ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 2, Issue – 4, July – August - 2019, Page No. : 246 - 253

Immunohistochemical Correlation and Comparison of Ki-67 and P53 Expression in Epithelial Cell Proliferation among Variants of Ameloblastomas.

[1]Dr Shashi Ranjan, Reader, Department of Oral & Maxillofacial Pathology, Dr.B.R.Ambedkar Institute of Dental Sciences & Hospital, Patna, Bihar.

[2]Dr Rashmi Issar, Reader, Department of Conservative Dentistry & Endodontics, Patna Dental College & Hospital, Patna, Bihar.

[3]Dr Deepa Hugar, Reader, Dept of Oral and Maxillofacial Pathology, Albadar Dental College and Hospital, Gulbarga, Karnataka, India.

[4]Dr K.M.K. Masthan, Professor and Head, Dept of Oral and Maxillofacial Pathology, Sree Balaji Dental College & Hospital, Chennai, Tamil Nadu.

[5]Dr N. Aravindha Babu, Dept of Oral and Maxillofacial Pathology, Sree Balaji Dental College & Hospital, Chennai, Tamil Nadu.

Correspondence Author: Dr Rashmi Issar, Reader, Dept of Conservative Dentistry & Endodontics, Patna Dental College & Hospital, Patna, Bihar.

Conflicts of Interest: Nil

Type of Publication: Original Research Paper

Conflicts of Interest: Nil

Abstract

Introduction: Ameloblastoma has a diverse clinicopathological appearance. Several treatment modalities have been implicated based on such morphology. In this study we are evaluating tumor marker p53 and cell proliferative marker Ki-67 in various histological and clinical subtypes of ameloblastomas.

Background: The primary aim of the study is to know the behaviour of various clinicopathological variants of ameloblastomas on the basis of Ki-67 and p53 expression in its epithelial cell proliferation.

Methods: Most common variants of ameloblastomas(n=20) consisting six follicular ameloblastomas(FA), six plexiform ameloblastomas(PA), two granular cell ameloblastomas(GCA) and six unicystic ameloblastomas(UA) with luminal proliferation were selected and examined morphologically and Immunohistochemically for changes in proliferative activity using p53 and Ki-67 markers.

Results: Both the Ki-67 and p53 labelling index was significantly higher in FA, PA, UA than GCA. But both markers mean labelling index difference was insignificant among FA, PA,and UA. Among various histopathological variants highest ki-67 mean labeling index was observed for unicystic ameloblastoma(46%) followed by plexiform(37.73%) and follicular ameloblastoma(37.6%) and was minimal in granular cell ameloblastoma(7.8%). For p53, the highest mean labeling index was observed in follicular ameloblastoma(70.4%) followed by unicystic ameloblastoma (65%), plexiform ameloblastoma(64.6%) and was minimal in granular cell ameloblastoma(6.2%).

The pearson correlation (which is significant at the 0.01 level) among ki-67 and p53 expression was insignificant.(.977)

Conclusion: The pattern of expression of ki-67 and p53 varies among variants of ameloblastomas so it clearly indicates that it is not fruitful to correlate and compare both markers in ameloblastomas despite individually both markers are useful to assess the neoplastic behavior and recurrence of ameloblastomas. Also it can be concluded that histopathological variants have no or little role in assessing the behavior and prognosis and all ameloblastomas should be assessed clinically for treatment modalities.

Keywords: Cyst, Immunohistochemistry, Mean Labelling Index, Odontogenic Tumors

Introduction

Ameloblastoma is the most frequently encountered benign epithelial odontogenic tumor(1,2) and is characterized by a locally invasive behaviour with a high risk of recurrence rate despite radical therapy.(3,4) Histopathologically, ameloblastomas show different variants including follicular, plexiform, acanthomatous, granular cell, clear cell, desmoplastic, basal cell, mucinous, hemangiomatous, papilliferous keratoameloblastoma and clinical variants like peripheral, metastasizing and unicystic ameloblastomas with variable growth pattern including luminal, intraluminal and mural proliferation (1,5).It has been reported that the aggressiveness of ameloblastomas may be related to the histological subtype(3), infact in a review of the literature, found for plexiform, follicular, acanthomatous and unicystic ameloblastoma recurrence rates, respectively, of 16.7%, 29.5%, 4.5%, and 13.7%.(4)Several studies have detected genetic and cytogenetic alterations in these epithelial odontogenic tumors; however the detailed mechanism of oncogenesis, cytodifferentiation remain unknown(6,7,8).

