Prevalence of BRAF V600E and NRAS (G12V, G13V) mutations in Iranian patients with melanoma and their association with tumor-related factors

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Received: 19 December 2022
Accepted: 17 March 2023

Background: NRAS and BRAF mutations are commonly reported in melanoma with various frequencies in different countries. However, their correlation with the development of malignant melanoma and tumor prognosis has not previously been studied in the Iranian population. This study determined the prevalence of these mutations and their association with tumor-related factors.

Methods: This cross-sectional study involved 50 patients admitted to two dermatology hospitals with a definitive diagnosis of melanoma (primary or metastatic) who received surgery as a selective therapeutic option. The genomics of the BRAF and NRAS mutations were determined with the real-time PCR technique.

Results: BRAF and NRAS mutations were presented in 30% and 26% of patients, respectively. The NRAS mutation correlated with mitosis ($P = 0.026$), while the BRAF mutation correlated with visceral involvement ($P = 0.023$). None of the mutations correlated with gender, age, melanoma type (primary vs. metastasis), ulcer, microsatellitosis, and lymph node involvement.

Conclusion: BRAF and NRAS mutations demonstrated relatively high prevalence in Iranian patients with melanoma, which may be valuable prognostic tools in predicting tumor prognosis and metastasis.

Keywords: mutation, BRAF, NRAS, melanoma, skin neoplasms

Iran J Dermatol 2024; 27: 1-5

DOI: 10.22034/ijd.2023.377452.1641

Please cite this article as: Ghanadan A, Shirkoohi R, Shahsiah R, Alihosseini S, Amiri R, Kianfar N, Hadadi Abianeh S, Sabbagh E, Khodaei B, Nasimi M. Prevalence of BRAF V600E and NRAS (G12V, G13V) mutations in Iranian patients with melanoma and their association with tumor-related factors. Iran J Dermatol. 2024; 27(1): 1-5.

INTRODUCTION

Melanoma is the deadliest form of skin cancer, accounting for more than 4% of malignancies worldwide [1]. Sun exposure and genetic susceptibility are the main risk factors associated with melanoma prognosis. Several specific genomic aberrations have been proposed to be important in tumorigenesis, namely those in B-raf proto-oncogene serine/threonine kinase (BRAF), neuroblastoma ras viral oncogene homolog (NRAS), phosphatase and tensin homolog deleted on chromosome 10 (PTEN), and cyclin-dependent kinase inhibitor 2A (CDKN2A) [2,3].

Combining immunotherapy with gene-targeting strategies enhances patients' response to treatment. Therefore, identifying the associated mutations in melanoma improves the selection of the best therapeutic combination for each patient [3-5].

Accordingly, recent research has concentrated on the expression of genes that impact BRAF kinase in melanoma [6]. BRAF gene mutations, particularly at the V600E site, are associated with an increased incidence of melanoma in several studies, decreasing the survival of patients. Prior studies have noted BRAF gene mutations in roughly 54% of patients with early-stage cutaneous melanoma [7]. Furthermore, about 80% of melanoma patients had glutamic acid-valine translocation (V600E mutation) [8-10]. Hence, the application of V600E mutation inhibitors in melanoma treatment in combination with nivolumab and ipilimumab has been of interest in recent years [9,11].

Likewise, NRAS proteins regulate intercellular signals and act as critical factors in signal transduction. In this regard, NRAS-activating mutations play an essential role in developing cancerous cells [12]. Mutant NRAS genes have been identified in about 35% of human tumors, although the frequency of mutations for different NRAS oncogenes varies considerably depending on cell types [13]. However, the correlation between NRAS mutation and malignant melanoma characteristics has not been sufficiently investigated.

Identifying NRAS and BRAF-type mutations in melanoma will be invaluable in determining the tumor's genetic profile, potentially guiding treatment choices; however, there is scarce data investigating mutations associated with melanoma. Herein, we assessed the frequency of NRAS and BRAF V600E mutations in patients with malignant cutaneous melanoma and investigated their correlation with tumor and patient characteristics.

