British Journal of Medicine & Medical Research
6(12): 1149-1158, 2015, Article no.BJMMR.2015.293
ISSN: 2231-0614

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Comparison of C-Myc Oncogene Expression in Benign and Malignant Odontogenic Tumors

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Authors' contributions

This work was carried out in collaboration between all authors. Author SEIB designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author IMA managed the literature searches. All authors performed the immunohistochemical staining, histological evaluation and read and approved the final manuscript.

Article Information

DOI:10.9734/BJMMR/2015/15701
Editor(s):
(1) Ibrahim El-Sayed M. El-Hakim, Ain Shams University, Egypt and Riyadh College of Dentistry and Pharmacy, Riyadh, Saudi Arabia.
Reviewers:
(1) Rodrigo Crespo Mosca, Energetic and Nuclear Research Institute (IPEN/CNEN – SP) CTR, Radiation Technology Center and CB, Biotechnology Center, University of São Paulo , Brazil.
(2) Angelo Troedhan, CMF-surgery Department, Health Science University Vientiane, Laos.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=911&id=12&aid=7926

Original Research Article

Received 12th December 2014
Accepted 16th January 2015
Published 28th January 2015

ABSTRACT

Aims: The aim of this study was to comparatively investigate the expression of c-Myc oncoprotein in benign and malignant odontogenic tumors and also to assess its potential prognostic role in these conditions.
Study Design: The c-Myc protein is a nuclear transcription factor which centrally regulates cell proliferation, arrest, cell differentiation and cell death. Ameloblastomas are benign uncommon tumors. They are the most frequently encountered odontogenic tumors arising from the epithelium of the odontogenic apparatus. They rarely have the potentiality to turn malignant in the form of either malignant ameloblastoma or ameloblastic carcinoma. The expression of c-Myc oncogene products were detected in normal tissue and cancer cells. It is related to the proliferation of odontogenic epithelial cells and its degree of differentiation.

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Materials and Methods: Eleven cases of amelobastomas were included in this study. Six cases were benign and five cases were malignant ameloblastomas. All the specimens were retrieved from Alexandria University, Maxillofacial and Oral Pathology department. Biopsy specimens were prepared, fixed in paraffin wax and processed for immunohistochemical staining using the standard streptavidin Biotin peroxidase complex method, and the primary monoclonal antibody specific for c-Myc.
Results: Results revealed strong staining reaction of the nucleus and cytoplasm of the peripheral columnar cells and to a lesser extent in the polyhedral cells. The intensity of the reaction was stronger in malignant cases and mild in the benign cases.
Conclusion: Immunoreactivity of the c-Myc oncogene was directly proportional with the degree of malignancy and was a valuable marker for elucidating the mode of growth and development of odontogenic epithelial cells from the pathological point of view.

Keywords: c-Myc oncogene; odontogenic tumor.

1. INTRODUCTION

Ameloblastoma is a slow growing odontogenic epithelial tumor of the jaw and accounts for about 1% of all oral tumors and about 18% of odontogenic tumors. It is primarily seen in adults in the third to fifth decade of life, with almost equal occurrence in both genders. The odontogenic epithelium is responsible for tooth development under normal physiological conditions, but it might cause odontogenic cysts or tumors when pathologically degenerated [1,2].

Ameloblastomas are the most frequently encountered tumors arising from epithelium of the odontogenic apparatus and its derivatives or remnant tissue and exhibits considerable histological variations [3-6]. They are benign but locally invasivetumors of the jaws with a variable rate of growth and high recurrence rate [7,8,9]. They appear to exhibitbiological heterogenity except in case of malignancy, their histological appearance does not always allow topredict their histopathological behavior [7].

Malignant ameloblastoma is defined as a tumour in which cytological features of malignancy are shown by the primary growth in the jaw and/ or by any metastatic growth [10,11]

The general acceptance, that increased cell proliferation plays a role in the development of odontogenic cysts and tumors is based upon a variety of reports, using different methods to study the proliferative activity including counting mitosis and immunohistochemical detection of proliferating cell by cell markers [12-17].

