Original article

IMMUNOHISTOCHEMICAL EXPRESSION OF KI-67 AND P63 IN AMELOBLASTOMA, ODONTOGENIC KERATOCYST AND DENTIGEROUS CYST

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ABSTRACT

OBJECTIVE: The aim of this study was to evaluate the immunohistochemical expressions of Ki-67 and P63 in the epithelial component of ameloblastoma, OKC and DC, and to determine if there is any correlation between the expressions of Ki-67 and P63 in these lesions.

METHODS: A total of 10 ameloblastoma, 4 odontogenic keratocyst and 9 dentigerous cyst were utilized. Archival tissue blocks were retrieved and processed for immunohistochemistry using the Avidin-Biotin immunoperoxidase method. The primary antibody utilized was mouse monoclonal anti-Ki67 and anti-P63 (Novocastra®, 1:100 dilution). Visualization was achieved using diaminobenzidine (DAB) solution. Stained sections were counterstained using Haematoxylin, dehydrated using ascending grades of alcohol, cleared in xylene and cover-slipped using DPX as mountant. They were then viewed under a microscope for colour reaction.

Data was entered into a spreadsheet and analyzed using Kruskal-Wallis test. Correlation between Ki-67 and P63 was done using Spearman's rank correlation coefficient test. P-values ≤ 0.05 were considered statistically significant. All statistical analysis was done using IBM SPSS version 23.

RESULTS: Immunohistochemical expression of Ki-67was seen in 100% of OKCs, 70% of ameloblastomas and 44.4% of dentigerous cysts. For P63, there was positive immunohistochemical expression in 50% of ameloblastomas, 25% of odontogenic keratocysts and in 11.1% of dentigerous cysts. However, the differences in expression of these two markers in these lesions was not statistically significant. Spearman's rank correlation coefficient test showed that there was no statistically significant correlation between the expression of Ki-67 and p63 in ameloblastoma ($\sigma = 0.541$, $P = 0.106$), odontogenic keratocyst ($\sigma = -0.333$, $P = 0.667$) and dentigerous cyst ($\sigma = 0.530$, $P = 0.142$).

CONCLUSION: The pattern of expression of Ki-67 and P63 by ameloblastoma, odontogenic keratocyst and dentigerous cyst appears to follow the clinical aggressiveness of the lesions, with no correlation of the expression of these two markers.

Keywords: immunohistochemical, Ki-67, P63, ameloblastoma, odontogenic keratocyst, dentigerous cyst

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INTRODUCTION

Cellular proliferation is an essential process in the growth and development of organisms. Markers of cellular proliferation, such as Ki-67, have been used in evaluating the proliferative activity of cells in both normal and tumoral tissues.[1] Ki-67 is a nuclear protein associated with cellular proliferation, and is expressed in all active phases of the cell cycle, but absent in resting cells (G0 phase). This, coupled with its short half-life and rapid degradation makes it a specific marker of cellular proliferation [2,3]

The tumour protein p63 is a member of the p53 family of genes located on chromosome 3q27-29.[4] Its major function appears to be the regulation of growth and development of several epithelial-derived structures, and it is important in keratinocyte proliferation and differentiation, cell adhesion, and epithelial-mesenchymal signaling.[4,5] Up to 10 different isoforms of p63 have been identified, and they are categorized into 2 main groups, the TAp63 isoforms and the ΔNp63 isoforms, which tend to have opposing actions. While the Tap63 isoforms activate p53 target genes, promoting apoptosis, the ΔNp63 isoforms inhibit transcription activation of the p53 gene and exert a dominant negative effect on the Tap63 isoforms, thus promoting the proliferation of keratinocytes and other epithelial cells. [4,6]

Ameloblastoma, odontogenic keratocyst and dentigerous cyst are all derived from epithelial structures involved in tooth development Ameloblastoma displays aggressive clinical behaviour, with a tendency for local recurrence if not adequately removed.[7,8] Odontogenic keratocyst preferentially grows within the jaw bones in an antero-posterior direction without causing significant bony expansion. However, it frequently recurs, especially when treated with simple enucleation. [7] Dentigerous cysts are clinically not aggressive, and usually do not recur following treatment. [7] Studying the expression of Ki-67 and p63 in these odontogenic epithelial lesions could help provide better understanding of the role of these markers in the aetiopathogenesis and biological behaviour of these lesions.

