

Proliferating Cell Nuclear Antigen and KI-67 Expression in Solid/Multicystic Ameloblastoma and Unicystic Ameloblastoma

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Abstract

Ameloblastoma is slow growing, locally invasive, epithelial odontogenic tumour of the jaws with high recurrence rate and quite common in Myanmar population. The proliferative activity of ameloblastoma is important to predict its biologic behaviour. The purpose of the study was to determine the proliferative activity of ameloblastoma in both SMA (Solid/ multicystic ameloblastoma) and UA (Unicystic ameloblastoma). Forty paraffin embedded tissue specimens from both SMA and UA were immunohistochemically performed by using monoclonal anti-PCNA (1:1000) and monoclonal mouse anti-human Ki-67 antigen (1:100). Immunoreactivity was evaluated by mean labeling index (LI). The highest mean LI of PCNA was presented in plexiform type of SMA ($75.50 \pm 4.10\%$) and in luminal type of UA ($73.35 \pm 21.89\%$). PCNA expression was significantly higher in peripheral ameloblast like cells in both SMA and UA ($p=0.000$). Mean LI of PCNA in SMA ($74.63 \pm 5.55\%$) was significant higher than UA ($67.30 \pm 14.25\%$) ($p=0.034$). The highest mean LI of Ki-67 was presented in granular type of SMA ($14.59 \pm 5.51\%$) and in luminal type of UA ($11.24 \pm 4.42\%$). Ki-67 expression could not exhibit remarkable differences between peripheral and central area of SMA ($p=0.674$) and also between basal and luminal portion of UA ($p=0.946$). No significant difference of mean LI of Ki-67 was found between SMA and UA ($p=0.289$). When compare

the mean LI of PCNA and Ki-67 between SMA and UA, PCNA was significantly higher than Ki-67 ($p=0.000$). Based upon the results, SMA demonstrated more aggressive biologic behaviour than UA and PCNA was found to be the reliable immunohistochemical marker than Ki-67 for evaluating cell proliferation in ameloblastic tumours.

INTRODUCTION

Odontogenic tumours are lesions derived from epithelial, ectomesenchymal, and/or mesenchymal elements that are or have been part of the tooth-forming apparatus. These tumours are found exclusively within the jawbones or in the soft mucosal tissue overlying tooth-bearing areas (Philipsen et al., 2005).

Ameloblastoma is the most frequently encountered odontogenic neoplasm of the jaws with a strong predilection for the posterior region of the mandible. Accounts for approximately half (48.9%) of the odontogenic tumours with female to male and maxilla to mandible ratios of 1:1.7 and 1:8 respectively (Laxmidevi et al., 2010).

According to the 2005 histological classification of tumours by the WHO, ameloblastomas are categorized into four distinct clinico-pathological variants such as solid/multicystic ameloblastoma (SMA), unicystic ameloblastoma (UA), extraosseous/peripheral ameloblastoma and desmoplastic ameloblastoma.

The two most common subtypes are SMA and UA.

These two common types have different recurrence rates after surgical treatment. Moreover, the UA differs in biologic behaviour from the SMA, with a considerably lower rate of recurrence. Even within the UA group, difference in biological behaviour and prognosis apparently exists, with the intramural lesion, which is characterized by intramural proliferation of neoplastic tissue, being more aggressive than their simple unicystic form or the intraluminal unicystic ameloblastoma. Based on these differences, it has been suggested that solid and multicystic ameloblastomas and intramural unicystic ameloblastomas should be treated by resection, whereas the simple and intraluminal unicystic forms of this tumour can be enucleated (Meer et al., 2003).

On the other hand, the assessment of cell proliferation activity in tumours has become a common tool used by histopathologists to provide useful information for assessing diagnosis, used as indicator of aggressiveness and prognostic information. Proliferation markers refers to specific proteins or other factors whose presence in actively growing and dividing cells serves as an indicator for such cells. Today, the most common method for determining proliferative activity is the use of immunohistochemical techniques, which are increasingly being applied in routine pathology (Bologna-Molina et al., 2013).

Currently, new markers are being added to evaluate cell proliferation. However, PCNA is still used as a first-choice marker of cell proliferation. Furthermore, many investigations of tumour cell proliferative activity have used PCNA and Ki-67 to evaluate cell proliferation in oral tumours (Salehinejad et al., 2011).