A number of studies have reported the expression of oncogene and oncogene products in human normal tissue and cancer cells. The oncogene c-Myc accelerates cells through G1 and S phases of the cell cycle, abrogating cell cycle checkpoints and increasing cell metabolism, and eventually renders genomic instability to the cells. C-Myc is not only related to the proliferation of cells but also plays an important role in the control of cell differentiation [18,19]. It is also suggested that its products are related to the malignancy of human cancer [20-23]. The product- protein of the c-Myc oncogene predominantly is located in the nucleus, but the sub-cellular distributions of this gene- products have been shown in squamous cell carcinomas of the oral region [23,24]. However, there are very fewreports of its expression in normal oral tissues and benign tumors [21]. To date, data on the immunohistochemical expression of c-Myc oncoprotein in odontogenic cysts do not exist with only 1 study which determines the expression of c-Myc mRNA in Odontogenic keratocyst using in situ hybridization [25]. Until now there have been no reports describing its expression in benign and malignant ameloblastomas.

The aim of this study was to determine whether c-Myc oncoprotein might be of value in determination of the pathophysiological behavior of ameloblastomas and the differentiation of their cells.

2. MATERIALS AND METHODS

Eleven surgical specimens of ameloblastomas were collected from the Oral Pathology and Maxillofacial department, Faculty of Dentistry, Alexandria University.

They were histologically identified as 6 cases of benign ameloblastomas and 5 cases of malignant ameloblastomas (follicular and

plexiform types), among which 2 cases were associated with cervical lymph node metastasis.

Biopsy specimens were prepared, fixed in paraffin wax and processed for immunohistochemical staining using the standard streptavidin Biotin peroxidase complex method, and the primary monoclonal antibody specific for C-myc (Univeral Kit, Labelled Streptavidin Biotin) (L SAB + Dako) [26].

2.1 Evaluation of staining

The intensity of c-Myc oncoprote in antibody was demonstrated as brown coloration and the intensity of the reaction was scored as mild, moderate and strong reaction.

3. RESULTS

Eleven cases of selected ameloblastomas were included in this study. Histologically, they were classified as 6 benign and 5 malignant ameloblastomas (follicular and plexiform type).

In benign ameloblastomas, c-Myc immunostaining reaction revealed a mild reactivity in the peripheral columnar cells and weak reactivity in the central polyhedral ones. The c-Myc products were detected in the cytoplasm as well as in the nuclei (Figs. 1, 2, 3).

There was no distinct difference in reaction between the two main types of ameloblastomas.

In malignant ameloblastomas, the c-Myc immunostaining reaction revealed a strong and diffuse reaction in the peripheral palisading cells and to a lesser extent in the central polyhedral ones. The staining reaction was seen in the nuclei and the cytoplasm of these cells (Figs. 4, 5, 6, 7).

One out of five cases of rare malignant ameloblastomas revealed metastases in the cervical lymph nodes. Interestingly, there was negative c-Myc reaction in the central cells, as compared to the peripheral cells which showed intense total (cellular and nuclear) immune-reactivity (Fig. 8).

4. DISCUSSION

Monoclonal antibodies (MAbs) have served as useful tools for studying the molecular basis of cell differentiation and provide means of improving our understanding of the histogenesis of tumors [27].

