Immunohistochemical Expression of Ki67 and P53 and Their Prognostic Role in Ameloblastoma: A Longitudinal Study

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Abstract

Objectives: Ameloblastoma is an odontogenic tumor which comprises about 11% of all odontogenic tumors and is locally aggressive with high recurrence rate. This study aimed to assess the immunohistochemical expression of Ki67 and P53 and their association with clinical and pathological factors among patients with ameloblastoma.

Methods: Follow-up data of patients who were confirmed with ameloblastoma histologically were retrieved retrospectively. Factors associated with Ki67 and P53 immunohistochemical expression were determined using one-way ANOVA. Chi-square and Fisher's exact statistical tests were used to assess the factors associated with recurrence. A two-tailed $p < 0.05$ was considered statistically significant.

Results: A total of 40 patients confirmed histologically with ameloblastoma were included in the analysis. Majority (65%) of the cases were of conventional type of ameloblastoma. The expression of Ki67 and p53 was 52.5% and 85%, respectively. Recurrence was found in 47.5% of all the patients and it was associated with conventional histological type ($p = 0.042$), segmental resection ($p < 0.001$), tumor size ($p < 0.001$), and high p53 expression ($p = 0.002$). **Conclusion**: Almost half the cases in this study had recurrence. The immunohistochemical expression of p53 was significantly higher than that of Ki67.

Keywords: Ameloblastoma, recurrence, Ki67, p53, immunohistochemical expression.

Introduction

Ameloblastoma is an odontogenic tumors (OT) which comprises about 11% of OTs and only 1% of all solid tumors involving the oral cavity.[1] The World Health Organization (WHO) defines ameloblastoma as a locally invasive and histologically heterogeneous tumor with high recurrence rate.[2] Ameloblastoma has a difficult clinical course in almost every patient due to lack of reliable clinical and pathologic factors that can help in predicting its biological behavior.[3] The use of cell proliferation biomarkers (e.g. Ki67 and PCNA) and tumor suppressor genes (e.g. p53 and BRCA1) has been reported to have ability of predicting the biological behavior of numerous tumors including ameloblastoma.[1-4] For instance, Migaidi et al. reported that increased expression of Ki67 was associated with increased microsatellite instabilities, high rate of recurrence, and short disease-free survival (DFS) among patients with ameloblastoma.[5] The quantity and quality of p53 expression involves a more aggressive behavior of cancers and therefore, its overexpression can promote cell proliferation in odontogenic lesions.[6]

Ki67 is a non-histone nucleoprotein in cells which increases its proliferation as cells prepare to divide into new cells.[7] There are two isoforms of Ki67 antigen namely 345 and 395 kDa that have been identified.[8] Ki67 positive nuclei in the ameloblastoma are mainly located in peripheral ameloblast-like cells in follicular as well as plexiform variants of the conventional type and in the basal cells of unicystic ameloblastoma.[9] Stellate reticulum-like cells in ameloblastoma and those in developing teeth tend to be negative for this biomarker. This staining pattern indicates that the cellular proliferation and consequently the growth of ameloblastoma are concentrated in the peripheral areas composed by ameloblast-like cells.[10] In one study it was found that, Ki67 expression was higher in amelobastic carcinoma compared to benign ameloblastoma, suggesting that this biomarker can be useful when considering diagnosis of malignancy, and perhaps could play a role in malignant transformation of ameloblastoma.[11]

P53 is a tumor suppressor gene located on chromosome 17p13.1 which codes for a protein that regulates the cell cycle and hence plays a role of tumor suppression.[12] In one review article it was reported that, expression of p53 in plexiform was 25% and in follicular pattern was 20%.[13] Also, Hirayama et al reported expression of p53 of 43% for plexiform pattern and 37% for follicular pattern.[14] One study reported that, p53 expression was higher in ameloblastic carcinoma compared to benign ameloblastoma, suggesting that this biomarker can be useful when considering diagnosis of malignancy, and perhaps could play a role in malignant transformation of ameloblastoma. Apart from unicystic histological subtype with well-established better prognosis by virtual of not being locally very aggressive Ong'uti, other clinical and pathologic factors have been reported to be controversial.[1]

There is a gap in knowledge and practice regarding establishment of serum biomarkers and/or molecular prognostic factors which can reliably predict the biological behavior of ameloblastoma. Therefore, this study aimed to determine the immunohistochemical expression of Ki67 and p53 and factors associated with their expression among patients with ameloblastoma.

Methods

This was a retrospective longitudinal study which was conducted at Makerere College of Health Sciences (MakCHS) in Kampala, Uganda. Retrospective data of patients who were confirmed histologically with ameloblastoma, treated surgically then followed-up for a period of 13 years (from January 2012 to December 2018) were extracted for analysis.

Patients with a confirmed histological diagnosis of ameloblastoma, complete clinical and follow-up data, and available formalin fixed paraffin embedded tissue block were included in the analysis. However, all patients with missing or spoiled tissue blocks and those with incomplete or missing clinical and follow-up data were all excluded from the analysis.

The sample size of the 40 cases was obtained conveniently and all the available cases meeting the inclusion criteria were recruited simultaneously and included in the study.

Recurrence was defined as confirmation of the disease either clinically or radiologically after surgical removal of the primary tumor. This work has been reported in line with the STROCSS criteria.[15] Histological classification of the subtypes of ameloblastoma was based on the current WHO classification system of the year 2017.[16]

Re-confirmation of the previous histological diagnosis using routine hematoxylin and eosin stains was done. Then we classified histologically the cases based on the new WHO classification ameloblastoma of 2017.[17] Immunohistochemical (IHC) staining for monoclonal mouse antibodies (DAKO) were used to evaluate the expression of the proteins of interest (p53 – clone D0–7 and Ki67 – clone MIB1) by adapting the protocol from a previous study.[6] The serial sections were cut at the thickness of 4 microns and then were de-waxed by heating them on a hot plate at $60\,^{0}C$ for 30 min followed by clearing in three changes of xylene. The sections were brought down to water by dipping them in descending concentration of alcohol and then rinsed in distilled water. Two drops of 3% H_2O_2 solution was added to each section for 15 min to block endogenous peroxidase, and then, the slides were rinsed in distilled water. The slides were incubated in the antigen retrieval solution citrate buffer of pH 6.0 within a pressure cooker at 95°C, from which the slides were removed after 2 min of full pressure.

