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OPEN ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

Correlation Between BRAF^{V600E} Positivity and Recurrence and Poor Prognosis in Preoperative Fine Needle Aspiration Biopsy of Papillary Thyroid Carcinoma

Papiller Tiroid Kanserlerinde Preoperatif İnce İğne Aspirasyon Biyopsisinde Braf^{V600E} Pozitifliğinin Nüks ve Kötü Prognozla İlişkisi

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ÖZET

Amaç: Tiroid maligniteleri epitelyal ve non-epitelyal olmak üzere iki ana gruptan oluşmaktadır. Epitelyal olanlar; papiller, folliküler ve anaplastik karsinomlardır. Non-epitelyal olan ise medüller karsinomlardır. Papiller tiroid kanseri (PTK) tüm tiroid maligniteleri içerisinde %80'lik bir kısmı oluşturmaktadır ve bu maligniteler içerisinde tedaviye en iyi yanıt alınanlardan birisidir. Papiller tiroid kanseri gelişiminde tümörogenezisin moleküler genetiğinde A-Raf, B-Raf (BRAF^{V600E}) ve C-Raf olmak üzere 3 farklı Raf kinaz mevcuttur. Çalışmamızda papiller tiroid kanserlerinde cerrahi sonrası oluşan nükslerde BRAF^{V600E} pozitifliğinin nüks açısından prognostik faktörlerle ilişkisini ortaya koymayı amaçladık.

Gereç ve Yöntem: Papiller tiroid kanseri nedeni ile daha önce ameliyat edilmiş ve nüks gelişmiş olan hastaların ameliyat öncesi yapılan ince iğne aspirasyon biyopsi preparatları temin edildi. Bu preparatlar üzerinden kanserli bölge işaretlendi ve işaretli alanlardaki hücrelerden DNA izole edildi. İzole edilen DNA üzerinde pyrosequence dizi analizi yöntemi ile BRAF^{V600E} mutasyonu varlığı araştırıldı. Hastaların papiller tiroid kanserinde nüks açısından diğer prognostik kriterleri de kayıt altına alınarak BRAF^{V600E} mutasyonu ile ilişkileri istatistiksel olarak analiz edildi.

Bulgular: Papiller tiroid kanserlerinde nüks nedeni ile ameliyat edilen hastalarda tüm çalışma grubunda BRAF^{V600E} pozitifliği oranı %70,8 olarak bulunmuştur. Ayrıca kapsül invazyonu, yumuşak doku invazyonu, evresi, nüks zamanı ve lenf nodu metastazı ile BRAF^{V600E} mutasyonu arasında pozitif korelasyon gösterilmiştir.

Sonuç: BRAF^{V600E} mutasyonu ile papiller tiroid kanserlerindeki bazı kötü prognostik kriterler arasında pozitif ilişki gösterilmiştir.

Anahtar Kelimeler: Tiroid, İnce iğne biyopsi, Papiller, Kötü Prognoz, BRAF^{V600E}, Sitoloji

ABSTRACT

Aim: Papillary thyroid carcinoma (PTC) accounts for 80% of all thyroid malignancies and is one of the most responsive thyroid malignancies to treatment. There are three different Raf kinases implicated in the molecular genetics of tumorigenesis for the development of papillary thyroid cancer: ARAF, BRAF (BRAF^{V600E}), and CRAF. Among the BRAF^{V600E} mutations, T1799A (V600E amino acid translocation) is the most common and also observed in thyroid cancer. In our study, we aimed to establish the association between BRAF^{V600E} positivity in preoperative fine needle aspiration biopsy and prognostic factors in patients with recurrence.

Methods: Preoperative fine-needle aspiration biopsy slides were obtained from patients who had previously undergone surgery for PTC and had recurrence. The cancerous areas on the slides were marked and DNA was isolated from cells within the marked areas. The presence of the BRAF^{V600E} mutation was established from the DNA using the pyrosequencing method. Other patient prognostic criteria regarding the recurrence of PTC was also recorded and their correlation with the BRAF^{V600E} mutation was statistically analyzed.

