



Immunohistochemical Expression of MCM-2 for Evaluation of Proliferative Activity in the Epithelial Lining of Radicular Cyst, Dentigerous Cyst, and Odontogenic Keratocyst: A Retrospective Study

¹Smita V Rathod, ²Shilpa Patel, ³Jigna Pathak, ⁴Niharika Swain

ABSTRACT

Aim: To evaluate and compare immunohistochemical expression of minichromosome maintenance protein-2 (MCM-2) for proliferative activity in the epithelial lining of radicular cyst (RC), dentigerous cyst (DC) and odontogenic keratocyst (OKC)

Materials and methods: This retrospective study was conducted on 45 formalin-fixed paraffin-embedded tissue blocks of odontogenic cysts, which were retrieved from the archives. Sections from RC, DC, and OKC were subjected to MCM-2 nuclear staining technique. For each of the specimens, intensity and extent of MCM-2 expressions were evaluated by a comprehensive scoring formula; 100 nuclei were assessed in the epithelial lining of each specimen, under 400× magnification. Scoring was done for positively stained nuclei and expressed as percentage.

Results: The mean (%) MCM-2 immunopositive nuclei in the epithelial lining were higher in OKC cases (10.93%) as compared with RC (10.40%) and DC (3.07%), and the difference was statistically significant between the OKC and DC ($p = 0.046$). The mean (%) MCM-2 expression in different histopathological grades of inflammation in RC showed a statistically significant difference of MCM-2 expression (%) between cases of mild ($n = 4$) and severe ($n = 2$) degrees of inflammation ($p = 0.012$) and also between cases of moderate ($n = 9$) and severe (2) degrees of inflammation ($p = 0.008$). The mean (%) MCM-2 expression in inflamed ($n = 8$) and noninflamed ($n = 4$) cases of OKC showed a statistically significant difference. The mean (%) MCM-2 expression in cases of DC with ($n = 7$) and without ($n = 7$) inflammation also showed a statistically significant difference ($p = 0.033$).

Conclusion: The mean (%) MCM-2 expression was higher in OKC cases as compared with RC and DC, which shows that the epithelium of OKC has a higher proliferative capacity than RC and DC. In present samples, the MCM-2 expression in epithelial lining increased, with increasing grades of inflammation, thus supporting the carcinogenic role of inflammation.

Keywords: Dentigerous cyst, Minichromosome maintenance protein, Odontogenic keratocyst, Radicular cyst.

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INTRODUCTION

Odontogenic cysts develop from the residues of odontogenic epithelium, such as reduced enamel epithelium, cell rests of Serres or cell rests of Malassez, or epithelial cells of dental follicle (DF).¹⁻³ The coronal part of follicle of fully developed tooth is entitled as pericoronal sac or pericoronal follicle and that is occasionally present around impacted tooth.⁴ It is an ectomesenchymal tissue and frequently comprises the epithelial remnants of odontogenesis, which might be the initiating point of pathology.^{1,5} Occasionally, DF adjacent to impacted teeth will persist and lead to the development of cysts and tumors, such as RC, DC, OKC [as of 2017, the World Health Organization (WHO) has reclassified keratocystic odontogenic tumor (KCOT) as OKC].⁶

The RCs, DCs, and OKCs show different growth patterns and biological behaviors. It is well known that the lining epithelium of both inflammatory and developmental cysts is primarily comprised of squamous epithelium.¹ The differences in cell proliferation and the proliferation rate of this odontogenic epithelium play a significant role in numerous biological and pathological events in it.^{1,7} Also, different literatures have shown that the chronic inflammation has a direct influence on these epithelial cells. The growth factors and cytokines of inflammatory infiltrates are also responsible for the higher proliferation ability of the residual epithelium.⁸ Inflammation may also increase the squamous changes in good, healthy DFs. The incidence of malignancy developing in odontogenic cyst is believed to be caused by long chronic inflammation and continuous intracystic pressure.⁹