Heat-Induced Epitope Retrieval (HIER) method in microwave oven for 30 min in Tris-EDTA buffer solution (TBS) pH 9 for p53 protein and in citrate buffer solution (CBS) pH 6.1 for Ki67 antigen. TBS was then drained from slides and a ring was made around the section using Pap pen (Sigma-Aldrich Labware, German) to limit spreading of antibody solutions. The sections were then incubated with MIB-1 monoclonal antibody (mouse monoclonal antibody, dilution of 1:200, Dako) and anti-p53 monoclonal antibodies mouse (clone DO-7, dilution 1:50, Dako) and for 3 min at room temperature simultaneously. Then 2,3-diaminobenzidine (DAB) was used as a chromogenic substrate and DAKO LSAB2 as detection system. The sections were washed in distilled water, counterstained in hematoxylin for 10 sec, and differentiated by 2 dips into 1% acid alcohol. The sections were blued in warm water for 2 min, dehydrated through 70%, 80%, 95%, and 100% ethanol, and then cleared in xylene for 10 min. The sections were finally cover-slipped using Distyrene Plasticizer Xylene (DPX) and were ready for scoring. Positive staining was defined by presence of brownish intranuclear staining in tumor cells for both Ki67 and p53. For validation of the IHC results, a known case of breast cancer was used as a positive control for both Ki67 and p53 proteins and removal of the primary antibodies was taken as negative control for Ki67 and p53 expression.[18]

The labeling index (LI) for both Ki67 and p53 was calculated as the number of positive cells x 100/total number of cells (positive + negative) at high magnification (x400) as previous.[19] Counting of the positive tumor cells was done from different compartments (basal, granular, squamous, stellate, and reticulum) manually. Ki67 LI of ≤20% was considered to be low and >20% was regarded to be high as done in a previous study.[20] The p53 LI was regarded high if the expression was ≥10% and low if <10% as previous.[21] This was followed by determining the staining intensity for both antibodies and the intensity was labelled as 1, 2, and 3 for weak, moderate, and strong intranuclear staining, respectively.

Analysis of the data was done using SPSS programme version 23.0 (IBM statistics, Chicago). Assessment of factors associated with Ki67 and p53 expression was done using one-way analysis of variance (ANOVA) and for variables with more than three groups, post hoc test was performed to determine which group was different from other groups. Factors associated with recurrence were assessed using Chi-square and Fisher's exact tests where appropriately. The level of statistical significance was set at 95% confidence interval with $p<0.05$.

Results

The sociodemographic and clinical characteristics of the patients and expression of Ki67 and P53 are presented in Table 1. A total of 40 patients who were confirmed histologically with ameloblastoma were analyzed in the present study. The mean age of the patients was 33.6 ± 14.4 years with age ranging from 9 to 67 years. Males were slightly higher (n = 23, 57.5%) than females with male to female ratio of 1.4:1. The mean duration from onset of symptoms to hospitalization was 9.2 ± 6.9 months (range: 2-30 months). Most (n = 23, 57.5%) of the patients sought medical services beyond 6 months since onset of the symptoms. The mean tumor size was 4.8 ± 2.2 cm and majority (n = 28, 70%) of the patients had tumor size greater than the mean tumor size. Also, over half (n = 22, 55%) of the tumors were located in the posterior part of the mandible.

Table 1: Clinical Characteristics, Ki67 and P53 Expression (N = 40).

Case	Age (years)	Sex	A*	Tumor size (cm)	Site	B*	Recurrence	C*	Surgery	Ki67%	Ki67 intensity	p53%	p53 intensity
1	38	F	3	3.0	MA	U	N	38.0	CT	0.0	0	3.2	3
2	21	M	4	3.2	MP	C	N	30.0	CT	10.6	1	0.0	0
3	34	M	2	4.2	MXP	U	N	49.0	CT	8.0	1	2.3	3
4	32	M	5	2.3	MP	U	N	100.0	CT	3.5	1	0.0	0
5	41	F	8	8.4	MP	C	Y	60.0	SR	22.2	3	79.5	3
6	48	F	2	3.1	MP	U	N	56.0	CT	0.0	0	3.4	3
7	20	F	4	3.4	MP	C	N	40.0	CT	3.6	2	2.3	3
8	18	M	5	3.5	MA	U	N	35.0	CT	2.8	3	3.6	3
9	62	F	3	2.4	MP	C	N	102.0	CT	15.8	3	3.4	3
10	29	M	2	4.1	MP	C	N	80.0	CT	3.6	3	24.7	3
11	56	M	6	6.8	MA	C	Y	48.0	SR	34.8	1	12.5	0