Results: In patients who underwent surgery for recurrence of papillary thyroid cancer, the BRAF^{V600E} positivity rate was 70.8% of the study group. Furthermore, capsular invasion, soft tissue invasion, stage, recurrence time, and lymph node metastasis positively correlated with BRAF^{V600E} mutation.

Conclusion: A positive correlation between BRAF mutation and some poor prognostic criteria in PTC was observed.

Keywords: Thyroid, papillary, fine needle biopsy, poor prognosis, BRAF^{V600E}, cytology

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INTRODUCTION

Papillary thyroid carcinoma (PTC) accounts for 80% of all thyroid malignancies and is one of the most responsive cancers to treatment. The survival rate for PTC is 90–95% over 5–10 years. The diagnosis of PTC is made by radiological imaging and fine needle aspiration biopsy (FNAB) (1). There are three different Raf kinases implicated in the molecular genetics of tumorigenesis for the development of PTC: ARAF, BRAF ($BRAF^{V600E}$), and CRAF. Many variations of $BRAF^{V600E}$ mutations have been reported, with T1799A (V600E amino acid translocation) the most common and occurring in thyroid cancer (2,3).

There are published studies that show that papillary carcinomas with a $BRAF^{V600E}$ gene mutation are aggressive and have a poor prognosis. For this reason, it is becoming increasingly important for preoperative treatment protocol for PTCs and postoperative follow-up to determine the presence of the $BRAF^{V600E}$ mutation (4).

In our study, we aimed to investigate $BRAF^{V600E}$ mutation positivity using FNAB preparations from patients diagnosed with and surgically treated for PTC who were followed-up for at least 1 year and who experienced recurrence during the follow-up period. Therefore, by examining the FNAB specimens, we aimed establish the presence of the $BRAF^{V600E}$ mutation before patients underwent surgery and to compare the positive mutation rate in patients with recurrence with the positive mutation rate reported in the literature in patients diagnosed with PTC.

MATERIALS AND METHODS

Patients who underwent surgery with a diagnosis of recurrent PTC at Necmettin Erbakan University Faculty of Medicine, Department of General Surgery were included in this study. The Ethics Committee of Necmettin Erbakan University Faculty of Medicine granted approval for the research using non-drug and non-medical devices (decision number 2018/1396). 96 patients who had undergone surgery for PTC between 2007 and 2017 and who had recurrence during the follow-up period were included in the study. The follow-up period was at least 12 months.

As a criterion in patients diagnosed with recurrent disease, Thyroglobulin (Tg) levels were classified as: Tg >1 ng/ml with suppressed thyroid stimulating hormone (TSH), and Tg >2 ng/ml with stimulated TSH or evidence of recurrent disease seen on the ultrasonograph of the neck, recurrence on cross-sectional imaging (CT/MRI) and/or nuclear medicine imaging (if performed). It was accepted that a lesion was present. All patients included in the study were diagnosed with PTC, underwent total thyroidectomy or regional lymph node dissection with total thyroidectomy at the first operation, were older than 18 years. The inclusion criteria was that Tg was negative (Tg<1ng/ml) and there was no suspicion of residual tissue on the neck ultrasonography. None of the patients underwent prophylactic lymph node dissection, except for lymph node dissection for treatment purposes. Patients who had residual tissue after the first surgery, who did not receive radioactive iodine (RAI) treatment after surgical treatment, who underwent prophylactic lymph node dissection, and who were younger than 18 years were excluded from the study. Patients who were Tg and anti-Tg positive were considered inadequately treated and were excluded from the study. Each patient in the study group was screened for the presence of a BRAFV600E mutation by isolating DNA from the FNAB samples taken from the lesion before primary surgery. To establish the presence of the mutation, the areas with malignant cells on the FNAB preparations were first labeled under a light microscope (figure 1). After labeling, DNA was isolated and BRAFV600E positivity was determined by PCR (Figure 2a, 2b).