¹Postgraduate Student, ²Professor and Head, ³Professor
⁴Lecturer

¹⁻⁴Department of Oral Pathology and Microbiology, Mahatma Gandhi Mission's Dental College and Hospital, Navi Mumbai Maharashtra, India

Corresponding Author: Smita V Rathod, Postgraduate Student Department of Oral Pathology and Microbiology, Mahatma Gandhi Mission's Dental College and Hospital, Navi Mumbai, Maharashtra India, Phone: +917710078828, e-mail: smitarathod2012@gmail.com



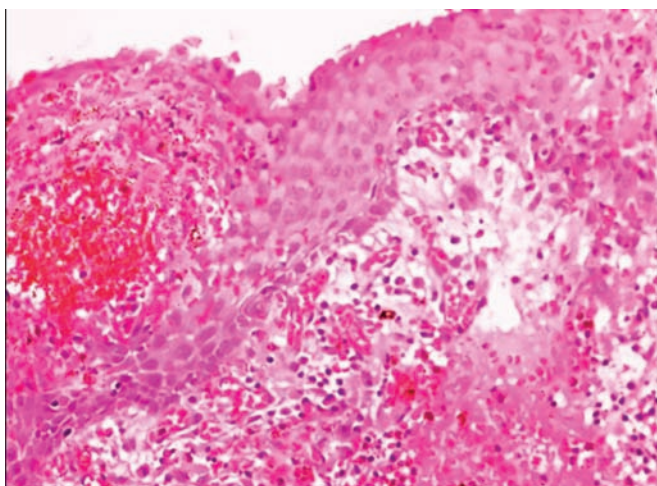


Fig. 1: Photomicrograph of H&E-stained soft tissue section of RC showing nonkeratinized stratified squamous epithelial lining and connective tissue capsule with mild inflammation (H&E, 400 \times). H&E: Hematoxylin and eosin

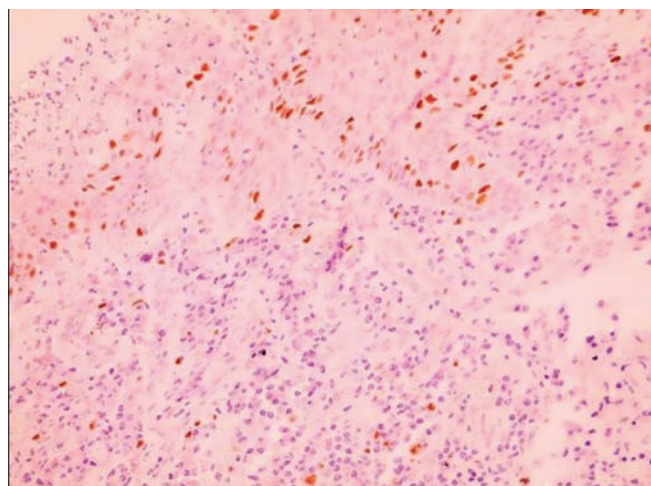


Fig. 2: Photomicrograph of MCM-2-stained soft tissue section of RC showing MCM-2 expression in the epithelial lining (400 \times)

This proliferative potential of epithelial cells can be evaluated by immunohistochemistry analysis using monoclonal antibodies. Several markers can be used to find out cell proliferation which has prognostic value along with the understanding of biological behavior of various cysts and tumors. One such marker is MCM-2.

The MCM family of proteins contains six major forms (MCM 2–7), which have similar molecular structure and biochemical functions. The MCM 2 to 7 proteins are equally important for continuous deoxyribonucleic acid (DNA) replication.^{1,10–14} The MCM-2 protein is necessary for maintaining genomic integrity and also for prevention of re-replication once per cell cycle. The MCMs are generally not detected in quiescent, differentiated, or senescent cells.¹⁰ However, high levels of MCM-2 can be observed in all active phase of cell cycle and also during the cellular proliferation in the healthy, preneoplastic, and neoplastic cells.^{1,2,13} Antibodies which are used next to MCMs recognize more cells in tissues than any other “proliferation” markers and hence, can be used to assess the proliferative potential of the residual epithelium in odontogenic cysts and tumors.