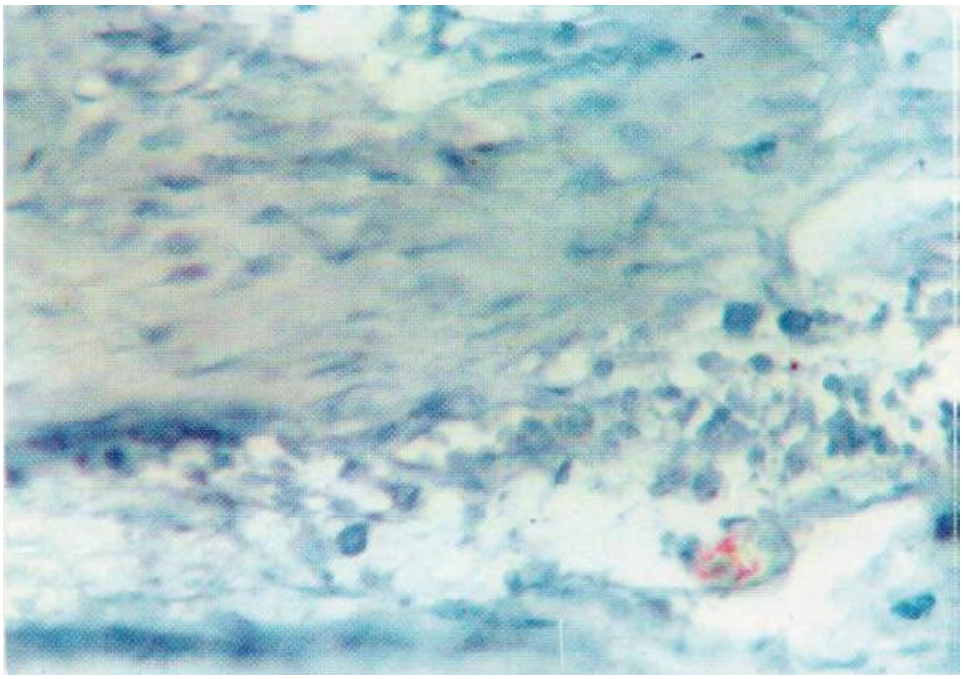

Fig. 1. Immunohistochemical staining for c-Myc showing mild focal reaction in the peripheral cells of a case of benign follicular ameloblastoma [ABC-DAB X250)

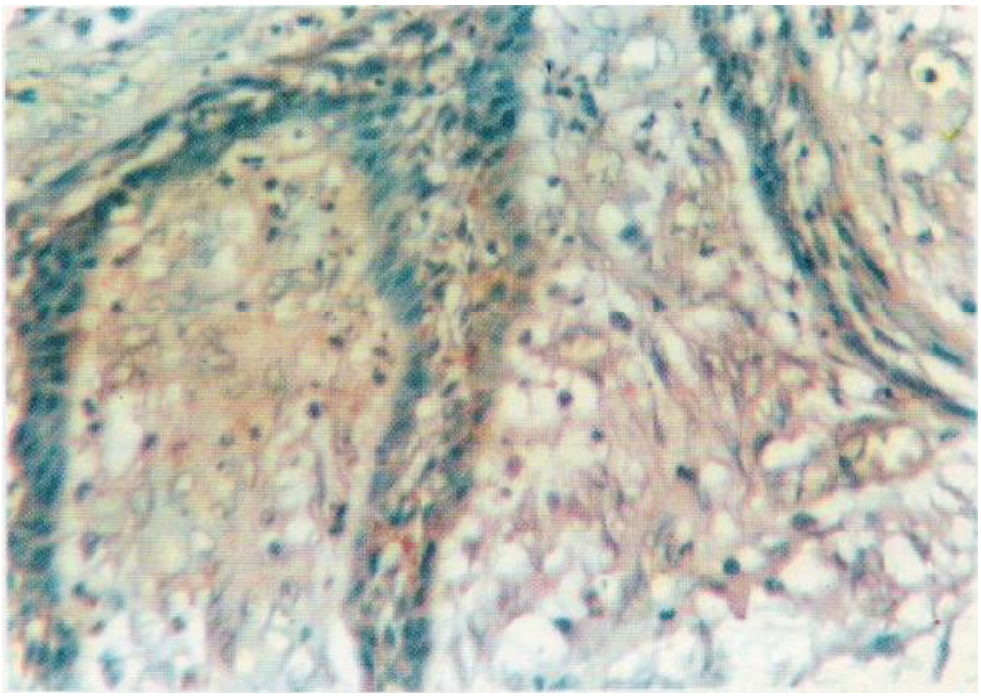

Fig. 2. Immunohistochemical staining for c-Myc revealing mild focal reaction in the peripheral cells of a case of benign follicular ameloblastoma [ABC-DA B X250]