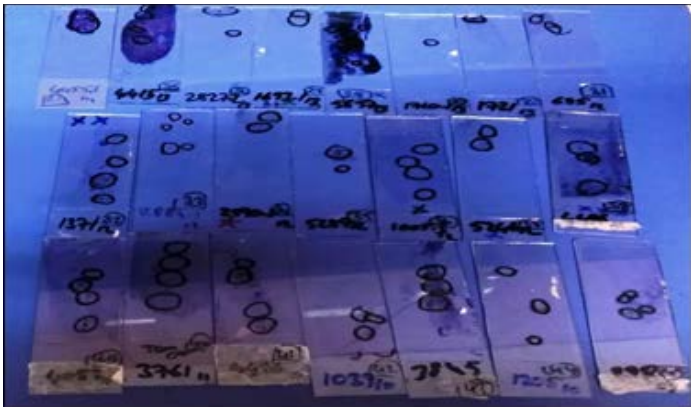

Figure 1. Labeled FNAB slides

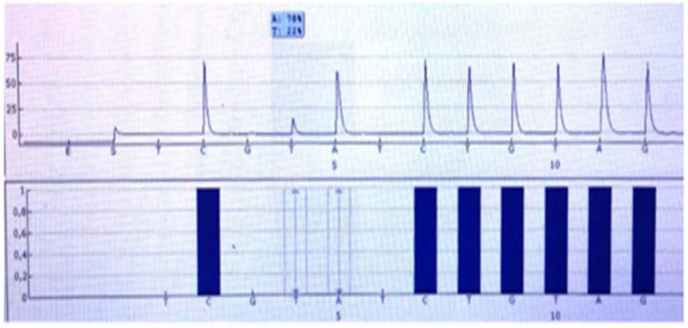

Figure 2a

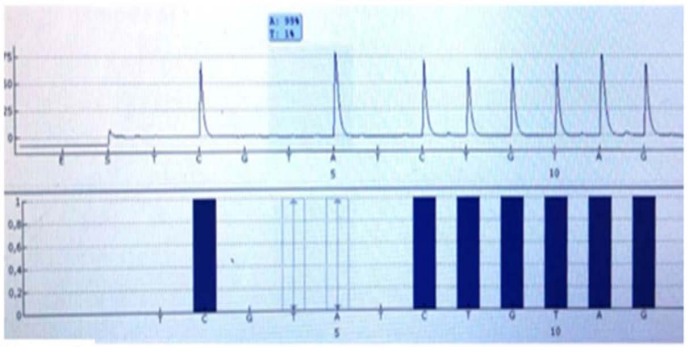

Figure 2b

Figure 2a. ABRAFV600E positive mutation
Figure 2b. BRAFV600E negative mutation

Table 1. Demographic and tumor characteristics of the study group

		Patients (n)
Total number of patients		96
Age (years)		45 (18–79)
Sex	Male	19 (20%)
	Female	77 (80%)
Tumor type	Classic	56 (58.3%)
	Follicular	30 (31.3%)
	Other	10 (10.4%)
Tumor diameter	1–2 cm	35 (36%)
	2,1–3 cm	31 (32%)
	3,1–4 cm	13 (13%)
	>4 cm	17 (15%)
Tumor location	Upper lobe	50 (52.1%)
	Lower lobe	46 (47.9%)
Multicentricity	Yes	64 (67.7%)
	No	32 (32.3%)
Capsular invasion	Yes	66 (77.6%)
	No	19 (22.4%)
Extracapsular invasion	Yes	43 (44%)
	No	53 (56%)
Lymph node metastasis at initial diagnosis	Yes	65 (67%)
	No	31 (33%)
BRAFV600E mutation	Yes	68 (70.8%)
	No	28 (29.2%)
Distant metastasis	Lung	3
	Bone and lung	1
AJCC stage	Stage 1	16 (16.6%)
	Stage 2	13 (13.5%)
	Stage 3	39 (40.6%)
	Stage 4	28 (29.3%)
Recurrence localization	Central area of neck	50 (52%)
	Lateral area of neck	18 (18.7%)
	Thyroidectomy loj	28 (29.3%)
Relapse time	<6 months	3 (3.1%)
	6–12 months	24 (25%)
	12–18 months	31 (32.2%)
	18–24 months	17 (17.7%)
	>24 months	19 (22%)

