Original article

IMMUNOHISTOCHEMICAL STUDY OF p53 AND α-SMA EXPRESSION TO DETERMINE BIOLOGIC AGGRESSIVENESS OF CLINICO-PATHOLOGIC TYPES OF AMELOBLASTOMA

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ABSTRACT

OBJECTIVE: p53-cell cycle regulation system plays a critical role in the development of ameloblastoma, a slow growing and locally invasive tumour with tendency to recurrence. High frequency of stromal myofibroblasts contributes to more aggressive behaviour of a tumour. The study evaluated the expression patterns of p53 and alpha smooth muscle actin (α-SMA) among clinico-pathologic types of ameloblastoma in relation to biological aggressiveness.

METHODS**:** Sixty-nine histologic sections of ameloblastoma stained with H&E were subjected to immunohistochemistry via treatment with antibodies to p53 and α-SMA (mouse polyclonal antibody, DAKO) utilizing positive and negative controls. All slides were evaluated for immunoreactivity in ameloblast-like and stellate reticulum-like cells and tumour front in four quadrants by two pathologists. Data was analyzed by one-way ANOVA and Chi square using STATA 11 software. Statistical significance was set at $p < 0.05$.

RESULTS: The 69 cases consist of 37 males and 32 females of ameloblastoma were in age range 12-70 (mean: 35.0 ± 15.5) years with mandibular predilection. Clinical and histopathologic parameters analyzed showed follicular and plexiform growth patterns in 27 and 23 cases respectively. Immunohistochemical analysis revealed p53 expression in ameloblast-like and stellate reticulum cells, with α-SMA expression within stroma around the tumour front with mean positivity of 28.3 ± 24.5 and 29.7 ± 20.1 respectively. There was no statistically significant difference in expression within the clinico-pathologic types of ameloblastoma to p53 ($p= 0.209$) and α-SMA ($p= 0.976$).

CONCLUSION: Plexiform ameloblastoma with highest mean positivity to α-SMA and p53, is reasonably more aggressive than other clinic-pathologic types of ameloblastoma.

Keywords: Ameloblastoma, p53, α-SMA, Immunohistochemistry, Odontogenic tumours

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INTRODUCTION

Ameloblastoma is characterized as a slow growing, non-metastatic and a locally invasive tumour with a high risk of recurrence and belongs to the group "Odontogenic epithelium with mature, fibrous stroma without odontogenic ectomesenchyme". Although it represents only about 1-3% of tumours and cysts of the jaws[1-3], it is the commonest clinically significant odontogenic tumour in Nigeria and Africa, but the second most common odontogenic tumour in the western world [4,5]. Usually diagnosed in the third and fourth decades of life (mean age, 33 years), over 80% of ameloblastomas occur in the mandible [6], and of these, 70% occur in the molar region and the ascending ramus, 20% in the premolar region, and 10% in the incisor region and about 10-15% are associated with unerupted teeth[6].

It is classified by WHO (2005) into solid/ multicystic, unicystic, desmoplastic and peripheral types each of them presenting a specific biologic behavior and thus different treatment and prognosis. There is histologic sub-variation into follicular and plexiform growth patterns which in turn may show acanthomatous, desmoplastic, basal cell, clear cell, granular cell and (keratoameloblastoma) papilleferous changes. Despite this histologic diversity, the multiple cellular patterns can coexist in the same lesion [7]. It has been asserted that the follicular ameloblastoma recurs more often than the plexiform type and that the unicystic ameloblastoma shows lower recurrence rate than the solid type [7].

