20/BRAF V600e/KRAS G13V variants as well as their predictive role in the response of DTC patients to RAI therapy.

METHODS

Patients and sampling

A total of 117 DTC patients were selected from those referred to a Ministry of Health hospital in Alexandria, Egypt, from October 2022 to December 2023. The study was approved by the ethical committee of the Medical Research Institute, University of Alexandria, Egypt (E.C. S/N. T2/2022) in accordance with the Declaration of Helsinki and subsequent modifications, and informed written consent for patients' participation in clinical research was collected before inclusion in the study protocol according to ethical guidelines. Demographic and clinicopathological data of patients were collected including age, gender, weight, type of surgery, type of TC, presence of vacular invasion. Additionally, patient clinical staging was done according to TNM classification (tumor size, lymph node involvement and presence of distant metastasis).

RAI treatment procedure

Before considering therapy, all patients were instructed to stop using any iodide-containing products, iodine supplements, thyroid hormones, or other drugs that might impair the thyroid tissue's capacity to accumulate iodide. Activity between 1.85 and 5.5 GBq (50 and 150 mCi) were used for postoperative ablation of thyroid bed remnant.

Sampling

Two venous blood samples were withdrawn from each TC patient prior to (3 ml) and 3 months after

RAI treatment (5 ml). Samples were immediately centrifuged at 2000 × g for 10 min and plasma was separated and stored at – 80 °C.

DNA extraction

For each patient, cell free DNA was extracted according to the manufacturer's instructions using Qiagen's DNeasy Blood & Tissue Kit (Hilden, Germany, cat# 69504) using a volume of 0.5 mL of plasma with an elution volume of 50 µL. DNA concentration and purity were determined by measuring optical absorbance at 230, 260, and 280 nm by a NanoDrop (R) ND-1000 UV-Visible Spectrophotometer (Thermo Fischer Scientific, USA).

Detection of EGFR, BRAF, and KRAS Mutations by qPCR

Detection of KRAS (G13V rs112445441 (C>A)), BRAF (V600e rs113488022 (T>A)), and EGFR (exon 20 rs1050171 (A>G)) mutations was carried out using predesigned Taqman SNP genotyping assay kits (Thermo Fisher Scientific, USA, cat# 4351379). Reactions were carried out in a total volume of 10 µl containing 5 µl of TaqMan Master Mix, 0.5 µl of assay working stock, and 2 µl of sample containing 2 ng of DNA, and the volume was completed to 10 µl by adding nuclease-free water. The thermal cycler (Bio-rad, USA) was used under the following conditions: 95 ºC for 10 mins followed by 40 cycles of 15 seconds at 95 ºC and 1 minutes, at 60 ºC. Data was analyzed according to the Fam/Vic dye detection patterns where the Vic prob indicates the wild-type allele, while the FAM probe indicates the mutant allele.

Assessment of thyroid functions

TgAb and TSH were evaluated prior to RAI treatment and after a follow-up period of 3 months using chemiluminescent microparticle immunoassay. Antithyroglobulin antibodies TgAb levels were assessed using Elecsys® anti-Tg reagent kit (Roche Diagnostic, Switzerland; reference interval 0.00- 115 IU/mL), and levels of TSH were assessed using Elecsys® TSH reagent kit (Roche Diagnostic, Switzerland; reference interval 0.27-4.2 uIU/L).

Statistical analysis

Statistical analysis of the data was analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using number and percent. The Shapiro-Wilk test was used to verify the normality of distribution. Quantitative data were described using mean ± standard error. Significance of the obtained results was judged at the 5% level . The used tests were Chi-square test for categorical variables, to compare between different groups. Mann Whiteny test was used to compare between two groups of abnormally distributed values. Pearson Correlation coefficient (R) was calculated for bivariate correlations. Hardy-Weinberg the population of the studied sample was explored to find its equilibrium with Hardy-Weinberg equation.

RESULTS

Demographic and clinicopathological data of TC patients

The demographic and clinicopathological data of patients included in this study is shown in Table (1). The mean of included TC patients' age was 42.1±9 years, ranging from 27 to 71 years and 84.6% were females. The majority of patients had papillary TC (94%) and received a mean RAI dose of 81.5 ± 21.4 ranging from 50 to 120 mCi. According to TNM staging, 87.2% presented with clinical stage I, 7.7% presented with clinical stage II, and 5.1% of patients presented with clinical stage IV. Seventy-five patients had T1 tumor size representing almost two thirds of the included patients, while one-quarter had zero lymph node involvement, and the majority of patients (75.2%) had no distant metastasis.

