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BRAF p.V600E Mutational Status Does Not Correlate with Biological Behavior in Conventional Ameloblastomas: A Disease-Free Survival Analysis

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Abstract

Background Dysregulation of the MAPK pathway appears to exert a pivotal role in the pathogenesis of ameloblastomas, since *BRAF* p.V600E has been reported in over 65% of the tumors. Therefore, the purpose of this study was to investigate whether the *BRAF* p.V600E is related to biological behavior and disease-free survival in patients with conventional ameloblastomas.

Methods This is a retrospective cohort study based on the STROBE (*Strengthening the Reporting of Observational Studies in Epidemiology*) recommendations. The study population consisted of individuals treated for conventional ameloblastomas. Clinical, imaging, histomorphological, immunohistochemical (Ki67 and CD138/syndecan-1), and molecular *BRAF* p.V600E mutation analyses were performed. Bivariate statistical analysis was performed through chi-square and Fisher's exact tests. Kaplan–Meier analysis with log-rank test and Cox proportional hazards regression were used to identify predictors of disease-free survival, with a significance level of 5%.

Results Forty-one individuals were included, with a male-to-female ratio of 1.15:1. *BRAF* p.V600E mutation was identified in 75.6% of the tumors. No association between the *BRAF* mutational status and other clinical, imaging, histomorphological, and immunohistochemical variables was observed. Only the initial treatment modality was significantly associated with a better prognosis in univariate ($p = 0.008$) and multivariate ($p = 0.030$) analyses, with a hazard ratio of 9.60 (95%IC = 1.24–73.89), favoring radical treatment.

Conclusion *BRAF* p.V600E mutation emerges as a prevalent molecular aberration in ameloblastomas. Nevertheless, it does not seem to significantly affect the tumor proliferative activity, CD138/syndecan-1-mediated cell adhesion, or disease-free survival outcomes.

Keywords Ameloblastoma · *BRAF* p.V600E · Antigen Ki67 · Syndecan-1 · Disease-free survival · Survival analysis

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Introduction

Ameloblastoma is a benign epithelial odontogenic tumor and represents one of the most clinically relevant neoplasms of odontogenic origin [1, 2]. In accordance with the 5th Edition of the Classification of Head and Neck Tumors by the World Health Organization (WHO) [1], this neoplastic entity is categorized into three variants with distinct clinical behaviors: conventional, unicystic, and peripheral. Notably, the conventional ameloblastoma stands as the most prevalent among these variants. Although benign, conventional ameloblastoma is a locally aggressive lesion with the potential to infiltrate and destroy adjacent tissues, and associated with high rates of recurrence [1, 2].

The molecular basis for the pathogenesis of ameloblastomas is not fully understood. Dysregulation of the mitogen-activated protein kinase (MAPK) pathway appears to exert a pivotal role in its development [1–3], since a missense activating mutation in the RAF proto-oncogene, specifically identified as *BRAF* p.V600E, has been reported in over 65% of ameloblastomas [4, 5]. This mutation results in the synthesis of an altered protein that disrupts cell signaling within the MAPK pathway and might compromise important cellular processes, such as growth, proliferation, and differentiation [6]. Interestingly, *BRAF* p.V600E has emerged as a driver in a number of neoplastic processes, and why this particular variant is such a ubiquitous driver is yet unexplained [4, 6].

Therefore, the purpose of the present study was to investigate whether the *BRAF* p.V600E mutational status is related to biological behavior and disease-free survival in patients with conventional ameloblastomas.

Materials and Methods

Study Design

This is a retrospective cohort study based on the STROBE (*Strengthening the Reporting of Observational Studies in Epidemiology*) recommendations for cohort studies [7] and approved by the Ethics Committee of the University of Pernambuco (#4.309.512; CAAE: 35920620.7.0000.5192).

Study Population

The study population was composed of individuals who underwent surgical treatment for ameloblastomas at the Department of Oral and Maxillofacial Surgery of a tertiary care hospital located in northeast Brazil (Recife, Pernambuco, Brazil), between January 2013 and December 2022. Potential participants were identified from medical records, selected for review, and assessed for eligibility.

This study included individuals of both sexes, all ages, a confirmed diagnosis of conventional-type ameloblastoma, and with a minimum postoperative follow-up period of