Perusal of studies[78] from the scientific literature shows that some studies to predict the biologic nature of odontogenic tumours and cyst using various proliferative markers have been done. However, to the best of our knowledge similar studies are yet to be conducted in Nigeria. The aim of this study was to evaluate the immunohistochemical expression of Ki-67 and P63 in the epithelial component of ameloblastoma, odontogenic keratocyst and dentigerous cyst, and to determine if there is any correlation between the expression of Ki-67 and P63 in these lesions.

MATERIALS AND METHODS

A total of 23 cases were utilized for this study, comprising 10 solid/multicystic ameloblastoma, (SMA) 4 odontogenic keratocyst and 9 dentigerous cyst. These represented the cases of these lesions reported six months before commencement of the study in the Oral Pathology Laboratory of University of Port Harcourt Teaching Hospital. Archival formalin-fixed, paraffin-embedded (FFPE) tissue blocks were retrieved and processed for immunohistochemistry using the Avidin-Biotin immunoperoxidase method as follows. Two µm thick sections were taken from the FFPE tissue blocks using a rotary microtome. Sections were placed on a hot plate at 70^0 C for 1 hour, deparaffinized by passing through two changes of xylene, and then rehydrated by passing them through descending grades of alcohol. Antigen retrieval was achieved by immersing the sections in citric acid solution (pH = 6.0), and then heating in a microwave for 15 minutes. Sections were allowed to cool for 5 minutes, following which endogenous peroxidase activity was blocked by covering the sections with 3% hydrogen peroxide (H2O2) solution for 15 minutes. Protein blocking was achieved by applying avidin for 15 minutes, following which sections were washed with PBS and endogenous biotin activity blocked by adding biotin and incubating for 15 minutes.

The primary antibody (mouse monoclonal anti-Ki67 and anti-P63; Novocastra®, 1:100 dilution) was then applied and sections were incubated for 60 minutes. Excess antibodies were washed off, and the biotinylated secondary antibody applied and incubated for 15 minutes. Sections were

washed before application of horseradish peroxidase for 15 minutes. Diaminobenzidine (DAB) solution was then applied and sections incubated for 5 minutes. Excess (DAB) solution and precipitate were washed off with water. Finally, the sections were counterstained using Haematoxylin for 2 minutes, dehydrated by passing through ascending grades of alcohol, cleared in xylene and cover-slipped using DPX as mountant. Stained sections were then viewed under a microscope for colour reaction. A distinct brown colouration of the nucleus of the cells was considered a positive staining. Stained sections were categorized semi-quantitatively as follows:

0 = No reactions
+ = When < 25 % of cells were stained when viewed under high power
++ = When between 25-50% of cells were stain when viewed under high power
+++ = When > than 50% of cells were stained when viewed under high power

Data was analyzed using Kruskal-Wallis test. Correlation between Ki-67 and P63 was done using Spearman's rank correlation coefficient test. P-values ≤ 0.05 were considered statistically significant. All statistical analysis was done using IBM SPSS version 23.

RESULTS

Ki-67 immunohistochemical expression was seen in all the cases of OKC examined, with the expression being mild (+) in most cases (75%). Seventy percent of ameloblastomas showed positive expression of Ki-67, while 44.4% of the cases of dentigerous cyst were positive for Ki-67. (Table 1) However, the difference observed in the expression of Ki-67 in these lesions was not statistically significant. Ki-67 immunopositive cells in ameloblastoma were located randomly in both the outer ameloblast-like cells and inner stellate cells of the epithelial islands, in OKC Ki-67 immunopositive cells were seen mostly in the suprabasal layer of the epithelium, whereas in DC, the Ki-67 immunopositive cells were located mostly in the basal layer (Fig 1).