Descriptive analysis of EGFR exon 20/BRAF V600e/KRAS G13V variants in DTC patients

The frequencies of the three variants are presented in Table 2. Of the included patients, 66.7% carried the mutant allele for EGFR exon 20 mutation, and 54.7% of them carried the mutant allele for BRAF V600e mutation. In 43.6% of the studied patients both EGFR exon 20 and BRAF V600e mutations co-existed, while the rest tested negative for both. Regarding the KRAS G13v, 64.1% tested positive for the mutant allele and when we analyzed the patients for the co-existence of all three mutations, we found that 38.5% were positive.

Association between EGFR exon 20/BRAF V600e/KRAS G13V variants and DTC patients' clinicopathological parameters

Statistical analysis showed that the presence of positive EGFR mutant allele in DTC patients was significantly associated with larger tumor size ($p<0.001$), distant metastasis ($p=0.005$), advanced clinical stage ($p=0.014$) and positive vascular invasion ($p<0.001$). Also, BRAF mutation showed a significant association with the same clinicopathological parameters except for vascular invasion ($p=0.047$, 0.018, 0.013 and 0.056 respectively). The KRAS mutation however showed a significant association with metastasis ($p=0.002$), advanced clinical stage ($p=0.008$) and positive

vascular invasion (p=0.006). The co-existence of EGFR/BRAF or all three mutations together was associated with all of the previously mentioned parameters. Regarding the association with radioactive iodine dose, neither EGFR nor BRAF mutant allele carriers showed a significant association with the required dose of radioiodine separately (p=0.110 and 0.149), however, the co-existence of both mutations in the same patient was significantly associated with higher radiation dose (p=0.018). KRAS mutant allele carriers required a significantly higher dose for RAI treatment (p<0.001), and hence the carriers of all three mutations as well (p<0.001) (Table 3).

EGFR exon 20/BRAF V600e/KRAS G13V variants and DTC patients' response to RAI

The response to RAI treatment was assessed by following up TgAb and TSH levels in sera of DTC patients before and 3 months after the completion of RAI treatment. The data of TgAb are represented in Figure (1a). At the beginning of the treatment, there was no significant difference in the TgAb between patients with the presence of the mutant alleles of any of the investigated mutations (p=0.212, 0.490 and 0.102 for EGFR, BRAF and KRAS respectively). Also, the co-existence of EGFR/BRAF and all three mutations EGFR/BRAF/KRAS was not associated with any significant increase in TgAb levels (p=0.656 and 0.391 respectively).

Three-month post RAI treatment, statistical analysis showed that patients with positive EGFR exon 20 mutant allele had a significantly higher TgAb post RAI treatment compared with those who tested negative (p<0.001). The mean values of TgAb in patients with positive BRAF V600e mutant allele also were significantly higher that its value in patients with negative BRAF mutation (p<0.001). Patients who carried both EGFR exon 20 and BRAF V600e mutant alleles had even higher mean value of serum TgAb compared to those with either one of these mutations and to also to those who tested negative for both (p<0.001). KRAS mutation was also associated with higher TgAb levels compared to patients with wildtype alleles (p<0.001). The co-existence of the three mutations hence showed similar results with even higher mean of TgAb compared to patients who didn't carry any of the three alleles (p<0.001).

Regarding the levels of TSH, which are presented in figure (1b), similarly, at the beginning of the treatment, there was no significant difference in the TSH between patients with the presence of the mutant alleles of any of the investigated mutations (p=0.160, 0.705 and 0.506 for EGFR, BRAF and KRAS respectively). Also, the co-existence of EGFR/BRAF and all three mutations EGFR/BRAF/KRAS was not associated with any significant increase in TSH levels (p=0.767 and 0.840 respectively). However, three months after RAI treatment, statistical analysis showed that patients with positive EGFR exon 20 mutant allele had a significantly higher TSH post RAI treatment compared with those who tested negative (p-0.032). Although the mean values of TSH in patients with positive BRAF V600e mutant allele was not significantly different than those of patients with negative BRAF mutation (p=752), patients with both EGFR exon 20 and BRAF V600e mutant alleles had significantly higher mean value of serum TSH compared to those with either one of these mutations and also to those who tested negative for both (p-0.039). KRAS mutation was not significantly associated with any change in the levels of TSH either when analyzed alone (p=0.123) or when co-existed with the other two mutations (p=294).