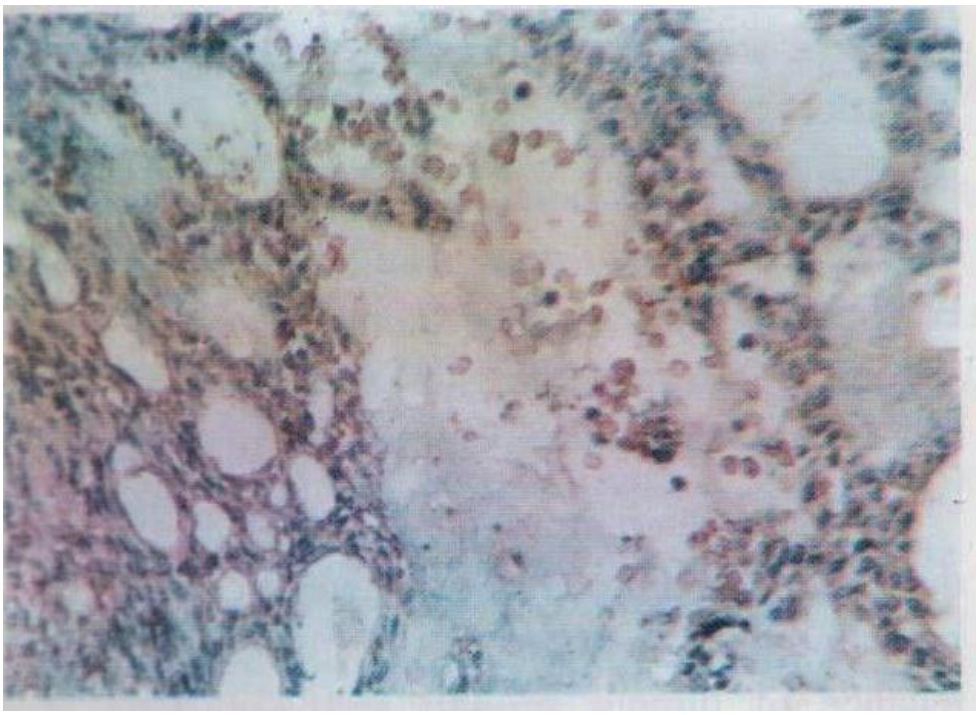

Fig. 3. Immunohistochemical staining for c-Myc oncogene revealing mild reaction in most of the peripheral cells in a case of benign plexiform ameloblastoma [ABC-DAB X250]

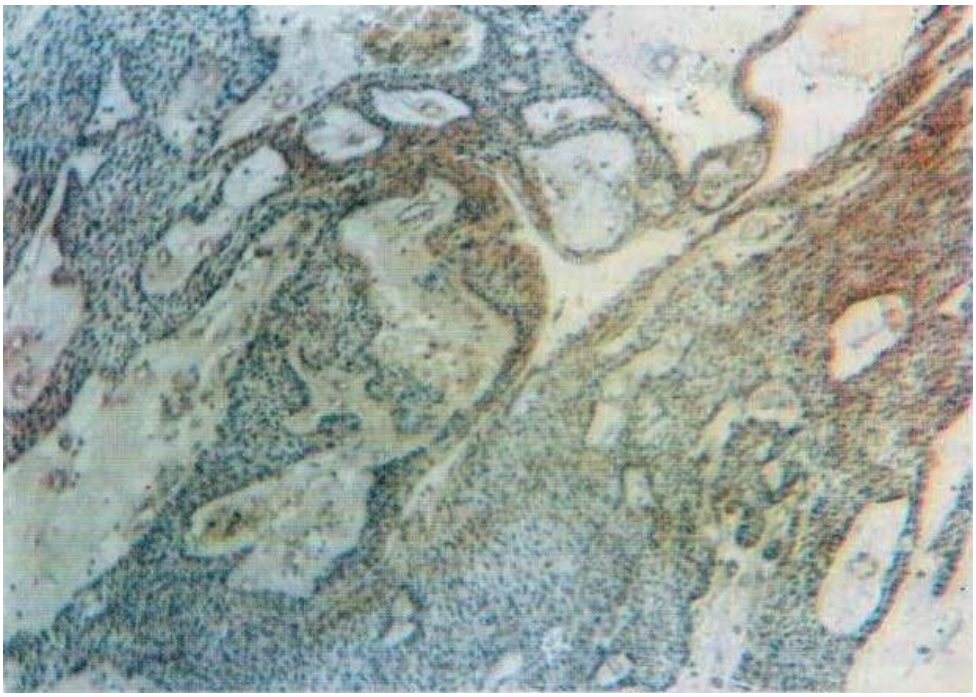

Fig. 4. Immunohistochemical staimng for c-Myc revealing focal distributed cytoplasmic reaction in malignant ameloblastoma (plexiform type) [ABC-DAB X250]