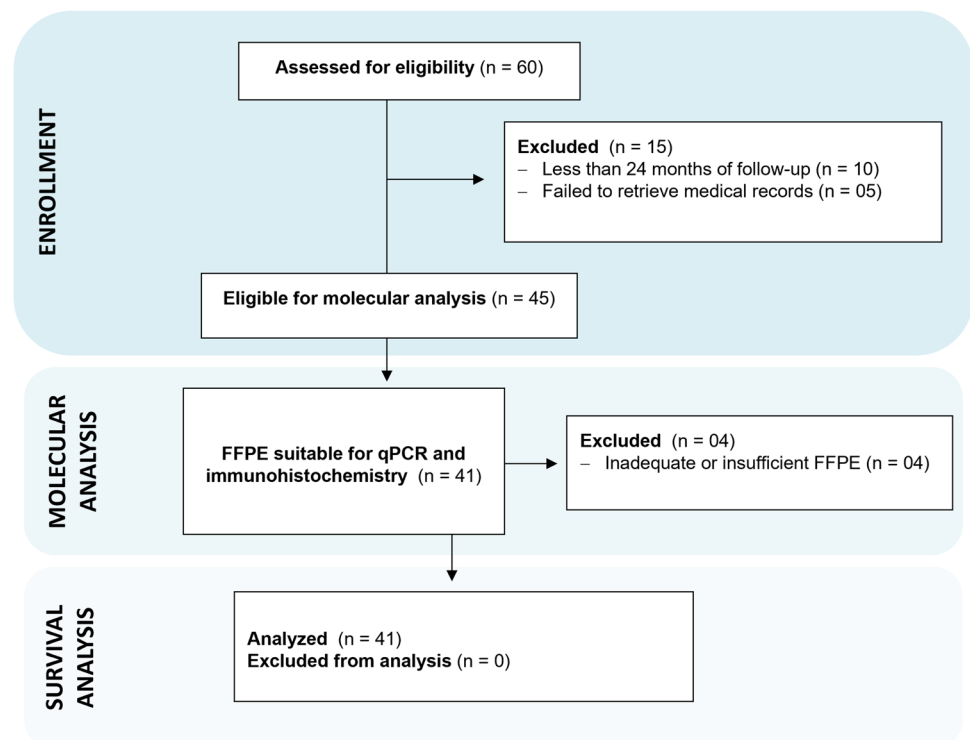**Fig. 1** Flow diagram of the study participants

Table 1 Clinical characteristics and follow-up of the study participants

Age at diagnosis	Years		
Age range	11–74		
Mean ± SD	34.51 ± 17.73		
		n	*%*
Sex			
Female		19	46.3
Male		22	53.7
Skin color			
Brown		30	73.2
White		07	17.1
Black		04	9.7
Anatomic location of the tumor			
Mandible		40	97.6
Maxilla		01	2.4
Symptomatology			
No		31	75.6
Yes		10	24.4
Initial surgical treatment			
Conservative		27	65.9
Radical		14	34.1
Episodes of recurrence			
No		22	53.7
Yes		19	46.3
	Months		
Time until the first recurrence			
Minimum–Maximum	12–120		
Median	47		
Mean time ± SD	46.63 ± 30.63		
Total follow-up			
Minimum–Maximum	24–318		
Median	41		
Mean time ± SD	77.22 ± 74.08		

SD standard deviation

24 months to control for possible late recurrence episodes, since ameloblastoma is a slow-growing tumor. Cases in which there was absence or inconsistency in the medical records data, or in which the biological material available was insufficient or inadequate to carry out the proposed molecular and immunohistochemical analyses were excluded from the study.

Clinical and Imaging Analysis

Demographic (gender, age, and skin color) and clinical data related to the tumor (associated symptoms, time of evolution, history of treatments, recurrences, and follow-up time) were retrospectively retrieved from medical records. For analytical purposes, surgical procedures were categorized as radical (e.g., marginal, partial or total resections) or conservative (e.g., enucleation and/or curettage, with or without adjuvant or neoadjuvant methods).

Digital images from computed tomography scans and/or panoramic radiographs archived at the hospital records were used for the imaging analysis. The images were analyzed by two oral and maxillofacial surgeons with experience in interpreting head and neck imaging exams (FACA and TFF). The following imaging aspects were assessed: anatomical location and extent of the lesion, locularity, margins, expansion and resorption of cortical bone, displacement or involvement of teeth, and resorption of adjacent tooth roots.

Table 2 Imaging, histomorphological, and immunohistochemical characteristics of the ameloblastomas included in the study

	n	%
Radiographic locularities		
Unilocular	11	26.8
Multilocular	30	73.2
Imaging margins		
Well-defined	33	80.5
Irregular	08	19.5
Imaging characteristics		
Cortical bone expansion	39	95.1
Cortical bone perforation	40	97.6
Adjacent teeth displacement	33	80.5
Root resorption	28	68.3
Impacted teeth	08	19.5
Histological subtype		
Plexiform	15	36.6
Follicular	13	31.7
Acanthomatous	08	19.5
Basal cell	05	12.2
Cell proliferation index (Ki67 nuclear immunoexpression)	%	
Range	0.00–14.52	
Mean ± SD	5.07 ± 3.19	
Cell proliferation index (Ki67 nuclear immunoexpression)		
< 5%	25	61.0
≥ 5% and < 10%	13	31.7
≥ 10%	03	7.3
CD138/Syndecan-1 immunoexpression		
Positive in neoplastic epithelium	24	58.5
Positive in tumor stroma	23	56.1

SD standard deviation

Histomorphological Analysis

For each included case, slides with 5 µm-thick histological sections of formalin-fixed, paraffin-embedded (FFPE) tumor tissue stained with hematoxylin and eosin were analyzed by two researchers with experience in Oral and Maxillofacial Pathology (AVMB and MVC) to confirm the diagnosis. The histological subtype was defined according to WHO criteria [1], and the cases were classified as follicular, plexiform, acanthomatous, granular cell, basal cell or desmoplastic.