Correlation between EGFR exon 20/BRAF V600e/KRAS G13V variants and clinicopathological parameters and I-131 Dose

Pearson correlations were done to correlate the studied variants with I-131 dose, markers of response to therapy (TgAb and TSH) and clinicopathological parameters (Table 4). Analysis indicated a significant direct correlation between EGFR exon 20 allele with the level of TgAbs, clinical stage, vascular invasion and tumor size (p=0.03, 0.011, <0.001 and <0.001 respectively). Regarding BRAF V600e was similarly correlated with those parameters in addition to I-131 dose (p<0.001, <0.001, <0.001, =0.013 and 0.014 respectively). For patients who had both mutations, significant correlations were observed only with TgAbs and tumor size (p<0.001 and =0.04 respectively). The KRAS mutation was correlated with all previously mentioned parameters in addition to tumor metastasis (p=0.049). Finally, the co-existence of all three studied mutations was directly correlated with TgAbs, clinical stage, vascular invasion, tumor size and higher I-131 dose (p<0.001).

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Table 1: Demographic and clinicopathological parameters of patients

Demographic and clinicopathological parameters	No. (%)
Age (years)	43.1 ± 9.0
Sex	
Male	18 (15.4%)
Female	99 (84.6%)
Tumor Type	
Papillary	110 (94%)
Follicular	7 (6%)
Tumor Size	
T1	75 (64.1%)
T2	21 (17.9%)
T3	15 (12.8%)
T4	6 (5.1%)
Lymph Node Involvement	
N0	30 (25.6%)
N1	39 (33.3%)
Nx	48 (41%)
Metastasis	
M0	88 (75.2%)
M1	14 (12%)
Mx	15 (12.8%)
Clinical Stage	
I	102 (87.2%)
II	9 (7.7%)
IV	6 (5.1%)
Vascular Invasion	
Negative	72 (61.5%)
Positive	45 (38.5%)
I-131 Dose (mci)	81.5 ± 21.4

Table 2: Frequencies of studied variants in DTC patients.

Mutation	No.	%
EGFR exon 20		
Negative	**39**	**33.3%**
Positive	**78**	**66.7%**
BRAF V600e		
Negative	**53**	**45.3%**
Positive	**64**	**54.7%**
EGFR/BRAF		
Negative	**66**	**56.4%**
Positive	**51**	**43.6%**
KRAS G13V		
Negative	**42**	**35.9%**
Positive	**75**	**64.1%**
EGFR/BRAF/KRAS		
Negative	**72**	**61.5%**

Positive	**45**	**38.5%**

Table 3: Association between EGFR exon 20/BRAF V600E/KRAS G13V Variants and Clinicopathological Parameters