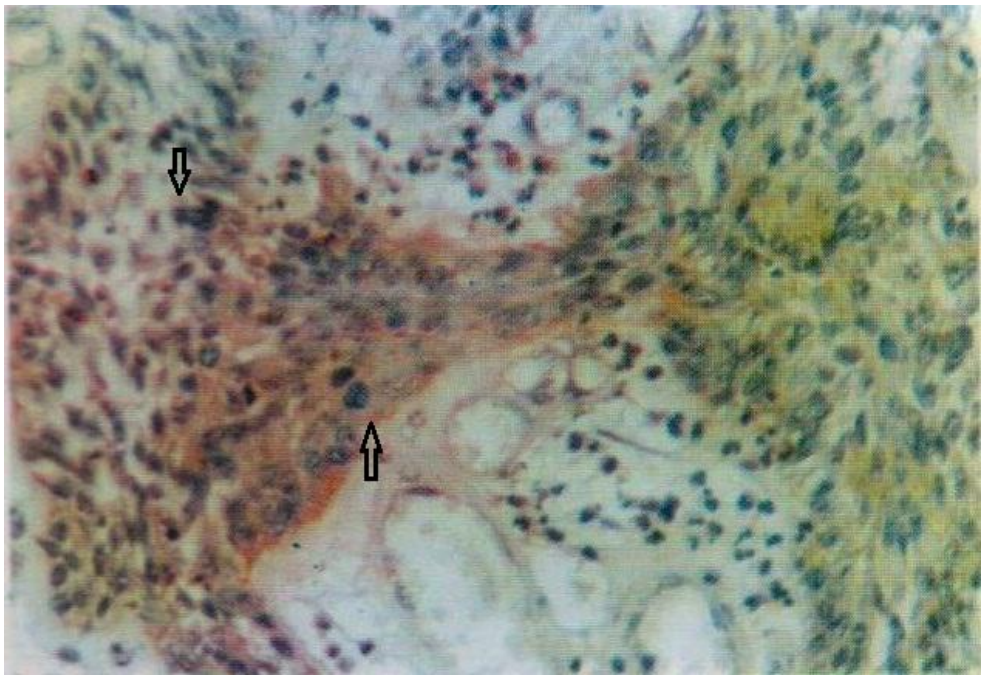

Fig. 5. Immunohistochemical staining for c-Myc revealing focaldistributed cytoplasmic reaction in malignant ameloblastoma (plexiform type) arrows showing cellular pleomorphism, hyperchromatism, increased mitotic activity, cellular proliferation and loss of polarity [ABC-DAB, X450]