12	40	F	7	7.1	MP	C	Y	48.0	SR	25.9	1	0.0	0
13	19	M	3	3.4	MP	U	N	50.0	CT	0.0	0	33.2	3
14	9	M	5	3.5	MP	C	N	45.0	CT	0.0	0	0.0	0
15	53	M	8	4.3	MXP	U	Y	12.0	SR	8.15	3	1.7	1
16	14	M	2	4.2	MXP	U	N	20.0	CT	17.9	3	76.5	3
17	21	F	15	6.2	MP	U	Y	84.0	CT	20.8	1	18.5	3
18	26	M	7	2.5	MA	U	N	45.0	CT	3.2	3	9.4	3
19	21	F	24	5.1	MP	C	Y	28.0	SR	9.55	3	35.4	0
20	21	M	9	3.4	MP	U	N	90.0	CT	11.2	3	26.0	3
21	19	F	12	10.0	P	C	Y	72.0	SR	15.0	3	25.0	3
22	14	M	8	10.0	MP	C	N	45.0	CT	2.2	1	10.4	3
23	52	M	10	3.7	MA	U	N	70.0	CT	6.7	1	21.1	1
24	30	F	9	3.0	MP	U	N	65.0	CT	0.0	0	26.3	3
25	42	M	24	8.3	MP	C	Y	30.0	SR	1.5	3	35.8	1
26	27	F	24	4.0	MXA	C	Y	60.0	SR	18.6	1	33.2	1
27	30	F	6	3.8	MA	C	N	45.0	CT	0.0	0	0.0	0
28	20	M	10	3.6	MA	C	Y	96.0	SR	26.8	3	40.1	3
29	20	M	9	4.6	MP	C	Y	36.0	SR	30.0	3	41.0	0
30	20	F	11	6.4	MP	C	Y	24.0	SR	5.1	3	34.2	3
31	45	M	5	3.3	MXP	C	N	104.0	CT	0.0	0	0.0	0
32	35	M	7	2.6	MP	C	N	24.0	CT	0.0	0	15.0	0
33	38	F	12	10.0	MP	C	Y	24.0	CT	10.8	3	55.2	0
34	38	F	9	6.7	P	C	Y	60.0	CT	11.0	3	40.0	1
35	55	M	12	4.6	MP	C	Y	8.0	CT	24.2	1	20.0	3
36	45	M	10	3.1	MA	P	Y	60.0	SR	18.2	1	25.8	0
37	45	M	26	6.7	MP	C	Y	156.0	SR	22.0	1	42.3	0
38	67	F	9	4.1	MXA	P	N	65.0	CT	0.0	0	23.4	2
39	45	M	30	3.8	P	C	Y	79.0	SR	34.6	2	44.0	2
40	33	F	20	4.2	MP	C	Y	34.0	CT	15.7	2	32.4	0

A*-Duration from onset of symptoms to hospitalization (months), B*-Histopathological subtypes, C*-Duration from initial removal of primary tumor to recurrence (months), MP-mandible posterior, MA-mandible anterior, MXP-maxilla posterior, MXA-maxilla anterior, P-palate, Y-yes, N-no, M-male, F-female, SR-surgical resection, CR-conservative therapy, U-unicystic, P-peripheral, C-conventional

Majority (n = 26, 65%) of the ameloblastoma cases were of conventional type followed by unicystic type which comprised (n = 12, 30%) of all the cases. Of all the conventional types, majority (n = 11, 61.1%) of them were follicular type. Peripheral type consisted of only (n = 2, 5%) (Table 1).

Majority (n = 26, 65%) of the patients were treated using conservative therapy and the remaining (n = 14, 35%) underwent segmental resection followed by reconstruction of the operated sites of the jaw bones with non–vascularized bone graft and titanium reconstruction plates. Postoperative complications were reported in (n = 7, 17.5%) of all patients which consisted of site surgical infections (n = 5, 12.5%) and facial deformity (n = 2, 5%). None of the patients had reconstruction plate exposure as a complication. The mean follow-up duration of the patients was 55.4 ± 2.9 months. Recurrence was reported among (n = 19, 47.5%) patients. The first patient developed recurrence after a period of 8 months following initial removal of the primary tumor and the last patient had recurrence after a period of 13 years (156 months). None of the patients died until the end of the follow-up time (Table 1).
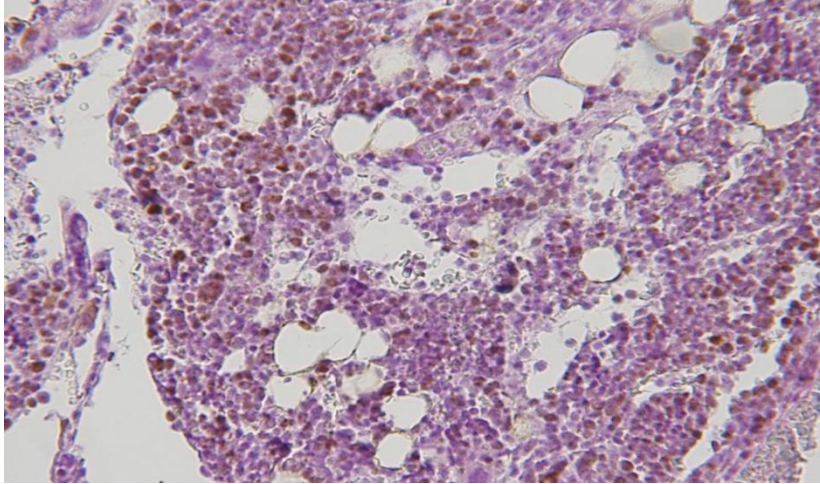
Table 2 presents the association of clinicopathological characteristics with expression of Ki67. Expression of Ki67 was observed in (n = 21, 52.5%) of all the cases with mean expression of 13.3 ± 1.2% (range: 0-34.8%). Of all the cases that expressed Ki67, those which stained weakly, moderately, and strongly were (n = 8, 38.1%), (n = 3, 14.3%, and (n = 10, 47.6%), respectively [Figure 1A-B]. The mean expression of Ki67 for cases with recurrence was significantly higher (23.4 ± 8.6%) than that of non-recurrent cases (4.2 ± 5.5%) (p<0.001). Also, we found that there was a significant increase in mean expression of Ki67 whereby patients with tumor size equal or greater than mean tumor size had higher mean expression (21.2 ± 10.5% than those with tumor size less than mean tumor size (10.0 ± 11.1% (p = 0.005).

