Immunohistochemical Expression of P53 In Different Types of Ameloblastoma

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Abstract

***Introduction*:** Authors agree that ameloblastoma is a benign but aggressive tumor develops in the jaw area either from tooth bud epithelium, or the epithelial remnants left after tooth eruption or extraction. The WHO classified the Ameloblastoma, in its 2005 tumors classification, as a group of tumors, each one of tumors of this group has its own clinical behaviour and radiographic appearance. The local invasion and destruction of the adjacent structures are the source of risk in ameloblastoma. Nevertheless, a high rate of recurrence is reported after surgery. A lot of immunohistochemical researches have been conducted in the last two decades in an attempt to elucidate the etiologic factors and treatment modalities for this tumor. P53 gene plays a key role in cell cycle regulation and it has been shown as an important factor in the genesis of some types of this tumor that affects its behavior.

Aims of the Study: This study was carried out to investigate the association between P53 positivity and some types of ameloblastoma.

Materials and Methods: Twenty specimens of ameloblastoma have been studied immunohistochemically with p53 expression using monoclonal antibody (according to instruction of the Immunotech, Marseille, Codex-9-France).

Results: Observed tumor types were as follows: 7, follicular type, 5, plexiform type and 8 with unicystic type.

P53 was found to be positively expressed in 17 (85%) cases, and negatively in 3(15%) cases.

Conclusion: The unicystic type showed higher intensity of p53 expression which indicates higher rate of invasion and recurrence.

***Keywords*: Ameloblastoma ; P53 .**

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Introduction

Ameloblastomas, also known as adamantinoma, are the most common odontogenic tumor (35%). They are benign, locally aggressive neoplasm arising from ameloblasts, which typically occur at the angle of the mandible and they are often associated with an unerupted tooth and must therefore be differentiated from a dentigerous cyst which will be centered around the crown.[1,2] When in the maxilla (less common), they are located in the premolar region, and can extend up in the maxillary sinus.[3]

According to the new WHO Classification of Odontogenic Tumors (2005) and analyses of their clinical and microscopic features, the lesion has a tendency to expand the bony cortices because slow growth rate of the lesion allows time for periosteum to develop thin shell of bone ahead of the expanding lesion. This shell of bone cracks when palpated and this phenomenon is referred to as "Egg Shell Cracking" or crepitus, as an important diagnostic feature.[4]

Ameloblastoma is tentatively diagnosed through radiographic examination and must be confirmed by histological examination (e.g., biopsy). Radiologically, 50% of ameloblastomas appear as multilocular radiolucent lesions with sharp delineation. Histologically, one-third are plexiform, one-third follicular; other variants such as acanthomatous ameloblastoma occur in older patients. Two percent of ameloblastomas are peripheral tumors. Unicystic ameloblastomas occurring in younger patients have been found in 6%.[5]. While these tumors are rarely malignant or metastatic (that is, they rarely spread to other parts of the body), and progress slowly. The resulting lesions can cause severe abnormalities of the face and jaw.

Additionally, because abnormal cell growth easily infiltrates and destroys surrounding bony tissues, wide surgical excision is required to treat this disorder. Further, dentist's caution is that wide surgical excision is not invasive enough to adequately treat this disorder[6]. It is now believed that neoplastic transformation, consisting of a multistep accumulation of adverse genetic events, occurs in a wide variety of human tumors over a large region of the genome[7].

Mutations in the p53 tumor suppressor gene are among the most common abnormalities in human cancer. It is implicated in the control of the cell cycle, DNA repair and synthesis, cell differentiation, genomic plasticity and apoptosis[8].

Aim of the Study

The purpose of this study was to detect the association between P53 positivity and some, types of ameloblastoma(solid intraosseous and unicystic intraosseous).

Materials and Methods

Selection of cases

Study group:

This retrospective study was conducted on twenty cases of ameloblastoma during(2007) from the archive paraffin blocks in the department of Oral Pathology in the Faculty of Dentistry, University of Damascus.

Control group:

Three specimens of mandibles from normal human fetuses were obtained from Oral Histology Department in the Faculty of Dentistry ,Damascus University. They were fixed in 10% neutral buffered formalin and then decalcified and prepared as paraffin blocks.