The proliferating tumour may infiltrate the marrow spaces without causing significant bone destruction. Occasionally an ameloblastoma may be left unattended for many years without intervention and yet does not break through the bone[8]. But for reasons unknown some ameloblastomas manage to penetrate the bone and extend into the surrounding soft tissues. p53 is a tumour suppressor gene with a short half-life in normal cells, and cannot be detected immunohistochemically[9,10] Its concentration increases as its half-life is extended, which may occur due to p53 gene mutation, association of wild type p53 with other proteins, or disruption of its degradation pathway[9,10]. With mutation,the p53 protein product is more stable and can be detected using by immunohistochemistry.

p53 cell cycle regulation system was proven to play a critical role in a variety of neoplasms including odontogenic tumours. The expression ratio of p53 in tooth germs was found to be significantly lower than those in ameloblastoma, suggesting that p53 might be associated with its oncogenesis, tissue structuring and cytodifferentiation[11]. p53 immunoreactivity signifies the prognostic status of the tumour where verification of more than 10% p53-positive cells may give an indication for a tendency to recurrence[12].

A combined study of p53 and ki-67 that was carried out by Slootweg[13] reported nuclear staining for p53 in 11 of 13 cases of OKC, 6 of 9 cases of ameloblastoma and 2 of 2 cases of odontogenic carcinoma. The expression of p53 antigen in ameloblastoma was significantly higher than AOT in another study carried out by Salehinejad et al[14]. Similarly, other authors confirmed the overexpression of p53 in ameloblastoma[13]. Barboza and colleagues indicated that ameloblastoma had higher p53 labeling index than AOT[11].

In wound healing and tumourigenesis, the trans-differentiation of fibroblasts to myofibroblasts (MF) marks the stromal changes which may contribute to tumour invasion[15]. A high frequency of stromal MF has been observed in known aggressive odontogenic lesions, such as parakeratinising OKC (KCOT) and solid ameloblastoma, implying that MF can contribute to variations in the biological behaviour of these odontogenic lesions[16]. This study therefore assessed the frequency of stromal myofibroblasts by means of alpha smooth muscle actin (α-SMA) and the expression patterns of p53 among clinico-pathologic types of ameloblastoma and the relationship to biological aggressiveness.

MATERIALS AND METHODS

Study Design

This was a retrospective study designed to determine the immunohistochemical profile of

clinicopathologic types of ameloblastoma seen over 20 years (1991-2010) at the Obafemi Awolowo University Teaching Hospitals Complex, (O.A.U.T.H.C). The study was approved by the Ethical Review Committee of the O.A.U.T.H.C, Ile-Ife, Nigeria.

Study Group

Cases of ameloblastoma, that were to be analysed immunohistochemically were selected from a pool of 187 biopsy cases previously diagnosed histologically as ameloblastoma over a 20year period (1991-2010). Clinical information regarding age, sex, location and duration of tumour with clinical and histologic diagnosis were retrieved from biopsy report registers. Paraffin wax blocks of all ameloblastoma cases were retrieved, recut and reconfirmed by haematoxylin and eosin (H&E) stained sections.

The ameloblastoma cases were subsequently analyzed using a panel of antibodies consisting of anti-SMA and anti-p53 antibodies. The positive and negative controls for each antibody were processed simultaneously along with the other slides for the same antibody to ensure validity. While ameloblastic carcinoma that contained the target antigen against which the primary antibody raised was used as positive control when staining with p53, tonsillar tissue was used as negative control for anti-SMA.

Immunohistochemical Assessment

The criterion for a positive reaction confirming the presence of p53 protein was a dark, brownish, intranuclear precipitate and cytoplasmic precipitate for α-SMA. A semiquantitative evaluation of the immunoreactivity to the p53 and α-SMA marker was assessed using the scoring criteria:[17]

+: <10% positive p53 & α-SMA cells
+: 10-25 % positive p53 & α-SMA cells
++: 26% - 50% positive p53 & α-SMA cells
+++: 51-75% positive p53 & SMA cells
++++: >75% positive p53 & SMA cells

Data Analysis

Data was analysed using Stata 11 (Statacorp College Station, Texas). Descriptive statistics were carried out for socio-demographic variables such as age and sex. Quantitative data were summarized using mean, standard deviation and confidence interval. Association between immunostaining and clinical / histological type of tumour as well as the immunoprofile were analysed using Chi-square statistics to compare proportions.