**Table 2:** Association of Clinical and Pathological Factors with Ki67 Expression (N = 40).

Variables	n	Ki67% Mean ± SD	95% CI for mean	p-value
Age (years)				0.083
≤50	23	10.1 ± 10.3	5.598-14.540	
>50	17	17.8 ± 12.9	11.143-24.357	
Sex				0.830
Male	23	13.0 ± 12.1	7.759-18.195	
Female	17	13.8 ± 12.1	7.569-20.059	
Anatomical location				0.076
Posterior mandible	22	12.3 ± 11.3	7.301-17.351	
Anterior mandible	9	12.9 ± 13.1	2.858-23.022	
Posterior maxilla	4	8.5 ± 7.3	3.122-20.122	
Anterior maxilla	2	9.3 ± 13.2	108.868-127.468	
Palate	3	31.0 ± 6.1	15.902-46.195	
Histological subtypes				0.097
Unicystic	18	17.5 ± 13.9	10.661-24.373	
Peripheral	9	5.2 ± 5.8	0.764-9.676	
Conventional	13	13.2 ± 9.8	7.206-19.106	
Surgical approach				0.063
Conservative therapy	26	16.6 ± 5.6	4.349-12.853	
Segmental resection	14	22.1 ± 9.3	16.789-27.490	
Tumor size (cm)				0.005
≤4.8 ± 2.2	28	10.0 ± 11.1	5.686-14.272	
>4.8 ± 2.2	12	21.2 ± 10.5	14.492-27.827	
Lag period (months)				0.071
≤9.2 ± 6.9	21	11.8 ± 8.2	2.548-10.061	
>9.2 ± 6.9	19	21.1 ± 10.6	16.013-26.241	
Recurrence status				p<0.001
Recurrent	19	23.4 ± 8.6	1.750-6.733	
Non-recurrent	21	4.2 ± 5.5	19.260-27.505	
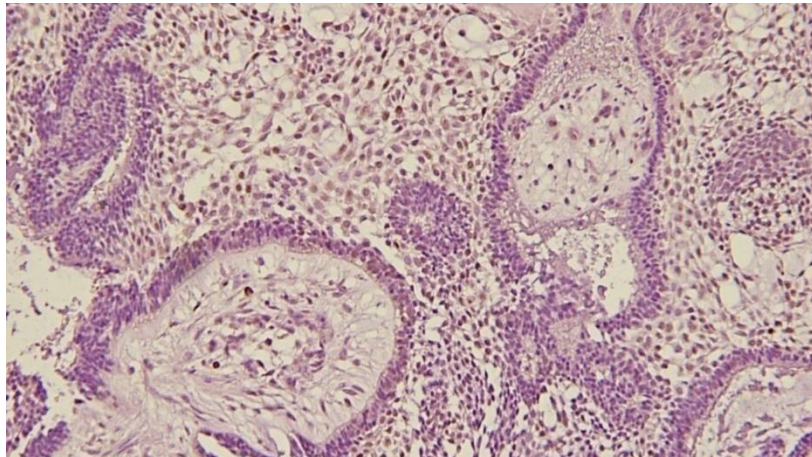
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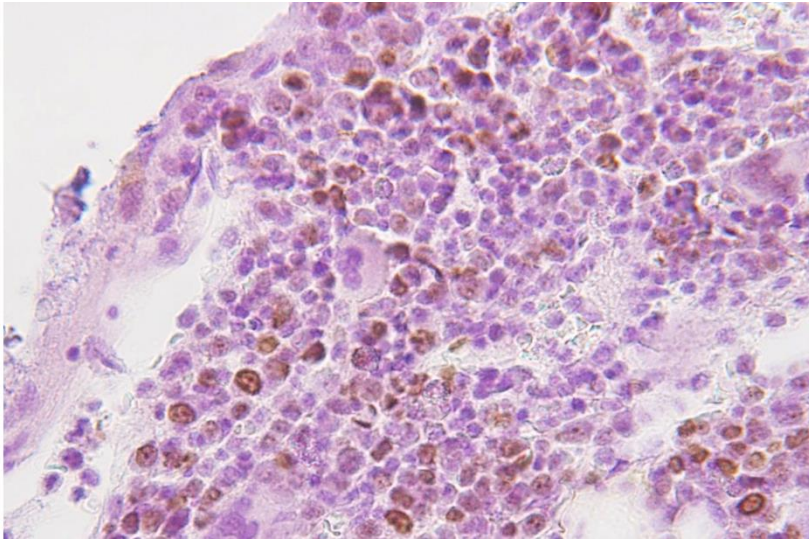
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Figure 1: (A) Weak intranuclear staining for anti-Ki67 for the case of follicular ameloblastoma (IHC, x100), (B) Moderate intranuclear staining for anti-Ki67 for the case of peripheral ameloblastoma (IHC, x100), and (C) Strong intranuclear staining for anti-Ki67 for plexiform ameloblastoma (IHC, x200)

Although the mean expression of Ki67 for older patients, patients that underwent segment resection, lag period above mean lag duration, and those with unicystic ameloblastoma was higher than their counter parts, but the difference did not reach statistical significance.

Table 3 presents the association of clinicopathological characteristics with expression of p53. Expression of p53 was found in (n = 34, 85%) of all the cases. The mean expression of p53 was $22.5 \pm 2.0\%$ (range: 0-79.5%). Weak, moderate, and strong nuclear staining was found in (n = 8, 23.5%), (n = 14, n = 41.2%), and (n = 12, 35.3%) cases, respectively (Figure 2A-C). The mean expression of p53 for recurrent cases was significantly higher ($32.5 \pm 18.3\%$) compared with that of non-recurrent cases ($13.5 \pm 18.1\%$) (p = 0.002). Also, there was a significantly high expression of p53 for cases with larger tumor size than mean tumor size ($32.4 \pm 21.5\%$) compared with that of cases whose tumor size was smaller than mean tumor size ($12.3 \pm 18.6\%$). There was a positive association (p = 0.041) between increased

mean expression of p53 for patients aged >50 years (24.2 ± 22.7%) and those aged ≤50 years (21.2 ± 18.8%). The mean expression of p53 for females was higher (24.4 ± 21.8%) than that of males (21.1 ± 20%) but the difference was insignificant (p = 0.615). Patients who were treated by segmental resection had higher mean expression of p53 (32.2 ± 19.8%) compared with that of patients who were treated conservatively (17.3 ± 2%) but the difference was insignificant (p = 0.076).

