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IMMUNOHISTOCHEMICAL EXPRESSION OF PCNA AND MCM2 IN AMELOBLASTOMA

Dental Science

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ABSTRACT

a) Background:- An odontogenic tumour is a neoplasm of the cells or tissues that initiate odontogenic processes. These are the most frequent osseous destructive lesions of the jaws. Ameloblastoma is a rare neoplasm that accounts for approximately 1% of all other oral tumors. Various cell proliferation markers are used as diagnostic and prognostic tools in oral lesions. Simultaneous evaluation of these markers can increase the precision of estimation of the proliferative status of different tissues.

b) Aim:- The aim of this study is to analyze the presence of, Proliferating cell nuclear antigen (PCNA) and the minichromosome maintenance-2 (MCM2) proteins in histopathologically diagnosed cases of ameloblastoma.

c) **Materials and method:-** 15 histopathologically diagnosed cases of ameloblastic variants are obtained from the archieves of the Department of Oral and Maxillofacial Pathology to study the expression of MCM2 and PCNA. The data was analysed by Kruskals ANOVA, Mann-Whitney U test and Post hoc analysis.

d) Results:- When analyzing the MCM-2 positivity among the 3 variants(Follicular, Plexiform and Unicystic), there was a significant difference between the mean percentages of their staining (p= 0.016), where Follicular Ameloblastoma displayed significantly higher rate than Unicystic Ameloblastoma.When analyzing cell proliferation with PCNA among the 3 variants (Follicular, Plexiform and Unicystic) of Ameloblastoma, there was no significant difference between the mean percentages of the three(p= 0.22). On overall comparison between the 2 marker in the 3 variants of Ameloblastoma MCM2 showed a significant difference than PCNA (P=0.002). A P-value < 0.001 was considered statistically significant

KEYWORDS

PCNA, MCM2, Ameloblastoma

INTRODUCTION

The word Ameloblastoma is derived from two languages where the English word "amel" means enamel and the Greek word "blastos" means the germ. It is a benign neoplasm of odontogenic epithelium, principally of enamel organ–type tissue that has not undergone differentiation to the point of hard tissue formation.1 It is generally present in the jaw bones (most common in the mandible), which is slow growing but locally invasive and can progress to greater size causing facial asymmetry, displacement of teeth, malocclusion and pathologic fractures. It accounts for about 1% of oral tumors and 9-11% of odontogenic tumors.1 It deserves special attention, because of its biological behaviour exhibiting great infiltrative potential, high recurrence rate and capacity to metastasize.[2,3]

The increased cell proliferation in the odontogenic epithelium can be a result of perturbations in the cell cycle regulators and mutations in tumor suppressor genes. Investigating cell kinetics of epithelial lining of these lesions may be helpful in assessing their biological behaviour.[4]

Cell proliferation is an essential process in all living organisms because of its role in cell growth and the maintenance of tissue homeostasis. Control of this process is dysregulated in some types of neoplasias.2 Proliferative markers are specific proteins whose presence in actively growing and dividing cells serves as an indicator for such cells.[5]

PCNA is 36kDa acidic non histone protein that is useful for DNA synthesis and is an accessory protein for Deoxy Ribose Nucleic Acid (DNA) polymerase alpha, whose levels are high in G1-S phase of the cell cycle.5,6 As the cells remain for a longer duration in G1-S phase when proliferating, PCNA can be used as a marker for cell proliferation.[5]

MCM proteins are the family of molecules that function in DNA synthesis of both prokaryotic and eukaryotic cells.2 It plays a role in cell differentiation, proliferation and replication complexes in eukaryotic cells.2,7 The function of this protein is still unclear as the individual role of each protein in helicase activity and chromatin organization is yet to be resolved. The regulation of MCM proteins is low during cell cycle arrest (G0-Phase), and is not detectable by immunohistochemistry (IHC). Till date, the expression of MCM2 and MCM3 proteins in ameloblastoma have not been assessed, so proliferation indexes and their associations with the clinical behaviour in ameloblastoma have not been determined.2

The aim of the present study is to evaluate and compare the role of PCNA and MCM2 in the variants of ameloblastoma to predict their biological behavior.

