

# Low frequency of BRAF V600E immunoexpression in mandibular ameloblastomas: An institutional study

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## Abstract

**Background:** Ameloblastoma is an uncommon, benign neoplasm of odontogenic epithelium commonly affecting the posterior mandible (almost 80%) particularly in the molar/ramus region, with occasional tumors involving the maxilla. Recently, there has been much interest generated after the reports of BRAF V600E mutations in ameloblastomas with a frequency of 46%–80% using both molecular as well as immunohistochemical (IHC) techniques. We sought to assess the presence of BRAF V600E expression in ameloblastomas in Indian patients and correlate the same with clinical behavior and histological variants by performing IHC analysis with anti-BRAF V600E antibody.

**Materials and Methods:** Thirty formalin-fixed paraffin-embedded tissues of mandibular ameloblastomas were examined by anti-BRAF V600E antibody and correlated with clinicopathologic and histological parameters. Cytoplasmic staining of neoplastic epithelium was considered positive for BRAF V600E expression.

**Statistical Analysis:** Data analysis was performed using Chi-square test and Student's *t*-test with statistical software IBM SPSS statistics 20.0.

**Results:** BRAF V600E antibody showed positive expression only in 33.3% (10/30) cases. About 66.7% (4/6) recurrent cases and 63.6% (7/11) plexiform cases showed statistically significant association of  $P = 0.05$  and  $P = 0.021$ , respectively, among cases with positive BRAF V600E immunoexpression.

**Conclusion:** We report the lowest frequency (33.3%) of BRAF V600E immunoexpression in mandibular ameloblastomas in Indian population. However, a significant association of BRAF V600E-positive immunoexpression with recurrence and plexiform pattern could underline its role as a therapeutic marker for ameloblastoma.

**Keywords:** BRAF V600E, immunohistochemistry, mandibular ameloblastomas

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## INTRODUCTION

Ameloblastoma is an uncommon, benign neoplasm of odontogenic epithelium commonly affecting the posterior mandible (almost 80%) particularly in the molar/ramus region, with occasional tumors involving the maxilla.<sup>[1,2]</sup>

The lesions generally advance slowly in a locally infiltrative manner and if unchecked may result in severe morbidity, rarely showing malignant behavior. The median age of presentation is approximately 36 years without any sex predilection.<sup>[2,3]</sup> Radical surgery which is presently an established treatment for ameloblastoma often results

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in facial disfigurement since there is a higher incidence of recurrence after conservative procedures such as enucleation and curettage.<sup>[1,3,4]</sup>

Recently, the WHO has simplified the ameloblastoma classification to ameloblastoma, unicystic ameloblastoma and extraosseous/peripheral types.<sup>[5]</sup> The year 2014 was a defining moment in our knowledge about etiopathogenesis of ameloblastoma since three independent reports identified BRAF V600E as the most prevalent mutation among recurring MAPK mutations in ameloblastoma by genomic analysis and immunohistochemistry. They reported a frequency of 63% (15/24), 64% (54/84) and 46% (13/28), respectively.<sup>[2,4,6,7]</sup> Following this, two more studies of BRAF V600E mutation in ameloblastoma reported a frequency of 80% (4/5) and 46.6% (34/73), respectively, the former using sequencing as well as BRAF V600E immunohistochemistry and latter employing only immunohistochemistry.<sup>[8,9]</sup>

V-raf murine sarcoma viral oncogene homolog B1, BRAF, an oncogene within MAPK pathway, is usually stimulated by somatic point mutation in human cancer. BRAF is a part of RAF family of serine/threonine protein kinases which are constituents of evolutionary highly conserved kinase cascade signaling downstream of RAS, which is activated by growth factors, hormones and cytokines. RAS first stimulates RAF which consecutively activates second protein kinase called MEK, which successively triggers third protein kinase ERK that synchronizes cellular response to extracellular signals. Thus, the RAS-RAF-MEK-ERK-MAP kinase pathway functions as a communicator between extracellular milieu and nucleus. Since BRAF is the most dominant activator of MAP/ERK Kinase (MEK), any dysregulation of this pathway induces tumorigenesis. The RAS/BRAF/MEK/ERK pathway is hyperactivated in ~30% of all cancers with BRAF gene mutations in almost 7% of cancers, identifying it as an important oncogene in this pathway.<sup>[10-13]</sup> BRAF gene mutation is present in cutaneous melanoma (50%), papillary thyroid cancer (46%), borderline ovarian tumor (34%), pleomorphic xanthoastrocytomas (66%), biliary tract tumor (11%), colorectal cancer (10%), nonsmall lung cell cancer (2%) and hairy cell leukemia (100%) among others.<sup>[14]</sup> Benign lesions showing BRAF mutation include melanocytic nevi,<sup>[15]</sup> melanotic neuroectodermal tumor of infancy<sup>[16]</sup> and polyps of the colon<sup>[17]</sup> as well as ameloblastic fibroma and ameloblastic fibro-odontomas.<sup>[6,18]</sup> This mutation causes critical stimulation of BRAF protein and subsequent MEK and ERK signaling, amplifying cell proliferation, survival and eventually neoplastic transformation.<sup>[2]</sup>