	EGFR exon20 (%)		P	BRAF V600E (%)		P	EGFR/BRAF (%)		P	KRAS G13V (%)		p	EGFR/BRAF/KRAS (%)		p
	Positive (n = 78)	Negative (n = 39)		Positive (n = 64)	Negative (n = 53)		Positive (n = 51)	Negative (n = 66)		Positive (n = 75)	Negative (n = 42)		Positive (n = 45)	Negative (n = 72)	
Dose (mci)	77.7±3.3	83.5±2.5	0.110	77.7±3.1	84.7±2.5	0.149	77.3±2.6	87.1±2.9	**0.018***	64.3±2.5	91.2±2.1	**<0.001***	75.4±2.5	91.3±2.6	**<0.001***
Tumor size															
T1	50.0	92.3	**<0.001***	53.1	77.4	**0.047***	47.1	77.3	**0.006***	60.0	71.4	0.142	40.0	79.2	**<0.001***
T2	26.9	0.0		21.9	13.2		29.4	9.1		24.0	7.1		33.3	8.3	
T3	15.4	7.7		17.2	7.5		17.6	9.1		12.0	14.3		20.0	8.3	
T4	7.7	0.0		7.8	1.9		5.9	4.5		4.0	7.1		6.7	4.2	
Lymph node involvement			0.244			0.165			0.877			0.117			
N0	23.1	30.3		18.8	34.0		23.5	27.3		24.0	28.6		26.7	25.0	
N1	38.5	23.1		37.5	28.3		33.3	31.8		40.0	21.4		40.0	29.2	0.357
Nx	38.5	46.2		43.8	37.7		41.0	40.9		36.0	50.0		33.3	45.8	
Metastasis															
M0	66.7	92.3	**0.005***	65.5	86.6	**0.018***	60.8	86.4	**<0.001***	65.3	92.9	**0.002***	55.6	87.5	**<0.001***
M1	17.9	0.0		18.8	3.8		27.5	0.0		18.7	0.0		31.1	0.0	
Mx	15.4	7.7		15.6	9.4		11.8	13.6		16.0	7.1		13.3	12.5	
Clinical stage															
I	80.0	100	**0.014***	79.7	96.2	**0.013***	70.6	100.0	**<0.001***	80.0	100.0	**0.008***	66.7	100.0	**<0.001***
II	11.5	0.0		14.1	0.0		17.6	0.0		12.0	0.0		20.0	0.0	
IV	7.7	0.0		6.3	3.8		11.8	0.0		8.0	0.0		13.3	0.0	
Vascular invasion															
Negative	50.0	84.6	**<0.001***	53.1	71.7	0.056	41.2	77.3	**<0.001***	52.0	78.6	**0.006***	40.0	75.0	**<0.001***
Positive	50.0	15.4		46.9	28.3		58.8	22.7		48.0	21.4		60.0	25.0	

Quantitative data were represented by mean ± S.E., qualitative data were represented by percentage, n=117, significance level p<0.05

Table 4: Correlation between EGFR exon 20/BRAF V600E/KRAS G13V variants and clinicopathological parameters and I-131 Dose

	EGFR	BRAF	EGFR/BRAF	KRAS	EGFR/BRAF/KRAS
TgAbs	**0.197 (0.033*)**	**0.407 (<0.001*)**	**0.417 (<0.001*)**	**0.292 (0.001*)**	**0.471 (<0.001*)**
TSH	0.138 (0.170)	0.080 (0.426)	0.012 (0.904)	0.002 (0.982)	0.058 (0.565)
Clinical stage	**0.234 (0.011*)**	**0.377 (<0.001*)**	0.154 (0.098)	**0.248 (0.007*)**	**0.419 (<0.001*)**
Vascular invasion	**0.335 (<0.001*)**	**0.368 (<0.001*)**	**0.190 (0.040*)**	**0.262 (0.004*)**	**0.350 (<0.001*)**
Metastasis	0.160 (0.084)	0.054 (0.562)	0.138 (0.138)	**0.182 (0.049*)**	0.104 (0.266)
Lymph node involvement	0.000 (1.000)	0.025 (0.791)	0.132 (0.157)	-0.056 (0.546)	-0.086 (0.357)
Tumor size	**0.343 (<0.001*)**	**0.229 (0.013*)**	0.254 (0.006)	0.015 (0.870)	**0.303 (0.001*)**
I-131 dose	0.127 (0.173)	**0.226 (0.014*)**	0.161 (0.082)	**0.603 (<0.001*)**	**0.361 (<0.001*)**

Pearson Correlations were represented as R (p value), n=117, significance level p<0.05

