

EVALUATION OF IMMUNOHISTOCHEMICAL ANALYSIS IN THE MANAGEMENT OF AMELOBLASTOMA

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ABSTRACT

Surgical management of ameloblastomas depends on radiographic extension of the lesion and the histological findings up till now. Meanwhile, there is controversy regarding the surgical techniques of different types of ameloblastomas. The aim of this study was to assess the degree of lesion aggressiveness, using p53, to determine the potential biologic behavior of those lesions. Patients and Methods, were conducted on 17 patients (10 males and 7 females), aged from 13 to 67 year. These patients divided initially as primary, recurrent, and of malignant ameloblastoma. Immunohistochemical analysis was used to classify ameloblastomas into aggressive and non-aggressive lesions. Surgical technique was done according the immunohistochemical result. Postoperative results were accepted for patients and no recurrence was observed after surgical excision in all cases. The present study concluded that p53 is good approach to detect mutation and the biological behavior of the cells upon which surgery should be addressed. This study provided that treatment strategy of the ameloblastoma must be preceded by immunohistochemical evaluation to detect the natural behavior of the cells. Moreover, the conventional histological and radiographical assessment is not enough for the final management of such cases.

INTRODUCTION

Tumors arising from epithelium of odontogenic apparatus or from its derivatives or remnants exhibit considerable variation and are classified into benign and malignant entities.⁽¹⁾ Ameloblastoma is the most frequently encountered tumor arising from odontogenic epithelium and is characterized by a benign but locally aggressive behavior with high risk of recurrence.⁽²⁾ It may become clinically evident as

early as the first decade or as late as the seventh. The lesion may occur in either jaw and in any area, but predilection are the molar-ramus region of the mandible and posterior maxilla with no sex or racial predilection. The mandible is the favored site over maxilla by about a 4.5: 1 ratio. Radiographically, it presents as unilocular or multilocular radiolucent appearance in several different sizes and shapes. Extensive distortions of normal mandibular and maxillary anatomic features are commonly appeared.

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Ameloblastoma may have a markedly aggressive clinical course from the time of discovery, others after years of quiescent chronicity, and some after irradiation treatment. Curettage and enucleation of unicystic and multicystic ameloblastoma have resulted in recurrence rates ranged from 18% to 100% respectively.⁽³⁾

Histologically, ameloblastoma shows considerable variations including follicular, plexiform, acanthometous, granular, basal cell, and desmoplastic types. However, two or more types may occur within the same tumor. Malignant ameloblastoma is a neoplasm in which the pattern of an ameloblastoma and cytological features of malignancy are shown by the primary growth in the jaws and/or by any metastatic growth.⁽⁴⁾ Malignant ameloblastoma has been subclassified into metastasizing ameloblastoma and ameloblastic carcinoma on the basis of metastatic spread and cytological malignant features.⁽⁵⁾

Several recent studies, detected genetic and cytogenic alterations in epithelial odontogenic tumors, however, the mechanisms of oncogenesis, cytodifferentiation, and tumor progression remain unknown. Due to its nature, extensive irradiation with safety margin is indicated and the recurrence may be detected after surgical excision. So that, another investigation was indicated to determine aggressive nature of the tumor.^(6,7)

Tumor suppressor gene (p53), may explain the aggressive nature of ameloblastoma, compared with adenomatoid odontogenic tumor.⁽⁸⁾ This gene located on the short arm of chromosome 17. p53 protein product is a 393 amino acid phosphoprotein which localizes to the nucleus of all mammalian cells. The normal (wild) p53 protein is an important cell cycle regulator and suppressor of uncontrolled cell division.⁽⁹⁾ Normally, it suppresses cancer cell proliferation at the G1- phase, preventing cells from entering the S- phase of cell cycle.⁽¹⁰⁾ Mutation of the normal p53 cancer suppressor gene allows uncontrolled cellular division and tumorigenesis. This gene

mutation has been reported in about 50% of all human cancer and it has been considered as a marker of both malignancy and tumor progression.⁽¹²⁾

Despite the fact that different histologic types are existed in the same lesion, surgery is still based on the size of the tumor. Some surgeons are still treating ameloblastoma by resection and/or Curettage and enucleation. Peripheral ostectomy depending on the histologic features of ameloblastoma regardless the presence of different histologic types in the same sample. Pre-surgical evidence of high or low destructive potentiality could result in more precise line of treatment.⁽¹³⁾ This work aimed to evaluate p53 expression in ameloblastomas in order to assess the degree of proliferation and aggressiveness of cells. Further, was to clarify the potential biologic behavior of those lesions. Moreover, this work is a trial to help in placing a plan to select the most appropriate line of the treatment of primary, recurrent and malignant ameloblastoma depending on the biological cell behaviors.