Ki-67 antigen is present in all active parts of cell cycle, rises during later half of S phase and reaches a peak in G2 and M phase and rapidly degrades after mitosis with a half life of detectable antigen being an hour or less. So it correlates with other variables of cell proliferation.(9)

p53 is a mutated product of tumor suppressor gene p53. It has a short half life in normal cells and cannot be detected immunocytochemically, but when it mutates, the p53 protein product is more stable and can be detected immunocytochemically. It has been widely used for diagnosis of malignant tumors.(10-12)

As we know that irreversible increased cellular proliferation is a phenomenon of tumor, so in our study we are using proliferative marker Ki-67 to assess correlation of epithelial cell proliferative index and to assess mutated p53 epithelial cell expression among histological variants of ameloblastomas including follicular, plexiform, granular cell ameloblastoma and unicystic ameloblatoma with luminal proliferation.

Materials and Methods

The study material comprised of archival formalin fixed paraffin embedded specimens of patients from private oral pathology laboratory as 20 cases of ameloblastomas. The cases were selected on the basis of most commonly encountered clinicopathological variants of ameloblastomas and included 6 cases of follicular ameloblastoma, 6 cases of plexiform ameloblastoma, 2 cases of granular cell ameloblastoma and 6 cases of unicystic ameloblastoma with luminal proliferation.

All selected cases had been routinely fixed in 10% neutral formalin (24-48 h), dehydrated in graded alcohols, cleared in xylene and embedded in paraffin. Using haematoxylin and eosin-stained sections, all the slides were reviewed, the quality of the material was checked and the slides for the quantitative evaluation of the material were selected. In each case the slides selected showed the peripheral and

© 2019 IJDSIR, All Rights Reserved

the central zone of the tumour. Each individual tissue block was cut for two individual slides. So each case was having two slides, one for Ki-67 and other for p53. 1 Control slide was also made for each group of cases. Standard immunohistochemistry staining protocol was performed (9) using primary antibody monoclonal mouse anti-human p53 (1:100 prediluted) and monoclonal mouse anti-human Ki-67 (1:100 prediluted) and super sensitive poly horse radish peroxidise (HRP) secondary antibody kit (Biogenex India private limited). Slides were checked for positive staining as crisp, brown nuclear staining for Ki-67 and p53 in respective sections. Positive controls were obtained from colonic adenocarcinoma for p53 and lymphoma for Ki-67. Negative controls were included by performing duplicate assays, in which the primary antibody was replaced with phosphate buffer saline.

Evaluation of p53 And Ki-67 Staining

Representative fields were selected in each case as randomly or without bias as possible. These areas included only labelled cystic or tumor epithelial areas and excluded areas of necrosis, inflammation, and stromal cells. Representative Fields were selected in each case of solid and multicystic ameloblastomas and along the cyst lining of unicystic variant. All cell counts were performed with a Olympus Ch20i microscope fitted with an eyepiece graticule, x10 oculars, a x40 objective, and a counting grid containing 400 blocks in conjunction with a hematology laboratory differential cell counter (Olympus India limited). The cases were scored by counting the positive cells per minimum of 500 tumor cells per specimen. The percentage labelling index (number of positive tumor cells/total number of tumor cells expressed as a percentage) was calculated per case. To determine intraobserver and interobserver reliability, the same examiner counted the cells in each case twice at one hour interval while a second examiner counted the cells independently in a sample of the cases using the same positions of the grid.