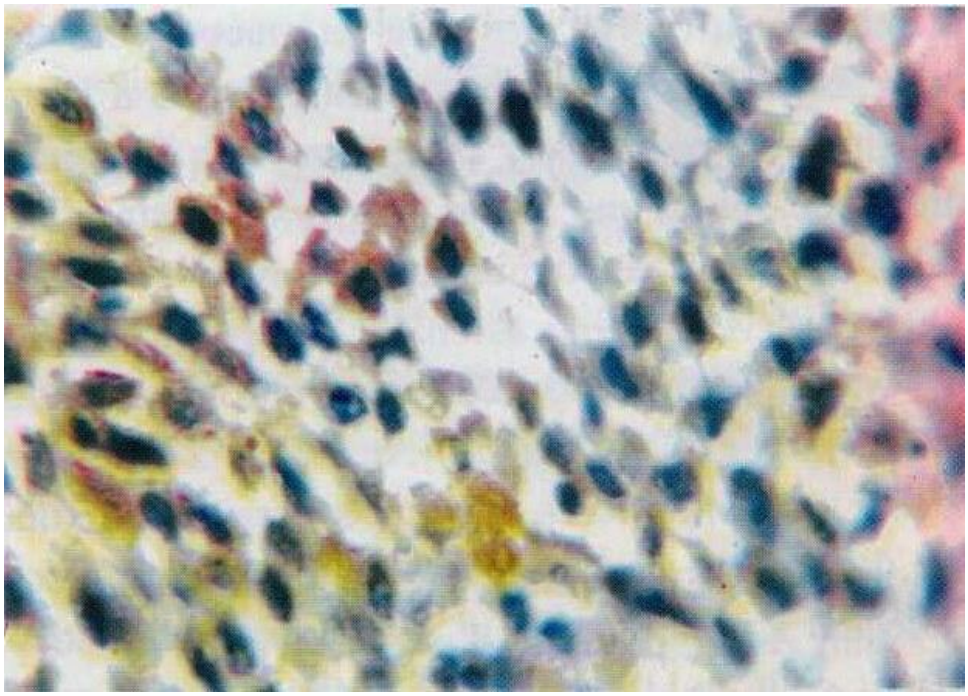

Fig. 6. Immunohistochemical staining for c-Myc revealing diffuse cytoplasmic reaction in malignant ameloblastoma (plexiform type) [ABC-DAB XOil immersion)

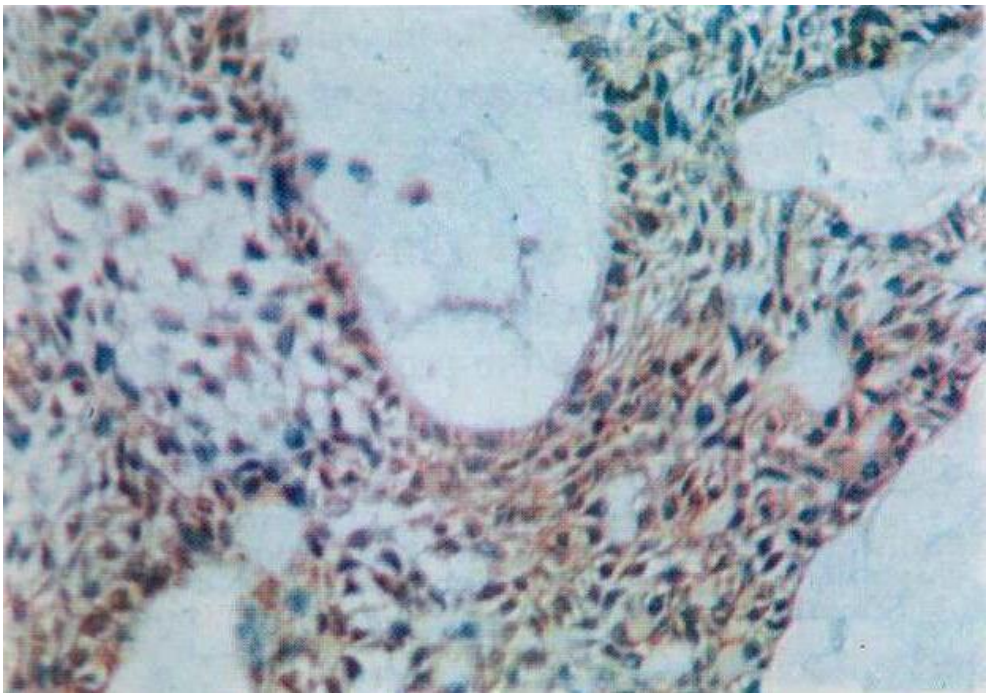

Fig. 7. Immunohistochemical staining for c-Myc revealing diffuse strong nucleus and cytoplasmic reaction in malignant ameloblastoma (follicular type) [ABC-DAB X250>