In Myanmar, although ameloblastoma is common odontogenic tumour there were limited studies and the studies were mainly based on clinical interest and not reflecting the biological behaviour. Therefore, the purpose of

this study is to predicts its biologic behaviour, as suggestion for more aggressive treatment when recurrence occur, as well as for regular follow up and prognostic value by using both monoclonal anti-proliferating cell nuclear antigen (PCNA) antibody and monoclonal anti Ki-67 antibody on SMA and UA ameloblastoma.

Proliferating Cell Nuclear Antigen (PCNA)

Proliferating cell nuclear antigen (PCNA) is a 36 KD protein identified as an auxiliary protein of DNA polymerase delta. Its distribution in the cell cycle, increasing through G1, peaking at the G1/S-phase interface, decreasing through G2, and reaching low levels which are virtually undetectable by immunocytochemical methods in M-phase and quiescent cells, makes it a useful marker for proliferating cells (Figure 1) (Coltrera and Gown et al., 1991).

The half-life of PCNA exceeds 20 hours and PCNA expression may be used as a marker of cell proliferation because cells remain a longer time in the G1/S phase when proliferating. Furthermore, this protein has an essential role in nucleic acid metabolism as a component of the DNA replication and repair mechanism (Oliveria et al., 2007).

Ki-67

The Ki-67 antigen (Ki-67) is a classic marker of cellular proliferation that has been widely applied in the diagnostic, research. Over 10 years later, immunostaining with antibodies to the Ki-67 antigen is well established as a quick and efficient method for evaluating growth fractions of various tumour types because of its distinctive reaction patterns that exclusively involve proliferating cells. The large number of citations evidences the extensive use of Ki-67 as a marker of proliferation in pathology, which is increasing year on year (Ross and Hall, 1995).

The Ki-67 antigen is preferentially expressed during the late G1, S, G2 and M phase of the cell cycle, whereas resting, non-cycling cells (G0 phase) lack Ki-67 expression. Because of its

absence in quiescent cells (G0 phase), this protein developed into a widely used tumour marker in the fields of research and pathology. The Ki-67 protein has been shown to have an extremely short half-life of around 20 minutes throughout the cycle, with major catabolism occurring at the end of mitosis, indicating that few cells that have left the cycle will contain the antigen. The standard antibody for the detection of Ki-67 is MIB-1. The fraction of MIB-1-positive tumour cells (the MIB-1/Ki-67 labeling index) is often correlated with the clinical course of cancer; Ki-67 is of prognostic value for many types of malignant tumours (Thmann et al., 2004).

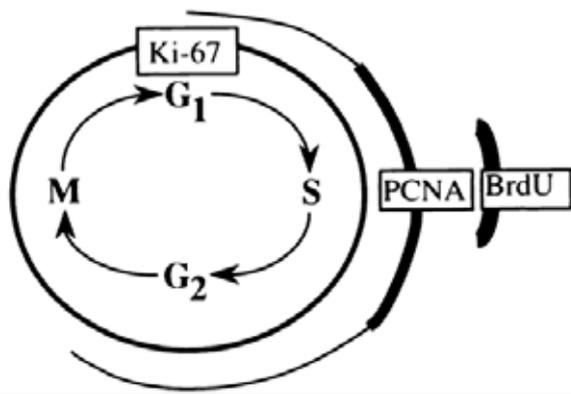


Figure 1. Theoretical cell cycle distributions for immuno-cytochemical staining of the three proliferation markers. The thickness of the line for PCNA staining refers to the peak staining observed in cells. (adapted from Coltrera and Gownet al., 1991)

This study was aimed to study the proliferative activity of ameloblastoma by using proliferating cell nuclear antigen (PCNA) and Ki-67 in Myanmar patients

MATERIALS AND METHODS

Materials

Study Population

(40) Cases of histopathologically proven ameloblastoma

Primary Antibodies

1. Anti-proliferating cell nuclear antigen (PCNA)

Code No-DAKO M 0879

1:1000 dilutions (DAKO, USA)

2. Monoclonal mouse anti-human Ki-67 antigen, Clone MIB-1

Code M 7240

Lot-00004924

1: 100 dilutions (DAKO, USA)

Secondary Antibody

Dako REALTM EnVisionTM/HRP, Rabbit/Mouse (ENV)

Dextran coupled with peroxidase molecules and goat secondary antibody molecules against rabbit and mouse immunoglobulins.