Three positive control blocks of a tissue were known to contain the target antigen against which the primary antibody raised. In this study the tissue was ductal breast carcinoma which was obtained from the Teaching Laboratories in Baghdad Medical City. The tissues had been fixed in 10% neutral buffered formalin. From each of the twenty paraffin-embedded blocks two tissue sections were cut at 5μm, one was used for histopathological diagnosis stained with Harris' hematoxylin & eosin while the other was used for immunohistochemical evaluation, so they were mounted on probe-on plus slides (Fischer brand), then sections were immersed in retrieval solution and heated in water bath at 95 C^{o} for 30 minutes, the sections then were cooled at room temperature ,followed by immersing the sections in PBS (Phosphate-buffered solution), then excess solution was removed by blotting off the slides, the non-specific primary and secondary antibody binding was inhibited by incubating the sections with power block reagents ,excess solution was blotted off. We have tried not to rinse the sections, the primary antibody were appropriately diluted and rinsed well with PBS for 10 minutes and wiped around the sections. This step was omitted in negative control slides.

Then diluted biotinylated goat-anti-mouse IgG (link) solution were added. After this labeled streptavidin (label) solution were added. Incubated and rinsed well with PBS, then chromogen solution was applied. While negative control sections were incubated with PBS instead of primary antibody; ductal breast carcinoma sections were served as positive controls. The above – mentioned IHC procedures were all done in teaching – Laboratories – Medical City of Baghdad (according to instruction of the Immunotech, Marseille, Codex-9-France).

Evaluation of IHC Stained Sections:

The criterion for a positive reaction confirming the presence of p53 protein was a dark, brownish, intranuclear precipitate. Whole tissue sections were examined with light microscope for P53-positive nuclear staining at x 100 and at x 400. The P53 expression was recorded as positive if > 10% of all the tumor cells positive for nuclear P53 staining and as negative if < 10% were positive cells based on[9] method which depends on the visual evaluation of the field.

The staining intensity in p53-positive cases was further categorized as dense or faint as follows:

• Dense: densely stained nuclei, easily seen at low magnification (objective 5´)

• Faint: faint nuclei staining that could only be detected by using higher magnification (objective 40´) **[9,10].**

Results

The 20 Ameloblastoma cases in this study included 14 males and 6 females, with ages ranging from 21years to 50 years with mean age of (35.5) as shown in table-1. All ameloblastoma cases were intraosseous, located in the mandible.

Table-1 Distribution of patients according to the age and sex.

Age group	No. of cases	%
20-30years	M 6 F 4	50
31-40years	M 3 F 2	25
41-50years	M 5 F -	25
Total	M 14 F 6	100

Clinical & Histological Results:

Light microscopic examination revealed that the twelve cases had well-known microscopic features of conventional and solid ameloblastoma(seven of them of follicular type and five of them of plexiform type). Eight cases were of the unicystic type as shown in figure-1.

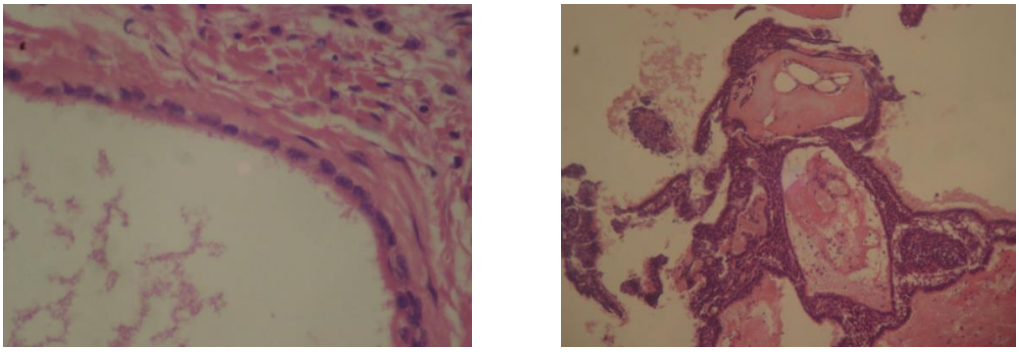

Figure-1 : unicystic type plexiform type
H&E Stain of Ameloblastoma X 100

Immunohistochemical Results

None of the examined sections of the control group showed positive staining for the p53 protein marker used in this study.

Expression of positive p53 protein immunoreactivity was present in 9 cases of the 12 (four from follicular and five from plexiform types) solid ameloblastoma cases (45%).

Table-2 Expression of p53 protein in different histological types of ameloblastoma and their intensities in appearance.

Clinical &Histological type	P53+ve expression	% of +ve expression	Intensity of expression
Follicular= 7	4	20	faint
Plexiform= 5	5	25	faint
Unicystic= 8	8	40	dense
Total= 20	17	85	

In unicystic ameloblastoma cases, positive cells were evident in all eight cases (40%) as shown in table-2. It was noticed that the positive cells for p53 were found in groups of epithelial cells lining the cystic space, and were confined to the basal layer.

The p53-positive cells among the reactive cases in solid ameloblastoma were mostly faintly stained, and the distribution of immunoreactive cells was noticeably in the peripheral, ameloblast-like cells and occasionally in the central, stellate reticulum-like cells as shown in figure-2.