METHODS

Study design

This cross-sectional study was conducted at the cancer clinics of Tehran University Hospitals, Tehran, Iran, over a year from March 2019 to March 2020. Patients with a confirmed diagnosis of melanoma were included. The diagnosis of melanoma was established according to the clinical and histopathological findings. The study was conducted according to the Helsinki Declaration, and ethical approval was provided by the Tehran University of Medical Sciences Ethics Committee (95-02-30-31602). Demographic data and tumor characteristics were collected, including the type of melanoma (primary or metastatic), mitosis, microsatellitosis, ulcer, lymph node, and visceral metastasis status.

Tissue sampling

Five micrometers of paraffined tissue blocks were prepared for the genomic investigation of the NRAS (G12V & G13V) and BRAF V600E mutations. The H&E staining for tumor cells was done on one of the cuts, while the other sections were deparaffinized, and their DNA was extracted using the salting-out technique. We investigated the quality of extracted DNA in optical absorption of 280/260. For BRAF mutation, the polymerase chain reaction (PCR) master mix solution was prepared and poured into two separate 15-microliter tubes. Furthermore, 5 µL of the normal probe/primer solution was added to the first tube, and 5 µL of the mutant probe/primer solution was added to another tube. The tubes were then placed in a real-time PCR machine for DNA amplification. Exons 11 and 15 were amplified using the following primers for BRAF:

5'-TCCCTCTCAGGCATAAGGTAA-3' (Forward)
5'-CGAACAGTGAATATTTCCTTTGAT-3' (Reverse)
5'-TCATAATGCTTGCTCTGATAGGA-3' (Forward)
5'- GGCCAAAAATTTAATCAGTGGA-3' (Reverse)

For the NRAS mutation, after preparing the PCR master mix (by adding nuclease-free water to sample DNA), 10 µl of PCR master mix was added to each tube. Then, we added 10 µl of codon 12, codon 13, and qPCR mix into the labeled tubes. Tubes were placed in a real-time PCR machine. Finally, the PCR results were sent for sequencing for confirmation.

Statistical analysis

Data are provided as mean ± standard deviation (SD) for quantitative variables and absolute frequencies and percentages for categorical ones. Data normality was assessed using the Kolmogorov-Smirnov test. Categorical variables were compared using the chi-squared or Fisher's exact test when more than 20% of cells with an expected count of less than five were observed. Quantitative variables were compared with the t-test or Mann-Whitney U test based on normality. We used SPSS version 24.0 software (SPSS Inc., Chicago, IL) for the statistical analysis. P-values of 0.05 or less were considered significant.

RESULTS

Fifty patients with confirmed melanoma were enrolled in this study. The mean age of the patients was 60.94 ± 14.47 years, and 60% were men. The scalp (20%) was the most common site of melanoma involvement, followed by the plantar region (16%), face (10%), legs (8%), and palms (6%). The mean lesion size was 6.56 ± 6.6 mm (range: 1 to 27 mm).

BRAF V600E mutations were observed in 15 cases (30%), with no gender preponderance ($P > 0.05$). The mean age in the groups with and without BRAF V600E mutations was 64.13 ± 16.33 and 59.57 ± 13.62 years, respectively, indicating no relationship between age and mutation occurrence ($P > 0.05$).

Table 1 demonstrates the patient's demographic and tumor parameters with regard to BRAF V600E and NRAS mutations. Accordingly, the BRAF V600E mutation correlated with visceral involvement ($P = 0.023$), while the NRAS mutation correlated with mitosis ($P = 0.026$). However, BRAF and NRAS mutations had no significant relationships with the type of melanoma (primary vs. metastatic), ulceration, microsatellitosis, and lymph node involvement ($P > 0.05$).

DISCUSSION

Several BRAF gene mutations are associated with dysplastic lesions transitioning into malignant melanoma and tumor metastasis [14]. The BRAF V600E mutation results in a glutamic acid to valine change in amino acid 600 and is associated with an increased risk of transforming pre-cancerous lesions into malignant melanoma [15]. The BRAF V600E mutation prevalence ranges from 25% to 72% in different countries, possibly due to ethnic factors.