The predominant mutation in the BRAF gene involves thymidine to adenosine T>A transversion at exon 15 nucleotide 1799 (T1799>A), resulting in replacement of valine (V) with glutamic acid (E) at position 600 of amino acid sequence (BRAF V600E), accounting for >90% mutations in BRAF.<sup>[4,11,13,19]</sup> There is a significant evidence for activated MAPK pathway being involved in the pathogenesis of ameloblastomas.<sup>[6]</sup> The fact that BRAF V600E expression was readily detectable by immunohistochemistry has diagnostic relevance.<sup>[1]</sup>

Based on the above information, we attempted to study BRAF V600E mutation by performing BRAF V600E immunohistochemistry in mandibular ameloblastomas and correlate the same with clinical behavior and histological subtypes in Indian population.

## MATERIALS AND METHODS

### Sample selection

This study was approved by the Institutional Ethics Committee (EC-48/O PATH-05 ND/2016). We retrieved the formalin-fixed paraffin-embedded (FFPE) and nondecalcified tissue blocks of 30 mandibular ameloblastoma cases from 2011 to 2016 from archives of the Department of Oral Pathology. Demographic data were obtained from the patients' histopathological records [Table 1].

### Immunohistochemical staining

Five micron sections were cut from nondecalcified FFPE tissue block and mounted on SuperFrost slides. Immunohistochemical (IHC) staining was carried out using polymer labeling technique. Sections were dewaxed and washed and antigen retrieval carried out in PT Link module with 1 mM ethylenediaminetetraacetic acid solution (pH 9) for 20 min. Endogenous peroxidase was blocked using 3% hydrogen peroxide in methanol at room temperature for 10 min. Immunostaining was carried out on the Dako autostainer. Sections were washed with phosphate-buffered saline (PBS) briefly and incubated with primary antibody against BRAF V600E (Tinto BRAF V600E Rabbit Monoclonal Antibody, Clone RM8, Bio SB, USA) for 60 min. Sections were washed with PBS and incubated with the EnVision polymer (Dako) for 30 min. Sections were washed with PBS. Diaminobenzidine was used as the chromogen in hydrogen peroxide for 10 min. Sections were then counterstained with Mayer's hematoxylin and mounted. Sections of papillary thyroid carcinoma treated with primary antibody served as positive control. Exclusion of primary antibody served as negative control.