Immunohistochemical Analysis of Proliferation and Invasiveness Markers

Immunohistochemical analysis was performed to analyze the expression of Ki67 and CD138/Syndecan-1. For this purpose, 3-µm-thick histological sections of the FFPE tumor tissue were submitted to automated processing for the immunohistochemical reaction on the Ventana BenchMark XT immunostainer (Ventana Medical Systems), using monoclonal primary antibodies specific for the Ki67 (clone 30-9)

(Ventana Medical Systems) and CD138/syndecan-1 (clone B-A38) (Cell-Marque) proteins, following the manufacturer's instructions. Diaminobenzidine (DAB) was used as the chromogen for the reaction using a biotin-free detection system, and counterstaining was performed with Mayer's hematoxylin. Positive controls (Ki67: lymph node; CD138/syndecan-1: oral mucosa) and a negative control (omission of the primary antibody) were carried out for each reaction.

For the analysis of Ki67 nuclear protein immunoexpression and calculation of the cell proliferation index, five randomly selected representative fields were photographed at ×400 magnification. The cell proliferation index was calculated for each case as the total percentage of neoplastic cells with positive nuclear immunostaining, counted in the photomicrographs using the cell counting tool in the ImageJ software [8].

The CD138 protein immunoexpression was analyzed semi-quantitatively at a magnification of 100x. The estimated percentage of neoplastic cells and stromal cells that showed positive immunostaining on the cell membrane was used to classify the cases according to the criteria
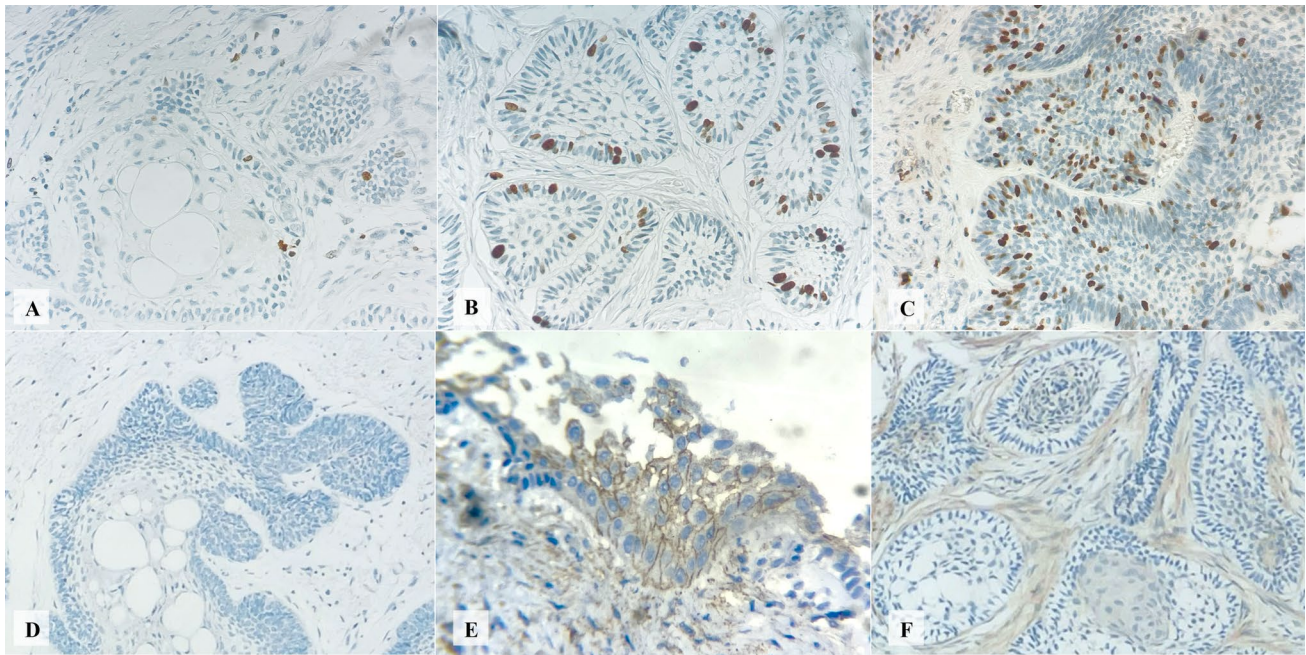
Fig. 2 Photomicrographs showing variations in the immunoexpression of Ki67 (**A**, **B**, **C**) and CD138/syndecan-1 (**D**, **E**, **F**) in ameloblastomas. A, low (> 1%) Ki67 nuclear immunoexpression (×250). B, medium (between 1 and 10%) Ki67 nuclear immunoexpression (×250). C, High (> 10%) Ki67 nuclear immunoexpression (×250). D, Absence of CD138/syndecan-1 immunoexpression (×250). E, CD138/syndecan-1 immunoexpression in neoplastic epithelial cell membrane (×400). F, CD138/syndecan-1 immunoexpression in tumor stroma (×250)

established by Nadalin et al. [9] as: negative, when the percentage of immunostaining was < 5% of the cells; or positive, when there was positive immunostaining in more than 5% of the cells.