**Table 3:** Association of Clinical and Pathological Factors with p53 Expression.

Variables	n	p53% Mean ± SD	95% CI for mean	p-value
Age (years)				0.041
≤50	23	21.2 ± 18.8	13.445-29.421	
>50	17	24.2 ± 22.7	12.503-35.877	
Sex				0.615
Male	23	21.1 ± 19.5	12.651-29.551	
Female	17	24.4 ± 21.8	12.205-35.665	
Anatomical location				0.060
Posterior mandible	22	20.9 ± 16.7	13.841-28.282	
Anterior mandible	9	21.7 ± 25.2	2.239-41.029	
Posterior maxilla	4	20.1 ± 37.6	-39.693-79.938	
Anterior maxilla	2	28.3 ± 6.9	-33.960-90.580	
Palate	3	36.3 ± 10.0	11.451-61.216	
Histological subtypes				0.094
Unicystic	18	24.2 ± 17.0	15.689-32.617	
Peripheral	9	15.3 ± 24.8	3.704-34.390	
Conventional	13	25.2 ± 21.8	12.051-38.391	
Surgical approach				0.076
Conservative therapy	26	17.3 ± 19.0	9.635-24.999	
Segmental resection	14	32.2 ± 19.8	20.750-43.601	
Tumor size (cm)				0.043
≤4.8 ± 2.2	18	12.3 ± 18.6	11.057-25.511	
>4.8 ± 2.2	12	32.4 ± 21.5	18.725-46.066	
Lag period (months)				0.065
≤3	21	31.2 ± 18.9	5.618-22.839	
>3	19	14.7 ± 18.2	22.918-40.441	
Recurrence status				0.002
Non-recurrent	21	13.5 ± 18.1	5.290-21.774	
Recurrent	19	32.5 ± 18.3	23.638-41.261	
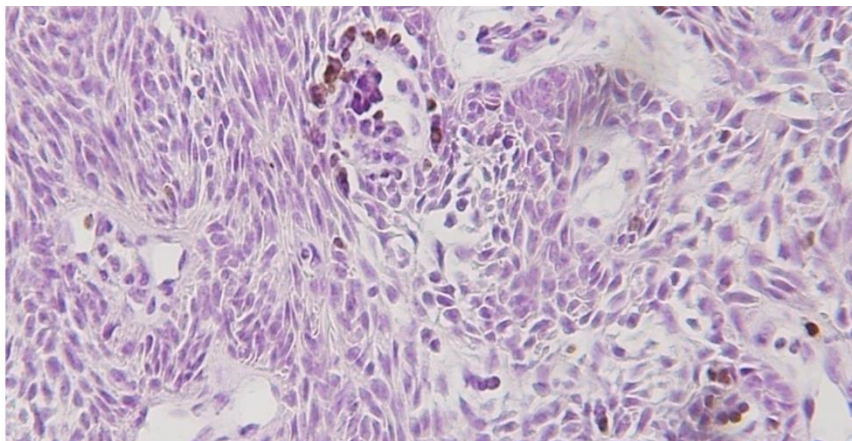
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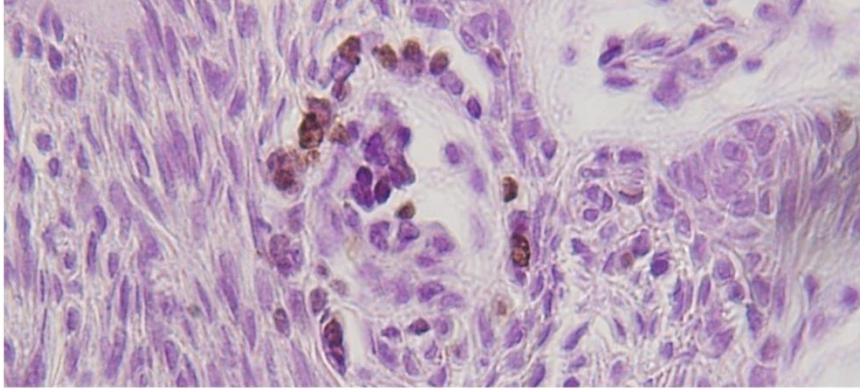
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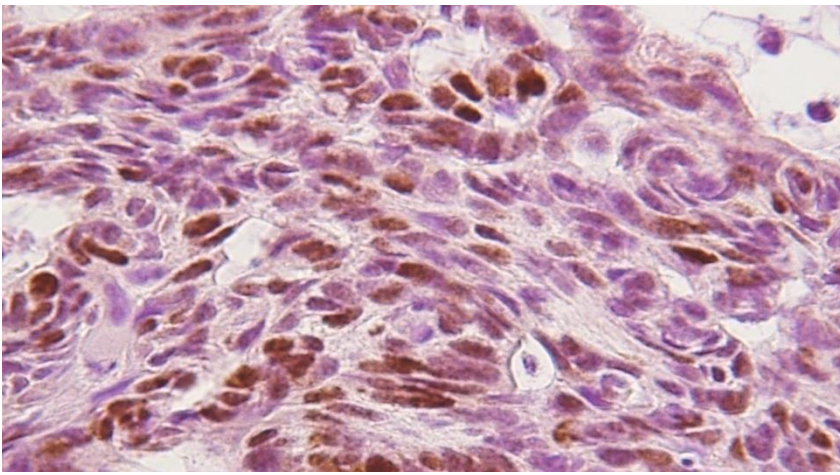
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Figure 2: (A) Weak intranuclear staining for p53 protein for the case of peripheral ameloblastoma (IHC, x100), (B) Moderate intranuclear staining for p53 protein for the case of basal ameloblastoma (IHC, x100), and (C) Strong intranuclear staining for p53 protein for plexiform ameloblastoma (IHC, x200)

Table 4 shows the association of clinicopathological factors with recurrence among patients. There were significantly more cases with high p53 LI that had recurrence compared with cases with low p53 LI that had recurrence ($p = 0.041$). Also, it was found that there were significantly more cases with tumor size ≥5 cm that had recurrence compared with cases with tumor size <5 cm which had recurrence ($p<0.001$). Furthermore, it was found that there were significantly more cases with recurrence which were treated with segmental resection compared with recurrent cases which were treated by conservative surgery ($p<0.001$). Additionally, we observed that there were more conventional histological subtypes with recurrence than other histological subtypes with recurrence ($p = 0.024$).