MF expression by the tumours was categorised into 0, 1, 2 3 and 4 based on the percentage of cells stained and comparison of MF expression within variants of ameloblastoma was analysed using Chi-square. One-way analysis of variance (ANOVA) was used to compare the difference in the p53 and α-SMA positivity in histologic variants of ameloblastoma. Statistical significance was inferred at $p<0.05$.

RESULTS

The 69 cases of ameloblastoma included 37 men and 32 women, with a mean age of 35.0 ± 15.5 years (range of 12-70 years) and predominated in the male gender and mandible (n= 65, 95.5%). There were 27 (39.1%), 23 (33.3%), and 10 cases of follicular, plexiform and unicystic ameloblastoma respectively, as well as 4 cases each of acanthomatous and granular cell ameloblastoma and 1 case of keratoameloblastoma (Table 1; Figure 1).

p53 expression was observed within epithelial tumour parenchyma, namely peripheral columnar and stellate reticulum cells while α-SMA expression was observed within the stroma around tumour front and as cytoplasmic staining of columnar cells in the periphery of tumour islands, and rarely in stellate reticulum-like cells 23 (33.3%) cases. There was moderate to strong positivity to p53 in 47.8% (33) and to α-SMA in 76.8% (53) of cases (Tables 1 and 2), with mean index of positivity (IP) of 28.3 ± 24.5 and 29.7 ± 20.1 respectively. The highest mean IP of 31.7 ± 26.6 (p53) and 32.9 ± 24.9 (α-SMA) was observed in plexiform ameloblastoma while the lowest scores of 22.3 ± 26.0 in α-SMA and15.4 ± 22.0 in p53 were observed in granular cell and unicystic ameloblastoma respectively [Table 3]. There was no statistically significant difference in expression within the clinico-pathologic types of ameloblastoma to p53 (p= 0.209) and α-SMA (p= 0.976) [Table 4].

Table 1: Clinico-pathologic parameters of ameloblastoma

	Frequency	%
Age (years)		
9-29	28	40.6%
30-49	26	37.7%
50-69	12	17.4%
70-89	01	1.4%
Gender		
Female	32	46.4%
Male	36	52.2%
Site		
Mandible	65	94.2%
Maxilla	4	5.8%
Histopathology		
Follicular	27	39.1%
Plexiform	23	33.3%
Unicystic	10	14.5%
Acanthomatous	4	5.8%
Granular cell	4	5.8%
Keratoameloblastoma	1	1.4%
p53		
+	12	17.4%
++	19	27.5%
+++	14	20.3%
_	24	34.8%
α-SMA		
+	17	24.6%
++	27	39.1%
+++	8	11.6%
_	16	23.2%

Table 2: Index of positivity to α-SMA and p53 in clinico-pathologic types of ameloblastoma

Intensity of Staining	α-SMA (%)	p53 (%)
0 (negative=<10%)	16 (23.2)	24 (34.8)
1+ (weak staining=10-25%)	17 (24.6)	12 (17.4)
2+ (moderate staining=26-50%)	27 (39.1)	19 (27.5)
3+ (strong staining=51-75%)	8 (11.6)	14 (20.3)
4+ Strong staining=>75%	1 (1.5)	0 (0.0)
Total	69 (100.0)	69 (100.0)

DISCUSSION

Many studies on odontogenic tumours (OTs) were carried out before the re-classification of odontogenic tumours by WHO in 2005 [18,19]. Substantial changes to ameloblastoma involved subdivision into 4 distinct clinico-histologic types: solid/multicystic; extraosseous/peripheral; desmoplastic and unicystic types due to significant differences in the surgical management and prognosis of the various subtypes.