The purpose of our study was to evaluate the MCM-2 expression in lining epithelium of different odontogenic cysts to determine the proliferative ability of epithelial cells and to determine whether there is any association between inflammation and the expression of MCM-2 in odontogenic cysts.

MATERIALS AND METHODS

This retrospective study was performed on 45 formalin-fixed paraffin-embedded tissue blocks of odontogenic cysts, 15 each of RC, DC, and OKC, which were selected from the archives of the Department of Oral Pathology,

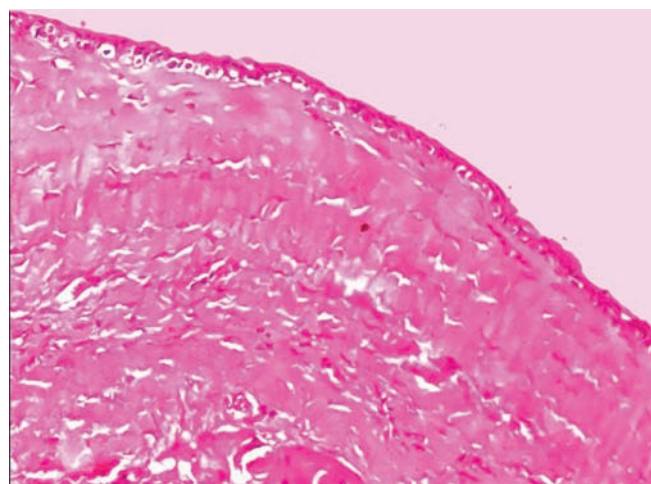


Fig. 3: Photomicrograph of H&E-stained soft tissue section of DC showing nonkeratinized stratified squamous epithelial lining and connective tissue capsule with no inflammation (H&E, 400 \times). H&E: Hematoxylin and eosin

MGM Dental College & Hospital, Navi Mumbai, India. All cases of RC were evaluated for the degree of inflammation which was further graded as mild, moderate, and severe. Epithelial component of odontogenic cysts, i.e., RC, DC, and OKC, was assessed based on the following criteria: Cell thickness, rete peg formation, squamous epithelium, and keratinization. All cases of DC and OKC were evaluated only for an existence or nonexistence of inflammation. The mesenchymal component of cysts, i.e., RC, DC, and OKC, was assessed for an existence or nonexistence of inflammatory infiltrate (Figs 1 to 3). Sections from each case were subjected to MCM-2 nuclear staining (immunohistochemical) procedures. For each of the RC, DC, and OKC specimens, extent and intensity of MCM-2 expression were calculated by a comprehensive scoring formula (Figs 4 to 6). Evaluations of 100 nuclei were done in the lining of each specimen, under 400 \times magnification. Scoring was done for

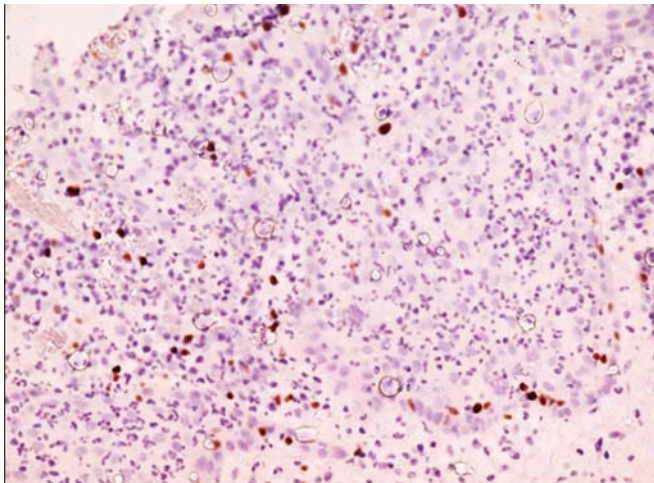


Fig. 4: Photomicrograph of MCM-2-stained soft tissue section of DC showing MCM-2 expression in the epithelial lining (400x)