AJCC=American Joint Committee on Cancer. Other=oncocytic, columnar, and diffuse sclerosing variants.

Table 2. Relationship between continuous variables and $BRAF^{V600E}$ positivity

	BRAFV600E positive		BRAFV600E negative		
	Median	(Min–max)	Median	(Min–max)	P
Age	50	(18–79)	46.5	(19–79)	0.448
Tumor diameter (cm)	2.65	(1–6)	1.65	(1–3.5)	0.002
Multicentricity	2	(1–4)	2	(1–4)	0.709
Time of recurrence (months)	14	(7–65)	20	(4–66)	0.041
RAI dose (mCi)	99	(49–159)	101	(51–156)	0.744

RAI=radioactive iodine.

Statistical analysis

The data were grouped and analyzed using IBM SPSS 20.0 software. In the Kaplan–Meier (K–M) survival analysis, the time of PTC diagnosis and surgery was taken as the starting point and the time at which the study ended was the overall follow-up period.

RESULTS

The demographic and clinical characteristics of the 96 cases included in our study are shown in table 1. The most common PTC variant in our study group was the classical type. In the BRAFV600E-positive group, 85% of the PTCs were the classic type. The tumor diameter was significantly higher in

Table 3. Relationship between AJCC clinical stage and BRAF^{V600E} mutation

			BRAFV600E positive	BRAFV600E negative	Total
ACCJ stage	Stage 1	n	6	10	16
		%	37.50	62.50	100.00
	Stage 2	n	9	4	13
		%	69.20	30.80	100.00
	Stage 3	n	28	11	39
		%	71.80	28.20	100.00
	Stage 4	n	25	3	28
		%	89.30	10.70	100.00
Total		n	68	28	96
		%	70.80	29.20	100.00

Pearson's chi–squared P value=0.004.

the group with BRAF mutation (table 2). The time to disease relapse was significantly shorter in the BRAF^{V600E}-positive group than in the negative group. There was no statistically significant difference between the BRAF^{V600E} positive and negative groups in terms of RAI dose given for treatment, age, and multicentricity. When tumor, node, and metastasis staging is performed according to the American Joint Committee on Cancer (AJCC) system, most patients were in stages 3 and 4. As the disease progresses towards stage 4, the rate of BRAF^{V600E} positivity increased. While the BRAF^{V600E} positivity rate in stage 1 was 37.50%, at stage 4 it was 89.30% (table 3). There was a statistically significant association between the high stage of patients at diagnosis and BRAF^{V600E} positivity (P=0.004). 18.9% of patients relapsed with lymph node metastasis in the cervical area, 29.50% in the thyroidectomy area, and 51.60% in the central area and underwent reoperation. Lymph node metastases were mostly seen in the central area as the localization of recurrence. BRAF^{V600E} mutation was present in 73.50% of patients with lymph node metastases in the central area. Although the number of recurrences in the cervical area was low in the overall distribution (18.90%), the highest rate of BRAF^{V600E} positivity was seen in recurrences detected in the cervical area (77.30%). As for BRAF^{V600E} mutation positivity, the distribution between groups was similar when analyzed by site of recurrence and there was no statistically significant difference (P=0.376). However, in BRAF^{V600E}-positive patients who had recurrence, the recurrence rate was highest in the central region at 53.70%. Kaplan–Meier analysis was performed to investigate the relationship between BRAF^{V600E} mutation and disease-free survival (figure 3).