Methods

Type and place of Study

Hospital and Laboratory based analytical study was conducted at:

- (1) Department of Oral Medicine, University of Dental Medicine, Yangon
- (2) Department of Oral and Maxillofacial Surgery, University of Dental Medicine, Yangon
- (3) Department of Oral Medicine, University of Dental Medicine, Mandalay
- (4) Department of Plastic and Maxillofacial Surgery, Yangon General Hospital, Yangon
- (5) Research Laboratory of School of Postgraduate Studies and Research of International Medical University, Kuala Lumpur, Malaysia

Selection of Cases

Histologically proven biopsy specimen of SMA and UA ameloblastoma cases were selected starting from January 2010 to December 2012. Previously treated and recurrent cases of SMA and UA ameloblastoma, cases of SMA and UA ameloblastoma if the patient did not agree to

involved in this study, cases of desmoplastic ameloblastoma and cases of peripheral ameloblastoma were excluded.

RESULTS AND DISCUSSION

Table 1. Distribution of PCNA labeling index in different Types of SMA

| Type of SMA | Number of cases | PCNA labeling index | |
|-------------|-----------------|---------------------|-------------|
| | | Mean% LI±SD | Range % |
| Follicular | 6 | 74.73±5.61 | 65.50-80.89 |
| Plexiform | 7 | 75.50± 4.10 | 67.96-79.94 |
| Mixed | 5 | 75.35± 8.10 | 65.81-83.52 |
| Granular | 2 | 69.48±2.09 | 68-70.96 |

The highest mean labeling index in plexiform type and the lowest labeling index in granular type of SMA

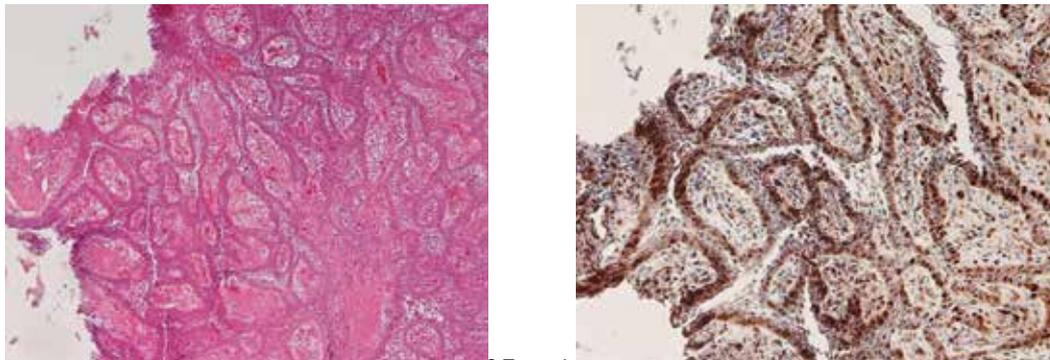


Figure 2. Plexiform type of SMA (A) H&E staining × 200 (B) immunohistochemical expression of PCNA × 200

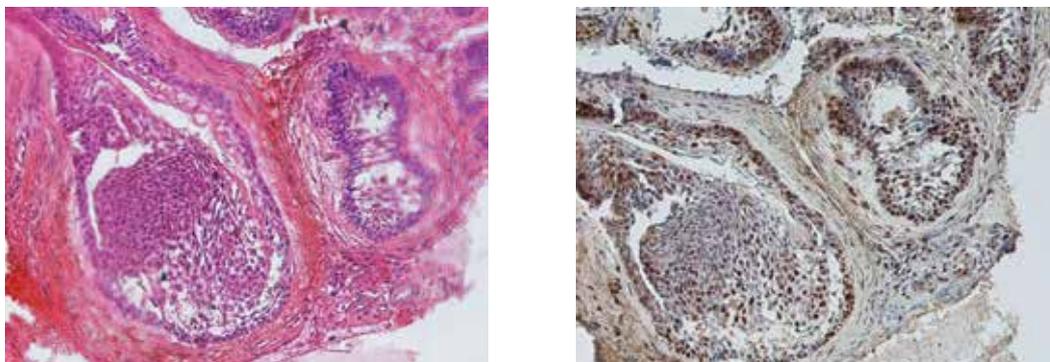


Figure 3. Granular type of SMA (A) H&E staining × 200 (B) Immunohistochemical expression of PCNA × 200