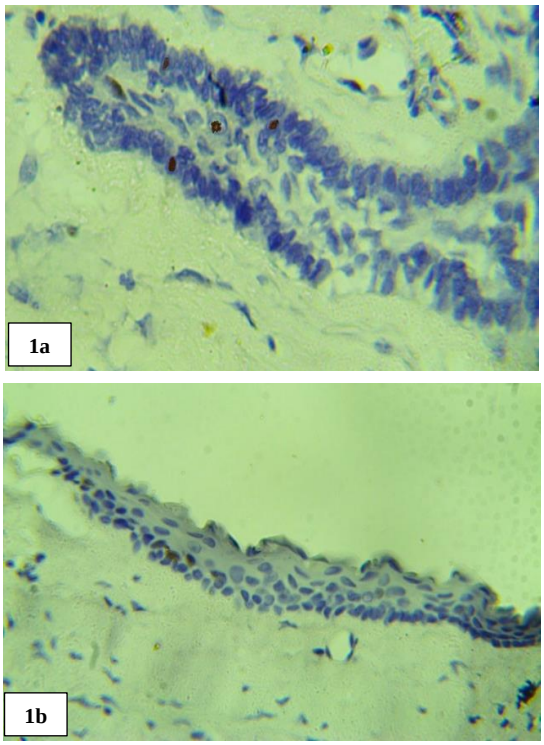


Fig 1a and b: Immunohistochemical expression of Ki-67 in (a) ameloblastoma and (b) odontogenic keratocyst (x 400 magnification)

Table 1: Ki-67immunohistochemical score distribution for the lesions

Histologic diagnosis	Ki-67 Score				
	0	**+**	**++**	**+++**	**Total**
Ameloblastoma	3 (30%)	3 (30%)	3 (30%)	1 (10%)	10 (100%)
Odontogenic Keratocyst	-	3 (75%)	-	1 (25%)	4 (100%)
Dentigerous Cyst	5 (55.6%)	2 (22.2%)	2 (22.2%)	-	9 (100%)
Total	**8 (34.8%)**	**8 (34.8%)**	**4 (17.4%)**	**2 (8.7%)**	**23 (100%)**

p= 0.139

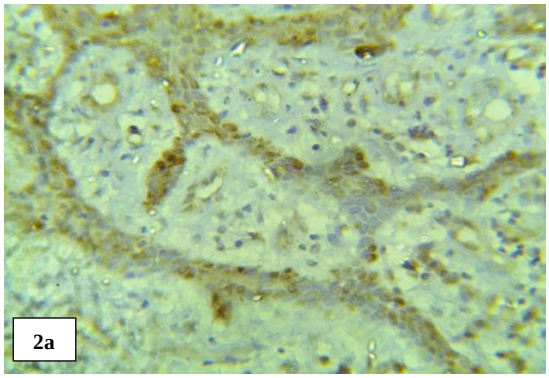
Table 2: P-63 immunohistochemical score distribution for the lesions

Histologic diagnosis	P-63 Score				
	0	**+**	**++**	**+++**	**Total**
Ameloblastoma	5 (50%)	1 (10%)	2 (20%)	2 (20%)	10 (100%)
Odontogenic Keratocyst	3 (75%)	-	-	1(25%)	4 (100%)
Dentigerous cyst	8 (88.9%)	-	-	1 (11.1%)	9 (100%)
Total	**16 (69.6%)**	**1 (4.3%)**	**2 (8.7%)**	**4 (17.4%)**	**23 (100%)**

p= 0.178

For P63, there was positive immunohistochemical expression in 50% of ameloblastomas, 25% of odontogenic keratocysts and in 11.1% of dentigerous cysts (Table 2). Again, this was not statistically significant. P63 positive cells were distributed diffusely across all epithelial layers in the lesions studied (Fig 2).