PATIENTS AND METHODS

During the period between February 2008 to September 2014, 164 patients were chosen from Outpatient Clinic of Oral and Maxillofacial Surgery Departments of Zagzig, and Al-Minia University Hospitals. They were complained of slowly growing swelling, facial asymmetry, and teeth mobility. Numbness of the lower lip and pain were complaints of some patients. The intraoral swelling was firm and sometimes with areas of fluctuation and ulceration (Fig.1).

Patients were subjected to routine clinical examinations for preoperative evaluation. Biopsy and histopathological examinations have been done. Out of these patients, 17 cases (10 males and 7 females with age ranged between 13 and 67 year) have proven to have ameloblastoma. Radiologic examinations of the facial bones with orthopantomogram and CT scan in axial, coronal and 3D views were done (Fig. 2).

Also, plain chest X-rays and abdominal ultrasonography were done. Complete laboratory investigation for liver, kidney, in addition to complete blood picture, bleeding & clotting time, erythrocyte sedimentation rate, blood sugar and electrocardiogram were done. Biopsy was taken for routine histopathological examinations with H&E stain (Fig.3)

For diagnosis and classification in this study, immunohistochemical analysis was done with the strept-avidin-biotin immune-peroxidase (Strept A-B staining) technique, using monoclonal antibodies against p53. The unstained paraffin block

sections were dewaxed in xylene for 15 minutes and rehydrated with ethanol. Endogenous peroxidase was blocked with 3% H_2O_2 for 30 minutes at room temperature. After 3 washes in phosphate buffered saline (PBS), PH was 7.4, antigen was retrieved by boiling the sections in buffered sodium citrate (PH: 6), in a microwave oven, twice for five minutes each. Then mouse monoclonal antibody against p53 oncoprotein at a dilution of 1:100 was added (DO-7, DSKO, USA) and incubated for overnight at 4°C. After washing in PBS, slides were incubated for 30 minutes with a peroxidase-conjugated anti mouse secondary antibody (DAKO-Hamburg, Germany). After being washed twice in

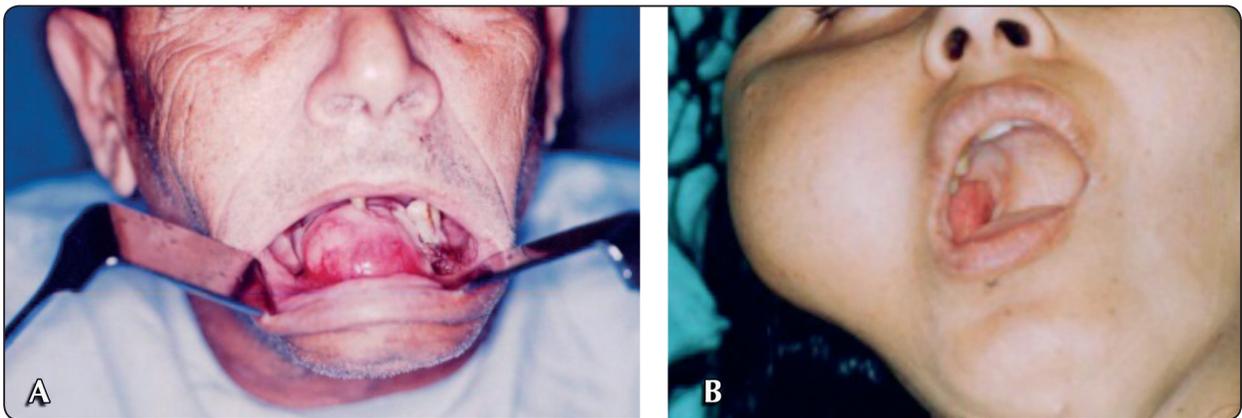


Fig. (1) Photograph showing male patient had mandibular swelling of ameloblastoma with mucosal ulceration(A) and female patient had massive right facial swelling of recurrent ameloblastoma up to temporal region and intraoral ulceration of palatal mucosa (B).

