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Molecular evaluation of *BRAF* V600 mutation and its association with clinicopathological characteristics: First findings from Indian malignant melanoma patients

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Abstract

Mutations in the *BRAF* gene have been described to occur in two-third of melanomas. The objective of the study was to establish the frequency of *BRAF* V600E/K/R mutation in a series of melanomas from Indian origin and to correlate mutation status with clinicopathological features. Seventy melanoma cases were evaluated for *BRAF* V600 mutation by pyrosequencing. Overall, *BRAF* mutations were detected in 30% of the patients. All mutations observed were missense type (GTG > GAG) resulting in p.V600E, while none showed V600K/R mutation. The frequency of *BRAF* V600E mutations were more in patients with onset age of 50 years. *BRAF* mutations were significantly associated with tumor site wherein more mutations were seen in tumors from head and neck and extremities region. Acral and mucosal tumor subtype showed a mutation frequency of 31% and 20%, respectively. Epithelial cell morphology tends to harbor frequent *BRAF* V600E mutation (36%) than other morphological subtypes. Tumors with ulceration and necrosis showed increased *BRAF* mutation rate (32.5% and 33%) respectively. In conclusion, this is the first study to report a mutation frequency of 30% in this cohort. Our results demonstrated that the *BRAF* V600E mutation is a frequent event in Indian melanomas, and represents an important molecular target for novel therapeutic approaches.

Keywords BRAF mutation, India, Melanoma, Pyrosequencing.

Introduction

Malignant melanoma is a complex heterogeneous disease with respect to tumor morphology, histopathology and molecular aberrations. It has the clinical features of poor prognosis, high metastatic rate, rapid development, and potentially fatal with high mortality rate [1]. Although uncommon in India compared with the other geographical locations such as Australia, America and Europe, its incidence is rising globally [2]. Interestingly, clinical and histopatholgical subtypes of malignant melanoma also vary among different ethnicities [3,4]. For examples, superficial spreading and nodular malignant melanomas are more commonly seen in Caucasians [5], whereas acral and mucosal malignant melanomas are often reported in Asian populations [6].

Recent genetic analyses have revealed that malignant melanomas show a high degree of molecular diversity and these molecular events can be utilized as a potential therapeutic target. Some of the molecular markers which have already been evaluated in malignant melanomas include *PIK3CA, PTEN, NRAS, KIT* and *IDH1* [5,7–10], while mutations in the *BRAF* have been extensively studied and implicated in majority of cases of melanomas [11–13]. Most of these genes are part of the mitogen-activated protein kinase pathway (MAPK),

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and plays an important role in the activation of the MAPK signaling cascade, which in turn regulates cell growth, differentiation, and apoptosis [14]. Somatic oncogenic mutations of *BRAF* have been reported to be as high as 70% in malignant melanomas, wherein majority of these mutations (≈90%) occurs due to a single substitution (T to A) of glutamate for valine at codon 600 (V600E). In addition, other rare variants such as p.V600K, p.V600D, p.V600E, etc are also reported in 5–7% of melanomas [15]. The use of Vemurafenib for treating patients with *BRAF*-mutated malignant melanoma has been proven to be milestone in the management of melanoma. The drug Vemurafenib produced improved rates of overall and progression-free survival in patients with previously untreated melanoma with the *BRAF* V600E mutation, thus identification of mutation status is very crucial [16].

While most of the reports on *BRAF* V600 mutation in malignant melanoma is available from the Western countries [5,11,17,18], there have been few studies from Asian patients [19–21]. Melanoma is rare in India and the frequency and distribution of *BRAF* V600 mutations are still unknown. Thus the aim of the current study was to establish the frequency of *BRAF* mutation in a series of melanomas from Indian origin and to correlate mutation status with various demographic and clinicopathological features. To our knowledge, this report is the first series of *BRAF* mutations reported using advanced pyrosequencing assay from Indian subcontinent.

Materials and methods

The current study was carried out at the Research and Development Division of SRL Ltd., Mumbai, India. In total, 70 formalin-fixed paraffin-embedded (FFPE) melanoma samples were included in the current study. Histopatholgical diagnosis of all the samples was performed by two independent experienced histopathologists as per criteria provided by American Joint Commission on Cancer (AJCC) [22]. The demographic and clinicopathological features included age, sex, tumor site, subtype, cell type, morphology, ulceration, necrosis and mitosis/10HPF. Treatment and outcome was not evaluated. The study is in accordance with declaration of Helsinki, approved by Institute Research Committee, with consent available from study participant.