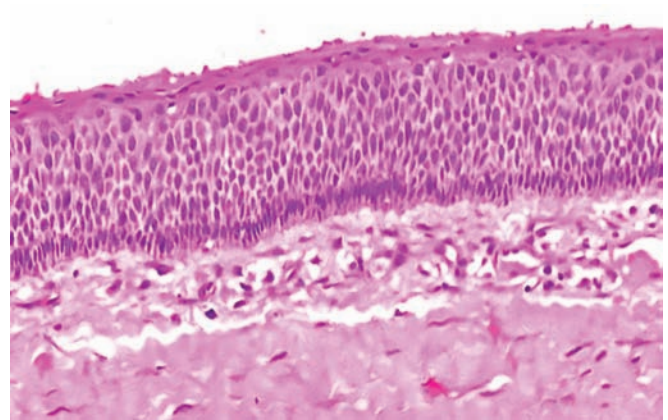


Fig. 5: Photomicrograph of H&E-stained soft tissue section of OKC showing parakeratinized stratified squamous epithelial lining with palisaded basal cell layer and connective tissue capsule without inflammation (hematoxylin and eosin, 400x)

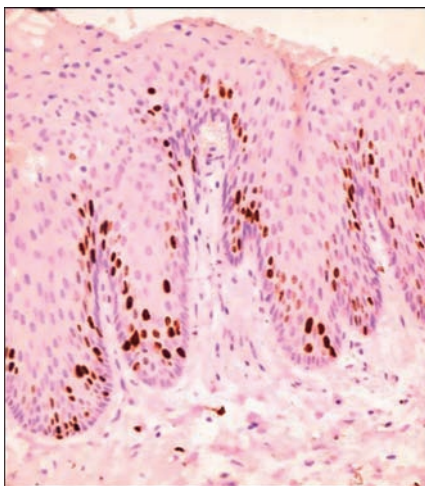
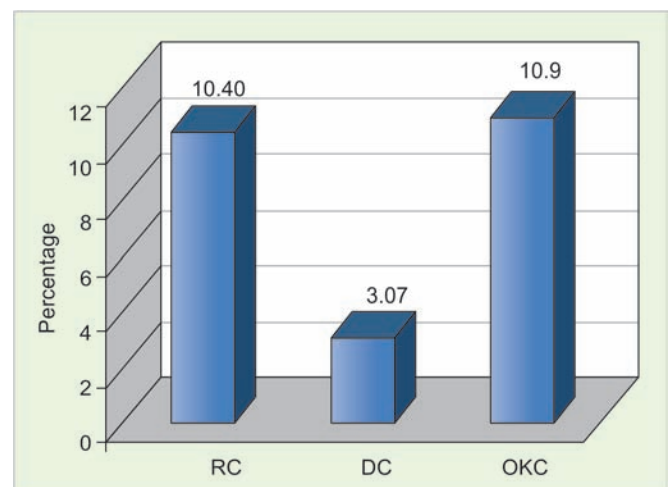


Fig. 6: Photomicrograph of MCM-2-stained soft tissue section of OKC showing MCM-2 expression in the epithelial lining (400x)