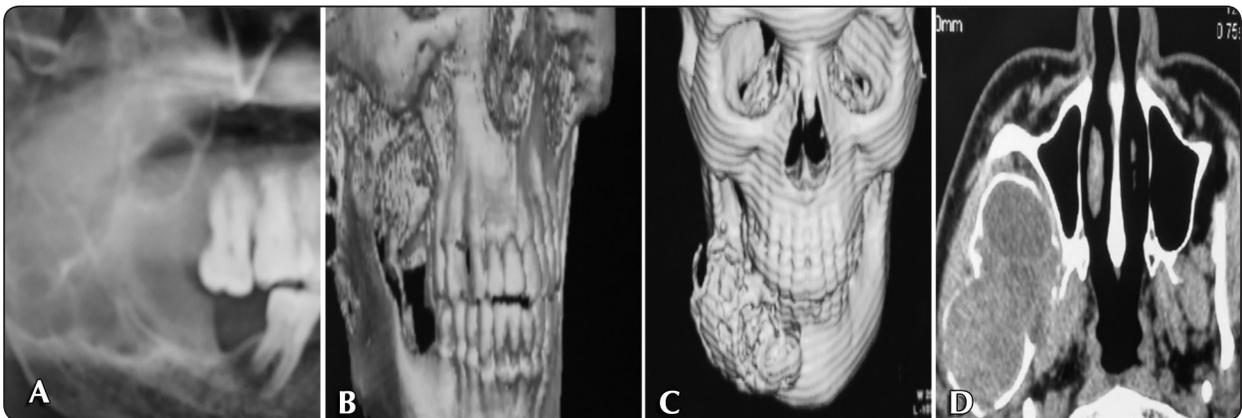


Fig. (2) Photographs of ameloblastomas showing; mandibular multilocular radiolucency and bone destruction of the angle and ramus in panoramic X- ray film (A), which extended to condyle & temporal bone in CT scan (B). Massive multilocular destructive right side of the mandible(C). Also, massive mass at the right side of maxilla observed in axial CT scan (D).

PBS, slides were then treated with the detection system (streptavidin- biotin- peroxidase complex) and a peroxidase reaction was performed using diaminobenzidine (DAB) as a chromogen. Sections were then counterstained with Mayer's hematoxylin for 30 seconds. A brown colored nuclear staining is denoting the presence of p53 protein by light microscope.⁽¹²⁾

The immunohistochemical results of p53 were graded on four point scale: negative (- ve); less than 10% of cells are immunoreactive, weakly positive(+); 10-< 25% of cells were stained, moderately positive (++) ; 25-50% of cells stained,

and strongly positive(+++) ; > 50% of cells are immunoreactive.⁽¹³⁾ (Figs: 4&5).

According to the obtained data of immunohistochemical analysis, ameloblastomas were classified to non-aggressive and aggressive lesions. The non-aggressive lesions has negative reaction to p53, were managed with curettage and enucleation and/or peripheral ostectomy with preservation of the inferior alveolar nerve (6,A). Another case, treated with complete lesion excision, without discontinuity and bone plate applied to avoid suspected mandibular fracture which may occur (Fig.6, B).