Table 4: Association of Clinical and Pathological Factors with Recurrence (N = 40).

	Recurrence		
Variables	**Yes: n (%)**	**No: n (%)**	**P-value**
Age (years)			0.061[†]
≤35	8 (34.8)	15 (65.2)	
>35	11 (64.7)	6 (35.3)	
Sex			0.218[†]
Male	9 (39.1)	14 (60.9)	

Female	10 (58.8)	7 (41.2)	
Anatomical location			0.379*
Posterior mandible	10 (45.5)	12 (54.5)	
Anterior mandible	4 (44.4)	5 (55.6)	
Posterior maxilla	1 (25.0)	3 (75.0)	
Anterior maxilla	1 (50.0)	1 (50.0)	
Palate	3 (100.0)	0 (0.0)	
Histological subtypes			0.024*
Unicystic	1 (11.1)	8 (88.9)	
Peripheral	6 (46.2)	7 (53.8)	
Conventional	12 (66.7)	6 (33.3)	
Surgical approach			<0.001*
Conservative therapy	5 (19.2)	21 (80.8)	
Segmental resection	14 (100.0)	0 (0.0)	
Tumor size (cm)			<0.001*
≤5	8 (28.6)	20 (71.4)	
>5	11 (91.7)	1 (8.3)	
Lag period (months)			0.074*
≤3	16 (84.2)	3 (15.8)	
>3	3 (14.3)	18 (85.7)	
Ki67 expression			0.061 [†]
Low	8 (34.8)	15 (65.2)	
High	11 (64.7)	6 (35.3)	
P53 expression			0.041*
Low	4 (26.7)	11 (73.3)	
High	15 (60.0)	10 (40.0)	

There were more cases with high Ki67 LI that had recurrence compared to cases with low Ki67 LI that had recurrence despite lack of statistical significance (p = 0.061). Similarly, patients who were aged >35 years showed more rate of recurrence compared to younger ones although the difference was not significant (p = 0.061).

Discussion

In this study, the expression of Ki67 and p53 and their association with recurrence using IHC method from formalin fixed paraffin embedded tissue blocks of patients who were confirmed histologically to have ameloblastoma, treated, and followed-up for a period of 13 years. The clinicopathological factors associated with the expression of Ki67 and p53 were assessed. Also, association of clinicopathological factors with recurrence was determined.

The key findings in this study included expression of p53 being quite higher than that of Ki67 (85% vs 52.5%), recurrence and larger tumor size than mean tumor size were the independent factors which were significantly associated with both Ki67 and p53 expression.

The expression of Ki67 using IHC method in ameloblastoma varies in different studies. This can be evidenced for example, by the percentage of Ki67 LI in this study which was lower than the rate of expression reported in other studies. Studies have reported Ki67 LI ranging from 82.3% to 100% in tissue blocks of patients with ameloblastoma.[6,10,16] Some factors may explain the difference in the expression of Ki67. For instance, it has been shown that cellular proliferation is subject to mutation of the cells. Therefore, it can be understood that, the variation of the level of mutations for patients with ameloblastoma may explain the difference of the Ki67 LI observed.[20,21] Also, Migaldi et al. found a positive association of microsatellite instabilities and increased proliferation of tumor cells which is detected by cellular proliferation biomarkers including Ki67.[5] Furthermore, difference of the methodological approach in scoring Ki67 LI in terms of cut-off due to lack of universal cut-off standards, may also explain the difference in the level of expression of Ki67 observed in various studies.

The mean Ki67 LI in this study was associated with recurrence and tumor size. Although the association of recurrence and Ki67 expression contradicts in reported studies, still there is a significant evidence of high Ki67 LI with possibility of recurrence of ameloblastoma.[22,23] The mean percentage of Ki67 LI in the present study was almost 5 times for cases with recurrence compared with non-recurrent cases despite lack of association. In 2015, Ahlem et al. also reported a positive association of Ki67 LI with recurrence and percentage of cases with high Ki67 LI that had recurrence was significantly different from cases with low Ki67 LI which had recurrence.[24] However, Ramon et al. reported contradicting finding in their study, in which the mean Ki67 LI for non-recurrent cases was higher (15.7 ± 13.6%) than that of recurrent cases (10.6 ± 4.5%) but the difference was insignificant (p>0.05).[11] Also, there was a significant association between tumor size and level of Ki67 LI. This is different from the finding in the studies of Alma et al. and Amol et al. who found no association between tumor size and Ki67 LI among patients with ameloblastoma.[6,16] Increased cell proliferation which is driven by mutation, usually leads to increased tumor size.[25] This may help in explaining the fact that, ameloblastoma cases with large tumor size are more likely to demonstrate high Ki67 LI.