AIMS AND OBJECTIVES

- To evaluate the expression of MCM2 and PCNA in the variants of Ameloblastoma.
- To compare the expression of MCM2 and PCNA in the variants of Ameloblastoma.

MATERIALS AND METHODS

A retrospective study was done to demonstrate the role of PCNA and MCM2 in formalin fixed paraffin embedded tissues specimens taken from the archives of the Department of Oral and Maxillofacial Pathology, Sri Sai College of Dental Surgery, Vikarabad.

The study sample comprised of 15 cases of Ameloblastoma. Out of which Follicular Ameloblastoma (n=6), Plexiform Ameloblastoma (n=4), Unicystic Ameloblastoma (n=4) and Ameloblastic Fibro Odontome (n=1) were included.

IMMUNOHISTOCHEMISTRY (IHC) PROCEDURE

Sections of 3-5µm thick from the paraffin embedded blocks were made and transferred onto the amino propyl epoxy silane (APES) coated slides. The slides were treated with two changes of xylene for 10mins each to remove paraffin wax, followed by descending grades of alcohol (3mins each) for rehydration. Peroxidase block was done using 3% hydrogen peroxide for 10 mins. Antigen retrieval was done by using TRIS-EDTA buffer (PCNA, pH= 9.0 and MCM2, pH=9.0) in a conventional microwave oven at 600watts for 20 mins (each cycle of 10mins), followed by peroxidase block again with 3% hydrogen peroxide and washed in TRIS wash buffer (PCNA, pH=7.6 and MCM2, pH=7.6). The slides were then incubated with primary antibodies (PCNA) and (MCM2) for 40mins each at room temperature followed by washing with TRIS wash buffer. Horse radish peroxidase (HRP) conjugated secondary antibody was used as an enchancer followed by DAB (3' Diaminobenzidine Tetrahydrochloride- a substrate chromogen) for 5 mins for both PCNA and MCM2. Two changes of TRIS buffer washes were performed after each step. The sections were countered stained with hematoxylin and mounted with DPX®

STATISTICALANALYSIS

- Stained slides were observed by a pathologist using with light microscopy under 40x magnification.
- Five fields were randomly selected on each slide and the labeling index (LI) was calculated by dividing the number of immuno positive cells by the total number of cells per case and multiplying with 100.
- Furthermore, expression levels were evaluated using the semi quantitative scale: Score 1 (<1%), Score 2 (1-10%), score 3 (11-33%), score 4 (34-66%), score 5 (67-100%).
- The obtained data from clinical and immunohistochemical studies was analyzed by the Statistical Package for the Social Sciences, version 16.0 (SPSS Inc., Chicago, IL, USA).
- A P-value < 0.001 was considered statistically significant.

RESULTS AND OBSERVATION

In the present study 15 cases of variants of Ameloblastoma were included out of which 6 cases were follicular ameloblastoma, 4 cases were plexiform ameloblastoma, 4 cases were unicystic ameloblastoma and 1 case was of Ameloblastic fibro odontome.

12 cases reported were males and 3 were females with sex ratio 4:1. Age ranged from 13 years to 62 years with mean age of 40.46 years.(Table 1)

POSITIVITY IN VARIANTS OF AMELOBLASTOMA

PCNA Immunoexpression was positive in all cases of variants of ameloblastoma where as, MCM 2 was negative in 2 cases (unicystic ameloblastoma) out of 15 cases of ameloblastoma.(Table 2)

When analysed for the positivity of PCNA in the variants of ameloblastoma, its distribution predominated in the nucleus of the basal and suprabasal cells of the epithelium. PCNA showed no significant difference in the mean percentages of staining of 3 variants of ameloblastoma (follicular ameloblastoma 61± 17.73, plexiform amelobastoma 47.5±24.44, unicystic ameloblastoma 15.62± 15.04). P-value was 0.22. However, the proliferation index was greater for Follicular ameloblastoma. (Table 3, Graph 1)