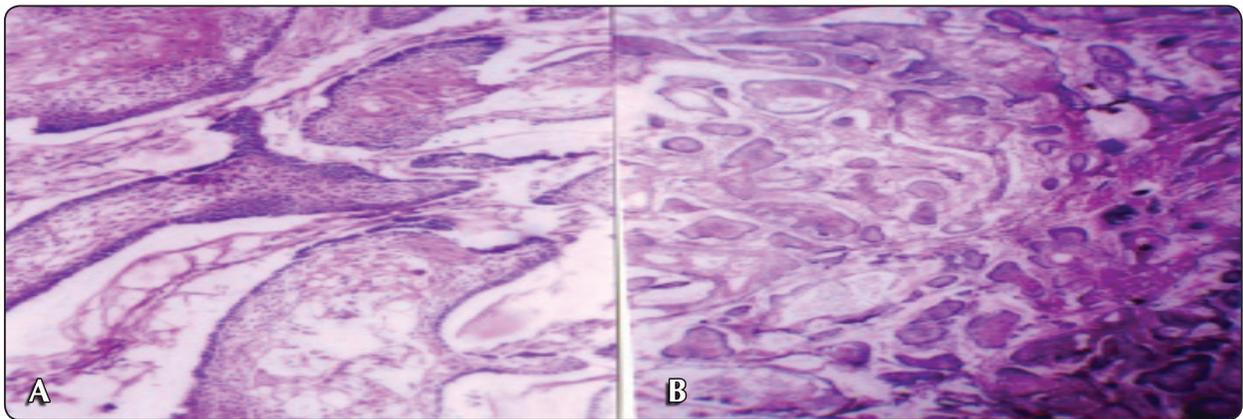


Fig (3): Histopathological Photographs of ameloblastoma showing; mixed follicular & acanthomatous type with H&E stain.

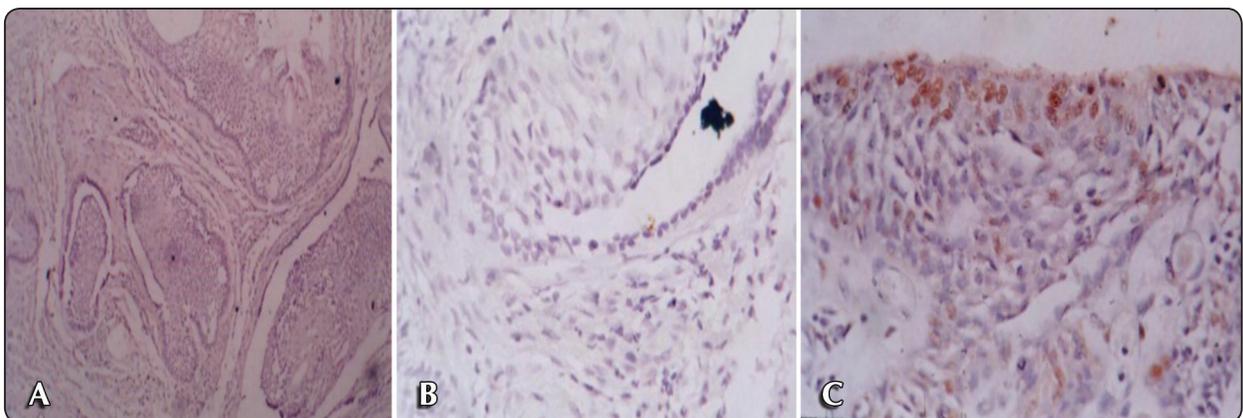


Fig (4): Photographs of ameloblastoma showing; 2 different types acanthomatous ameloblastoma (A&B) showing -ve staining for P53 (Avidin - Biotin Complex (ABC), Meyer's Hx counter stain) $\times 200$. A case of plexiform ameloblastoma showing weak peripheral staining for P53 (ABC $\times 400$) (C).