BRAF p.V600E Mutation Detection in Ameloblastoma Samples

From each included case, 10 μm sections of FFPE tumor tissue were processed using the MagMax™ FFPE DNA/RNA Ultra Kit (Applied Biosystems Thermo Fisher Scientific™), according to the manufacturer's instructions, to isolate genomic DNA. The double-stranded DNA concentration and purity were measured in NanoDrop Lite spectrophotometer (Thermo Scientific™) and stored at − 20 °C until molecular analysis.

BRAF p.V600E detection in DNA samples was performed by real-time PCR using 40 ng of template DNA combined with castPCR™ somatic mutation detection assays containing TaqMan™ probes specific for the c.1799 T > A mutant *BRAF* allele (BRAF_476_mu) and for the reference *BRAF* gene (BRAF_rf) (Applied Biosystems Thermo Fisher Scientific™). The PCR amplification conditions followed the manufacturer's recommendations. Amplification curves of cfDNA and genomic DNA reactions were separated and imported to Mutation Detector™ software (Thermo Fisher Life Technologies), where the mutational status of the *BRAF* gene was analyzed. Positive control (confirmed *BRAF* p.V600E template DNA) and No Template Control (NTC) were included in the reactions.

Statistical Analysis

The database was built on the SPSS® software platform in version 20.0.0. Descriptive and inferential statistics were analyzed to associate the *BRAF* p.V600E mutation with categorical predictive variables using the chi-square test or Fisher's exact test, when applicable.

Kaplan–Meier survival analysis was used to estimate disease-free survival (DFS) rates, considering the first tumor recurrence as the main event. Patients who were alive and disease-free were censored at the time of the last follow-up contact recorded in the hospital records. For statistical purposes, the quantitative variables were dichotomized based on the median in this analysis. Statistical differences between Kaplan–Meier curves for disease-free survival in relation to *BRAF* p.V600E mutational status and other clinicopathological characteristics were analyzed using the log-rank test. Prognostic variables with a *p* value < 0.20 in the univariate analysis were introduced into a multivariate Cox proportional hazards regression model to identify independent

Table 3 Inferential statistical analysis of *BRAF* p.V600E mutation in ameloblastomas

	BRAF wild-type	*BRAF V600E*	*p* value
Sex			0.469
Female	06 (31.6%)	13 (68.4%)	
Male	04 (18.2%)	18 (81.8%)	
Age at diagnosis			1.000
≤ 28 years	05 (23.8%)	16 (76.2%)	
> 28 years	05 (25.0%)	16 (75.0%)	
Skin color			0.660
White	01 (14.3%)	06 (85.7%)	
Brown and black	09 (26.5%)	25 (73.5%)	
Anatomic location of the tumor			1.000
Mandible	10 (25.0%)	30 (75.0%)	
Maxilla	00 (0.0%)	01 (100.0%)	
Symptomatology			0.683
No	07 (22.6%)	24 (77.4%)	
Yes	03 (30.0%)	07 (70.0%)	
Radiographic localities			0.413
Unilocular	04 (36.4%)	07 (63.6%)	
Multilocular	06 (20.0%)	24 (80.0%)	
Imaging margins			0.378
Well-defined	07 (21.2%)	26 (78.8%)	
Irregular	03 (37.5%)	05 (62.5%)	
Cortical bone expansion			0.433
No	01 (50.0%)	01 (50.0%)	
Yes	09 (23.1%)	30 (76.9%)	
Cortical bone perforation			0.244
No	01 (100.0%)	00 (0.0%)	
Yes	09 (22.5%)	31 (77.5%)	
Adjacent teeth displacement			0.653
No	01 (12.5%)	07 (87.5%)	
Yes	09 (27.3%)	24 (72.7%)	
Root resorption			1.000
No	03 (23.1%)	10 (76.9%)	
Yes	07 (25.0%)	21 (75.0%)	
Impacted teeth			0.082
No	06 (18.2%)	27 (81.8%)	
Yes	04 (50.0%)	04 (50.0%)	
Histological subtype			0.198
Plexiform	04 (26.7%)	11 (73.3%)	
Follicular	02 (15.4%)	11 (84.6%)	
Acanthomatous	01 (12.5%)	07 (87.5%)	
Basal cell	03 (60.0%)	02 (40.0%)	
Ki67 cell proliferation index			0.237
< 5%	08 (32.0%)	17 (68.0%)	
≥ 5% and < 10%	01 (7.7%)	12 (92.3%)	
≥ 10%	01 (33.3%)	02 (66.7%)	
CD138/syndecan-1 in neoplastic epithelium			0.480
Positive	07 (29.2%)	17 (70.8%)	
Negative	03 (17.6%)	14 (82.4%)	
CD138/syndecan-1 in tumor stroma			1.000
Positive	06 (26.1%)	17 (73.9%)	
Negative	04 (22.2%)	14 (77.8%)	

Table 4 Inferential statistical analysis of recurrence and treatment modality in ameloblastomas