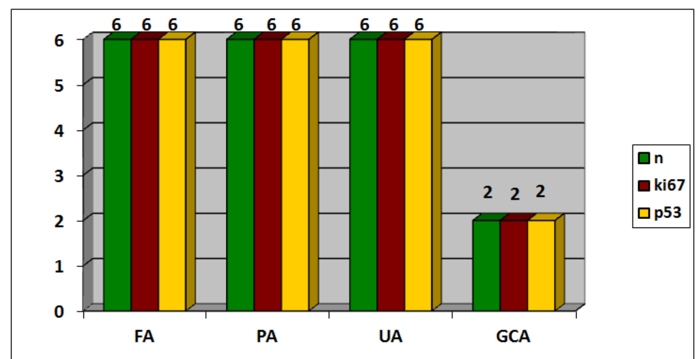
Statistical analysis

Descriptive statistical analysis was performed for each group of lesions by statistical software program SPSS (version 16.0). The mean labeling indices were compared by using an analysis of variance test (ANOVA) for both p53 and Ki-67. Kruskal-Wallis Test were then performed to determine statistically significant differences between p53 and ki-67 expression overall. Probability levels of < 0.05 were regarded as being statistically significant. To satisfy the statistical assumptions of the tests, all data were square root transformed before analysis.

Results

Positive cases were selected on basis of positive staining by both markers in same cases.(Graph 1) There was no significant difference found among follicular, plexiform and unicystic ameloblastomas for mean labelling index of both the markers but these variants shows significantly higher mean labelling index than granular cell ameloblastomas for both the markers. (p=.041)

The pearson correlation (which is significant at the 0.01 level) among ki-67 and p53 expression was insignificant.(.977)

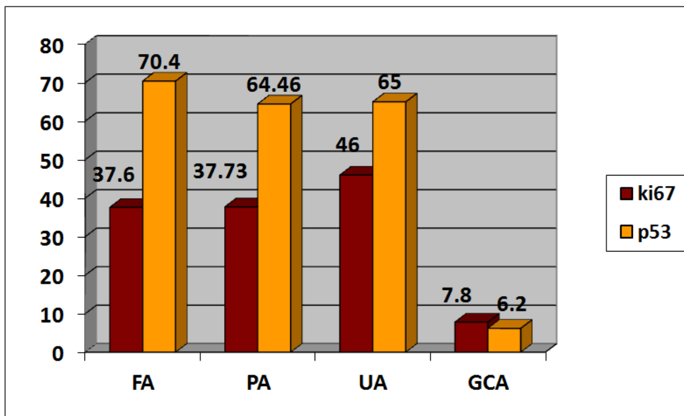


Graph 1. Immunopositive Samples Of Ameloblastomas (FA-Follicular Ameloblastoma, PA- Plexiform Ameloblastoma, UA- Unicystic Ameloblastoma, GCA- Granular Cell Ameloblastoma, n=Number Of Cases)

© 2019 IJDSIR, All Rights Reserved

Nuclear p53 reactivity was exhibited by ameloblastomas, and it shows more and dense expression mostly in basal and parabasal odontogenic epithelial cells in all ameloblastomas. Highest mean labelling index was observed in follicular ameloblastoma followed by unicystic and plexiform ameloblastoma and was minimal in granular cell ameloblastoma.

Nuclear Ki-67 reactivity was exhibited by ameloblastomas, mostly in basal and parabasal odontogenic epithelial cells in many areas of different variants of ameloblastomas. Among various histopathological variants highest ki-67 mean labelling index was observed for unicystic ameloblastoma followed by plexiform and follicular ameloblastoma and was minimal in granular cell ameloblastoma.



Graph 2. Mean Labeling Index For Ki-67 And P53 In Ameloblastomas

(FA-Follicular Ameloblastoma, PA-Plexiform Ameloblastoma, UA- Unicystic Ameloblastoma, GCA- Granular Cell Ameloblastoma)