Spearman's rank correlation coefficient test showed that there was no statistically significant correlation between the expression of Ki-67 and p63 in ameloblastoma ($\sigma = 0.541$, $P = 0.106$), odontogenic keratocyst ($\sigma = -0.333$, $P = 0.667$) and dentigerous cyst ($\sigma = 0.530$, $P = 0.142$).



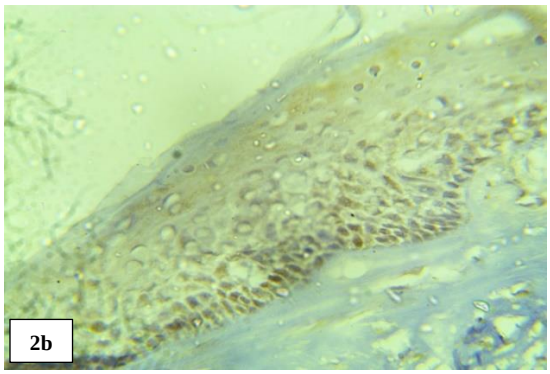


Fig 2a and 2b: Immunohistochemical expression of P-63 in (a) ameloblastoma and (b) odontogenic keratocyst (x 400 magnification).

DISCUSSION

Ameloblastoma is a an aggressive odontogenic tumour which is commonly seen among Nigerians.[8] It is known to have a high proliferation rate.[1] Odontogenic keratocyst is also a relatively common lesion whose nature and properties have remained controversial. This lesion was initially classified by the WHO as a cyst (1972), was later categorized as a tummor in 2005 and most recently (2017), reclassified as a cyst in 2017.[7] Its categorized as a tumour was due to its aggressive clinical nature and high proliferation rate similar to that of other odontogenic tumours.

In this study, all the odontogenic keratocysts (100%) expressed Ki-67 (a known marker of aggressiveness) while 70% of ameloblastomas expressed this marker. This is similar to the report of Kim et al,[9] Kaplan and Hirshberg,[10] and Kichi et al,[11] that reported over expression of Ki-67 by odontogenic keratocyst. However, 75% of the expression in odontogenic keratocysts were mild while 40% of the expression in ameloblastomas were either moderate or marked. The expression of Ki-67 by both odontogenic keratocyst and ameloblastoma supports the fact that both lesions are aggressive in nature. The difference in the expression of Ki-67 by both lesions could indicate that solid/multicystic ameloblastoma (SMA) is more aggressive in nature than odontogenic keratocyst and corroborates the study of Jaafari-Ashkavandi et al, who reported a similar finding. [12]

Only 44.4% of dentigerous cyst showed mild to moderate Ki-67 expression, indicating that this lesion is far less aggressive than odontogenic keratocyst and SMA. This obsernation is similar to that of Jaafari-Ashkavandi et al, who reported about 50% expression of Ki-67 among dentigerous cyst, with the expression limited to the basal and suprabasal layers only.[12]

In this study, 50% of amoloblastomas expressed P63, with 20% each showing moderate and marked expressions, while only 25% of odontogenic keratocysts and 11% of dentigerous cysts expressed P63. The pattern of expressionin this study showed that the more clinically aggressive lesions had higher expressions of the P63 marker. This observation is an agreement with reports from previous studies that showed that aggressive lesions such as ameloblastoma and odontogenic keratocyst tend to express P63 more frequently than less aggressive lesions. [13, 14, 15]

In this study, only a small percentage of the lesions (ameloblastoma, odontogenic keratocyst and dentigerous cyst) expressed P63 compared to Ki-67. The correlation between the expression of

Ki-67 and P63 in these three lesions was not statistically significant. A similar finding has been reported by Jaafari-Ashkavandi et al.[12]

In conclusion, the pattern of expression of Ki-67 and P63 by ameloblastoma, odontogenic keratocyst and dentigerous cyst appears to follow the clinical aggressiveness of the lesions. However, the correlation of the expression of these two markers shows no statistical difference.