Genomic DNA extraction

QIAamp DNA FFPE tissue *KIT* was used for extraction of genomic DNA as per instructions from the manufacture with slight modification in the protocol. Before extraction, each sample was evaluated for the presence of atleast 50% tumor on hematoxcylin and eosin sections. Briefly, five FFPE sections of 5 µm thicknesses was deparaffinized and then lysed in DNA tissue lysis buffer supplemented with Proteinase K. This was followed by addition of DNA binding buffer and ethanol to the samples and the lysates were placed on spin column tubes. Samples were than washed twice and the DNA was eluted with DNA elution buffer. The extracted DNA was checked for quality on 0.8% agarose gel and quantitated using Qubit (Invitrogen) and stored at −20 °C.

PCR amplification for codon 600 of the *BRAF* gene

Approximately 100 ng of extracted genomic DNA was subjected to PCR amplification using primers as per previous study with slight modifications [23]. Briefly, the PCR master mix was prepared using 10 picomole of each *BRAF* primers (forward 5′-TGAAGACCTCACAGTAAAAATAGG-3′ and reverse: 5′- Biotin-AAAATGGATCCAGACAACTGTTC-3′), 1X of HotStarTaq Master Mix (Qiagen) in a final volume of 50 µl. The cycling of the PCR mix was performed on ProFlex 3 × 32-well PCR system (Thermo Fisher) under the following conditions: 95 °C for 15 min followed by 45 cycles at 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min, and a final extension step at 72 °C for 5 min. Appropriate no template control and a positive control was used in each batch. Post PCR amplification, 15 µl of PCR product was loaded on a 3% agarose gel to confirm the presence of 100-bp PCR product.

Pyrosequencing analysis

Pyrosequencing analysis for detecting *BRAF* V600E/K/R mutation was performed on PyroMark Q24 Qiagen system using PyroMark Gold reagents (Qiagen). In short, the sequencing reagents contained single stranded DNA template prepared from 15 µL of biotynylated PCR product with streptavidin-coated Sepharose beads (GE Healthcare) and 0.5 mM of the sequencing primer: 5′-GGTGATTTTGGTCTAGC-3′ using the PyroMark Vacuum Prep Tool (Qiagen). Sequences surrounding the site of interest served as normalization and reference peaks for quantification and quality control. The dispensation order was as follows: GTACACGATGAT. Two different "sequence to analyze" were used: TACAGT/AGAAAT or TACA[GT]GAAAT for manual analysis of V600E and V600K/R, respectively. Samples with 10% mutated alleles or more were scored as mutation positive. All positive results were reconfirmed by repeat analyses starting from amplification and sequencing. In addition, a negative widltype sequencing control was used in each run for comparison of abnormal results.

Statistical analysis

The data was analyzed by Fischer's exact test or chi-square test as a measure of statistical association between *BRAF* mutation status and the range of clinicopathological and demographic variables. All the p-values were two-tailed and the statistical significance was set at $p < 0.05$.

Result

Clinico-pathological and demographic data of melanoma cases

A total of 70 melanoma specimens were evaluated for the presence of *BRAF* mutation. The demographic and clinicopathological findings of all the cases are demonstrated in Table 1. Among the 70 cases included, there were 33 (47%) females and 37 (53%) males with a median age of 62 years

Table 1 Clinicopathological data and their correlation with *BRAF* gene mutation.