Expression of Proliferating Cell Nuclear Antigen (PCNA) in SMA and UA

In the present study, the plexiform type of ameloblastoma presented the strongest PCNA labeling index, while the granular type of SMA showed the weakest labeling index. Table 1, Figure 2(B), 3(B)

The results were compatible with the studies of Hirayama et al. (2004). Maya et al., (2012). Proliferative activities between the different histologic types of the SMA were not statistically significant. These findings were consistent with Broboza et al. (2005), Salehinejad et al. (2011), Bologna-Molina et al. (2013), who did not find the significant differences of proliferative activity among the difference histological types of SMA.

Table2. PCNA expression in peripheral cell layer and central portion of SMA

| Cytological pattern | Number of cases | PCNA labeling index | |
|---------------------|-----------------|---------------------|-------------|
| | | Mean% LI±SD | Range % |
| Peripheral cell | 20 | 82.978±5.59 | 78.95-91.48 |
| Central cell | 20 | 67.41±6.50 | 59.80-77.32 |

PCNA expression is significantly increased in peripheral cells layer of SMA
Paired sample t test (p=0.000)

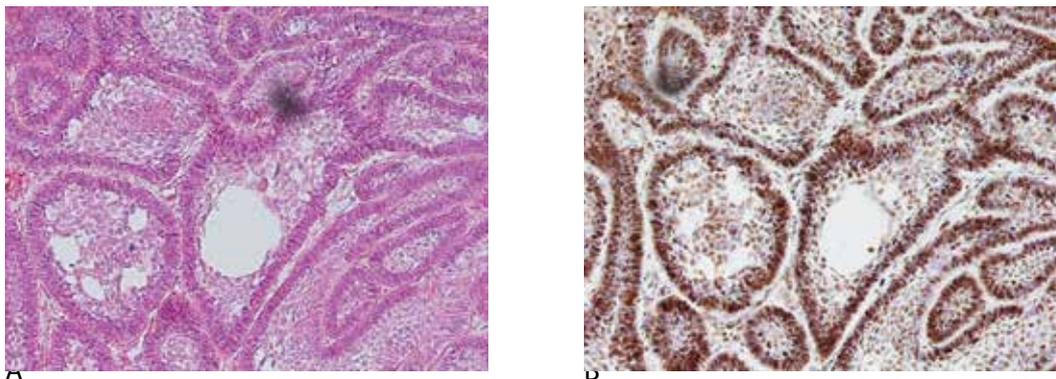


Figure 4. Mixed type of SMA showing follicular as well as anastomosing strands

(A) H&E staining × 200 (B) Immunohistochemical expression of PCNA × 200

The peripheral epithelial cells of the nests of the SMA showed strong PCNA labeling index than that of central cells. (Table 2, Figure 4.B) These results were also in accordance with the studies of Miyake et al. (2004), Ahmed and El-Azab (2008), Salehinejad et al. (2011) and Maya et al. (2012), they verified the higher PCNA labeling index were seen in the basal and suprabasal layers with the stellate reticulum area showing less number of positive cells. This result may be interpreted that the cellular proliferation and consequently the ameloblastoma growth are concentrated in the peripheral areas composed by ameloblast like cells.

Table 3. Distribution of PCNA labeling index in different types of UA

| Type of UA | Number of cases | PCNA labeling index | |
|------------------------------------|-----------------|---------------------|--------------|
| | | Mean% LI±SD | Range % |
| Luminal | 3 | 73.35±21.89 | 52.91- 96.45 |
| Luminal & intraluminal | 2 | 70.99±13.36 | 61.54-80.44 |
| Luminal, intraluminal & intramural | 3 | 57.21±14.58 | 47.94-74.02 |
| Luminal & intramural | 12 | 67.69±12.97 | 46.20-80.71 |

The highest mean labeling index in luminal unicystic ameloblastoma and lowest labeling index in luminal, intraluminal & intramural type of UA