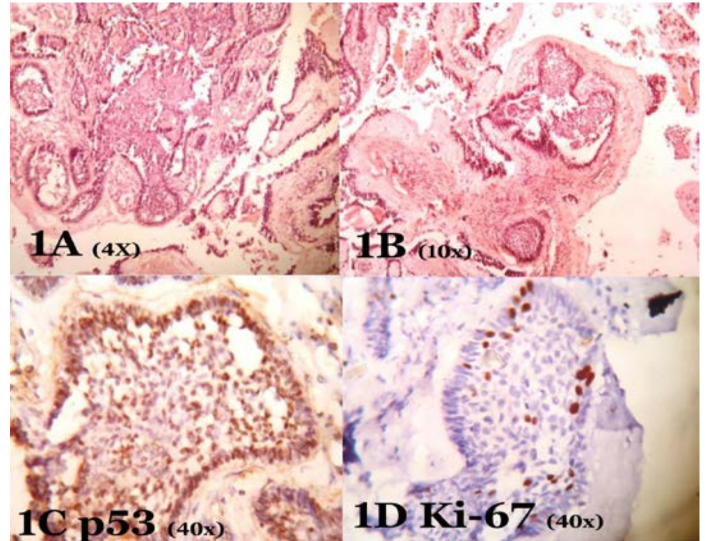


Figure 1: A-D Follicular ameloblastoma ,1A-H&E section of follicular ameloblastoma (4x),1B- H&E section of follicular ameloblastoma (10x), 1C- Positive p53 expression in peripheral & central cells of follicular ameloblastoma(40x) ,1D- Positive Ki-67 expression in peripheral & central cells of follicular ameloblastoma(40x)

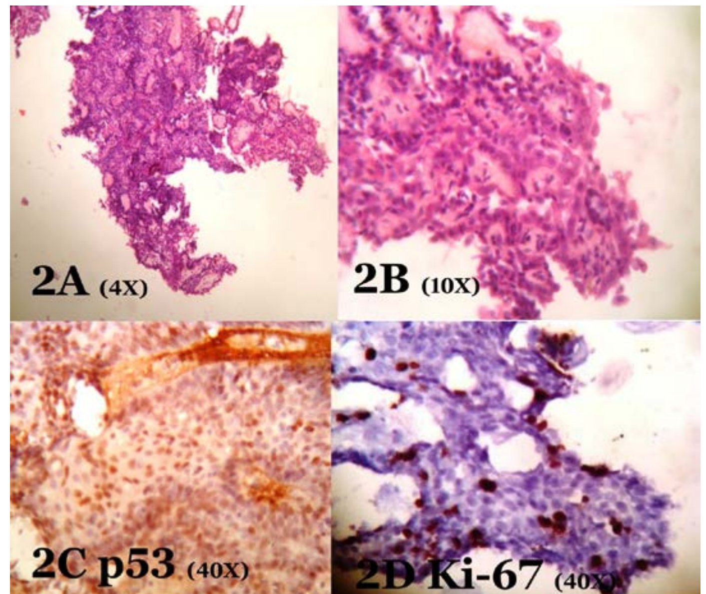


Figure 2: A-D Plexiform ameloblastoma,2A-H&E section of plexiform ameloblastoma (4x),2B-H&E section of plexiform ameloblastoma (10x), 2C-Positive p53 expression in peripheral & central cells of plexiform ameloblastoma(40x),2D-Positive Ki-67 expression in

© 2019 IJDSIR, All Rights Reserved

peripheral & central cells of plexiform ameloblastoma(40x)

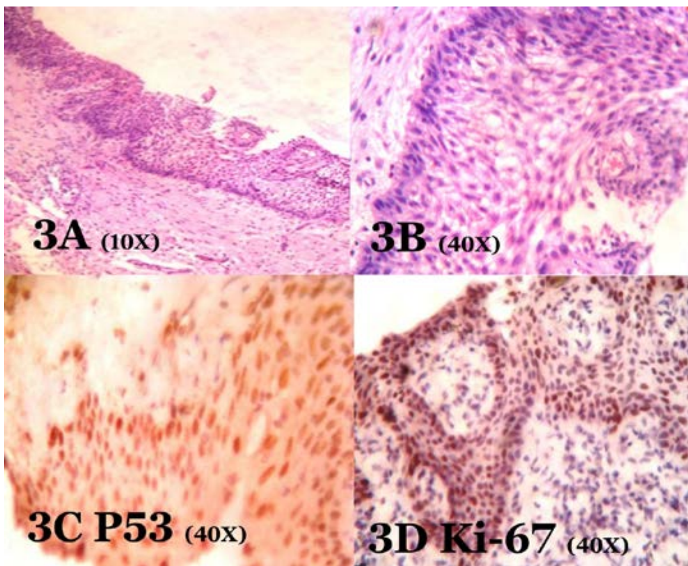


Figure 3: A-D Unicystic Ameloblastoma,3A-H&E section of unicystic ameloblastoma (10x),3B-H&E section of unicystic ameloblastoma (40x),3C-Positive p53 expression in basal & suprabasal cells of unicystic ameloblastoma(40x),3D-Positive Ki-67 expression in basal & suprabasal cells of unicystic ameloblastoma(40x)