Despite few studies that have shown low p53 LI in patients with ameloblastoma, the vast majority of studies have reported high p53 LI. For example, Barboza et al. and Gadbail et al. showed 100% of p53 LI among patients with ameloblastoma.[6,24] This indicates that, expression of p53 is not only associated with malignant transformation but also increased aggressiveness and also in locally invasive tumors including ameloblastoma.[26] This is in agreement with the finding in this study in which it was observed that, there were more recurrent cases with high level of p53 LI compared with non-recurrent cases which had low level of p53 LI. Similar observation was also reported in the study of Florescu et al. in 2012 who observed that, increased tendency of expression of p53 was found more in recurrent cases than non-recurrent cases, although the proportion of expression of the protein was low (52.9%).[18] Evidence of mutation of p53 gene was also reported in the study of Sharifi-Sistani et al. in which detection of mutation of the gene was done using polymerase chain reaction (PCR).[27] Additionally, there was also a positive correlation between expression of p53 with age of the patients, in which high p53 LI was significantly found more in elderly patients than younger patients (p = 0.041). This may be due to the reason that, aging is associated with accumulation of various forms of mutations including those of p53 gene.[28]

Furthermore, there was a positive association between tumor size and recurrence in this study. Cases with larger tumor size than mean tumor size had higher recurrence rate than cases with smaller tumor size than mean tumor size. Although the association between recurrence and tumor size seems to be contracting, still the association of the two variables has also been reported in another study of Yang et al. in which there was a positive association between the two variables.[29] In a study which was done by Fregnani et al. it was found that there was no association between tumor size and recurrence of ameloblastoma.[30]

There was a significant association of conservative surgery with increased recurrence rate in this study. This is similar to the finding in other previous studies[29-31]. In one systematic review study, it was reported that surgical margin for patients with ameloblastoma ranging from 2.3-8 mm confers better prevention of recurrence.[32] It was also argued that use of intraoperative CT scan to assess for adequacy surgical margin operatively would help to prevent or reduce high chance of recurrence.

Furthermore, it was observed that there was a significant association between conventional subtypes (follicular) of ameloblastoma and recurrence. However, there is contradicting information regarding association of recurrence with histological variants of ameloblastoma.[33] Other studies have also reported significant association of follicular variant of ameloblastoma with recurrence.[32,34,35] However, Au et al. and Bi et al. found no association between follicular type and increased risk of recurrence.[29,31] The difference in the criteria used to classify the various histological types in different studies may help to explain the observed discrepancy. Additionally, lack of agreement across studies reported previously may in one way or another justify lack of clinical association despite the statistical association that has been reported in some studies.

The prognostic role of p53 protein in ameloblastoma has been studied extensively and evidence has shown that there is derangement in the p53 gene and even its product both for the benign and malignant transformed forms of ameloblastoma.[36] Recurrence in ameloblastoma indicates local aggressiveness of the tumor and it has been associated with mutation of the p53 gene.[37] In this study, a significant positive association between increased expression of p53 protein and increased risk of recurrence was also found.

The strength of this study is based on the use of two potential biomarkers used to study the biological behaviors of tumors (cellular proliferation, initiation and progression of tumors). However, the study faced a number of methodological limitations including the following: small sample size due to limited fund to purchase enough primary antibodies for detecting Ki67 and p53 which would have helped us to stain many cases, reduced the power of the study and weakened the strength of the conclusions. Also, inability to perform molecular tests for example, p53 gene mutation due to lack of funds contributed to failure to have enough evidence regarding the link between recurrence of ameloblastoma and expression of the biomarkers that were investigated.

Conclusion

In conclusion, although the expression of both Ki67 and p53 in this study was relatively low, however, their expression has shown that they can predict possibility of recurrence of ameloblastoma particularly p53 which was significantly associated with recurrence. Further studies in future with large sample size involving survival analysis and molecular analysis of these biomarkers would help in explaining their role in determining recurrence of ameloblastoma.

References

1. Bwambale P, Yahaya JJ, Owor G, Wabinga H. Histopathological patterns and biological characteristics of ameloblastoma: A retrospective cross-sectional study. J Taibah Univ Med Sci 2021 Nov;17(1):96-104.

2. Dandriyal R, Gupta A, Pant S, Baweja HH. Surgical management of ameloblastoma: Conservative or radical approach. Natl J Maxillofac Surg 2011 Jan;2(1):22-27.

3. Ong'uti MN, Cruchley AT, Howells GL, Williams DM. Ki-67 antigen in ameloblastomas: correlation with clinical and histological parameters in 54 cases from Kenya. Int J Oral Maxillofac Surg 1997 Oct;26(5):376-379.

4. Amsi PT, Yahaya JJ, Kalungi S, Odida M. Immunohistochemical expression of BRCA1 and BRCA2 in a cohort of Ugandan men with prostate cancer: an analytical cross-sectional study. Afr J Urol 2020;26:••• .

5. Migaldi M, Sartori G, Rossi G, Cittadini A, Sgambato A. Tumor cell proliferation and microsatellite alterations in human ameloblastoma. Oral Oncol 2008 Jan;44(1):50-60.

6. Gadbail AR, Patil R, Chaudhary M. Co-expression of Ki-67 and p53 protein in ameloblastoma and keratocystic odontogenic tumor. Acta Odontol Scand 2012 Dec;70(6):529-535.

7. Xiaoming Sun, a Aizhan Bizhanova, a Timothy D. Matheson, a Jun Yu, A. & Lihua Julie Zhu, a, b, c P. D. K. Ki-67 Contributes to Normal Cell Cycle Progression and Inactive X Heterochromatin in p21 CheckpointProficient Human Cells.

8. Shields CL, Shields JA. Basic understanding of current classification and management of retinoblastoma. Curr Opin Ophthalmol 2006 Jun;17(3):228-234.

9. Sathi GA, Tamamura R, Tsujigiwa H, Katase N, Lefeuvre M, Siar CH, et al. Analysis of immunoexpression of common cancer stem cell markers in ameloblastoma. Exp Ther Med 2012 Mar;3(3):397-402.

10. Lee SK, Kim YS. Current concepts and occurrence of epithelial odontogenic tumors: I. Ameloblastoma and adenomatoid odontogenic tumor. Korean J Pathol 2013 Jun;47(3):191-202.

11. Carreón-Burciaga RG, González-González R, Molina-Frechero N, Bologna-Molina R. Immunoexpression of Ki-67, MCM2, and MCM3 in Ameloblastoma and Ameloblastic Carcinoma and Their Correlations with Clinical and Histopathological Patterns. Dis Markers 2015;2015:683087.