No conflict of interest declared.

REFERENCES

1. Bologna-Molina R, Mosqueda-Taylor A, Molina-Frechero N, Mori-Estevez AD, Sánchez-Acuña G. Comparison of the value of PCNA and Ki-67as markers of cell proliferation in ameloblastic tumors. Med Oral Patol Oral Cir Bucal. 2013; 18 (2):e174-179.
2. Slootweg PJ. p53 protein and Ki-67 reactivity in epithelial odontogenic lesions. An immunohistochemical study. J Oral Pathol Med. 1995; 24: 393-397.
3. Li TJ, Browne RM, Matthews JB. Expression of proliferating cell nuclear antigen (PCNA) and Ki-67 in unicystic ameloblastoma. Histopathology. 1995; 26 (3):219-228.
4. Kumamoto H, Ohki K, Ooya K. Expression of p63 and p73 in ameloblastomas. Journal of Oral Pathology & Medicine: Official Publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology. 2005 Apr;34(4):220-226. DOI: 10.1111/j.1600-0714.2005.00284. x.
5. Guerrini L, Costanzo A, Merlo GR. A symphony of regulations centered on p63 to control development of ectoderm-derived structures. Journal of Biomedicine & Biotechnology. 2011; 2011:864904. DOI: 10.1155/2011/864904.
6. Alsaegh MA, Altaie AM, Zhu S. p63 Expression and its Relation to Epithelial Cells Proliferation in Dentigerous Cyst, Odontogenic Keratocyst, and Ameloblastoma. Pathology Oncology Research: POR. 2019 Jun DOI: 10.1007/s12253-019-00680-7.
7. El-Naggar, A.K., Chan, J.K.C., Grandis, J.R., Takata, T., Slootweg, P.J. (Eds). (2017) Odontogenic and maxillofacial bone tumours. In: WHO Classification of Head and Neck Tumours (4th edition). IARC: Lyon.
8. Lawal AO, Adisa AO, Olusanya AA. Odontogenic tumours: A review of 266 cases. J Clin Exp Dent. 2013; 5: e13-17.
9. Kim D, Ahn S, Kim J, Yoon J. Comparative Ki-67 expression and apoptosis inodontogenic keratocyst associated with or without an impacted tooth in addition to unilocular and multilocular varieties. Yonsei Medical J 2003; 44: 841-846.
10. Kaplan I, Hirsberg A. The correlation between epithelial cell proliferation and inflammation in odontogenic keratocyst. Oral Oncol 2004; 40: 985-991
11. Kichi E, Enokiya Y, Muramatsu T, Muramatsu T, Hashimoto S, Inoue T et al. Cell proliferation, apoptosis and apoptosis –related factors in odontogenic keratocysts and in dentigerous cysts. J Oral Pathol Med 2005; 34: 280-286.
12. Jaafari-Ashkavandi Z, Geramizadeh B, Ranjbar MA. P63 and Ki-67 expression in dentigerous cyst and ameloblastomas. Dent Shiraz Univ Med Sci. 2015; 16 (4): 323-328.
13. Lo Muzio L, Santerelli A, Caltabiano R, Rubini C, Pieramici T, Fior A et al. P63 expression in odontogenic cyst. Int J Oral Maxillofac Surg. 2005; 34: 668-673.
14. Seyedmajidi M, Shafaee S, Shafigh E, Bijani A, Hamidi H. P63 expression in randomized odontogenic cysts. Saudi Med J. 2011; 32: 463-466.
15. Goncalves CK, Fregnani ER, Leon JE, Silva-Sousa YT, Perez DE. Immunohistochemical expression of p63, epidermal growth factor receptor (EGFR) and notch-1 in radicular cyst, dentigerous cyst and keratocystic odontogenic tumours, Braz Dent J. 2012; 23: 337-343.