Characteristic	Total samples, *n* (%)	*BRAF* Status		*P* value
		Mutation, *n* (%)	Wildtype, *n* (%)	
Total	70 (100)	21 (30)	49 (70)	
Age (y)				
≤50	22 (31.4)	5 (23)	17 (77)	0.4
> 50	48 (68.6)	16 (33.3)	32 (66.6)	
Sex				
Female	33 (47)	11 (33)	22 (66)	0.6
Male	37 (53)	10 (27)	27 (73)	
Tumor site				
Trunk	15 (21.4)	3 (20)	12 (80)	**0.04**
Head and Neck	7 (10)	5 (71.4)	2 (28.6)	
Extremity	48 (68.6)	13 (27)	35 (73)	
Tumor subtype*				
Acral	39 (56)	12 (31)	27 (69)	0.5
Mucosal	15 (21.4)	3 (20)	12 (80)	
Others	16 (22.6)	6 (37.5)	10 (62.5)	
Tumor morphology				
Epithelial	39 (56)	14 (36)	25 (64)	0.7
Spindle	19 (27)	4 (21)	15 (79)	
Pleomorphic	9 (13)	2 (22)	7 (78)	
Others	3 (4)	1 (33)	2 (67)	
Tumor ulceration				
Present	40 (57)	13 (32.5)	27 (67.5)	0.75
Absent	15 (21)	4 (27)	11 (73)	
Tumor Necrosis				
Present	6 (8.5)	2 (33)	4 (67)	1
Absent	60 (86)	18 (30)	42 (70)	
Mitosis/10HPF				
<1/HPF	43 (61)	13 (30)	30 (70)	1
≥1/HPF	27 (39)	8 (30)	19 70)	

* Subtype on the basis of origin of malignant melanoma. Acral subtype includes melanomas on acral skin (soles, fingers, palms, and nail bed); mucosal subtype includes melanomas on mucosal membranes. Others subtype includes melanomas originating at miscellaneous anatomical sites (non-acral and non-mucosal).

ranging from 26 to 85 years. The disease was predominantly seen in older patients, (69%, > 50 years) in comparison to those who were lesser than 50 years (31%). Considering the anatomical site of the malignant melanomas, tumors were more frequently derived from extremities parts of the body ($n=48$, 68.6%), followed by trunk ($n=15$, 21.4%) and Head and neck ($n=7$, 10%). Histopatholgical analysis of tumors revealed that Acral was the most common tumor subtype (56%), followed by mucosal (21.4%), and other miscellaneous malignant melanomas (22.6%). Morphological examination under the microscope revealed that bulk of the cases were epithelial in nature ($n=39$, 56%), followed by spindle ($n=19$, 27%), pleomorphic ($n=9$, 13%) and other types. Notably, tumor ulceration and necrosis was noted in 57% and 8.5% of the cases respectively. Furthermore, mitotic figures counted in 10 consecutive high-power fields revealed that larger number of cases had < 1 mitosis/10 HPF ($n=43$, 61%), while remaining cases showed ≥ 1/10HPF ($n=27$, 39%).

BRAF mutation status and its correlation with clinico-pathological data

Of the 70 specimen included in the study, 21 cases showed presence of *BRAF* mutation and the remaining 49 cases had widltype *BRAF*, giving an overall mutation rate of 30% [Table 1]. All twenty one mutations observed were missense type as a result nucleotide substitution (GTG > GAG) that predicted to change the amino acid from valine at codon position 600 to glutamic acid. None of the cases in the current cohort showed V600K/R mutation. All V600E positive specimens were heterozygous and retained a wild-type allele. Figs. 1 and 2 show the representative pyrogram for *BRAF* V600 wildltype and mutant (c.1799T > A; p.V600E) case, respectively. The summary of the various clinicopathological findings and mutation pattern of *BRAF* gene is depicted in Table 2. The prevalence of *BRAF* mutations was more in patients with onset age of 50 years or older (16/48; 33.3%) as compared with those younger than 50 years (5/22; 23%) ($p=0.4$). Also, female patients tend to harbor more *BRAF* mutation in comparison to their male counterparts (33% vs. 27%, $p=0.6$). Correlation between *BRAF* V600E mutation and tumor site revealed that significantly more number of tumors from head and neck and extremities region harbored *BRAF* mutation in comparison with tumors from trunk (combined $p=0.04$). Notably, *BRAF* V600E mutations were uniformly detected across different tumor subtype with a frequency of 31% in acral, 20% in mucosal and 37% in other miscellaneous subtype. Malignant melanomas with epithelial cell morphology showed

Table 2 Summary of the various clinicopathological findings and mutation pattern of *BRAF* gene.