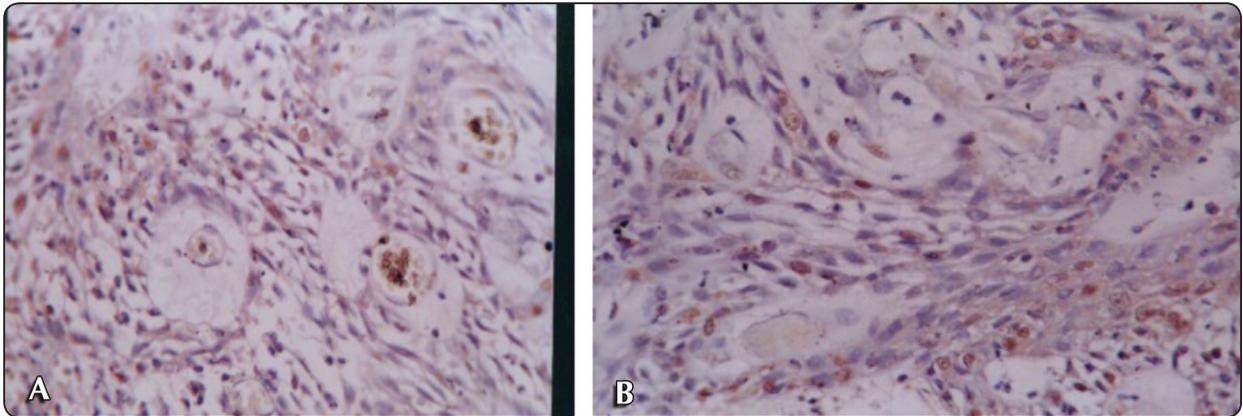


Fig (5): Photomicrograph of ameloblastomas showing a case of follicular type with a moderate staining for P53 (ABC \times 400) (A) and a case of malignant ameloblastoma showing marked staining for P53 (ABC \times 400) (B).

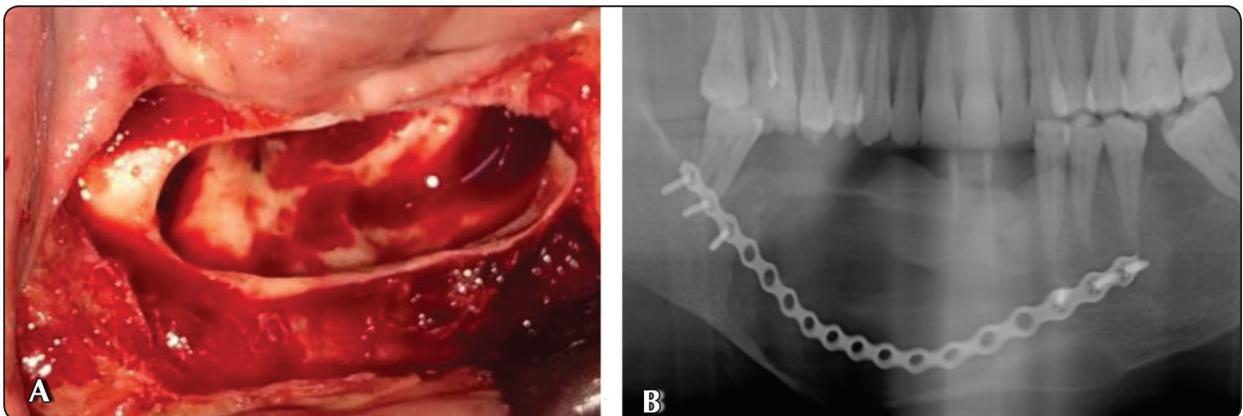


Fig.6, Photograph of non-aggressive ameloblastoma showing, good curettage only and enucleation of mandibular lesion (A) and panoramic view of another patient has huge lesion at right side of mandible, treated without resection and bone plate applied to avoid mandibular fracture (B).

The aggressive one exhibited marked cellular proliferations and positive reaction to p53. These lesions were managed with resection with adequate margin (1 cm) of normal bone and soft tissue. The inferior alveolar neurovascular bundle was removed with the resected bone in these cases. Immediate bony reconstruction with iliac crest or rib bone grafts accompanied with reconstructive plate was made for the resected primary or recurrent ameloblastoma (Fig. 7). Reconstruction was postponed for the cases of malignant ameloblastoma. Maxillary cases that were resected received immediate prefabricated obturator when the oronasal and/or orontral areas were violated.

Operations were performed under general anesthesia. Prophylactic antibiotic cover was recommended intraoperatively using intravenous third generation cephalosporin and metronidazole. Submucous and subcutaneous infiltration of 1/100,000 concentration epinephrine in sterile normal saline was used to define the tissue planes, facilitate the dissection and provide hemostasis. A mucoperiosteal flap was elevated up to the inferior and posterior border of the mandible through intraoral approach in non-aggressive case and /or extra oral submandibular approach in aggressive cases which need resection. Resection of the tumor of the aggressive cases was made by cutting in the healthy bone. The size of the resected area depends on extent of the tumor involvement (Fig. 7).