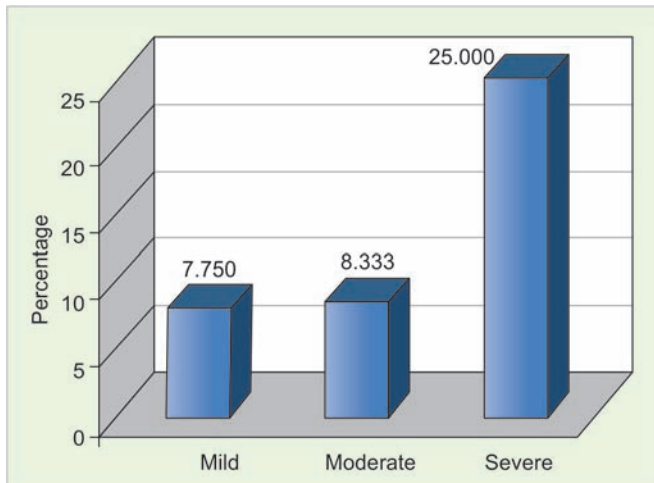
Graph 1: Descriptive statistical analysis of mean (%) MCM-2 immunopositive nuclei in epithelial lining of RC, DC, and OKC

positively stained nuclei and expressed as percentage. The data obtained were presented using descriptive statistics, such as mean, standard error, and standard deviation with appropriate graphs and charts. All collected data were entered into Statistical Package for the Social Sciences version 20.0 worksheet. Further statistical analysis was conducted by using one-way analysis of variance (ANOVA) followed by least significant difference (LSD) *post hoc* analysis and unpaired t-test. A significance level of 0.05 was applied to decide the statistical significance of the hypothesis being tested.

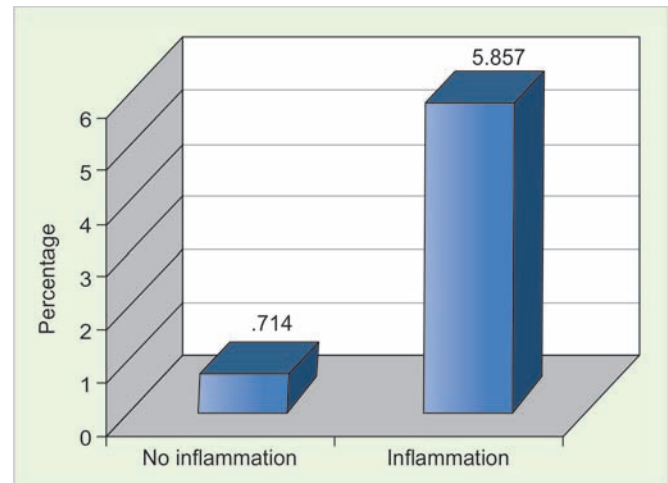
RESULTS

On evaluating MCM-2 (%) expression in the cystic lining of RC, we found that out of 15 cases (n = 15), 1 case (n = 1) showed immunonegativity and the remaining cases of RC (n = 14) showed immunopositivity ranging from 2 to 29%. The mean (%) immunopositivity of MCM-2

in RC (n = 15) was 10.4%. On evaluating MCM-2 (%) expression in the cystic lining of DC, we found that out of 15 cases (n = 15), 7 cases (n = 7) showed immunonegativity, and the remaining cases of DC (n = 8) showed immunopositivity ranging from 1 to 13%. The mean (%) immunopositivity of MCM-2 in DC (n = 15) was 3.07%. On evaluating MCM-2 (%) expression in the cystic lining of OKC, we found that out of 15 cases (n = 15), 7 cases (n = 7) showed immunonegativity, and the remaining cases of OKC (n = 8) showed immunopositivity ranging from 4 to 44%. The mean (%) immunopositivity of MCM-2 in OKC (n = 15) was 10.93% (Graph 1). Statistically, on multiple comparisons of mean (%) MCM-2 immunopositive nuclei in lining epithelium of RC, DC, and OKC by using one-way ANOVA test followed by LSD *post hoc* analysis, we found that there was a significant difference among mean (%) immunopositive nuclei of OKC and DC (p = 0.046).