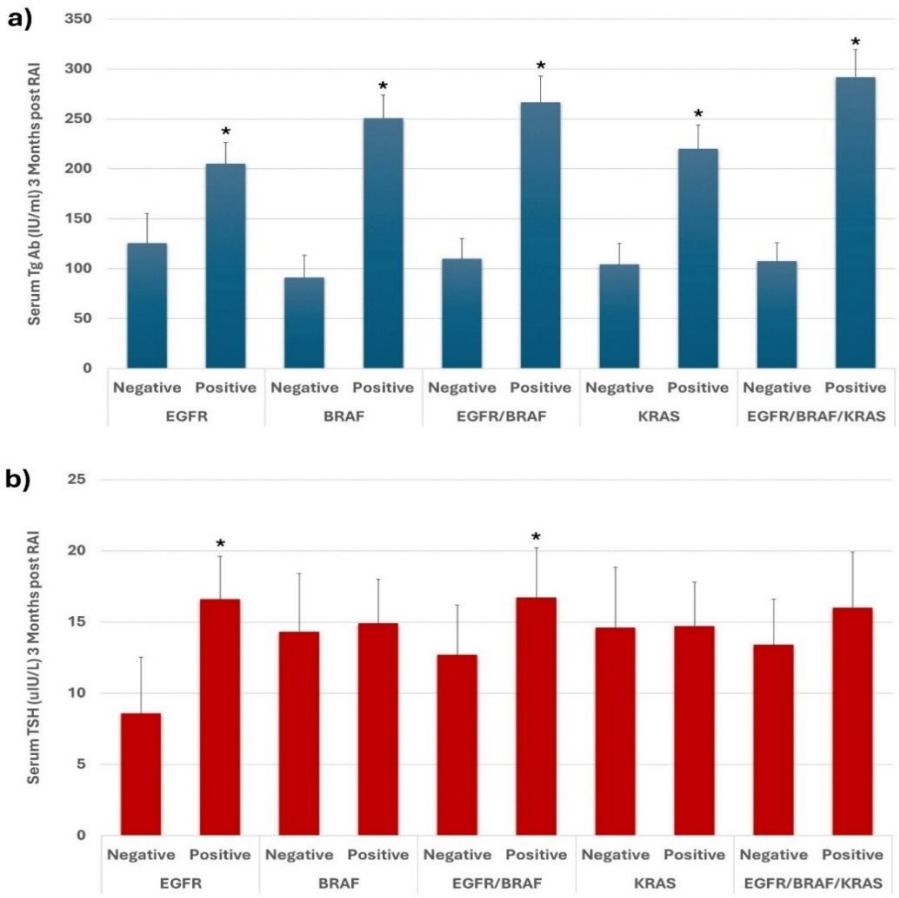

Figure 1: a) Serum Tg Ab (IU/ml) and b) TSH (uIU/L) three months after I-131 treatment in DTC patients classified according to mutation status.

DISCUSSION

Radioactive iodine can be a useful diagnostic and therapeutic tool for treating patients with thyroid cancer of which approximately ninety percent of thyroid cancer patients can benefit [18]. Clinical follow up of patients' response to RAI therapy is usually dependent on a number of imaging modalities as well as thyroid markers including levels of thyroid-stimulating hormone and anti-thyroglobulin antibodies [19]. However, the understanding of the genetic mutations of signaling pathways involved in the progression and response of TC represents an important role in development of treatment protocols. The activation of MAPK

pathway has been reported to be associated with thyroid tumorigenesis and many drugs have been developed to target its components over the past few years [20].

EGFR mutations have been reported to be prevalent in TC patients with a high likelihood of EGFR mutations in females compared to males [21]. Our investigation of EGFR exon 20 mutation indicated that two-thirds of the included DTC patients carry at least one copy of the mutant variant. EGFR mutations were previously thought to be less prevalent as a number of reports have also suggested a 30% rate in TC patients [11, 22]. Our results indicated a significant correlation with more advanced clinical stage, positive vascular invasion and larger tumor size. The significance of EGFR as a therapeutic target in PDTC is suggested by Lote et al.'s description of a case of metastatic PDTC with an EGFR mutation that was responsive to therapy and treated with a selective EGFR tyrosine kinase inhibitor [23]. EGFR mutations have been linked to rearranged during transfection (RET) kinase activation with lead to enhanced tumor proliferation and metastasis [24].

Previous researches have demonstrated that mutations in EGFR/ERK-MAPK pathway have been linked to dedifferentiation of thyroid cells leading to loss of their ability to retain iodine [25, 26]. EGFR has been one of the targets of MAPK inhibitors like Vandetanib. These tyrosine kinase inhibitors have been reported to lead to re-differentiation of thyroid cancer cells leading to restoration of their ability to uptake radioiodine and enhancement of patients' response to RAI [27, 28]. Furthermore, tyrosine kinase inhibitors interfere with angiogenic growth factors induced by EGFR [29]. This emphasizes the importance of identification of EGFR mutation and their role in planning patients' treatment strategies.