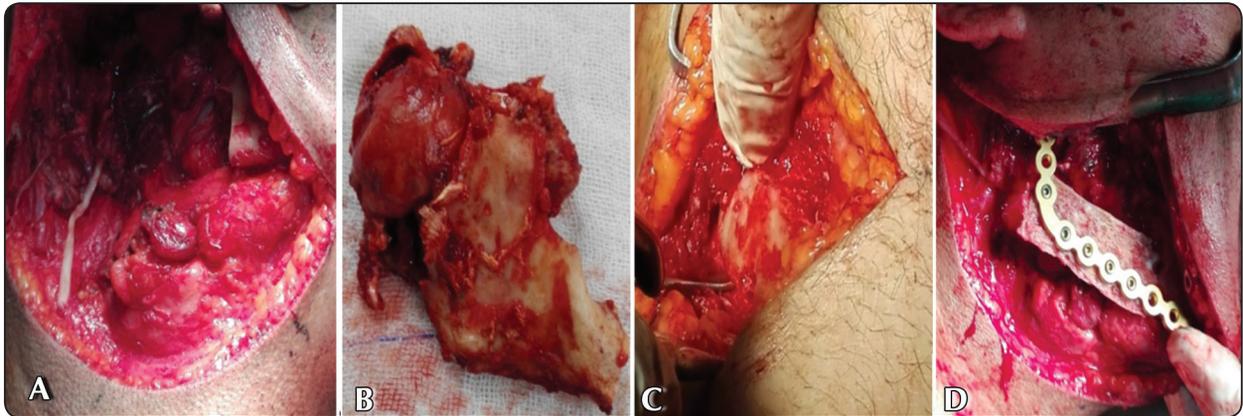


Fig. (7) Photographs of surgical excision of aggressive mandibular ameloblastoma extending to condyle and temporal region showing; submandibular incision (A), resected angle, ramus and condyle of the mandible (B) Incision for rib grafts and palpated (D) Rib graft applied and fixed at proximal end of mandible with bone plate (D).

Bleeding was controlled by bipolar coagulating diathermy and pressure packing using gauze soaked with epinephrine. The mucosa was then repaired with 3-0 interrupted silk sutures. Pro-vac hemosuction were inserted as a drain and was then removed one or two days after the operation. The extraoral incisions were closed in layers. Antibiotic coverage was continued for 7 days postoperatively. Oral feeding was avoided for at least 48 hours after the operation except with soft diet, and then the patient gradually returned to normal diet. Follow up of all patients was continued clinically and radiographically for two years.

RESULTS

The age of the patients subjected to this study ranged between 13 to 67 years, ten of them were males and seven were females. The clinical pictures of the studied patients showed that swelling and facial asymmetry were the main symptoms of the presentation of ameloblastoma. While pain, lip parasthesia and teeth mobility was shown in some cases. Of 17 cases, 12 had primary ameloblastoma (6 plexiform, 3 follicular, and 3 acanthometous types), recurrent ameloblastoma (3 cases), and two cases had malignant ameloblastoma.

Multilocular expansile radiolucent areas were observed radiographically in ten cases, while

unilocular expansile radiolucency was detected in seven cases. Bony perforation and soft tissue involvement was observed in eight cases and nine cases were without cortical perforation. Teeth involved in the lesions showed displacement up to the sigmoid notch in some cases. Ten cases had invasive growth and/or resorption of teeth roots. Ten cases were in right side and seven were left sided. Twelve cases were in the mandible and five were in the maxilla. Of 12 mandibular cases, two cases had recurrent extension that was reached the temporal and pterygoid area (As the patient in Fig.1).

The immunohistochemical results showed that the reactivity of P53 was detected in the nuclei of the neoplastic epithelial cells of the different types of ameloblastomas (Table 1). P53 immunoreactivity was detected in the peripheral columnar or cuboidal cells in 2 of 3 cases of follicular type (weak positive) and in all 6 cases of plexiform type (2 cases; weak positive and 3 cases; moderately positive, and one case showed strong positivity). The keratinizing cells in acanthometous ameloblastoma (3 cases) were not reactive with anti- P53 antibody. Expression of P53 in recurrent ameloblastoma (3 cases) and malignant ameloblastoma (2 cases) was detected in most of the malignant epithelial cells of all cases (strong positive +++).