	Non-recurrent	Recurrent	p value	Conservative treatment	Radical treatment	p value
Sex			1.000			1.000
Female	10 (52.6%)	09 (47.4%)		13 (68.4%)	06 (31.6%)	
Male	12 (54.5%)	10 (45.5%)		14 (63.6%)	08 (36.4%)	
Age at diagnosis			0.536			0.078
≤ 28 years	10 (47.6%)	11 (52.4%)		17 (81.0%)	04 (19.0%)	
> 28 years	12 (60.0%)	08 (40.0%)		10 (50%)	10 (50%)	
Skin color			1.000			1.000
White	04 (57.1%)	03 (42.9%)		05 (71.4%)	02 (28.6%)	
Brown and black	18 (52.9%)	16 (47.1%)		22 (64.7%)	12 (35.3%)	
Anatomic location of the tumor			0.463			0.341
Mandible	22 (55.0%)	18 (45.0%)		27 (67.5%)	13 (32.5%)	
Maxilla	00 (0.0%)	01 (100.0%)		00 (0.0%)	01 (100.0%)	
Symptomatology			0.727			0.064
No	16 (51.6%)	15 (48.4%)		23 (74.2%)	08 (25.8%)	
Yes	06 (60.0%)	04 (40.0%)		04 (40.0%)	06 (60.0%)	
Radiographic locularities			0.499			0.064
Unilocular	07 (63.6%)	04 (36.4%)		10 (90.9%)	01 (9.1%)	
Multilocular	15 (50.0%)	15 (50.0%)		17 (56.7%)	13 (43.3%)	
Imaging margins			0.249			0.012
Well-defined	16 (48.5%)	17 (51.5%)		25 (75.8%)	08 (24.2%)	
Irregular	06 (75.0%)	02 (25.0%)		02 (25.0%)	06 (75.0%)	
Cortical bone expansion			1.000			1.000
No	01 (50.0%)	01 (50.0%)		01 (50.0%)	01 (50.0%)	
Yes	21 (53.8%)	18 (46.2%)		26 (66.7%)	13 (33.3%)	
Cortical bone perforation			1.000			1.000
No	01 (100.0%)	00 (0.0%)		01 (100.0%)	00 (0.0%)	
Yes	21 (52.5%)	19 (47.5%)		26 (65.0%)	14 (35.0%)	
Adjacent teeth displacement			1.000			0.411
No	04 (50.0%)	04 (50.0%)		04 (50.0%)	04 (50.0%)	
Yes	18 (54.5%)	15 (45.5%)		23 (69.7%)	10 (30.3%)	
Root resorption			0.313			0.734
No	05 (38.5%)	08 (61.5%)		08 (61.5%)	05 (38.5%)	
Yes	17 (60.7%)	11 (39.3%)		19 (67.9%)	09 (32.1%)	
Impacted teeth			0.249			1.000
No	16 (48.5%)	17 (51.5%)		22 (66.7%)	11 (33.3%)	
Yes	06 (75.0%)	02 (25.0%)		05 (62.5%)	03 (37.5%)	
Histological subtype			0.619			0.260
Plexiform	09 (60.0%)	06 (40.0%)		11 (73.3%)	04 (26.7%)	
Follicular	05 (38.5%)	08 (61.5%)		10 (76.9%)	03 (23.1%)	
Acanthomatous	05 (62.5%)	03 (37.5%)		03 (37.5%)	05 (62.5%)	
Basal cell	03 (60.0%)	02 (40.0%)		03 (60.0%)	02 (40.0%)	
Ki67 cell proliferation index			0.233			0.427
< 5%	12 (48.0%)	13 (52.0%)		16 (64.0%)	09 (36.0%)	
≥ 5% and < 10%	07 (53.8%)	06 (46.2%)		08 (61.5%)	05 (38.5%)	
≥ 10%	03 (100.0%)	00 (0.0%)		03 (100.0%)	00 (0.0%)	
CD138/syndecan-1 in neoplastic epithelium			0.216			0.742
Positive	15 (62.5%)	09 (37.5%)		15 (62.5%)	09 (37.5%)	
Negative	07 (41.2%)	10 (58.8%)		12 (70.6%)	05 (29.4%)	
CD138/syndecan-1 in tumor stroma			1.000			1.000
Positive	12 (52.2%)	11 (47.8%)		15 (65.2%)	08 (34.8%)	

Table 4 (continued)

	Non-recurrent	Recurrent	*p* value	Conservative treatment	Radical treatment	*p* value
Negative	10 (55.6%)	08 (44.4%)		12 (66.7%)	06 (33.3%)	
BRAF mutational status			0.075			0.267
BRAF p.V600E	14 (45.2%)	17 (54.8%)		22 (71.0%)	09 (29.0%)	
BRAF wild-type	08 (80.0%)	02 (20.0%)		05 (50.0%)	05 (50.0%)	

predictors of DFS and calculate their hazard ratio (HR) and respective 95% confidence intervals. A statistical significance level of 5% ($p \leq 0.05$) was established.