Reports also vary as regards prevalence and frequency of each type of OT. Previous African[20] and Asian[21] studies have found ameloblastoma to be the most common OT. Studies from Asia showed that ameloblastoma represented 58.6% [21] and 68.9% of OTs [22] while Ladeinde *et al* [23], Adebayo *et al* [24], Chidzonga *et al* [25] and Tawfik [3] all from Africa reported that ameloblastoma represented 63%, 73%, 79.1% and 41.5% of OTs respectively. In our study, ameloblastoma accounted for 74.8% of OTs which was within the range of most reports from Asia and Africa. A male to female ratio of 1.2:1, in agreement with previous Nigerian studies [6,19,20] slightly contrasted by Olaitan *et al* who reported a 1.5:1 ratio [26] was observed. While earlier studies reported ameloblastoma between the first and ninth decades of life, the cases in this study were limited to the first and eighth decades of life. The peak incidence of second and third decades observed in this study also differs from the universal figures of third and fourth decades which previous Nigerian studies had corroborated [20,26.] This may be due to the fact that our centre is a referral centre in our catchment area.

p53-cell cycle regulation system has been proven to play a critical role in a variety of neoplasms including odontogenic tumours with expression ratio of p53 in tooth germs found to be significantly lower than those in ameloblastoma, suggesting its association with oncogenesis, tissue structuring and cytodifferentiation [11]. Analysis of p53 expression revealed weak to strong positivity in over 60% of the ameloblastomas. p53 protein expression does not necessarily imply an association with malignant disease, but expression in a quantitatively and

qualitatively increasing manner indicates a more aggressive behavior of cancers (tumours). Therefore, p53 inactivation or overexpression can promote cell proliferation in odontogenic lesions [28].

Our findings revealed a stronger overall index of positivity (IP) to p53 in plexiform ameloblastoma than the follicular subtype, in agreement with studies from Brazil[11], Japan [27] and Egypt [3], but contrary to an Iranian study[14] which found the highest mean IP for p53 in acanthomatous ameloblastoma. A strong IP to p53observed in plexiform ameloblastoma has been attributed to the higher level of cellular activity owing to greater genomic damage or cell stress in plexiform than in follicular ameloblastoma [27]. The least IP for p53 was found in unicystic ameloblastoma, suggesting that the unicystic ameloblastoma is less aggressive than the multicystic ameloblastoma.

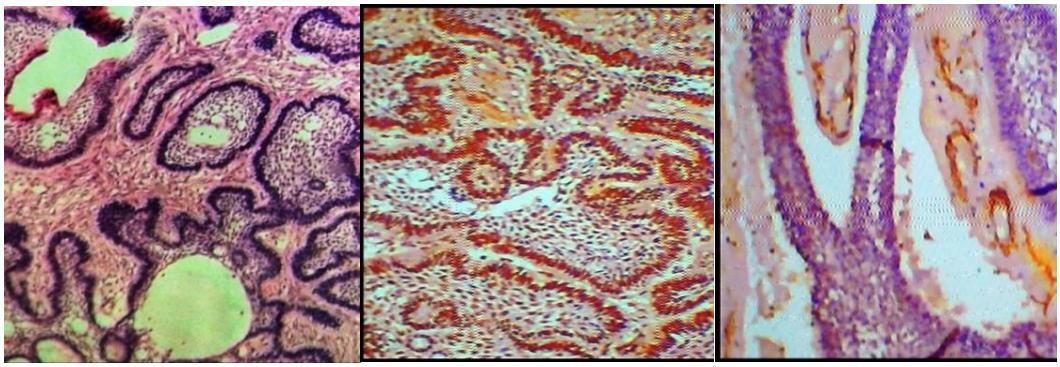

Figure 1- Immunohistochemical profile of ameloblastoma (a) haematoxylin and eosin (H&E) (b) intense nuclear staining of peripheral cells with p53 (c)staining of mature connective tissue near the islands with strong positivity for blood vessels and myofibroblasts with α-SMA. All immunohistochemical sections were counterstained with hematoxylin. Magnification is x100 except α-SMA (x400).