Table 1. The prevalence of BRAF V600E and NRAS mutations according to patient and tumor parameters

Parameter	n (%)	Frequency of mutation		*P*-value	
		BRAF	NRAS	BRAF	NRAS
Gender					
Male	30 (60%)	26.7%	24.1%	0.529	0.724
Female	20 (40%)	35.0%	28.6%		
Type of melanoma					
Primary	44 (88%)	27.3%	27.3%	0.348	0.578
Metastatic	6 (12%)	50.0%	16.7%		
Ulcer					
Present	18 (36%)	28.3%	22.2%	0.700	0.746
Absent	32 (64 %)	33.3%	28.1%		
Mitosis					
Present	30 (60%)	25.0%	44.4%	0.125	0.026
Absent	20 (40%)	33.3%	15.6%		
Microsatellitosis					
Present	5 (10%)	40.0%	25.0%	0.629	0.999
Absent	45 (90%)	28.9%	26.1%		
Lymph node involvement					
Present	12 (24%)	41.7%	25.0%	0.471	0.999
Absent	38 (76%)	26.3%	26.3%		
Visceral extension					
Present	3 (6%)	100%	33.3%	0.023	0.999
Absent	47 (94%)	25.5%	25.5%		

BRAF, B-raf proto-oncogene serine/threonine kinase; NRAS, neuroblastoma ras viral oncogene homolog.
The bold values indicate a significant P-value of < 0.05.

Furthermore, various study designs and diagnostic techniques can also affect the results. In this study, the BRAF V600E mutation prevalence in Iranian patients with melanoma was about 30%, similar to a Chinese (25.5%) study [16]. However, the BRAF V600E mutation was more prevalent in Turkish and Brazilian nationals [17,18] and less prevalent in a study on Mexican patients [19].

In the study of Can and colleagues in Turkey, the occurrence of the BRAF V600E mutation was related only to the age of the patients and did not correlate with the characteristics of the tumor tissue [17]. Despite the previous study, ours demonstrated a significant association of the BRAF mutation with visceral extension, which can be an essential predictor of tumor spread to adjacent and distant viscera and disease prognosis. Thiel *et al.* found a significant association of the BRAF mutation with lymph node metastases [20]. Inumaru *et al.* reported no relationship between mutation prevalence and clinical or prognostic parameters [18]. Furthermore, Si *et al.* concluded that patients with the V600E mutation had a greater ulcerative pattern than the non-mutated group [16]. These findings indicate that the prognostic value of the mutation varies in different regions and might be specific to each community. Ultimately, identifying this mutation in our population could have a high prognostic value, mainly predicting visceral organ involvement.

Likewise, the NRAS mutation varies in prevalence among different populations. Our study demonstrated a 26% prevalence in patients with melanoma, similar to studies from New Zealand (21.9% to 38.3%) [21], the United States (26.2%) [22], and France (27%) [23]. However, other authors investigating Australian and Taiwanese patients reported a lower prevalence of NRAS mutation in their patients [24-26]. This difference might also be associated with disease severity, methods and study design, and genetic variation among study populations. The NRAS mutation has been associated with demographic characteristics, such as patients' geographical regions [21]. We detected a significant association of the NRAS mutation with high mitosis ($P = 0.026$), similar to Thomas *et al.*'s study, in which the NRAS mutation was significantly associated with higher mitosis and lower-grade tumor-infiltrating lymphocytes [24]. Sheen *et al.* also reported a higher prevalence of lymph node metastases in NRAS mutant patients [25]. In another study, the NRAS mutation had a significant relationship with central nervous system involvement [26]. Therefore, NRAS mutations might be associated with various tumor characteristics in different societies.

Ultimately, this study was not without limitations. The study sample size was small, which could affect the power of analysis. This study was performed at two tertiary referral hospitals in Iran, and the results might differ in other provinces. Finally, the association of BRAF and NRAS mutations was not assessed with patients' immunological and other clinical conditions. More studies with more cases are needed to shed further light on the association of BRAF and NRAS mutation with tumor and patient characteristics and to confirm our findings.

CONCLUSION

About one-third of patients with malignant melanoma exhibited the BRAF V600E mutation. This mutation had a high prognostic value in predicting visceral expansion. The NRAS mutation was present in 26% of melanoma patients, demonstrating a significant association with high rates of mitosis.

Authors contributions

Study conception: AG, RS, MN, SH; data collection: RS, SA, RA; analysis and interpretation of results: RA, NK; and manuscript preparation: NK, ES, BK.

Acknowledgment

None.

Financial disclosure

None.

Conflicts of Interest: None declared.

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