**Table 1: Clinical details and BRAF V600E immunohistochemical status of mandibular ameloblastomas**

Age	Sex	Location	Radiographic appearance	Treatment	Case source	Histopathology	BRAF V600E IHC status
54	Male	Mandible left	Multilocular	Conservative	NR	Follicular	Negative
40	Male	Mandible left	Multilocular	Aggressive	NR	Follicular	Negative
13	Male	Mandible right	Multilocular	Aggressive	NR	Follicular	Negative
16	Male	Mandible right	Unilocular	Conservative	NR	Follicular	Negative
32	Female	Mandible left	Unilocular	Aggressive	NR	Follicular	Negative
23	Male	Mandible left	Multilocular	Conservative	Recurrent	Follicular	Negative
55	Female	Mandible left	Unilocular	Conservative	NR	Follicular	Negative
24	Female	Mandible right	Unilocular	Aggressive	NR	Follicular	Negative
35	Male	Mandible left	Multilocular	Conservative	NR	Follicular	Negative
26	Female	Mandible right	Multilocular	Conservative	NR	Follicular	Negative
54	Male	Mandible right	Multilocular	Aggressive	2 <sup>nd</sup> recurrence	Plexiform	Positive
12	Male	Mandible right	Multilocular	Aggressive	NR	Plexiform	Positive
10	Male	Mandible left	Unilocular	Conservative	NR	Plexiform	Positive
13	Female	Mandible left	Unilocular	Aggressive	NR	Plexiform	Negative
35	Male	Mandible left	Unilocular	Conservative	NR	Plexiform	Positive
24	Male	Mandible right	Unilocular	Aggressive	NR	Plexiform	Positive
15	Female	Mandible left	Unilocular	Conservative	NR	Plexiform	Positive
20	Male	Mandible right	Unilocular	Conservative	NR	Plexiform	Negative
63	Male	Mandible left	Unilocular	Aggressive	NR	Plexiform	Negative
23	Female	Mandible right	Multilocular	Aggressive	Recurrent	Plexiform	Positive
32	Male	Mandible right	Unilocular	Conservative	NR	Plexiform	Negative
36	Male	Mandible left	Multilocular	Aggressive	NR	Desmoplastic	Negative
44	Female	Mandible right	Unilocular	Aggressive	NR	Desmoplastic	Positive
50	Female	Mandible left	Multilocular	Aggressive	NR	Desmoplastic	Negative
60	Male	Mandible left	Multilocular	Aggressive	NR	Desmoplastic	Negative
44	Female	Mandible right	Multilocular	Aggressive	3 <sup>rd</sup> recurrence	Granular	Positive
55	Female	Mandible left	Multilocular	Aggressive	NR	Granular	Negative
20	Female	Mandible right	Multilocular	Aggressive	2 <sup>nd</sup> recurrence	Granular	Negative
32	Male	Mandible right	Multilocular	Aggressive	NR	Granular	Negative
30	Male	Mandible right	Multilocular	Conservative	2 <sup>nd</sup> recurrence	Granular	Positive

IHC: Immunohistochemical, NR: Nonrecurrent

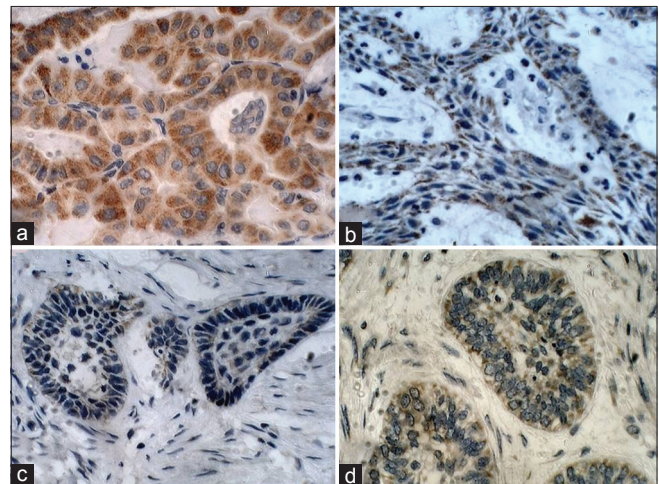
### Immunohistochemical analysis

Immunoreactivity was independently assessed by two authors. Cytoplasmic staining of neoplastic epithelium was considered positive for BRAF V600E expression [Figure 1].

The positive staining intensity and proportion were scored with slight modification of criteria given by Reiner *et al.* and Barnes *et al.*<sup>[20,21]</sup> Intensity was scored by evaluating average intensity of entire tissue section as 0 (no staining), 1 (visible at high-power magnification, ×40), 2 (visible at low-power magnification, ×10) and 3 (visible at scanner view, ×4). The total proportion of cell staining positively at any intensity was scored by screening five fields per tissue section at random as 0 (no cell staining), 1 (when 1%–5% cells stained), 2 (when 6%–25% cells stained), 3 (when 26%–50% cells stained) and 4 (when >50% cells stained). The results of intensity and proportion of cell staining positively were combined to give “quick score” as follows 2 and 3 points = low, 4 and 5 points = intermediate and 6 and 7 points = high positivity for BRAF V600E immunoexpression.<sup>[6,9,20,21]</sup>

### Statistical analysis

Chi-square analysis was used to find the significance of study parameters on categorical scale. Student’s *t*-test



**Figure 1:** Immunohistochemical expression of BRAF V600E, ×40. (a) Positive control-papillary thyroid carcinoma, (b) plexiform variant, (c) desmoplastic variant and (d) granular cell variant of mandibular ameloblastomas

(two-tailed, unpaired) was used to find the significance of study parameters on continuous scale between two groups. Level of significance was fixed at  $P = 0.05$  and any value  $\leq 0.05$  was considered to be statistically significant. The Statistical software IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses of the data.

## RESULTS

We reviewed 30 cases of mandibular ameloblastoma using antibody against BRAF V600E by immunohistochemistry. Table 1 illustrates demographic data with BRAF V600E expression status.