Table 3: Mean Positive cells for α-SMA and p53 in clinico-pathologic types of Ameloblastoma

Marker	FA (mean±SD)	PA (mean±SD)	AA (mean±SD)	GA (mean±SD)	KA (mean±SD)	UCA (mean±SD)	P value
α-SMA	31.3±25.2	32.9±24.9	25.0±21.2	22.3±26.0	30.0±0.0	28.5±20.7	0.976
p53	29.9±23.9	31.7±26.6	28.3±21.2	28.8±16.5	30.0±0.0	15.4±22.0	0.209

*FA= follicular ameloblastoma, PA= plexiform ameloblastoma, AA= acanthomatous ameloblatoma, GCA= granular cell ameloblastoma, KA= keratoameloblastoma, UCA= unicystic ameloblastoma

Table 4: Mean Labelling Indices of Histologic Subtypes of Unicystic Ameloblastoma

	Histologic Subtypes			
Marker	**Mural (5) mean±SD**	**Intraluminal (3) mean±SD**	**Luminal (2) mean±SD**	**P value**
SMA	36.0±19.2	29.0±27.6	19.0±24.7	0.679
P53	12.0±14.4	4.5±1.7	11.0±4.0	0.652

Most similar studies communicate the reduced p53 positivity in solid ameloblastomas, in less than half of the analyzed casuistry [29-31.] Moderate or weak p53 expression could be due to slow and expansive growth of tumours. Changes in DNA can be initiated in these cells following the appearance of mutant forms of p53 that negatively regulate expression of p53 wild form, in the sense of p53 mutant–p53 wild complex formation, leading to mutual inactivation of the two proteins [32].

Some authors have postulated that there is no correlation between the histologic types of ameloblastoma and clinical behavior (and consequent prognosis) because more than one cellular configuration can be seen in a single lesion [33,34]. Finding no statistical significance between clinic-pathologic subtypes of ameloblastomas ($p = 0.209$), in this series may support this statement as was also reported by Barboza *et al* [11]. On the contrary Ueno *et al* [35] and Reichart *et al* [7] opined that follicular ameloblastoma presents a higher recurrence rate compared with plexiform ameloblastoma. Within the subtypes of UCA, the mural variant had the highest mean IP for p53 but there was no statistically significant difference, $p = 0.652$.

α-SMA expression pattern was observed in both cells within the stroma (around the blood vessels). About a third of ameloblastomas showed α-SMA activity at the tumour front, clearly indicating the pivotal role of α-SMA positive myofibroblasts in tumour progression. α-SMA positivity was also observed within odontogenic epithelium with stronger expression in the stellate reticulum-like than the peripheral cells. IP for α-SMA was highest in plexiform ameloblastoma and least in granular cell ameloblastoma as reported by Vered *et al* [15], but highest in the mural subtype of UCA, than other UCA subtypes suggesting its greater aggressiveness. This supports the general consensus for a conservative approach to the treatment of luminal and intraluminal UCA and a more aggressive treatment for mural unicystic ameloblastoma. However, mural UCA was found to have a higher α-SMA but lower p53 LI than either of plexiform or follicular ameloblastoma.

Vered *et al* also demonstrated α-SMA positive myofibroblasts very close to the ameloblastomatous islands [15] as was observed in our study where epithelial islands showed positivity to α-SMA with stronger expression in the stellate reticulum-like than the peripheral cells. This is incongruent with reports of Bello *et al* [36] and Roy and Garg [37] in which expression of α-SMA was consistently seen within the epithelial island cells of ameloblastic carcinoma and less so in solid multicystic ameloblastoma, while the marker was well expressed in the stroma of both lesions. The reason for the myofibroblasts being associated with neoplastic epithelium is still not clear except for an association with distant metastasis and an epithelial-mesenchymal transition [38].

In conclusion, plexiform ameloblastoma had the highest mean IP for α-SMA and p53 among the clinic-pathologic types of ameloblastoma. It is reasonable to conclude that plexiform ameloblastoma is more aggressive than follicular ameloblastoma. As a high index of p53-positivity may give a prognostic indication for a tendency to recurrence, long term follow up of cases of plexiform ameloblastoma is therefore required.