From the resulting data, ameloblastoma were classified into aggressive and non-aggressive lesions. According to this classification, resection with safety margin was the line of treatment for the aggressive lesions (who's with strong positivity whether presented for the primary, recurrent, and malignant ameloblastomas). While the non-aggressive ones underwent to more conservative modality in the form of curettage and/or peripheral ostectomy. The postoperative follow-up was accepted and no recurrence was observed in all cases. (Fig.8,A,B,C&D).Also, good result observed in the patient of (fig.7), which treated with resection and rib graft(Fig.8,E).

Operatively, five tumors were adequately resected with safety margins about 1cm all around. Twelve cases were managed with enucleation and peripheral ostectomy with preservation of the

inferior alveolar nerve. In the postoperative follow up period, there were three cases with intraoral mucosal dehiscence and the patients subjected to resuturing after 3 weeks of the first operation. Ten cases had mild pain site to which they were received anti-inflammatory drugs in an intermittent doses. Seven cases suffered from parasthesia to which they received neurotonic drugs for 6 months then the sensation improved except for five cases still complaining through the follow up period. There was one case with oroantral fistula and the patient subjected to surgical closure by buccal pad fat and stent in another setting. One patient with malignant ameloblastoma had unilateral nodal metastasis (N₁) and he was subjected to unilateral supra-omohyoid block neck dissection. He was followed up radiographically for 24 months without recurrence or metastasis.

TABLE (1): Comparison of P53 reactivity in all studied cases of ameloblastomas.

Ameloblastoma Type	Cases No.	Negative - ve	Week + ve	Moderate + + ve	Strong + + + ve
Follicular type	3	1	2	-	-
Plexiform type	6	-	2	3	1
Acanthometous type	3	3	-	-	-
Recurrent type	3	-	-	-	3
Malignant type	2	-	-	-	2

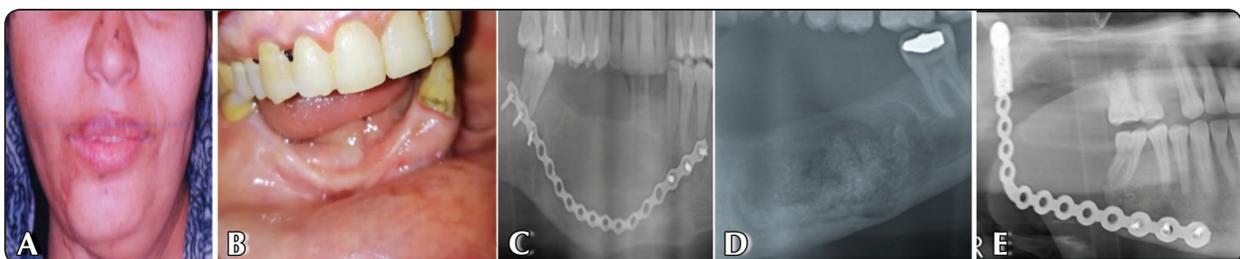


Fig. (8): Postoperative follow-up of patients after 2 years showing; good healing and no recurrence observed as; in frontal view photograph of female patients (A) and intraoral view(B, panoramic view of the patient, which treated with lesion excision only without resection (C&D), and patient of mandibular resection reconstructed with rib graft and reconstructive plate(E).

DISCUSSION

Ameloblastoma is the most common odontogenic neoplasm, which shares histological similarities to other malignant tumors as basal cell carcinomas.⁽¹⁴⁾ It may arise from the epithelial lining of the dentigerous cyst, from the remnants of the dental lamina, and enamel organ or from the basal layer of the oral mucosa.⁽¹⁵⁾ Ameloblastoma is a locally invasive tumor with a strong tendency to recur, and it rarely has a malignant behavior. Several histologic variants have been reported, including follicular, plexiform, acanthomatous, granular, and basal cell form. The tumor tissue of ameloblastomas consists of epithelial cell islands and trabeculae in connective tissue stroma. Cyst formation is common and varies from microcysts within a predominantly solid tumor to a cyst that envelops the main tumor mass.⁽¹⁶⁾