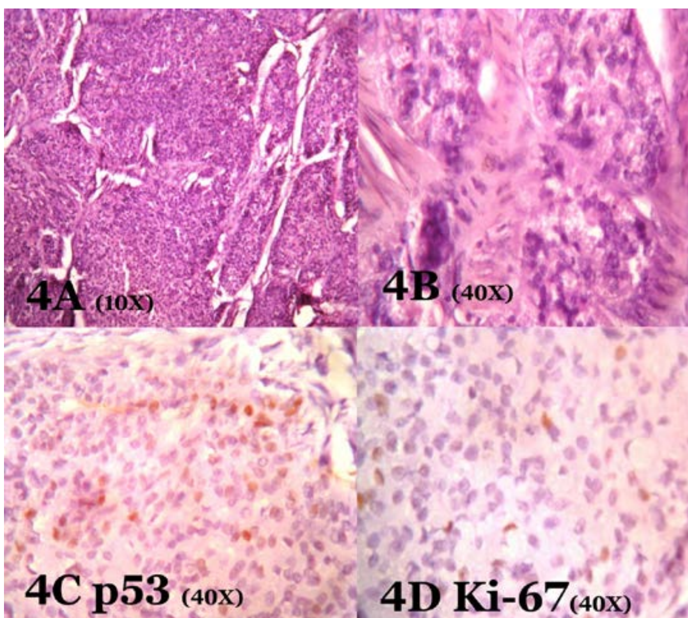


Figure 4: A-D Granular cell ameloblastoma,4A-H&E section of granular cell ameloblastoma (10x),4B-H&E section of granular cell ameloblastoma (40x),4C-Sparse positive p53 expression in basal & central cells of granular cell ameloblastoma(40x),4D-Sparse positive Ki-67 expression in basal & central cells of granular cell ameloblastoma(40x)

Discussion

Many investigation of tumor cell proliferative activity and role of p53 in tumorigenesis have been evaluated using Ki-67 and p53 in oral and other systemic tumors.(10-13) Kumamoto et al found no or little p53 expression in tooth germ tissue whereas nuclear positivity was seen in benign and malignant ameloblastomas and suggested that p53 expression is associated with oncogenesis of odontogenic epithelium. (10)However many studies on p53 has shown that it is not consistently expressed in odontogenic lesions and whatever expression occur is due to over expression and not due to mutation (14).

In this study, assessment of cellular proliferation markers was shown to be accurate, reliable and reproducible and produced excellent comparable results. The use of labeling indices has, however, proven to be extremely tedious for routine application in the assessment of cellular proliferation.

Keeping the concept of tumor in mind the most common property is excessive proliferation of tumor cell thought to be arising from mutation of p53 tumor suppressor gene or its product, we carried out this study with selection of Ki-67 epithelial proliferative marker and p53 marker to put the concept of tumor proliferation due to mutated p53 gene in tumorigenesis by comparing the expression of Ki-67 and p53 in epithelial component of these groups.(13)

Variable expression of Ki-67 and p53 noted among histopathological variants of ameloblastomas. p53 expression was higher in follicular type than plexiform and unicystic type suggesting that tissue structuring of ameloblastomas might be affected by p53 expression.(10) High proliferative activity seen in all the variants except granular cell ameloblastoma. Granular cell ameloblastoma

© 2019 IJDSIR, All Rights Reserved

showed minimal p53 expression and it has been shown that granular cells shows increased apoptotic cell death as compared with other neoplastic cells. So apoptosis of these cells was not induced by a p53-dependent pathway.(10,15,16) Granular cell variant minimal cellular proliferation and p53 expression can also be explained on the basis of loss or inactivation of signaling pathways related to cell proliferation and differentiation in the granular cells, suggesting that these cells are functionally inactive and their synthesis and secretion activities have become irregular (17).