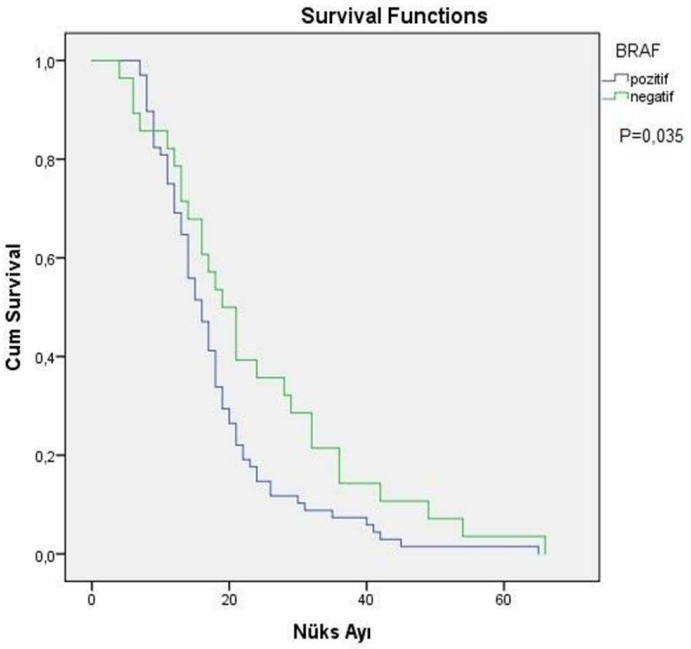

Figure 3. Relationship between disease-free survival and BRAF^{V600E} mutation

Mean and median values of disease-free survival time (months) were calculated. Since the recurrence time data were not normally distributed, Kaplan–Meier analysis was calculated with the median values. With a 95% confidence interval, the expected disease-free survival time in the BRAF^{V600E}-negative group was 19 months, while it was 16 months in the BRAF^{V600E}-positive group and this difference was statistically significant (P=0.035).

DISCUSSION

The BRAF^{V600E} gene mutation is known to be associated with PTC. In this study, patients who had previously undergone surgery with a diagnosis of PTC were in remission at evaluation after primary treatment and patients who developed recurrence during follow-up were evaluated. In this group of patients with recurrence, the association between prognosis-related parameters, such as tumor diameter, capsular invasion, soft tissue invasion, lymph node metastasis, and pathological subtype with BRAF^{V600E} mutation was investigated. A positive correlation between capsular invasion, soft tissue invasion, stage, recurrence time, and lymph node metastasis were seen with BRAF^{V600E} mutation.

The mutation rate of the BRAFV600E gene in our study group was 70.8%. In the literature, BRAF^{V600E} mutation rates in patients with PTC were between 25–81% in various studies. Kurtulmuş et al. (5) found a BRAF^{V600E} mutation rate of 31.7% in their study, Khan et al. (6) reported a rate of 25%, and Chunping Liu et al. (7) in a meta-analysis of 34 studies, the range of

$BRAF^{V600E}$ mutation positivity was 14–81%. Although there are varying rates of $BRAF^{V600E}$ positivity associated with PTC in the literature, the average prevalence is generally reported to be around 45%. Since our study group consisted of patients with complete recurrence, a significantly higher $BRAF^{V600E}$ mutation positivity was found compared with the literature, with a rate of 70.8% compared to a study group in which $BRAF^{V600E}$ was investigated with a diagnosis of PTC. A reason for this increase might be because we studied a group with poor prognosis and possible recurrence rather than all patients diagnosed with PTC.