Graph 2: Descriptive statistical analysis of the mean (%) MCM-2 immunopositive nuclei in RC with different grades of inflammation



Graph 3: Comparative statistical analysis of the mean MCM-2 immunopositive nuclei (%) in epithelial lining of DC with and without inflammation

However, when the mean (%) immunopositive nucleus of RC was compared with DC and OKC, results were found to be not significant; on multiple comparisons of mean (%) MCM-2 immunopositive nuclei with different grades of inflammation in RC ($n = 15$) by using one-way ANOVA test followed by LSD *post hoc* analysis, we found results to be statistically significant among mean (%) immunopositive nuclei of mild and severe degrees of inflammation ($p = 0.012$), and also, it was found between moderate 8.33% and severe degrees of inflammation ($p = 0.008$).

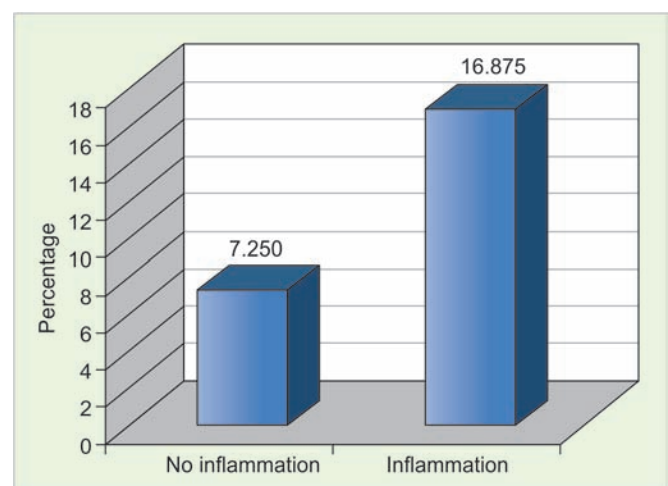
No significant difference was found among mild and moderate degrees of inflammation ($p = 0.887$) (Graph 2). Statistically, on comparison of the mean (%) MCM-2 immunopositive nuclei in lining epithelium of inflamed and noninflamed DC ($n = 14$) by using independent t-test, we found results to be significant between mean (%) MCM-2 expression in cases of DC with and without inflammation ($p = 0.033$) (Graph 3).

Statistically, on comparison of the mean (%) MCM-2 (%) immunopositive nuclei in the lining epithelium of inflamed and noninflamed OKC ($n = 12$) by using independent t-test, we found results to be significant between mean (%) MCM-2 expression in the inflamed and noninflamed cases of OKC ($p = 0.05$) (Graph 4).

DISCUSSION

Variations in the proliferation rates of oral epithelial cells could be the initiating point in the pathology of odontogenic cysts like RC, DC, and OKC.^{2,9,15} This proliferative potential of oral epithelial cells can be evaluated by immunohistochemical analysis using proliferation markers of monoclonal antibodies.

Several markers like Ki-67 and proliferating cell nuclear antigen (PCNA) can be used to assess cell proliferative



Graph 4: Comparative statistical analysis of the mean MCM-2 immunopositive nuclei (%) in OKC with and without inflammation

potential which has prognostic value along with the understanding of biological behavior of various cysts and tumors. One such marker is MCM-2. The MCM proteins were discovered first in yeast; they are a family of proteins with striking sequence homology.¹² Similar classes of proteins have been found in murine, xenopus, and human cells with significant maintenance of gene sequences.^{12,16} Human MCM-2 (BM28) was first recognized as a nuclear protein.¹⁷ The MCM-2 protein is the part of the family of six MCM (2 to 7) proteins with similar molecular structure and biochemical functions and are equally important for continuous DNA replication.¹⁶⁻¹⁸ In our study, on evaluating the mean (%) MCM-2 expression immunohistochemically, in the lining epithelium of RC, DC, and OKC, we observed that there was higher expression in OKC followed by RC and DC. The results were found to be statistically significant between the mean (%) MCM-2 expression of OKC and DC ($p = 0.046$).