A protein belonging to the RAF family, BRAF binds RAS and initiates the MAPK cascade. BRAF V600e mutation is one of the most extensively studied BRAF mutations that has been connected to a number of malignancies, including thyroid, colorectal, lung, and melanoma [4]. In the current study, 54.7% of included patients carried BRAF V600e mutant allele. BRAF V600e mutation was significantly correlated with clinicopathological parameters in DTC patients including patients' advanced clinical stage, larger tumor size and vascular invasion. Additionally, patients with positive B600e mutation had significantly higher levels of TgAbs. Consistant with our results, BRAF V600e mutation has been detected in 45.7% of papillary thyroid microcarcinomas patients of Middle Eastern origin that was also correlated with tumor relapse occurring and lung metastasis [30]. Similar findings have also been reported in a study of 90 PTC cases in the UAE in which BRAF V600e mutations significantly correlated with PTC with a larger tumor diameter, a positive surgical margin, and lymph node metastasis [31]. This is attributed to the persistent role of BRAF V600e mutation in MAPK signaling pathway activation, which encourages tumor growth, invasion, and metastasis [32]. Although the frequency of BRAF V600e mutations varies amongst cancer types, the mutation is always associated with a poor prognosis [33].

We also observed that patients with BRAF V600e mutation had to receive a significantly higher dose of radioactive iodine through their treatment which indicates a significant association between this mutation and worse clinical picture. The link between BRAF V600e mutation and higher doses of radioactive iodine during RAI may be attributed to the role of this mutation in modifying iodine retention in thyroid cells. Thyroid cancer patients with BRAF mutation lose their ability to concentrate iodine in their follicular cells which may result in radioiodine refractory disease (RAIR) [34]. The presence of BRAF V600e mutation has been associated with silencing a number of genes responsible for iodine uptake, particularly the sodium iodine symporter (NIS) gene. This would lead to impaired NIS expression and mis-localization of NIS in the cytoplasm and eventually loss of cellular ability to retain iodine and resistance to RAI [35].

Despite the well-established role of BRAF V600e mutation in the development and progression of TC, previous reports have shown that the BRAF V600e mutation is not the only factor contributing to the development of iodine resistance in thyroid cancer; therefore, further mutations needed to be investigated [36]. Mutations in the RAS family genes, especially KRAS are amongst the most frequent genetic abnormalities linked to thyroid cancer. The activated RAS protein functions as a molecular stabilizer and is crucial for cell division and proliferation [37]. Statistical analysis indicated a significant correlation between KRAS G13V mutation and higher levels of TgAb, advanced clinical stage, larger tumor size, positive vascular invasion and higher mean dose required for RAI treatment. Additionally, it was the only studied mutation that was directly correlated with distant

metastasis. The percentage of PTC carrying the KRAS G13V mutation in the current study was high compared to previous recent studies in different ethnicities. Our investigations reported a 64.1 % incidence of KRAS mutation. These results while comparable to those reported by Heriyanto et al in Indonesia [38], they are higher than other reports for different ethnicities [39].

Like EGFR exon 20 and BRAF V600e mutations, KRAS G13V also showed a significant association with higher doses administered during RAI therapy indicating a role in poor treatment outcomes that might be attributed to its role in tumorigenesis. Previous reports have indicated that KRAS codon 12/13 mutations co-exist with BRAF mutations particularly in DTC, however, no reports have confirmed the specific role of KRAS mutations in RAI resistance yet.

Unlike TSH, TgAb was significantly correlated with all studied mutations. These results are consistent with resent research that it is a good predictor of responses to therapy in DTC patients [40].

The co-existence of all three studied mutations showed stronger correlations with clinicopathological parameters than the individual mutations and presented with higher TgAb levels and higher I-131 dose as well. These results emphasized the role of this cluster of mutations in disease progression of DTC patients and its impact on their response to RAI therapy. Testing patients for studied mutations therefore might be a promising strategy for predicting patients' response to RAI therapy.

Conflict of interest: None.
Financial Disclosures: None.

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Citation

El Feky, S. E., Salem, T., Zahra, O., Ali, M., Abo El Azm, I., Dosouky, H., Morsi, M. The Predictive Role of Key MAPK Pathway Variants in Differentiated Thyroid Cancer Patients' Response to Radioactive Iodine Therapy. *Zagazig University Medical Journal*, 2025; (543-554): -. doi: 10.21608/zumj.2024.342234.3720