In our study, patients were categorized into four groups at preoperative assessment according to the AJCC staging system. Chunping Liu et al. (7) in a meta-analysis, reported the rate of patients with advanced stage and $BRAF^{V600E}$ positive gene as 37.5%. A correlation was found between mutation positivity and advanced stage. Tufano et al. (8) showed a strong association between AJCC stage 3 and stage 4 and the $BRAF^{V600E}$ mutation. In our study, a significantly higher $BRAF^{V600E}$ mutation was found in advanced stage patients (stage 3–4). Caroli Li et al. (9) showed a positive correlation with the positivity of BRAF mutation in patients with a tumor diameter of more than 1 cm. In our study, the correlation between the increase in tumor diameter and $BRAF^{V600E}$ positivity was statistically significant ($P<0.05$). While the median tumor diameter in the $BRAF^{V600E}$-positive group was 2.65 cm, it was 1.65 cm in the $BRAF^{V600E}$-negative group. A close correlation was found between $BRAF^{V600E}$ positivity and capsular and soft tissue invasion. The risk of capsular invasion was 5.61 times higher in the $BRAF^{V600E}$-positive group than in the negative group.

The expected disease-free survival time in the $BRAF^{V600E}$-negative group was 19 months, while it was 16 months in the $BRAF^{V600E}$-positive group, which was statistically significant ($P=0.035$) and it was observed that the mutation positive patients relapsed earlier than the $BRAF^{V600E}$-negative patients. However, it is possible that relapse occurs earlier in the $BRAF^{V600E}$-positive group and that other prognostic factors not directly related to the mutation are also associated with $BRAF^{V600E}$. It is known in the literature from studies conducted independently of BRAFV600 that the duration of disease-free survival is shortened for reasons such as an increase in tumor diameter, capsular invasion, multicentricity, and extra-thyroidal spread (10). Since $BRAF^{V600E}$ mutation is associated with parameters, such as capsular invasion, soft tissue invasion, and tumor diameter in our study, the results are indirectly associated with disease-free survival.

Patients were grouped by site of recurrence as central, cervical, or locus. There was no statistically significant difference between the areas of recurrence and the presence of $BRAF^{V600E}$ mutation status. However, in the $BRAF^{V600E}$-positive group, relapse occurred mostly in the central area. There are no studies in the literature on the relationship between the location of the primary tumor and $BRAF^{V600E}$; however, a study by T. Zhang et al. (11) found a higher rate of lymph node metastasis in the central area for tumors in the lower lobe when comparing tumor location and lymph node metastasis location. In our study, no statistically significant differences were found with respect to $BRAF^{V600E}$ mutation and tumor localization in the upper and lower lobe, which has a high rate of tumor localization. However, tumors were proportionally more frequent in the upper lobe. Assuming that the $BRAF^{V600E}$ mutation, which is a somatic mutation, is influenced by environmental factors, a panoramic X-ray of the jaw in the neck area often used by dentists, is performed. It is thought that PTC is more common in the upper lobes, partly due to exposure to radioactivity. However, a larger series of studies with controlled patient groups should be conducted to clarify this opinion.

Although many studies have found a significant association between the $BRAF^{V600E}$ mutation and poor prognostic criteria, the $BRAF^{V600E}$ mutation has not yet been definitively included in the globally accepted PTC management algorithms. However, if PTC can be detected preoperatively by FNAB, which is a minimally invasive method, and if it is accepted as a prognostic criterion by new and large-scale multicenter studies, the role of $BRAF^{V600E}$ mutation in the management of PTC will increase further.

CONCLUSION

In our study, the rate of $BRAF^{V600E}$ positivity was high in patients who underwent surgery for recurrence of PTC. Moreover, there was a positive correlation between $BRAF^{V600E}$ mutation and poor prognostic criteria, such as capsular invasion, soft tissue invasion, stage, time to recurrence, and lymph node metastasis. It is a fact that new studies from larger comparative series are needed to reveal the prevalence of $BRAF^{V600E}$ mutation and its relationship with poor prognostic criteria in PTC. As such, it is likely that the $BRAF^{V600E}$ mutation will be included in future guidelines with the contribution of new studies that will provide more definitive evidence.

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