However, only one study was performed to detect and evaluate the expression of MCM-2 protein in odontogenic cysts, such as RC, DC, and OKC.¹ Guler et al,¹ on investigating the expression of MCM-2 in the lining epithelium of RC (n = 6), found the mean MCM-2 expression significantly higher in RC ($p < 0.01$) as compared with OKC and DC. This result is inconsistent with our present study in which the mean MCM-2 (%) expression was higher in OKC as compared with RC and DC.

Moreover, rather than MCM-2, few other studies have evaluated variable expression of cell proliferation markers in RC, such as Ki-67 and laminin-1. Ayoub et al¹⁸ studied Ki-67 and laminin expression in RC, and they concluded that long-standing inflammatory reaction could act as stimulators causing epithelial proliferation.

Furuyama et al¹⁹ stated that in the presence of inflammatory cytokines which secrete matrix metalloproteinase (MMP)-9 and MMP-2, the ability of epithelial cells to form continuous basement membrane is lost. Gadbaill et al⁷ in 30 cases of RC noted the Ki-67 positive cells in relation to elongated rete pegs and in increased thickness of epithelial lining, and they stated that the proliferative activity in epithelial lining might be related to the grade of inflammatory reaction. Guler et al¹ investigated an expression of MCM-2 in the lining epithelium of seven cases of DC, and they found mean MCM-2 expression significantly increased in DC ($p = 0.423$) when compared with DF. However, it was significantly less than RC and KCOT. This is in accordance with our study.

Apart from MCM-2 expression, few other studies have assessed the expression of cell proliferation marker in DC, such as Ki-67 and Silver stained nucleolar organizer regions (AgNOR). Gadbaill et al⁷ studied Ki-67 and AgNOR expression immunohistochemically in DC (n = 30), and they observed that Ki-67-positive cells were predominant in basal cell layer and very few in suprabasal cell layers. This suggested that the Ki-67 is related to the maintenance of 2 to 3 cell thickness layer of epithelium.

On the contrary, he observed that AgNOR count was less in DC with respect to suprabasal cell layer and complete epithelium. Kichi et al²⁰ explained that this could be because the suprabasal cells of DC may undergo apoptosis on a relatively rapid basis, which seems to participate in maintaining regular thickness of cystic lining. Guler et al¹ investigated the MCM-2 expression in the lining epithelium of OKC (n = 7) and observed the mean MCM-2 expression to be significantly increased when compared with the DF and DC ($p = 0.586$). This is in accordance with our study where the MCM-2 (%) expression was significantly high in OKC as compared with DC. Moreover, rather than MCM-2, few other studies have assessed the expression of cell proliferation markers in OKC, such as Ki-67, laminin-1, and AgNOR.

Ayoub et al¹⁸ studied Ki-67 and laminin-1 expression in OKC; they observed that all cases of OKC (n = 12) included in their study are immunopositive for Ki-67. He explained that superior expression of Ki-67 is because of aggressive behavior of OKC. Li et al²¹ observed that the Ki-67-positive cell number is high in OKC than in DC and RC. This reveals a higher turnover rate and mitotic count in OKC epithelium.

Moreover, the irregular expression of Ki-67 positive cells within the residual epithelial component reveals the heterogeneity in the growth pattern of OKC epithelium, and also reflects the infiltrative growth in OKC in comparison with the expansive growth in other cysts.¹⁵ In OKC, the immunopositive expression of Ki-67 is expressed most commonly in the suprabasal layer, but in other cysts, it is mostly in the basal layer of epithelium.

The characteristic expression of proliferation marker suggests that the differentiation and maturation course of epithelium in OKC is different from other odontogenic cysts. Thus, the distinctive cell proliferation in OKC may determine its distinct growth and behavior.¹⁵ On evaluating the mean (%) MCM-2 expression in different grades of inflammation of RC (n = 15), we found that in 4 cases (n = 4) with mild inflammation, the mean (%) MCM-2 expression was 7.75%. In 9 cases (n = 9) with moderate degree of inflammation, it was 8.33% and the remaining 2 cases (n = 2) with severe degree of inflammation showed 25% mean MCM-2 expression.