Sr no.	Sample ID	Age	Sex	Tumor site	Tumor subtype	Tumor morphology	Tumor ulceration	Tumor necrosis	Mitosis/10HPF	*BRAF* gene mutation	
										Nucleotide change	Amino acid change
1	B4	75	M	Head and Neck	Other	Epithelial	Absent	Absent	$<$1/HPF	c.1799T $>$ A	p.V600E
2	B6	50	M	Extremities	Acral	Epithelial	Present	Absent	$<$1/HPF	c.1799T $>$ A	p.V600E
3	B8	70	F	Extremities	Acral	Epithelial	Present	Absent	$\geq$1/HPF	c.1799T $>$ A	p.V600E
4	B20	60	M	Extremities	Acral	Spindle	Absent	Absent	$\geq$1/HPF	c.1799T $>$ A	p.V600E
5	B24	60	F	Head and Neck	Mucosal	Other	Present	Absent	$<$1/HPF	c.1799T $>$ A	p.V600E
6	B27	61	F	Extremities	Acral	Spindle	Present	Absent	$<$1/HPF	c.1799T $>$ A	p.V600E
7	B37	62	F	Trunk	Other	Epithelial	NA	Absent	$\geq$1/HPF	c.1799T $>$ A	p.V600E
8	B41	60	M	Trunk	Other	Epithelial	NA	Present	$\geq$1/HPF	c.1799T $>$ A	p.V600E
9	B47	65	M	Extremities	Acral	Pleomorphic	NA	NA	$\geq$1/HPF	c.1799T $>$ A	p.V600E
10	B54	41	M	Head and Neck	Other	Pleomorphic	Present	Present	$<$1/HPF	c.1799T $>$ A	p.V600E
11	B55	76	M	Extremities	Acral	Epithelial	Present	Absent	$<$1/HPF	c.1799T $>$ A	p.V600E
12	B57	40	F	Extremities	Other	Epithelial	NA	Absent	$<$1/HPF	c.1799T $>$ A	p.V600E
13	B58	65	F	Extremities	Acral	Spindle	Present	Absent	$<$1/HPF	c.1799T $>$ A	p.V600E
14	B59	80	F	Head and Neck	Other	Spindle	Absent	Absent	$<$1/HPF	c.1799T $>$ A	p.V600E
15	B60	50	M	Extremities	Acral	Epithelial	Present	Absent	$\geq$1/HPF	c.1799T $>$ A	p.V600E
16	B62	62	M	Trunk	Mucosal	Epithelial	Absent	Absent	$\geq$1/HPF	c.1799T $>$ A	p.V600E
17	B65	54	F	Head and Neck	Mucosal	Epithelial	Present	Absent	$<$1/HPF	c.1799T $>$ A	p.V600E
18	B72	60	F	Extremities	Acral	Epithelial	Present	Absent	$\geq$1/HPF	c.1799T $>$ A	p.V600E
19	B77	57	F	Extremities	Acral	Epithelial	Present	Absent	$<$1/HPF	c.1799T $>$ A	p.V600E
20	B78	70	M	Extremities	Acral	Epithelial	Present	Absent	$<$1/HPF	c.1799T $>$ A	p.V600E
21	B79	50	F	Extremities	Acral	Epithelial	Present	Absent	$<$1/HPF	c.1799T $>$ A	p.V600E

*NA: Not Available.