Unicystic ameloblastoma showed statistically significantly higher ki-67 labelling indices than solid variants. It was similar to results of Meer et al(9) and this variation can be attributed to difference in methodology, especially the counting protocol, used. Unfortunately it is very difficult to compare these parameters because published reports frequently fail to provide sufficient detail or explanation. Another possible reason might be the difference in the morphology of the tumors, with the solid lesions providing large follicles or plexiform sheets for analysis, whereas only a thin lining is available in the unicystic cases. This may have resulted in the inclusion of greater numbers of basal and parabasal cells in the unicystic group, thus resulting in higher mean labeling indices. This is unlikely as cells in the entire thickness of the epithelial linings of the unicystic lesions were included in the count (9). Inclusion of granular cell variant has resulted in decreased overall expression of both markers.(17)

Ki-67 proliferating marker was observed predominantly in peripheral cells of tumor island and cystic lining. This could explain the locally infiltrating growth of ameloblastomas and its aggressive behaviour. Difference in morphology of ameloblastomas facilitates more difficulty in surgical accessibility of conventional ameloblastomas than unicystic ameloblastomas. So it can explain recurrence and worse prognosis in conventional ameloblastomas than unicystic ameloblastomas. (9)

Individually, result of p53 and Ki-67 expression for follicular, plexiform and unicystic variants were significantally higher than granular cell ameloblastoma. However correlation among both the markers was insignificant. Not all cells containing the Ki-67 antigen are actively proliferating cells, and the use of Ki-67 to assess proliferative activity is not advised in tissues over expressing p53.(18)

It has been proposed that odontogenic tumor may follow p53 independent pathway for tumorogenesis, so doesn't express p53.(15,16) Recent advancement has shown various molecular markers in tumor growth and its behaviour including vascular endothelial growth factors (VEGF) which has been found to be minimally expressed in acanthomatous and granular cell ameloblastoma than other histological variants. Also mutation in PTEN(phosphatase and tensin homolog deleted on chromosome 10) has been shown to occur in ameloblastomas and in carcinogenesis , loss of PTEN allows for overactivity of the phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) pathway inducing an upregulation of mammalian target of rapamycin (mTOR) and its downstream effector ribosomal subunit-6 kinase (S6K); allowing for uncontrolled cell proliferation, apoptosis inhibition and cell cycle deregulation. Aberrant signalling in the PI3K/AKT/m TOR pathway may be the cause of aggressiveness of ameloblastomas.(8)

Conclusion

So overall result clearly indicates the pattern of expression of ki-67 and p53 varies among variants of ameloblastomas so it is not fruitful to correlate and compare both markers in ameloblastomas despite individually both markers are useful to assess the neoplastic behaviour of ameloblastomas. p53 overexpression may be involved in

© 2019 IJDSIR, All Rights Reserved

pathogenesis of ameloblastomas and can be used to assess aggressive behaviour of various clinicopathological variants of ameloblastomas. Also anti p53 agent could be considered to reduce the size of large tumors and to treat unresectable tumors that are in close proximity to vital structures.

Ki-67 on the other hand could provide useful prognostic markers for proliferative activity and good prognostic indicators for recurrence of ameloblastomas. Our study clearly indicated that except granular cell ameloblastom, other variants of ameloblastomas have high proliferative potential. Granular cell ameloblastoma shows minimal proliferation and can be explained on molecular and pathogenetic basis as initially we use term granular cell changes in ameloblastoma suggesting that these changes are initiating in central stellate reticulumn like cells of either foliicular or plexiform ameloblastoma and in course of time granular cell changes involves even peripheral ameloblastoid cells and presents as extensive granular cell changes in whole tumor mass and at this time term granular cell ameloblastoma should be applied.(14-16,19) So granular cell ameloblastom can be considered as mature tumor showing minimal proliferation based on our results as well as previous molecular studies. So apart from proliferative potential, it is the ease with which a tumor can be removed will decide the tumor behaviour. So all ameloblastomas should be assessed clinically for treatment modalities.

However further study with large sample size of different clinicopathological variants and other relevant tumor marker analysis will be required to confirm these findings and to make a definite cause and effect relationship.