12. Freed-Pastor WA, Prives C. Mutant p53: one name, many proteins. Genes Dev 2012 Jun;26(12):1268-1286.

13. Fayad DW. -. Immunohistochemical Expression of P53 In Different Types of Ameloblastoma Conclusion : *Al- Anbar*. Med J (Ft Sam Houst, Tex) 2010;8:66-73.

14. Hirayama T, et al. Immunohistochemical analysis of cell proliferation and suppression of ameloblastoma with special reference to plexiform and follicular ameloblastoma. Acta Histochem Cytochem 2004;37:391-398 .

15. Agha R, Abdall-Razak A, Crossley E, Dowlut N, Iosifidis C, Mathew G; STROCSS Group. STROCSS 2019 Guideline: Strengthening the reporting of cohort studies in surgery. Int J Surg 2019 Dec;72:156-165.

16. Wright JM, Soluk-Tekkeşin M. ODONTOGENIC TUMORS: WHERE ARE WE IN 2017? Odontojen Tümörler: 2017 Yılında Neredeyiz? *J. Istambul Univ. Fac. od*. Dent 2017;51:10-30.

17. Maria, A. *et al.* Ameloblastomas : current aspects of the new WHO classification in an analysis of 136 cases. 4–9 (2019).

18. Florescu A, Simionescu C, Ciurea R, Pitru A. P53, Bcl-2 and Ki67 immunoexpression in follicular solid ameloblastomas. Rom J Morphol Embryol 2012;53(1):105-109.

19. Bologna-Molina R, Mosqueda-Taylor A, Lopez-Corella E, Almeida OP, Carrasco-Daza D, Garcia-Vazquez F, et al. Syndecan-1 (CD138) and Ki-67 expression in different subtypes of ameloblastomas. Oral Oncol 2008 Aug;44(8):805-811.

20. Abdel-Aziz A, Amin MM. EGFR, CD10 and proliferation marker Ki67 expression in ameloblastoma: possible role in local recurrence. Diagn Pathol 2012 Feb;7:14.

21. Sharifi-Sistani N, Zartab H, Babakoohi S, Saghravanian N, Jamshidi S, Esmaili H, et al. Immunohistochemical comparison of the expression of p53 and MDM2 proteins in ameloblastomas and keratocystic odontogenic tumors. J Craniofac Surg 2011 Sep;22(5):1652-1656.

22. Viner-Breuer R, Yilmaz A, Benvenisty N, Goldberg M. The essentiality landscape of cell cycle related genes in human pluripotent and cancer cells. Cell Div 2019 Dec;14:15.

23. Levine MS, Holland AJ. The impact of mitotic errors on cell proliferation and tumorigenesis. Genes Dev 2018 May;32(9-10):620-638.

24. Ahlem B, Wided A, Amani L, Nadia Z, Amira A, Faten F. Study of Ki67 and CD10 expression as predictive factors of recurrence of ameloblastoma. Eur Ann Otorhinolaryngol Head Neck Dis 2015 Nov;132(5):275-279.

25. Holland-Frei. Cancer Medicine. in (ed. Kufe DW, Pollock RE, Weichselbaum RR, et al., E.) (BC Decker; 2003., 2003).

26. Zhong Y, Guo W, Wang L, Chen X. Molecular markers of tumor invasiveness in ameloblastoma: An update. Ann Maxillofac Surg 2011 Jul;1(2):145-149.

27. Al-Salihi K, Li LY, Azlina A. P53 gene mutation and protein expression in ameloblastomas. Braz J Oral Sci 2006;5:1034-1040.

28. Richardson RB. p53 mutations associated with aging-related rise in cancer incidence rates. Cell Cycle 2013 Aug;12(15):2468-2478.

29. Yang, R. *et al.* Recurrence and cancerization of ameloblastoma : multivariate analysis of 87 recurrent craniofacial ameloblastoma to assess risk factors associated with early recurrence and secondary ameloblastic carcinoma. **29**, 189–195.

30. Fregnani ER, da Cruz Perez DE, de Almeida OP, Kowalski LP, Soares FA, de Abreu Alves F. Clinicopathological study and treatment outcomes of 121 cases of ameloblastomas. Int J Oral Maxillofac Surg 2010 Feb;39(2):145-149.

31. Au SW, Li KY, Choi WS, Su YX. Risk factors for recurrence of ameloblastoma: a long-term follow-up retrospective study. Int J Oral Maxillofac Surg 2019 Oct;48(10):1300-1306.

32. De Silva I, Rozen WM, Ramakrishnan A, Mirkazemi M, Baillieu C, Ptasznik R, et al. Achieving adequate margins in ameloblastoma resection: the role for intra-operative specimen imaging. Clinical report and systematic review. PLoS One 2012;7(10):e47897.

33. Ajila V, Hegde S. Ameloblastomas vs recurrent ameloblastomas: A systematic review. J. Oral Med. Oral Surg. 2022;28:1-8 .

34. Bi L, Wei D, Hong D, Wang J, Qian K, Wang H, et al. A retrospective study of 158 cases on the risk factors for recurrence in ameloblastoma. Int J Med Sci 2021 Jul;18(14):3326-3332.

35. Milman T, Ying GS, Pan W, LiVolsi V. Ameloblastoma: 25 Year Experience at a Single Institution. Head Neck Pathol 2016 Dec;10(4):513-520.

36. Kumamoto, H., Izutsu, T., Ohki, K., Takahashi, N. & Ooya, K. Proteins in Ameloblastomas. 292–299 (2004).

37. Barboza CA, Pereira Pinto L, Freitas RdeA, Costa AdeL, Souza LB. Proliferating cell nuclear antigen (PCNA) and p53 protein expression in ameloblastoma and adenomatoid odontogenic tumor. Braz Dent J 2005;16(1):56-61.