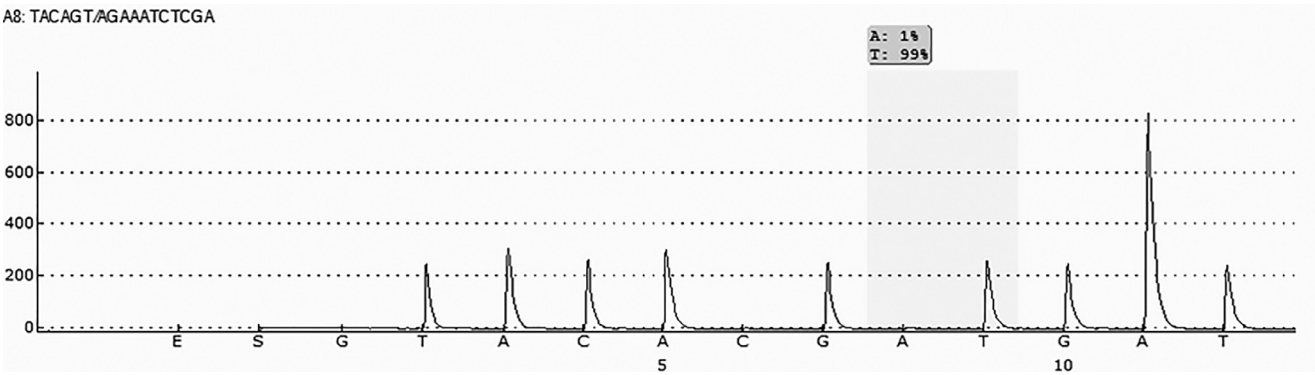

Fig. 1 *Representative Pyrogram depicting a normal or wild-type BRAF sequence:* For detecting V600E mutation due to substitution of GTG by GAG, the pyrosequencing assay was selected with the following sequence in 'Sequence to Analyze': TACAGT/AGAAAT.

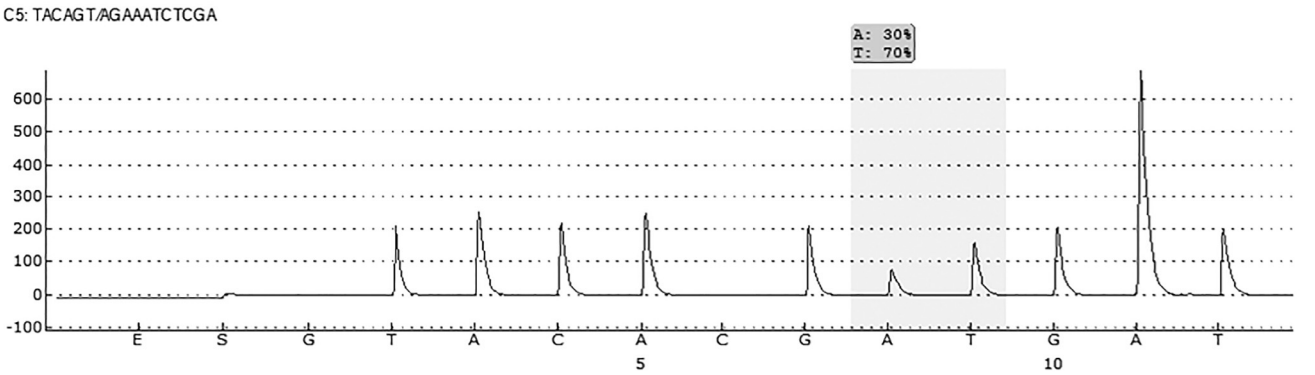

Fig. 2 Representative Pyrogram depicting a heterozygous mutant *BRAF* (V600E) sequence due to substitution of T > A at codon 600.

the most frequent *BRAF* mutation V600E (36%), followed by pleomorphic (22%), and spindle cell (21%). Interestingly, 13 patients with *BRAF* V600E mutations showed evidence of tumor ulceration on microscopic examinations. Although presence of tumor necrosis was lesser in this cohort, 2 out of 6 cases did show presence of *BRAF* V600E mutation. Lastly, *BRAF* V600E mutations were equally distributed in tumors with either < 1 mitosis/10 HPF or ≥ 1/10HPF (30% each, $p = 1.0$).

Discussion

The proto-oncogene *BRAF* is a well-known component of the MEK/MAPK pathway that plays a crucial role in the progression of malignant melanomas by regulating cell proliferation, differentiation and survival [14]. Available literature suggests that nearly 70% of malignant melanomas harbor activating *BRAF* mutations [15]. Among the *BRAF* mutations observed in melanoma, over 90% are at codon 600 resulting in substitution of glutamic acid for valine (p.V600E). Other uncommon mutations are p.V600K, p.V600R and p.V600D which are seen in 5–7% of malignant melanomas [15]. These mutations have been demonstrated to be activated by the RAS guanosine triphosphate (GTP) protein, leading to ERK activation and stimulating the growth of melanoma cells [11,24].