REFERENCES

1. Small LA, Waldron CA. Ameloblastoma of the jaws. Oral Surg Oral Med Oral Pathol 1955;8:281 297.
2. 2. Ajagbe HA, Daramola JO. Ameloblastoma:A survey of 199 cases in the University College Hospital, Ibadan, Nigeria. J Nat Med Assoc 1987;79:324-327.
3. Tawfik MA, Zyada MM. Odontogenic tumors in Dakahlia, Egypt: analysis of 82 cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:67-73.
4. Regezi JA, Kerr DA, Courtney RM. Odontogenic tumours: Analysis of 706 cases.J Oral Surg 1978; 36: 771-778.
5. Daley TM, Wysocki GP, Pringle GA. Relative incidence of odontogenic tumours and oral and jaw cysts in a Canadian population. Oral Surg Oral Med Oral Path01 1994; 77: 276-280.
6. Ghandhi D, Ayoub AF, Pogrel MA, MacDonald G, Brocklebank LM & Moos KF. Ameloblastoma: a surgeon's dilemma. J Oral MaxillofacSurg 2006; 64: 1010–1014.

7. Reichart PA, Philipsen HP, Sonner S. Ameloblastoma: biological profile of 3677 cases. Eur J Cancer B Oral Oncol 1995;31(2):86-99.
8. Al-Sahili KA, Li YL, Azlina A. p53 gene mutation and protein expression in ameloblastoma. Braz J Orl Sci 2006; 5 (17) : 1030-1040.
9. Levine AJ. p53, the cellular gatekeeper for growth and division. Cell 1997;88(3):323-331.
10. Wynford-Thomas D. P53 in tumour pathology: can we trust immunocytochemistry. J Pathol 1992 ;166(4):329-330.
11. Barboza CA, Pereira Pinto L, Freitas Rde A, Costa Ade L, Souza LB. Proliferating cell nuclear antigen (PCNA) and p53 protein expression in ameloblastoma and adenomatoid odontogenic tumor. Braz Dent J 2005;16:56-61.
12. Appel T, Gath R, Wernert N, Martini M, Bergé S. Molecular biological and immunohistochemical anal- ysis of tp53 in human ameloblastomas. Mund Kiefer Gesichtschir. 2004, 8 (3): 167-172.
13. Slootweg PJ. p53 protein and Ki-67 reactivity in epithelial odontogenic lesions: An immunohistochemical study J Oral Pathol Oral Med 1995;24:393-397.
14. Salehinejad J, Zare-Mahmoodabadi R, Saghafi S, Jafarian A-H, Ghazi N, Rajaei AR, et al. Immunohistochemical detection of p53 and PCNA in ameloblastoma and adenomatoid odontogenic tumor. Journal of Oral Science. 2011;53(2):213-217.
15. Vered M, Shohat I, Buchner A, Dayan D. Myofibroblasts in stroma of odontogenic cysts and tumors can contribute to variations in the biological behavior of lesions. J Oral Oncol 2005;41: 1028-1033.
16. Adebiyi KE, Ugboko VI, Omoniyi-Esan GO, Ndukwe KC, Oginni FO. Clinicopathological analysis of histological variants of ameloblastoma in a suburban Nigerian population. Head Face Med. 2006;2(42):1-8.
17. van Diest PJ, van Dam P, Henzen-Logmans SC, Berns E, van der Burg ME, Green J, et al. A scoring system for immunohistochemical staining: consensus report of the task force for basic research of the EORTC-GCCG. European Organization for Research and Treatment of Cancer-Gynaecological Cancer Cooperative Group. J Clin Pathol 1997; 50:801–4.
18. Philipsen HP, Reichart PA, Slootweg PJ, et al. Odontogenic tumours in Pathology and Genetics of head and neck tumours. 2005:283–318.
19. Jing W, Xuan M, Lin Y, Wu L, Liu L, Zheng X. Odontogenic tumours: a retrospective study of 1642 cases in a Chinese population. Int J Oral Maxillofac Surg 2007;36:20-25.