Statistically, on multiple comparisons of mean (%) MCM-2 immunopositive nuclei in different grades of inflammation of RC (n = 15), we found the results to be significant between mean (%) MCM-2 expression in mild 7.75% (SD = 7.3205) and severe 25% (SD = 5.657) degree of inflammation ($p = 0.012$) and also it was found between moderate 8.33% (SD = 6.557) and severe degree of inflammation ($p = 0.008$). Kaplan and Hirshberg²² concluded that the cyst epithelial lining with adjacent inflammation had a localized effect, which induced increase in the expression of the proliferation marker.

Proliferative activity in lining epithelium of RCs is different from those in normal epithelium and that cell turnover rate or cellular kinetics of cyst epithelium might be associated with the inflammatory changes. Ayoub et al¹⁸ concluded that the number of immunopositive cells in the epithelium increased with the grade of inflammation in the connective tissue component.

This could be explained on the assumption that long-standing inflammatory reaction could act as stimulators causing epithelial proliferation. On evaluating the mean (%) MCM-2 expression in DC with and without inflammation, we found that out of 14 cases of DC (n = 14), 7 cases of DC without inflammation

(n = 7) showed the mean (%) MCM-2 expression to be 0.71% (SD = 0.36). In the remaining 7 cases of DC with inflammation (n = 7), the mean (%) MCM-2 expression was 5.85% (SD = 5.58).

Statistically, on comparison of the mean (%) MCM-2 expression in DC (n = 14) with and without inflammation, we observed that there was a significant difference among mean (%) MCM-2 expressions in inflamed and noninflamed cases of DC (p = 0.033). Similarly, on evaluation of the mean (%) MCM-2 expression in OKC with and without inflammation, we found that out of 12 cases of OKC (n = 12), 4 cases of OKC without inflammation (n = 4) showed the mean (%) MCM-2 expression to be 7.25% (SD = 6.397).

In the remaining 8 cases of OKC with inflammation (n = 8), the mean (%) MCM-2 expression was 16.87% (SD = 8.765). Statistically, on comparison of the mean (%) MCM-2 expression in OKC (n = 12) with and without inflammation, results were to be found statistically significant among mean (%) MCM-2 expression in inflamed and noninflamed cases of OKC (p = 0.05).

The biologic behavior of an OKC has an aggressive nature and a tendency to recur following conservative surgical treatment. Most studies attribute this behavior to its epithelial lining. When evaluated to other cysts, the lining epithelium of OKC has an increased growth potential, which is expressed by a higher proliferation and mitotic index.^{9,23}

In the presence of inflammation, the morphologic alterations in the lining epithelium of OKC may also be associated with changes in the proliferative potential, thus affecting its biological behavior. de Paula et al⁹ found the total numbers of cells, as well as the PCNA and Ki-67 positive cell count, to be notably high in inflamed OKC compared with noninflamed OKC.

It is suggested that the inflammatory infiltrate present in the fibrous tissue capsule of OKC, which releases growth factors and cytokines, may be responsible for the greater proliferation ability in inflamed lesions compared with noninflamed lesions.

CONCLUSION

The results of our study showed that MCM-2 expression in the lining epithelium of inflamed cases of OKC is more.

The OKC is a clinicopathologically different form of odontogenic cyst that is noted for its high recurrence rate and aggressive behavior. The RC is the inflammatory jaw cyst associated with necrotic pulps in the infected teeth. It is possible that inflammation may not only alter the morphology of epithelium but also the proliferative potential of the lining epithelium. The incidence of malignancy developing in odontogenic cyst is believed

to be caused by long-standing inflammation, continuous intracystic pressure, and keratinization of cystic epithelium. Thus, further studies should be carried out on cyst showing inflammation to ascertain the malignant transformation.

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