References

1. Merva SOLUK-TEKKESIN, John M. WRIGHT. The world health organization classification of odontogenic lesions: a summary of the changes of the 2017 (4th) edition. Turkish Journal of Pathology; 2018:34(1-16)
2. Funaoka K, Arisue M, Kobayashi I, Iizuka T, Kohgo T, Amemiya A, Totsuka Y. Immunohistochemical detection of proliferating cell nuclear antigen (PCNA) in 23 cases of ameloblastoma. Oral Oncol, Eur J Cancer 1996;32B:328±332.
3. Kim J, In Yook J. Immunohistochemical study on proliferating cell nuclear expression in ameloblastomas. Oral Oncol, Eur J Cancer 1994;30B:126±131.
4. Reichart PA, Philipsen HP, Sonner S. Ameloblastoma: biological profile of 3677 cases. Oral Oncol, Eur J Cancer 1995;31B:86±99.
5. SB RAHMAN, SMA SADAT, IA HAIDER, M AHMED:Analysis of Histological Variants of Ameloblastomas of Jaws in Relation to their Clinical Presentations : Journal of Bangladesh College of Physicians and Surgeons Vol. 35, No. 2, April 2017.
6. A. Piattelli, M. Fioroni, A. Santinelli, C. Rubini. Expression of proliferating cell nuclear antigen in ameloblastomas and odontogenic cysts. Oral Oncology 34 (1998) 408-412.
7. Alma Florescu, Cristiana Simionescu, Raluca Ciurea, Allma Pitru:P53, Bcl-2 and Ki67 immunoexpression in follicular solid ameloblastomas ; Rom J Morphol Embryol(2012), 53(1):105–109
8. Ravleen Nagi, Shashikant Sahu, N Rakesh; Molecular and genetic aspects in the etiopathogenesis of ameloblastoma: An update: Journal of Oral and Maxillofacial Pathology, Sep- Dec 2016 (Vol 20)Issue 3
9. Shabnum Meer, Jacqueline S. Galpin, Mario Altin, Hedley Coleman, Hasiena Ali. Proliferating cell nuclear antigen and Ki-67 immunoreactivity in

© 2019 IJDSIR, All Rights Reserved

ameloblastomas. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;95:213-21.

10. Hiroyuki Kumamoto,Takashi Izutsu, Kousuke Ohki,Nobuhiro Takahashi, Kiyoshi Ooya . p53 gene status and expression of p53,MDM2, and p 14 ARF proteins in ameloblastomas. J Oral Pathol Med (2004) 33 :292-9.
11. Slootweg PJ. p53 protein and Ki-67 reactivity in epithelial odontogenic lesions. An immunohistochemical study. J Oral Pathol Med (1995) 24: 393–397.
12. JN Carvalhais, MCF de Aguiar, VC de Araujo, NS de Araujo, RS Gomez. p53 and MDM2 expression in odontogenic cysts and tumours. Oral Diseases (1999) 5, 218–222.
13. Amol Ramchandra Gadbail , Rahul Patil & Minal Chaudhary :Co-expression of Ki-67 and p53 protein in ameloblastoma and keratocystic odontogenic tumor ; Acta Odontologica Scandinavica, 2012; 70: 529–535
14. Li TJ, Browne RM, Matthews JB. Quantification of PCNA+ cells within odontogenic jaw cyst epithelium. J Oral Pathol Med (1994) 23:184-189.
15. Kumamoto H. Detection of apoptosis-related factors and apoptotic cells in ameloblastomas:analysis by immunohistochemistry and an in situ DNA nick-end labelling method. J Of Oral Pathol Med 1997; 26: 419-25.
16. Kumamoto H, Kimi K, Ooya K. Immunohistochemical analysis of apoptosis related factors (Fas, Fas ligand, caspase-3 and single stranded DNA) in ameloblastomas. J Of Oral Pathol Med 2001; 30: 596-602.
17. Sathi Gul San Ara, Phuu Pwint Han, Ryo Tamamura, Hitoshi Nagatsuka, Hailong Hu, Naoki Katase, Noriyuki Nagai. Immunolocalization of cell signaling molecules in the granular cell ameloblastoma. J of oral pathol med (2007) 36; 609-14.
18. Ayman Hegab, Mohmmad Shuman, Mohammed Abd El-Akher, Delaram Arwlan. Ki-67 immunohistochemical expression in mandibular ameloblastoma: A prognostic indicator for local recurrence; Open Journal of Stomatology, 2013, 3, 520-526
19. Neville BW, Damm DD, Allen CM, Bouquot JE. Oral and maxillofacial pathology. 3rd edition. Saunders; 2009. 706.

© 2019 IJDSIR, All Rights Reserved