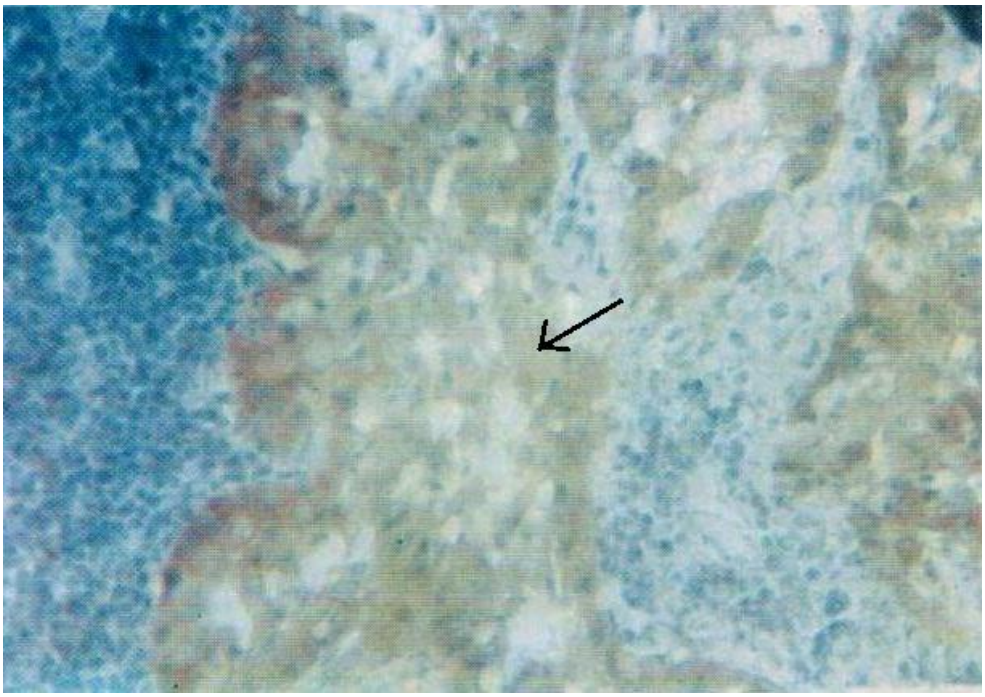

Fig. 8. Immunohistochemical staining of a rare case of ameloblastic carcinoma metastases in the cervical lymph node, revealing the strong total immune reactivity of c-Myc in the peripheral cell cytoplasms; Notice the mild to negative central cells reactivity revealing foci of necrotic cellular degeneration (arrow) associated with high grade of malignancy [ABC- DAB, X450]

In the recent immunohistochemical studies using c-Myc monoclonal antibodies, the expression of these oncogene products are noted not only in the cancer tissues, but also in normal tissues and non-cancerous lesions [21-24].

In the present study, genes of c-Myc protein were detected at high rates in the proliferating odontogenic epithelial cells of both benign and malignant ameloblastomas, but at different staining intensities.

Placarz et al. [22] have reported various staining patterns of c-Myc oncogene products in neoplastic tissues. The reactive products were extraordinarily localized in the nucleus, and to a lesser extent in the perinuclear cytoplasm with fine granular appearance, and in the entire cytoplasm of the cells. Concomitant staining of each reactive pattern were observed and in some occasions, the reactive product was strongly deposited on the chromatin undergoing mitotic phase. Their results reveal a close similarity to our present study. They concluded that the different staining patterns of the gene products reflected a modification of the biology of the neoplastic cells. They also concluded that the detection of the products in the cytoplasm around the nucleus is a transitional form of staining activity, in which the products of c-Myc oncogene diffuse into whole cells according to the stage of maturation.

Therefore, it appears that these products were related to the proliferation and differentiation of the odontogenic epithelial cells. Besides the fact that the oncogenic products were found more in the nuclei of the peripheral cell layer of ameloblastomas than the polyhedral ones- may suggest these products are closely related to the tumorous epithelial cells- and the quantity of these products might be related to the biological behavior of these cells.

These results indicate that Myc protein is considered to play a role in maintaining the stem-cell population in the peripheral layers of the tumour nests from which proliferating cells can be recruited. Also, Myc family proteins might function primarily as anti-apoptotic factors reflecting proliferative activity and may be predictor of rapid growth.

Malignant epithelial odontogenic tumors are very rare, they may arise from the epithelial components of the odontogenic apparatus (enamel organ) or the lining of odontogenic cysts which represent the precursor cells for malignant transformation.

Genetic alterations leading to a protein production with abnormal relative amount or structure, may result in malignant transformation, maintenance of tumor growth and metastatic propensity.