Results

Clinicopathological Characteristics of Study Participants

The flow diagram of the study participants is shown in Fig. 1. This study included 41 individuals diagnosed with conventional ameloblastoma. The mean age at diagnosis was 34.51 ± 17.73 years, ranging from 11 to 74 years. The tumors were more frequent in brown-skinned individuals (73.2%), with a male-to-female ratio of 1.15:1. Most cases of ameloblastomas were asymptomatic (75.6%) and located in the mandible (97.6%), with only one case affecting the maxilla (Table 1).

The imaging, histomorphological, and immunohistochemical characteristics of the cases are described in Table 2. Multilocular appearance, well-defined margins, expansion and disruption of cortical bone, displacement and root resorption of adjacent teeth were common findings in most ameloblastomas.

Plexiform and follicular were the most frequent histological subtypes, comprising 36.6% and 31.7% of cases, respectively. The cell proliferation index, calculated according to the nuclear immunoexpression of the Ki67 protein, ranged from 0% to 14.52%, with a mean of $5.07\% \pm 3.19$. In most cases, immunohistochemical expression of the CD138/syndecan-1 protein was positive in the neoplastic epithelium (58.5%) and/or in the tumor stroma (56.1%). Variations in immunoexpression of Ki67 and CD138/syndecan-1 are illustrated in Fig. 2.

BRAF p.V600E Mutational Analysis

The *BRAF* p.V600E mutation was detected in 31 out of 41 ameloblastomas, accounting for 75.6% of the tumors. No association between the mutation and other clinical, imaging, histomorphological, and immunohistochemical variables was observed, as shown in Table 3.

History of Treatments and Clinical Outcomes

Among the included cases, 27 (65.9%) were initially treated with conservative surgical approaches, while the other 14 (34.1%) received radical surgical resection. Nineteen individuals developed tumor recurrence during the follow-up period, with a recurrence rate of 46.3%. However, only one of the individuals treated with radical surgery had recurrence. The time until the first recurrence ranged from 12 to 120 months, with a mean of 46.63 ± 30.63 months. Detailed data on surgical treatment, recurrence and clinical follow-up are available in Tables 1 and 4.

Disease-Free Survival Analysis

All cases were included in the survival analysis. The estimated 5-year DFS rate was 59.5%. Only the initial treatment modality was associated with DFS, in both univariate ($p = 0.08$) and multivariate analyses ($p = 0.030$) (Table 5), with a HR of 9.60 (95%IC = 1.24–73.89) favoring radical treatment. Multivariate Cox regression showed no statistically significant differences in the DFS curves regardless of the *BRAF* mutational status, and other clinical, imaging, histopathological and immunohistochemical predictive variables (Fig. 3).

Discussion

While numerous clinical and molecular parameters have been proposed to potentially correlate with the prognosis of ameloblastomas, their implementation in clinical practice remains very limited [10, 11]. The recent identification of a heightened frequency of *BRAF* mutations in ameloblastomas raises pertinent questions about the possible impacts of this molecular alteration on the clinical behavior of these tumors. This prompts consideration of the utility of *BRAF* p.V600E mutation as a reliable prognostic marker and its potential implications for clinical decision-making in the management of ameloblastomas [5, 10, 12, 13].

Although the 5th Edition of the Classification of Head and Neck Tumors considered that *BRAF* p.V600E mutation could be associated with later recurrence [1],

Table 5 Univariate and multivariate disease-free survival analysis

	n (%)	Events (recurrences)	Univariate (log rank)	Multivariate HR (95% CI)
Sex			$p=0.302$	–
Women	19 (46.3%)	09		
Men	22 (53.7%)	10		
Age at diagnosis			$p=0.650$	–
≤ 28 years	21 (51.2%)	10		
> 28 years	20 (48.8%)	08		
Skin color			$p=0.129$	$p=0.290$
White	07 (17.1%)	03		2.17 (0.51—9.15)
Brown and black	34 (82.9%)	16		1.00
Anatomic location of the tumor			$p=0.962$	–
Mandible	40 (97.6%)	18		
Maxilla	01 (2.4%)	01		
Symptomatology			$p=0.502$	–
No	31 (75.6%)	13		
Yes	10 (24.4%)	03		
Radiographic locularities			$p=0.626$	–
Unilocular	11 (26.8%)	04		
Multilocular	30 (73.2%)	15		
Imaging margins			$p=0.769$	–
Well-defined	33 (80.5%)	17		
Irregular	08 (19.5%)	02		
Cortical bone expansion			$p=0.799$	–
No	02 (4.9%)	01		
Yes	39 (95.1%)	18		
Cortical bone perforation			$p=0.636$	–
No	01 (2.4%)	00		
Yes	40 (97.6%)	19		
Adjacent teeth displacement			$p=0.880$	–
No	08 (19.5%)	04		
Yes	33 (80.5%)	15		
Root resorption			$p=0.573$	–
No	13 (31.7%)	08		
Yes	28 (68.3%)	11		
Impacted teeth			$p=0.029$	$p=0.209$
No	33 (80.5%)	17		4.36 (0.43—43.48)
Yes	08 (19.5%)	02		1.00
Histological subtype			$p=0.850$	–
Follicular	13 (31.7%)	08		
Plexiform	15 (36.6%)	06		
Acanthomatous	08 (19.5%)	03		
Basal cell	05 (12.2%)	02		
Ki67 cell proliferation index			$p=0.982$	–
≤ 4.15%	21 (51.2%)	10		
> 4.15%	20 (48.8%)	09		
CD138/syndecan-1 in neoplastic epithelium			$p=0.581$	–
Positive	24 (58.5%)	09		
Negative	17 (41.5%)	10		
CD138/syndecan-1 in tumor stroma			$p=0.046$	$p=0.059$
Positive	23 (56.1%)	11		2.93 (0.95—8.99)