The surgical treatment of ameloblastomas has been controversial, and treatment modalities have included enucleation, curettage, and cryotherapy or radical treatment.⁽⁵⁾ There are three forms of ameloblastomas, namely, peripheral, unicystic, and multicystic tumors. Peripheral tumors are odontogenic tumors, with the histological characteristics of intraosseous ameloblastomas that occur solely in the soft tissues covering the tooth-bearing parts of the jaws. Unicystic tumors include those that have been variously referred to as mural ameloblastomas, luminal ameloblastomas, and ameloblastomas arising in dentigerous cysts.⁽¹⁷⁾

Mutation of P53 gene results in accumulation of a conformationally altered and functionally defective protein.⁽¹⁷⁾ Hiroyuki et al,⁽¹⁸⁾ suggested that expression of p53 homologs such as p63 and p73 in tooth germs and in benign and malignant ameloblastomas, play a role in differentiation and assess proliferation of odontogenic epithelial cells and might differentially function in both developing and neoplastic odontogenic tissues. The present study used a monoclonal antibody reactive with mutant type P53 protein which was recognized in primary,

recurrent and malignant ameloblastomas. These features confirmed that P53 expression is associated with oncogenesis of odontogenic epithelium. Reactivity for P53 was higher in Plexiform type than follicular type, suggesting that tissue structuring of ameloblastomas might be affected by P53 expression with varying degrees. However, no P53 expression was detected in keratinizing cells in acanthomatous ameloblastoma. With respect to recurrent and malignant ameloblastoma, strong expression of p53 was detected in those cases and in one case of primary ameloblastoma denoting the aggression nature of each individual type of ameloblastoma. These results are in agreement with previous studies.⁽¹⁹⁻²¹⁾

Feinberg and Steinberg, in 1996⁽²²⁾ reported that when the tumor involves the periphery of the connective tissue wall of the cyst, peripheral ostectomy should be considered. However, the possibility of tumor cell invasion into adjacent bone cannot be excluded. They added that in such cases, more aggressive therapy should be appropriate. Chana et al., (2004)⁽²³⁾ added that the multicystic or solid lesions represent the source of most controversy regarding surgical management and resection is indicated because the conservative approaches were not appropriate and when there is doubt, resection is preferable. These results are consistent with other previous studies^(24,25), where they reported recurrence rates in 90% of mandibular multicystic ameloblastomas which treated with conservative approaches. The present study confirmed that the biologic behavior for each individual case of ameloblastoma should be considered solely in its management regardless the radiologic and histologic features. Moreover, the proliferation indices will be significantly valuable than the traditional investigations depending on radiographic appearance of unicystic or multicystic features. These results are consistent with Adriano et al (2002)⁽²⁶⁾ in which the biologic behavior of different histologic types of unicystic ameloblastomas was the same. The results of the

present study are consistent with previous studies, in which it revealed that some cases of unilocular ameloblastoma had aggressive course in the form of cortical perforation, cell proliferation and they were treated with resection. In contrast, some cases of multilocular ameloblastomas had relatively less aggression and quiescent course and they were treated with conservative treatment without recurrence for two years follow up period.

Generally, ameloblastomas are regarded as benign tumors, though their propensity for recurrence and locally aggressive behavior similar to malignant tumors are well recognized, and there have been reports of metastasizing ameloblastomas showing typically benign morphological features in both primary and metastatic lesions.⁽²⁷⁾ Hayashi et al, (1997)⁽²⁸⁾ classified ameloblastomas into two types: one that follows morphologically malignant transformation and the other that shows typically benign morphological features at both the primary lesion and the metastatic lesion which occurs several years later. The results of the present study suggested that the high recurrence rate arising from inadequate management may increase the chance of metastasis and it seems very difficult to predict the propensity of recurrence and even metastasis from the gross characteristics including radiological and histopathological features. Therefore, the final presurgical evaluation must include immunohistochemical evaluation and those with strong positivity (even primary lesions) should be considered as aggressive lesions.⁽²⁹⁾

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