The highest frequency of p53-positive cells and dense staining was observed among unicystic ameloblastoma cases. These p53-positive cells were focally distributed in collections of neoplastic cells.

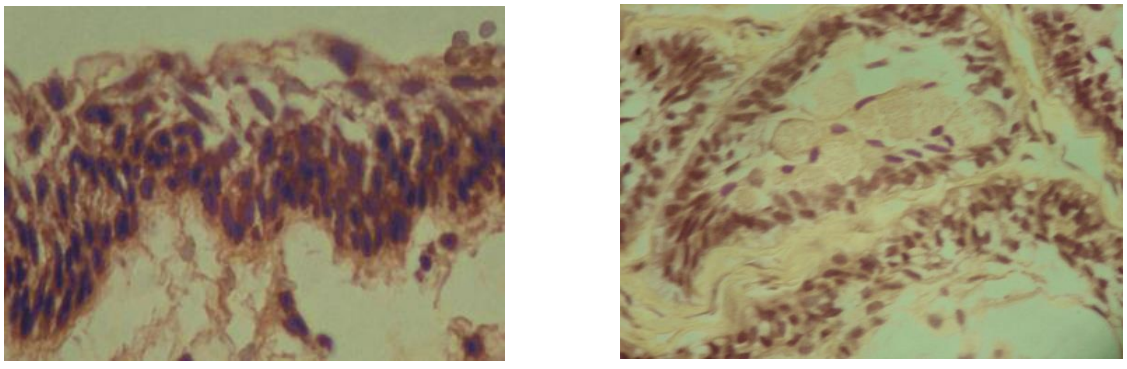

Figure-2

P53 Appearance In Immunohistochemistry Brown Stained Nuclei Result From Condensed Mutated P53 Protein With Different Intinsities. x 400

Discussion

The mean age for ameloblastomas cases in this study was (35.5) years old and this agreed with most researches and studies[11,12,13].

It is now believed that neoplastic transformation, consisting of a multistep accumulation of adverse genetic events, occurs in a wide variety of human tumors over a large region of the genome [14]. The p53 gene is a 53kD nuclear phosphoprotein encoded by a 16-20 kb gene on the short arm of chromosome 17 at position p13.1 . It has been implicated in the control of the cell cycle, DNA repair and synthesis, cell differentiation, genomic plasticity and apoptosis [15,16]. The odontogenic epithelial cells of the control tissues investigated failed to reveal any immunoreactivity to the p53 marker used. These results match results from other normal tissue in other studies which have also proved negative for p53 [7,15,17].

This can be explained by the fact that the wild type protein of p53 does not normally accumulate to amounts detectable by immunohistochemical methods, probably because of its short half-life (6-20 minutes[18].

In agreement with El-Sissy(1999), we found, in our study that the positive staining for the p53 marker in the solid ameloblastoma cases was examined which might indicate the existence of certain stimuli due to disturbed growth regulation,which triggered neoplastic transformation[7,18]. It has been suggested that detectable p53 proteins may reflect stabilization of the protein via interactions with other intracellular proteins[19,20].

The proto-oncogene product, p53, is a cellular protein expressed at low levels in non-transformed cells and acts as a negative regulator of cell division. In tumor-derived and transformed cell lines, the levels of p53 are often elevated and several investigators have suggested that inactivation of the p53 gene confers a selective advantage for the development of the tumourigenic phenotype with its subsequent impact on changing cellular activity) [21,22].

The active and accumulating p53 wild type might contribute to the more benign course and low recurrence rate commonly reported for unicystic ameloblastoma in comparison with solid ameloblastom[23]. Increased density, rather than increased number of p53-positive cells which has been reported as related to proliferation in odontogenic epithelium[24]. Migaldi et.al.,in 2008, found that the proliferating rate of the tumor cells indicated the prognosis of the tumor after treatment and it's recurrence rate[25,26].

In Conclusions Immunohistochemistry is a valuable technique for the localization of p53 over expression in the various forms of ameloblastoma. The altered p53 protein metabolism, occurs in both solid/ multicystic types and less frequently in unicystic ameloblastoma. Thus alterations in the p53 protein might be an early event in the pathogenesis of ameloblastoma. Over expression of p53 in multicystic ameloblastoma may indicate the high recurrence rate of such a tumor which requires long follow up to the patient.

The immunohistochemical detection of p53 over expression is recommended for cystic odontogenic conditions to disclose possible neoplastic transformation, and for conventional variants of ameloblastoma to reveal malignant changes that the inexperienced eye might miss, particularly in borderline cases. We need to focus our studies in the future on the immune reaction of the body towards these tumors and the effect of tumor supressor genes mutation on odontogenic tumors.