Our results showed a slight male predilection (M:F = 18:12), with a mean age of 33 years. Cases were equally distributed on the right and left sides of the mandible. Radiographically, multilocular pattern (56.7%,  $n = 17$ ) was more common than unilocular (43.3%,  $n = 13$ ). Aggressive treatment (60%,  $n = 18$ ) was preferred over conservative therapy (40%,  $n = 12$ ). Based on specimen source, majority of cases were nonrecurrent (80%,  $n = 24$ ) compared to (20%,  $n = 6$ ) recurrent cases. Histologically, plexiform variant (36.7%,  $n = 11$ ) was most common, followed by follicular (33.3%,  $n = 10$ ), granular (16.7%,  $n = 5$ ) and desmoplastic (13.3%,  $n = 4$ ) variety.

The present study demonstrated only 10 cases (33.3%) showing BRAF V600E-positive cytoplasmic expression in neoplastic epithelium (which included 9 cases staining strongly and 1 case with intermediate staining) whereas 20 cases (66.7%) were BRAF V600E negative.

Of these 10 positive BRAF V600E cases, recurrent source cases showed statistically significant association ( $P = 0.05$ ) with BRAF V600E expression as 66.7% (4/6) of recurrent source cases were BRAF V600E positive compared to only 25% (6/24) cases positive from nonrecurrent source. Statistically significant association ( $P = 0.021$ ) of BRAF V600E expression was observed on comparing histological variants of ameloblastoma as plexiform type had 63.6% (7/11) positive cases, followed by granular type with 40% (2/5) positive cases and desmoplastic type showing 25% (1/4) positive cases. Surprisingly, all cases of follicular type (10/10) were negative for BRAF V600E expression.

The present study examined five cases of granular cell ameloblastoma for BRAF V600E immunopositivity of which three were from recurrent source. Among these three recurrent cases, two expressed BRAF V600E.

Statistical analysis of clinicopathological features and BRAF V600E immunopositivity is summarized in Table 2.

## DISCUSSION

Ameloblastoma is a remarkably rare, benign epithelial odontogenic tumor which is locally invasive and highly recurrent, seldom displaying malignant behavior.<sup>[22]</sup> The

**Table 2: Analysis of clinicopathological data and BRAF V600E expression in mandibular ameloblastomas**

Features	Cases, $n$ (%)	BRAF V600E status		$P$
		Positive (%)	Negative (%)	
Sex				
Male	18 (60)	6 (33.3)	12 (66.7)	1.0
Female	12 (40)	4 (33.3)	8 (66.7)	
Total	30 (100)	10 (33.3)	20 (66.7)	
Mean age (years)				
<33	17 (56.67)	6 (35.3)	11 (64.7)	0.79
≥33	13 (43.33)	4 (30.8)	9 (69.2)	
Total	30 (100)	10 (33.3)	20 (66.7)	
Radiographic appearance				
Unilocular	13 (43.3)	5 (38.5)	8 (61.5)	0.60
Multilocular	17 (56.7)	5 (29.4)	12 (70.6)	
Total	30 (100)	10 (33.3)	20 (66.7)	
Treatment				
Aggressive	18 (60)	6 (33.3)	12 (66.7)	1.0
Conservative	12 (40)	4 (33.3)	8 (66.7)	
Total	30 (100)	10 (33.3)	20 (66.7)	
Specimen source				
Nonrecurrent	24 (80)	6 (25)	18 (75)	0.05*
Recurrent	6 (20)	4 (66.7)	2 (33.3)	
Total	30 (100)	10 (33.3)	20 (66.7)	
Histopathological variant				
Follicular	10 (33.3)	0	10 (100)	0.021*
Plexiform	11 (36.7)	7 (63.6)	4 (36.4)	
Desmoplastic	4 (13.3)	1 (25)	3 (75)	
Granular	5 (16.7)	2 (40)	3 (60)	
Total	30 (100)	10 (33.3)	20 (66.7)	

\*Statistically significant

current molecular approach of understanding odontogenic tumors has provided insights into their evolution and molecular pathogenesis.<sup>[12]</sup> Most of the odontogenic tumors including ameloblastomas show monoclonal pattern suggesting their origin from one altered clone of odontogenic cells.<sup>[22,23]</sup>