When analysed for the expression of MCM2 in the variants of ameloblastoma, its distribution was higher in the peripheral cells with columnar morphology, the expression was either low or absent in the central polyhedral cells. There was a significant difference in the mean percentages of the staining intensity of MCM2 marker in the variants of ameloblastoma (follicular ameloblastoma 22.5±13.41, plexifrom ameloblastoma 11.45±6.00 and unicystic ameloblastoma 2.77±3.44). The mean percentage of the follicular variant was significantly higher than the unicystic variant where P-value was 0.002. (Table 4, Graph 2) Overall comparison between the expression of PCNA and MCM2 in the variants, revealed the mean percentages of PCNA (47.34±28.21) was significantly greater than MCM2 (15.48±13.81). (Table 5, Graph 3)

DISCUSSION

IHC is an important tool that can help to determine the biological differences in the behaviors of different tumors. The study of proteins involved in tumor cell proliferation is relevant because these proteins can be used as valuable biomarkers of clinical and biologic behavior.

In our study, proliferative indices for MCM 2 and PCNA were assessed. MCM 2 are expressed throughout the cell cycle including the cells leaving G0 to enter into the early G1 phase. The PCNA expression may be used as a marker of cell proliferation because cells remain longer time in G1 to S phase when proliferating.

In the present study, PCNA has shown significant higher labeling index in follicular ameloblastoma (mean ± SD:61± 17) than plexiform (mean± SD: 47.5±24.44) and unicystic (mean± SD :15.62± 15.04) which is in accordance with the study conducted by *Funaoka et al. in 1996, Reichart et al 1995,* who stated that the high expression of PCNA in follicular ameloblastoma can explain its increased potential of recurrence. [9]

In our study MCM 2 has shown significant higher labellling index in follicular variant of ameloblastoma (mean± SD 22.5±13.41) than plexiform (mean± SD: 11.45 ± 6.00) and unicystic (mean± SD: 2.77 ± 3.44) which is similar to the study conducted by *Rogelio Gonzalez-Gonzalez et al. in 2015* and suggested that which indicate aggressive, invasive and metastatic neoplasm.[2]

Our study, in contrast with PCNA, the immunohistochemical levels of MCM 2 were lower in variants of ameloblastoma. These immuno expression differences may be related to the cell cycle phase.

In literature, it has been suggested that MCM 2 are expressed in the early G1 phase and throughout the cell cycle. Though, it may involve the entire cell cycle, the role of these proteins in the helicase activity and chromatin organization are yet to be determined.[2]

However, higher PCNA expression probably results not only form cellular proliferation, but also from other sources, including DNA repair and factors influencing the increase of autocrine and paracrine rates in m-RNA of the PCNA.

In the present study 1 case of ameloblastic fibro odontome was taken and expression of PCNA was evaluated and it was in consistent with the study conducted by *Yamamoto et al. in 1995* who had evaluated the expression of PCNA in Ameloblastic fibroma, AOT, Ameloblastic fibro odontoma, and Odonto-ameloblastoma. They reported that PCNA positive cells were rarely observed in these tumours. [11]

Present study also evaluated the expression of MCM 2 in Ameloblastic fibro odontoma and the ameloblast like cells in it showed positivity for MCM 2.

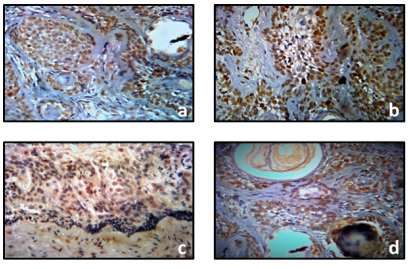
CONCLUSION

This study demonstrated that PCNA immunoexpression was more sensitive than MCM 2 immunoexpression in ameloblastoma. Additionally, expression levels of both the markers where higher in follicular ameloblastoma.

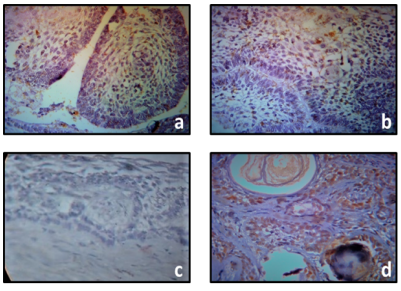
Therefore , PCNA may be used as predictive marker of more aggressive tumor behaviour and potentially as important marker to predict the risk of malignant transformation.