References

1- Brazis PW. et al. "Neuro-ophthalmologic Aspects of Ameloblastoma". Skull Base Surg.1995; 5 (4): 233–44. PMID 17170964.

2- Reichart PA. et al. "Ameloblastoma: biological profile of 3677 cases". Eur J Cancer B Oral Oncol. Mar; 1995; 31B (2): 86-99. PMID 7633291.

3-Stafine EC. et al."Giant ameloblastoma of jaw successfully treated by radiotherapy".Oral Oncology Extra 42 (1): 22-25. 2006; doi:10.1016/j.ooe.2005.08

4-Barnes L. et al. World Health Organization Classification of Tumours. Pathology and Genetics of Head and Neck Tumours. IARC Press: Lyon 2005.

5-Jing W. et al. Odontogenic tumours: a retrospective study of 1642 cases in a Chinese population. Int J Oral Maxillofac Surg. 2007; Jan;36(1):20-5. Epub 2006 Dec6.

6- Li TJ. et al. Unicystic ameloblastoma: a clinicopathologic study of 33 Chinese patients. J Surg Pathol. 2000;24:1385-1392.

7- El-Sissy N.A. Immunohistochemical detection of p53 protein in ameloblastoma types. Eastern Mediterranean Health Journal.1999; 5:487-89.

8- Gimenez-Conti IB et al. p53, Rb and cyclin D1 expression in human oral verrucous carcinomas. Cancer.1996; 78(1):17-23.

9- Ibrahim, S. O. and Anne, C. Johannessen "Immunohistochemical detection of P53 in non-malignant and malignant oral lesions associated with snuff Dipping in the Sudan and Sweden". Int. J. Cancer.1996; 68, 749-753.

10- Hsu SM. et al.Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. Journal of histochemistry and cytochemistry. 1981; 29(4):577-80.

10- Mandard AM et al. Expression of p53 in esophageal squamous epithelium from surgical specimens resected for squamous cell carcinoma of the esophagus with special reference to uninvolved mucosa. Journal of pathology. 1997; 181:153-7.

11-Gardner DG. and Corio RL. Plexiform unicystic ameloblastoma: a variant of ameloblastomas with a low recurrence after enucleation. Cancer. 1984; 53:1730

12-Ackerman GL. et al. The unicystic ameloblastoma: a clinicopathologic study of 57 cases. J Oral Pathol. 1988; 17:541

13-Gold L. Biologic behavior of ameloblastoma. Clin Oral Maxillofac Surg. 1991; 3:21

14- Lou SK. et al. Ameloblastic carcinoma of the jaws. Oral surgery, oral medicine, oral pathology, oral radiology and endodontics. 1996; 85:79-81.

15- Imamura J. et al. p53 in hematologic malignancies. Blood. 1994; 84:2412-21.

16- Lehman TA et al. p53 mutations, ras mutations and p53-heat shock 70 protein complexes in human lung carcinoma cell lines. Cancer research.1991; 51:4090-6.

17- Alfayad D. W. :P53 as an indicator for the prognosis of oral squamous cell carcinoma. Damascus University journal for health sciences. 2008; Vol.24-No.2: 411-21

18- El-Sissy N.A. and Saleh SMA. Expression of p53 gene product in acinic cell carcinoma of salivary glands. Cairo dental journal. 1997; 13(2):283-8.

19- Finlay C. et al. The p53 proto-oncogene can act as a suppressor of transformation. Cell. 1989; 57:1083-93.

20- Furihata M. et al. Detection of p53 and bcl-2 protein in carcinoma of the renal pelvis and ureter including dysplasia. Journal of pathology. 1996; 178:133-9.

21- Levine AJ, Momand J, Finaly CA. The p53 tumour suppressor gene. Nature. 1991; 351(6326):453-6.

22- Maass, J.D. et al. "High rate of P53 over-expression in head and neck carcinomas detected with a refined ELISA".Anticancer- Res. 1997; 17(1A): 473-8.
23- Kishino M. et al. immunohistochemical study of the peripheral ameloblastoma. Oral Dis. 2007; Nov;13(6):575-80.
24-Slootweg PJ. p53 protein and ki-67 reactivity in epithelial odontogenic lesions. An immunohistochemical study. Journal of oral pathology and medicine. 1995; 24(9):393-7.
25- Migaldi M. et al. Tumor cell proliferation and microsatellite alterations in human ameloblastoma. Oral Oncol. 2008; 44(1):50-60. Epub 2007 Feb 16.
26- Levine AJ. et al. The 1993 Walter Huber Lecture: the role of the p53 tumour-suppressor gene in tumor-genesis. British journal of cancer. 1994; 69(3):409-16.