Over expression of c-Myc protein in ameloblastomas indicates the increased mild type Myc in the tissues and denotes the early event of neoplastic transformation probably from a previous odontogenic cyst. Indeed, the strong nuclear staining pattern has a tendency to be found more frequently in highly anaplastic cells or poorly differentiated ones. Furthermore, nuclear staining occasionally showed a marked cellular atypism. The stronger reactivity of the cells in the mitotic phase may show higher proliferative activity of tumor cells, and was found only in some high grade carcinomas and metastatic cases and appeared to be correlated with the later phases of carcinogenesis and metastasis. Thus the expression of c-Myc oncogene is considered to be related to the biologic behavior of proliferating malignant tumor cells, and might reflect a mutational oncogenic role, promoting tumor growth.

Over expression of c-Myc protein products may either be due to the increased production or decreased break down of the protein which is the result of growth regulation. This could be a valid screening method for predicting underlying malignant genetic changes in ameloblastoma types, through increased frequency of immunoreactive cells or increased staining density.

Amplification is one of the most frequent genomic abnormalities and with respect to certain proto-oncogenes, its presence is linked particularly to their poor prognostic outcome. C-myc amplification has been reported in cases of squamous cell carcinomas of oral and laryngeal mucosa, cervical carcinomas and in neck metastasis [28]. Oncogene amplification in neck metastasis may indicate an increased metastatic propensity for individual tumor cells demonstrating c-Myc amplification.

In agreement with the above, Fig. 8-metastasizing ameloblastic carcinoma shows intense peripheral immunoreactivity due to aggressiveness and over expression of c-Myc amplification. While the central cells revealed mild to negative staining which may suggest terminal cellular differentiation or malignant necrosis like that seen in comedo-necrosis associated with high grade of malignancy.

The combined evaluation of the proliferation status together with the changes of c-Myc oncoproteins might constitute useful markers for the prognostic evaluation of potentially malignant, as well as malignant tumors, through elucidating the mode of growth or the mode of development of oraltumors from the pathologic point of view. Also, the evaluation of role of the oncogene products in the occurrence, proliferation and development of odontogenic epithelial tumour cells, might be a useful prognostic marker.

5. CONCLUSION

1. The expression of c-Myc oncogene products were detected in human normal tissue and cancer cells.
2. The oncogene c-Myc is related to the proliferation of odontogenic epithelial cells and plays an important role in the control of cell differentiation.
3. The product protein of c-Myc oncogene predominantly locates in the nucleus, to a lesser degree in the perinuclear area and the entire cytoplasm.
4. Moderate to weak reaction of the c-Myc oncoprotein was detected in benign ameloblastomas, and strong reaction was denoted in high grade carcinomas and metastasis.
5. The intensity of the nuclear reaction is correlated with the later phases of carcinogenesis and metastasis.
6. Amplification is one of the most frequent genomic abnormalities and with respect to the c- Myc oncoprotein, its expression is closely related to the biological behavior of human neoplastic cells.
7. The c-Myc oncogene products are useful for elucidating the mode of growth or the mode of development of odontogenic epithelial cells from the pathologic point of view.
8. We add to the literature, a very rare case of ameloblastic carcinoma metastasizing to the regional cervical lymph nodes with intense c-Myc over expression denoting high rate of proliferation with metastatic propensity as well as aggressiveness.

9. Last but not the least, determination of c-Myc oncogene products might enhance diagnosis of biopsies of suspicious cysts or conventional ameloblastomas of the jawbones, their neoplastic potentialityand for further malignant degradation in the clinical routine.

By this, it might also enhance the more precise clinical planning of radicalism and aggressiveness of the surgery adjunct with prophylactic resection of cervical lymph nodes. Based on these results, further studies can be undertaken to find a method to achieve standardized staining results for colorimetric analysis in which c-Myc can be a useful diagnostic and prognostic biological marker for evaluating, planning and monitoring the treatment modalities for these neoplasms.

CONSENT

All authors declare that 'written' informed consent was obtained from the patients for publication of these case reports and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that this study was approved by the Ethical Research Board of the Institution.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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