Table 5 (continued)

	n (%)	Events (recurrences)	Univariate (log rank)	Multivariate HR (95% CI)
Negative	18 (43.9%)	08		1.00
BRAF mutational status			p = 0.159	p = 0.759
BRAF p.V600E	31 (75.6%)	17		1.38 (0.17—11.–0)
BRAF wild-type	10 (24.4%)	02		1.00
Initial surgical treatment			p = 0.008	p = 0.030
Conservative	27 (65.9%)	18		9.60 (1.24—73.–9)
Radical	14 (34.1%)	01		1.00

HR hazard ratio, *CI* confidence interval

a recent systematic review and meta-analysis [4] showed that the *BRAF* p.V600E mutation alone seems to not affect the global recurrence of ameloblastomas, in accordance with the results presented in the current study. However, most of the studies included in the review presented major limitations in patient follow-up, and the reported results for recurrence outcomes varied widely among them. In fact, only a few studies suggested that the presence of the *BRAF* p.V600E mutation in ameloblastomas could be associated with higher recurrence rates and tumor aggressiveness [10, 14]. Additional biological modulatory roles for *BRAF* p.V600E, such as cell proliferation and invasiveness, were not further explored and remain to be better elucidated [10, 15–18].

The Ki67 nuclear antigen is a protein expressed during active phases of the cell cycle and is absent in quiescent cells. The assessment of the cell proliferation index based on Ki67 immunoreactivity is valuable for the diagnosis and prediction of the prognosis of various neoplasms, as disruptions in the cellular proliferation process are essential events in oncogenesis [19, 20].

Recent studies have shown that the presence of *BRAF* p.V600E was associated with high proliferative activity in thyroid carcinomas and congenital melanocytic nevi [21, 22]. Nevertheless, to the authors' knowledge, only one available study conducted by Fregnani et al. [10] assessed the relationship between *BRAF* p.V600E and cell proliferation in ameloblastomas. They found that all tumors with high Ki67 proliferation index (> 11%) harbored *BRAF* p.V600E. In the present study, however, it was observed that although the mean of Ki67 proliferation index in *BRAF*-mutant ameloblastomas was slightly higher than in *BRAF* wild-type ameloblastomas (5.44 ± 3.14 vs. 3.91 ± 3.21, respectively), this difference was not statistically significant. Thus, the role of *BRAF* p.V600E in mediating proliferative activity remains unclear.

The loss of cell adhesion is a molecular event associated with tissue invasiveness and tumor growth in epithelial neoplasms. CD138/syndecan-1 is a transmembrane proteoglycan that plays an important role in the regulation of several biological processes, such as cytoskeleton organization and cell adhesion [23, 24]. Physiological expression of CD138/syndecan-1 was observed in several epithelial tissues, including in those of odontogenic origin [25]. Compared to normal epithelial tissue and odontogenic cysts, ameloblastomas may present a marked reduction in CD138/syndecan-1 immunoexpression in neoplastic cells, associated with variable immunoexpression in the tumor stroma [24, 25]. Although the present survival analysis suggests a trend which may not have reached statistical significance due to the limited sample size, the role of the CD138/syndecan-1 in the pathogenesis of ameloblastoma and other epithelial odontogenic tumors is still unclear, and its immunoexpression levels seem to have little or no correlation with the presence of *BRAF* p.V600E or with tumor biological behavior.

In this scenario, none of the clinical, imaging, histomorphological, or immunohistochemical features analyzed in this study were associated with the *BRAF* p.V600E nor with the DFS in ameloblastomas, thus possessing limited value in predicting its prognosis. Nevertheless, individuals with ameloblastomas who were subjected to conservative surgical treatment exhibited a significantly higher risk of recurrence compared to those treated with radical surgical procedures, representing the only variable that reliably predicted DFS in these tumors. Despite this, it is imperative to emphasize that therapeutic decisions for ameloblastomas should not solely rely on the tumor's recurrence potential, as radical surgical treatment is linked to a higher incidence of postoperative complications and sequelae, significantly impacting quality of life [26–28].