Since 2014, few studies on ameloblastomas have reported BRAF V600E mutations ranging from 46% to 80%.<sup>[4,6-9]</sup> Although these studies employed molecular techniques, they also evaluated the usefulness of immunohistochemistry using anti-BRAF V600E antibody in ameloblastomas and unequivocally substantiated that BRAF V600E immunopositivity significantly correlated with BRAF V600E mutation status in ameloblastomas.<sup>[4,6-8]</sup>

Excellent concurrence of BRAF V600E immunohistochemistry with gene mutation has been established as the presence of mutated protein can be detected at a single cell level.<sup>[6,24]</sup>

Detailed review of exclusively IHC data of ameloblastomas in previous studies revealed that 11/20 (55%) cases of Kurppa *et al.*,<sup>[4]</sup> 47/77 (61%) cases of Brown *et al.*,<sup>[6]</sup> 12/28 (42.9%) cases of Sweeney *et al.*,<sup>[7]</sup> and 4/5 (80%) cases of Chang *et al.*<sup>[8]</sup> were positive for BRAF V600E expression.

Similar to the present study, Fregnani *et al.*<sup>[9]</sup> reported a positive frequency in 34/73 (46.6%) cases using only BRAF V600E immunohistochemistry. We observed a positive BRAF V600E immunoexpression in 10/30 (33.3%) cases of mandibular ameloblastomas as summarized in Table 3.

Kurppa *et al.*<sup>[4]</sup> found no significant association of BRAF V600E mutation with age or sex, tumor histology and tumor recurrence. Brown *et al.*<sup>[6]</sup> observed a significant correlation of BRAF V600E mutations with younger age, but we did not obtain any significant association between age and sex with BRAF V600E expression, a finding coinciding with Kurppa *et al.*<sup>[4]</sup>

Most of the previous studies on BRAF V600E mutation also had majority of cases from mandibular region. Similar to Kurppa *et al.*,<sup>[4]</sup> we only had mandibular cases for the present study. Brown *et al.*<sup>[6]</sup> and Sweeney *et al.*<sup>[7]</sup> reported that BRAF V600E mutation showed greater predilection for the mandible. However, Fregnani *et al.*<sup>[9]</sup> and Diniz *et al.*<sup>[12]</sup> found no association of BRAF mutation and tumor location.

Fregnani *et al.*<sup>[9]</sup> reported a significant association of BRAF V600E expression with multilocular radiographic appearance, a finding not consistent with our results.

Contrary to Brown *et al.*<sup>[6]</sup> who reported that earlier recurrences were associated with BRAF-negative cases, Fregnani *et al.*<sup>[9]</sup> demonstrated that positive BRAF V600E immunoexpression significantly correlated with the presence of recurrences, a finding in accordance with our study.

Kurppa *et al.*,<sup>[4]</sup> Fregnani *et al.*,<sup>[9]</sup> and Diniz *et al.*<sup>[12]</sup> found no significant association between BRAF V600E and histological pattern. An analysis of data of Brown *et al.*<sup>[6]</sup> and Sweeney *et al.*<sup>[7]</sup> showed plexiform histology to be significantly common among BRAF-negative tumors. Chang *et al.*<sup>[8]</sup> reported strong association of BRAF V600E expression with follicular type of ameloblastomas. However, we found a significant correlation ( $P = 0.021$ ) of BRAF V600E expression with plexiform type of ameloblastoma which is in complete contrast to findings of all previous studies.

Our results indicate the possible role of BRAF V600E immunohistochemistry as a predictive marker for mandibular ameloblastoma recurrence.

An interesting finding of the present study is the lowest frequency of BRAF V600E positive immunoexpression in mandibular ameloblastomas