20. Arotiba JT, Ogunbiyi JO, Obiechina AE. Odontogenic tumours: a 15-year review from Ibadan, Nigeria. Br J Oral Maxillofac Surg 1997; 35:363-367.
21. Lu Y, Xuan M, Takata T, et al. Odontogenic tumours: a demographic study of 759 cases in a Chinese population. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 86:707-714.
22. Okada H, Yamamoto H, Tilakaratne WM. Odontogenic tumors in Sri Lanka: analysis of 226 cases. J Oral Maxillofac Surg. 2007;65:875-882.
23. Ladeinde AL, Ajayi OF, Ogunlewe MO, Adeyemo WL, Arotiba GT, et al. Odontogenic tumors. A review of 319 cases in a Nigerian teaching hospital. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005;99:191-195.
24. Adebayo ET, Ajike SO, Adekeye EO. Odontogenic tumours in children and adolescent: a study of 78 Nigerian cases. J Craniomaxillofac Surg 2002; 30:267-272.
25. Chidzonga MM, Lopez Perez VM, portilla-Alvarez AL. Ameloblastoma: the Zimbabwean experience over 10 years. oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996;82:38-41.
26. Olaitan AA, Adeola DS, Adekeye EO. Ameloblastoma: Clinical Features and Management of 315 cases from Kaduna,Nigeria. J Craniomaxillofac 1993;21:351-355.
27. Kumamoto H, Ohki K, Ooya K. Expression of p53 and p73 in ameloblastomas. J Oral Pathol Med 2005; 34:220-226.
28. el-Sissy NA, Immunohistochemical detection of p53 protein in ameloblastoma types, East Mediterr Health J, 1999, 5(3):478–489.
29. Gadbail AR, Patil R, Chaudhary M, Co-expression of Ki-67 and p53 protein in ameloblastoma and keratocystic odontogenic tumor, Acta Odontol Scand, 2012; 70: 529–535
30. de Vicente JC, Torre-Iturraspe A, Gutiérrez AM, Lequerica- Fernández P, Immunohistochemical comparative study of the odontogenic keratocysts and other odontogenic lesions, Med Oral Patol Oral Cir Bucal, 2010, 15(5):e709–e715.
31. Kumamoto H, Ooya K, Immunohistochemical analysis of bcl-2 family proteins in benign and malignant amelo- blastomas, J Oral Pathol Med, 1999, 28(8):343–349.
32. Ahmed MM, el-Azab SM. Evaluation of cell-cycle related indicators in plexiform ameloblastoma Journal of Egyptian Nat.Cancer Inst. 2008;20(3):294-301.
33. Gardner DG. Some current concepts on the pathology of ameloblastomas. J Oral Surg Oral

Med Oral Pathol Oral Radiol Endod 1996; 82:660-669.
34. Thompson IO, van Rensburg LJ, Phillips VM. Desmoplastic ameloblastoma: correlative histopathology, radiology and CT-MR imaging. J Oral Pathol Med 1996; 25:405-410.
35. Ueno S, Mushimoto K, Shirasu R. Prognostic evaluation of ameloblastoma based on histologic and radiographic typing. J Oral Maxillofac Surg 1989; 47:11-15.
36. Bello IO, Alanen K, Slootweg PJ, Salo T. Alpha-smooth muscle actin within epithelial islands is predictive of ameloblastic carcinoma. Oral Oncol. 2009; 45(9):760-765.
37. Roy S, Garg V. Alpha Smooth Muscle Actin Expression in a Case of Ameloblastic Carcinoma: A Case Report. J Oral Maxillofac Res 2013;4(1):1-6
38. Kamath KP, Vidya M, Shetty N, Karkera BV, Jogi H. Nucleolar Organizing Regions and a-Smooth Muscle Actin Expression in a Case of Ameloblastic Carcinoma. Head and Neck Pathol 2010; 4:157-162.