With regards to ameloblastic fibro dontome, due to the rarity of this tumor, this study could only include one case, for which our suggestions and hypotheses must be tested with a large sample.

This study is the first to try to establish a relationship between PCNA and MCM 2 expression and suggests that the functions of MCM are still unclear and are yet to be determined.



Images showing expression of PCNA in variants of Ameloblastoma a) Follicular Ameloblastoma b) Plexiform Ameloblastoma c) Unicystic Ameloblastoma d) Ameloblastic fibro odontome



Images showing expression of MCM2 in variants of Ameloblastoma a) Follicular Ameloblastoma b) Plexiform Ameloblastoma c) Unicystic Ameloblastoma d) Ameloblastic fibro odontome

Table 1: Distribution according to Gender and Age

GROUP	GENDER Male n %	Female n %	M:F ratio	Age range (years)	Mean age ± SD, years
AMELOBLASTOMA	12 80	3 20	4:1	13-62	40.46 ± 12.62

Table 2: Positivity in variants of Ameloblastoma

GROUP	PCNA POSITIVE	PCNA NEGATIVE	MCM 2 POSITIVE	MCM 2 NEGATIVE
Foliicular (6)	6	0	6	0
Plexiform (4)	4	0	4	0
Unicystic (4)	4	0	2	2
Ameloblastic fibro odontome (1)	1	0	1	0

Table 3: Mean labeling index of PCNA in Variants of Ameloblastoma

GROUP	Mean	Std - deviation	P - value
Follicular ameloblastoma	61	17.73	0.22,NS
Plexiform ameloblastoma	47.5	24.44	
Unicycstic ameloblastoma	15.62	15.04	

ANOVA TEST, P VALUE NON SIGNIFICANT

Table 4 : Mean labeling index of MCM 2 in variants of Ameloblastoma

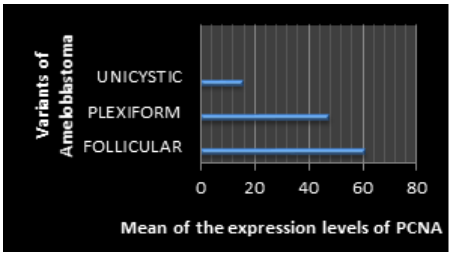
Variant/PCNA	Mean	Std - deviation	P - value	Post hoc
Follicular	22.5	13.41	0.016*,Sig	Follicular >
Plexiform	11.45	6.00		Unicystic
Unicycstic	2.77	3.44		

ANOVA TEST, P VALUE SIGNIFICANT, ON FURTHER POST HOC ANALYSIS FOLLICULAR > UNICYSTIC

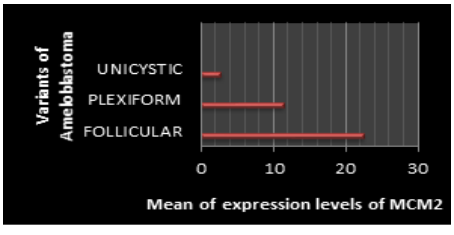
Table 5 : Overall Comparision of Mean between PCNA and MCM 2

Stain	Mean	Std - deviation	P - value
PCNA	47.34	28.21	0.002*,Sig
MCM 2	15.48	13.81	

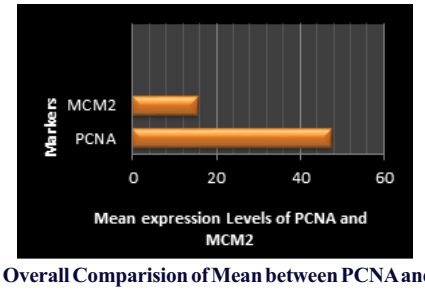
MANN-WHITNEY U TEST ,P VALUE SIGNIFICANT PCNA>MCM2



Graph 1: Mean labeling index of PCNA in Variants of Ameloblastoma



Graph 2: Mean labeling index of MCM2 inVariants of Ameloblastoma



Graph 3: Overall Comparision of Mean between PCNA and MCM 2

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