**Table 3: Previous studies of BRAF V600E frequency in ameloblastomas**

Author; cases; location Mandible/ maxilla/NA	Histopathological variant						Specimen source NR/recurrent/ residual/NA	BRAF V600E positive status	
	Follicular	Plexiform	Desmoplastic	Granular cell	Mixed	Other types/ Histology NA		Molecular analysis, n (%)	V600E IHC, n (%)
Kurppa <i>et al.</i> <sup>[4]</sup> Cases=24 Mandible=24	12	11	-	-	1	-	NR=16 Recurrent=7 Residual=1	15/24 (63)	11/20 (55%)
Brown <i>et al.</i> <sup>[6]</sup> Cases=84 Mandible=68 Maxilla=16	37	34	6	-	-	7 (Unicystic=5 Peripheral=1 Metastatic=1)	-	31/50 (62)	54/84 (64%) Multicystic=47/77 (61.03%) Unicystic=5/5 Peripheral=1/1 Metastatic=1/1 12/28 (42.9%)
Sweeney <i>et al.</i> <sup>[7]</sup> Cases=28 Mandible=14 Maxilla=11 Other jaw region=3	8	10	3	-	2	Histology NA=5	NR=10 Recurrent=8 NA=10	13/28 (46) 12 cases V600E 1 case L597R	-
Diniz <i>et al.</i> <sup>[12]</sup> Cases=17 Mandible=13 Maxilla=4	6	1	2	1	-	Unicystic=6/ Histology NA=1	NR=9 NA=8	14/17 (82) Multicystic=7/9 (78) Unicystic=5/6 (83) Desmoplastic=2/2 4/5 (80)	-
Chang <i>et al.</i> <sup>[8]</sup> Cases=5	3	1			1			4/5 (80)	4/5 (80%)
Fregnani <i>et al.</i> <sup>[9]</sup> Cases=73 Mandible=63 Maxilla=10			Plexiform=42 Nonplexiform=31				NR=58 Recurrent=15	-	34/73 (46.6%)
Present study Cases=30 Mandible=30	10	11	4	5			NR=24 Recurrent=6		10/30 (33.33%)

NA: Not available, NR: Nonrecurrent, IHC: Immunohistochemical

reported till now at 33.3% as compared to previous studies (range : 46% - 80%).<sup>[4,6,7,8,9]</sup> However, BRAF V600E immunopositivity showed a significant association with recurrent cases and plexiform histology. Granular cell ameloblastomas, second most common for BRAF V600E-positive immunoexpression, might have some yet undetermined association yet to be proven statistically significant.

Since there were no data on BRAF V600E mutation in the Indian population, we made a sincere attempt to study the same. To our surprise, we found the lowest frequency of BRAF V600E immunoexpression in our study. Previous studies have reported differing mutation frequencies ranging from 46% to 80% in different populations, and our study could represent the lowest end spectrum of BRAF V600E expression in Indian population. Only using IHC method for identification of BRAF-mutated protein in a small sample size could have resulted in low expression in Indian cohort. Cells having BRAF V600E-mutated protein below levels detectable by immunohistochemistry can also be the reason for the lowest frequency of BRAF V600E expression in our study. Furthermore, negative IHC expression probably may arise from loss of mutant antigen expression.<sup>[25]</sup> We evaluated BRAF V600E expression by stringent scoring criteria, which could be one more reason for the lowest frequency of BRAF V600E expression in our study.

The present study was an IHC study to determine BRAF V600E immunoexpression in Indian population. To the best of our knowledge, this is the first study to be carried out in Indian population. We do feel that employing molecular techniques may have probably influenced observed frequency of BRAF V600E expression in our study. Mutation at the DNA level without expression at the mRNA level is a common event, reported by the Cancer Genome Atlas studies which may have caused low BRAF V600E expression.<sup>[26]</sup> Furthermore, the possibility of ethnic and geographic variations having a role in low expression of BRAF V600E in Indian population cannot be entirely ruled out.

We want to highlight some potential limitations commonly associated with retrospective studies. Our study included only mandibular ameloblastomas as our institute receives very few maxillary, unicystic and peripheral ameloblastoma cases. Some previous studies<sup>[6,8,24]</sup> have demonstrated a perfect match of BRAF V600E immunoexpression with genetic BRAF detection, but we were unable to carry out BRAF V600E mutational analysis. Although few studies have reported discordance among the IHC data and molecular assays in melanomas,<sup>[27,28]</sup> colorectal carcinoma<sup>[26]</sup>

as well as unicystic ameloblastomas,<sup>[29]</sup> the molecular characterization of ameloblastoma for BRAF status has rationalized ameloblastoma treatment using BRAF inhibitors, vemurafenib and dabrafenib,<sup>[30,31]</sup> justifying BRAF V600E as therapeutic marker.

## CONCLUSION

The present study demonstrated the lowest frequency of positive BRAF V600E immunoexpression (33.3%) compared to other studies till date; hence, the dependency only on immunohistochemistry for assessing BRAF V600E mutational status in mandibular ameloblastomas remains questionable.

Based on our findings, we suggest that BRAF V600E immunopositive mandibular ameloblastoma cases should be kept under strict follow-up as there are greater chances of recurrence, especially in plexiform and granular variants.

With readily available material for BRAF V600E immunohistochemistry, extensive data on clinicopathological relation will be available for review in due course of time. Future multicentric studies with large sample size are required to substantiate the role of BRAF V600E expression as a reliable therapeutic and predictive marker for ameloblastomas.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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