Most of the present understanding of melanoma is derived from the study of patients from populations of Caucasian origin [5,11,17,18], while data from Asian patients is limited [19–21] [Table 3] [1,5–13,18–20,25–44]. In view of no data availability from India, we investigated the frequency and distribution pattern of *BRAF* mutation in malignant melanoma, and set out to compare the data with the existing literature. In the present study, *BRAF* V600E mutations were detected in 30% (21/70) of malignant melanoma, a mutation rate similar to some previous reports from Japan, USA and Italy (26%−34%) [6,28,32], lower than that observed in Swedish, German, and Brazilian studies (53%–70%) [25,27,37], and higher than reports from Korea, Taiwan and China (12%–15%) [20,41,44]. This difference in the frequency can be largely attributed to the sample sizes, melanoma subtypes, geographical location, and methodology used for mutation detection [6,18,20,40,42]. The *BRAF* mutation rate from Caucasian population was higher than in other studies of Asian melanoma patients, which may be due to higher number of superficial spreading melanomas (65%) in their study population [5]. In the current study, around 77% of the total cases were acral and mucosal subtype indicating these two types are predominant subtype in this cohort. Notably, Acral and mucosal types only account for a small proportion of malignant melanomas, but these two are the most common subtypes in Asian population [1,44], including the cases from the current study (77%). This is in sharp contrast to patients from Caucasian origin, wherein, acral and mucosal types are less frequently seen (< 10%) [5,19]. Apart from Acral and Mucosal subtype, chronic sun-induced damage (CSD) and

Table 3 Worldwide reported frequency of *BRAF* mutation in malignant melanomas.

Geographical region	Country	Year	Total cases	*BRAF* mutation (%)	Reference
Western countries	UK	2002	34	66.0	[11]
	USA	2003	77	40.0	[7]
	Italy	2005	35	63.0	[12]
	USA	2005	90	48.0	[8]
	USA	2006	69	57.0	[5]
	Sweden	2006	219	53.0	[25]
	UK	2006	59	37.2	[17]
	Australia	2007	251	45.0	[13]
	Canada	2007	37	24.3	[26]
	Germany	2007	97	55.0	[27]
	Italy	2009	35	34.2	[28]
	Hungary	2009	74	36.5	[29]
	USA	2010	350	49.7	[30]
	Germany	2010	179	20.7	[31]
	USA	2010	138	30.4	[32]
	USA	2011	223	49.0	[33]
	USA	2011	52	44.0	[10]
	Australia	2011	197	48.0	[34]
	USA	2012	150	38.0	[35]
	Germany	2013	187	45.0	[36]
	Brazil	2014	77	70.0	[37]
	Australlia	2016	414	39.0	[38]
	Italy	2017	100	62.0	[39]
	Germany	2017	217	40.0	[18]
Asian countries	Japan	2004	35	26.0	[6]
	Korea	2011	4493	40.0	[19]
	Japan	2011	39	10.2	[9]
	China	2011	180	15.0	[20]
	China	2012	432	25.5	[40]
	Japan	2012	79	25.3	[41]
	China	2012	86	16.3	[42]
	Korea	2013	202	12.0	[43]
	Korea	2014	36	19.4	[44]
	Japan	2015	79	42.0	[45]
	Taiwan	2016	119	14.3	[46]
	Taiwan	2016	73	24.6	[47]
	China	2018	60	23.2	[1]
	India	**2018**	**70**	**30.0**	**Present study**

Non- chronic sun-induced damage (Non-CSD) types are the two tumor subtypes which are reported in the literature [1]. However, we were unable to comment on this due to inadequate information related to this subtype, and represent one of the limitations of the current study. Nevertheless, larger prospective study is warranted to understand the prevalence of CSD and non-CSD subtype in Indian patients. In addition to melanoma subtype and geographical origin of patients, use of different technologies such as Sanger sequencing [32], Pyrosequencing [18], Realtime PCR [42], Single strand conformation polymorphism [27], and High resolution melting [26] may also significantly contribute to the overall difference in the mutation rate. In the current study, we used Pyrosequencing method for *BRAF* mutation detection which enables characterization of mutations and quantification of mutated alleles in samples with low tumor cell density and gives mutation detection with high accuracy rates [18]. The analytical sensitivity of the assay was established using DNA mixing studies, wherein, heterozygous tumor DNA was mixed with normal genomic DNA to get different level of tumor dilutions (100%, 80%, 60% 40%, 30%, 20%, 10% and 0% tumor dilution). The pyrosequencing could clearly detect 5% mutant peak in the 10% tumor dilution indicating the assay to be highly sensitive for detecting BRAF mutations. In comparison to other assay types, pyrosequencing appears to be more sensitive than Sanger sequencing and lesser sensitive than real-time PCR and Next generation sequencing (NGS). Nevertheless, pyrosequencing is less labor intensive and cost effective in comparison to Sanger sequencing, Realtime and NGS based assays.