Given that this is a single-center retrospective study with a limited sample size, it is susceptible to specific limitations inherent in its methodological design. Consequently, the results should be taken in the context of these limitations. The collection of secondary data from hospital records may introduce biases related to the type and quality of available data, which included the impossibility to assess reliable data
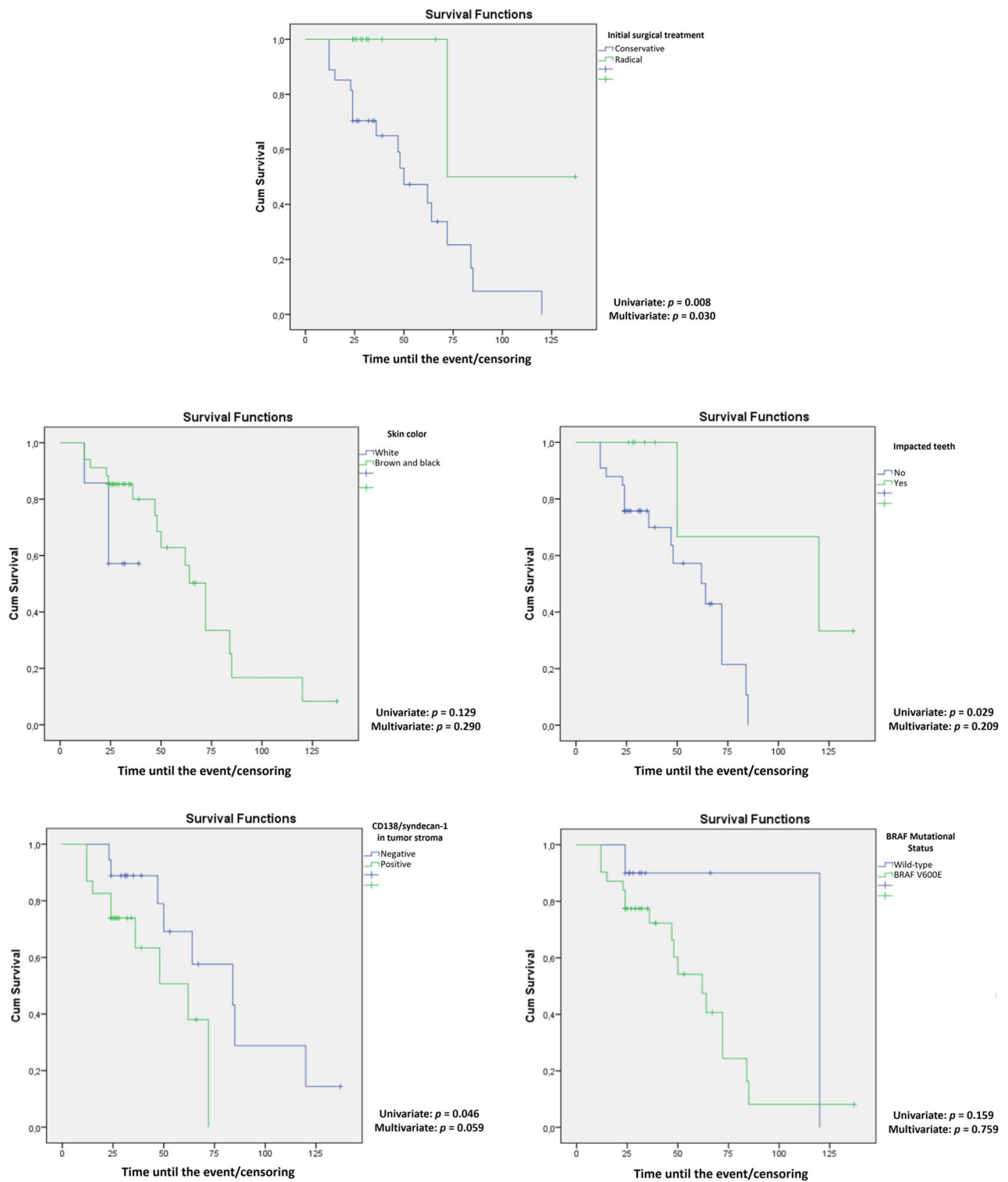
Fig. 3 Disease-free survival Kaplan–Meier curves stratified by prognostic variables introduced into multivariate model

to calculate tumor size. For this reason, this variable was not included in the present study. Furthermore, a considerable heterogeneity in the total follow-up time among patients was observed, which can distort the recurrence analysis. To control for this limitation, a minimum follow-up period of 24 months was established as eligibility criteria for this

study, given that ameloblastoma is a tumor characterized by slow growth and progression, with recurrence possible even decades after initial treatment [2].

In conclusion, the *BRAF* p.V600E mutation emerges as the predominant molecular aberration in ameloblastomas, with promising diagnostic and therapeutic implications for clinical practice. Nevertheless, it does not seem to exert a significant influence on the tumor proliferative activity and CD138/syndecan-1-mediated cell adhesion. Furthermore, the tumor recurrence rates were not affected by *BRAF* mutational status, thereby suggesting that the *BRAF* p.V600E mutation lacks a significant effect on disease-free survival outcomes in patients with ameloblastomas.

Declarations

Conflict of interest The authors have no conflict of interest to declare.

Ethical Approval This study was approved by the Ethics Committee of the University of Pernambuco (#4.309.512; CAAE: 35920620.7.0000.5192).

Informed Consent The Ethics Committee of the University of Pernambuco granted an exemption for patient consent.

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