Consistent with previous findings, this study further confirmed that *BRAF* mutations concentrated in exon 15 (p.V600E) wherein, the valine at amino acid 600 is replaced by glutamate (V600E) through mutation of a single nucleotide (GTG to GAG) [1,44]. However, we did not observe any uncommon mutations (p.V600K and p.V600R) which are seen in 5–6% of malignant melanomas in other study [15]. Correlation of the *BRAF* V600E mutation status with

clinicopathological data suggested that *BRAF* mutation occurred more frequently among patients who were 50 years of age or older when compared to those below 50 years (33% vs. 23%, $p=0.4$). This is in contrast to previous report wherein younger patients tend to show increased frequency of *BRAF* V600E mutation [45]. The mutation rate in female was 33% and that in male was 27% ($p=0.6$), suggesting no sex difference in the mutation rates which is in agreement with previous reports [1,44]. Recent research investigation has revealed that there exist site-specific molecular alterations in malignant melanoma [19]. In this context, findings from our study suggest that the *BRAF* V600E mutation is significantly associated with the melanomas arising on the extremities or head and neck in comparison to trunk, which is in agreement with earlier report [45]. However, differing from these observations, a recent study from Spanish and Austrian case series reported that melanomas arising on the trunk more frequently gave rise to *BRAF*-mutant melanomas and those arising on the head and neck were less likely to harbor *BRAF* mutations [46]. Correlation of *BRAF* V600E mutation with tumor subtype revealed that acral tumors tend to have frequent *BRAF* V600E mutation (31%) than mucosal malignant melanomas (20%). This finding is in contrast to a recent Chinese study, wherein *BRAF* mutation was noted only in 15.7% of the acral tumor subtype. Interestingly, the same study reported similar mutation frequency of 20% in mucosal malignant melanomas [1]. Nevertheless, previous studies have reported overall less *BRAF* mutations in acral lentigous and mucosal melanomas [6,44].

Furthermore, with respect to tumor morphology, it was noted that *BRAF* V600E mutated tumors tend to display a propensity for demonstrating epithelial cell morphology (36%), followed by pleomorphic (22%) and spindle cell (21%) melanomas. Notably, study from Kim and colleagues (2012) reported *BRAF* mutation in 30% of spindle cell melanomas, suggesting much higher mutation rate in Spindle cell tumors in their cohort [47]. Earlier studies have demonstrated that patients with genetic mutations in *BRAF* gene are more likely to have ulceration as compared to patients without *BRAF* mutations [25,34]. Even though statistically insignificant, this notion is further confirmed in this study, which found that patients with ulcerated malignant melanoma had higher *BRAF* V600E mutation rates compared with non-ulcerated tumors. Similar findings have also been previously reported from other Asian country [1]. Interestingly in contrast to this, a recent study from Taiwan reported a reverse finding, wherein, more patients without ulceration had significantly higher *BRAF* mutation rate [44]. There was a trend of increased *BRAF* mutation in tumors associated with necrosis than those without tumor necrosis which differs from another study, which reported increased *BRAF* mutation in tumors without necrosis [48]. Mitotic count is considered to be the one of strongest prognostic factor after tumor thickness in cases with localized melanoma [49]. No significant association of *BRAF* V600E mutation and tumor mitotic count was noted in this study, which is in disagreement from a German series reported a short time ago, wherein, the detection of at least 1 mitosis/mm^2 was associated with mutant *BRAF* in the entire cohort of patients [50].

To summarize, this is the first study to evaluate the frequency and distribution pattern of *BRAF* mutation in Indian melanoma cases. The mutation frequency of 30% in the Indian patients seems to be at par with the worldwide reports. The frequency of *BRAF* V600E mutation varies across different studies, possibly due to sample selection, sample size, and geographical location. Although pathogenetic mechanisms underlying melanoma initiation and evolution are numerous and still largely unidentified, classification of melanoma patients through the assessment of the molecular profile is becoming mandatory. In clinical practice, a potential role of the mutational status as a prognostic factor needs to be investigated in larger Indian patient cohort.

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Declarations of interest

None.

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