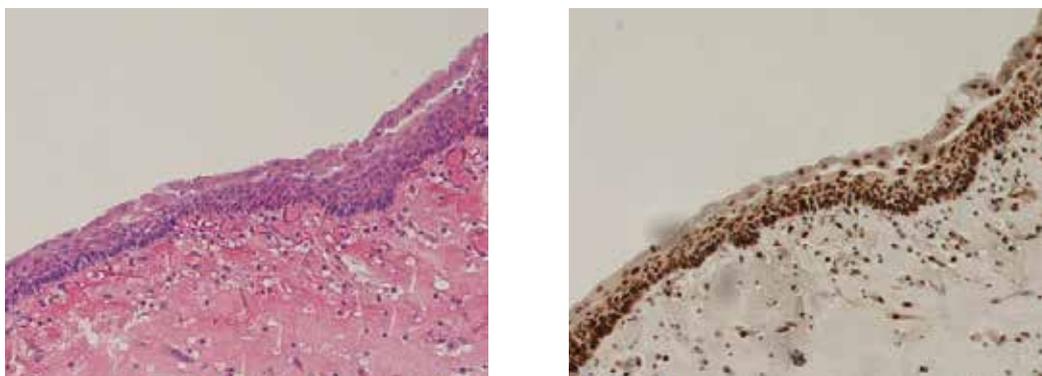


Figure 5. Luminal type of UA (A) H&E staining × 200 (B) Immunohistochemical expression of PCNA × 200

As for Unicystic Ameloblastoma (UA), differences in mean labeling indices for PCNA was observed among the various subtypes. The highest mean labeling index was detected in luminal unicystic ameloblastoma proper and the lowest in combination of luminal, intraluminal and intramural type (Table 3, Figure 5, B)

Proliferative activities between the different histologic types of the UA were not statistically significant. This finding is similar to the findings of Bologna-Molina et al. (2013) who showed that the luminal unicystic ameloblastoma had higher PCNA labeling index than the intraluminal unicystic ameloblastomas and mural unicystic ameloblastoma but there were no statically difference among them.

Kim and Yook (1994) found that the area of plexiform intraluminal growth showed the highest proliferating activity among the unicystic variants. Meer et al. (2003) reported that the highest mean labeling index in the intraluminal and intramural unicystic ameloblastoma types within the unicystic group. However, because the number of cases of each subtype was limited, we would be reluctant to draw any definite conclusions from current data.

Table 4. PCNA labeling index in basal cell layer and luminal portion of UA

| Cytological pattern | Number of cases | PCNA labeling index | |
|---------------------|-----------------|---------------------|---------|
| | | Mean% LI±SD | Range % |
| Basal cell | 20 | 72.56±14.78 | |
| Luminal cell | 20 | 62.20±15.03 | |

PCNA expression is significantly increased in basal cells layer of UA
Paired sample t test (p=0.000)

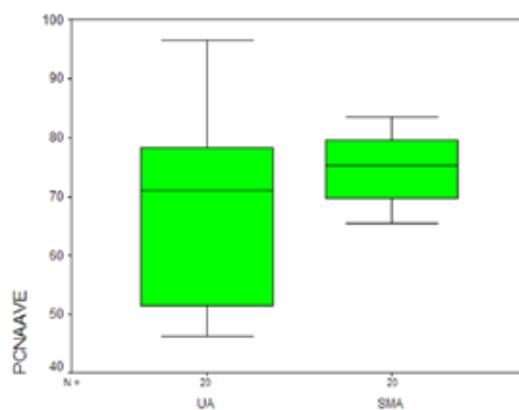


Figure 6. Comparison of PCNA labeling index between SMA and UA

The mean PCNA labeling index of basal cells were significantly higher proliferating index than that of luminal cells (Table 4). This result may be interpreted that the cellular proliferation and consequently the ameloblastoma growth in UA are concentrated in the basal cells layer.

Table 5. PCNA labeling index in SMA and UA

| Type of ameloblastoma | Number of cases | PCNA labeling index | |
|-----------------------|-----------------|---------------------|-------------|
| | | Mean% LI±SD | Range (%r) |
| SMA | 20 | 74.63± 5.55 | 65.50-83.52 |
| UA | 20 | 67.30±14.25 | 46.20-96.45 |

Higher PCNA labeling indices in SMA compared to the UA
Independent sample t test (p = 0.034)

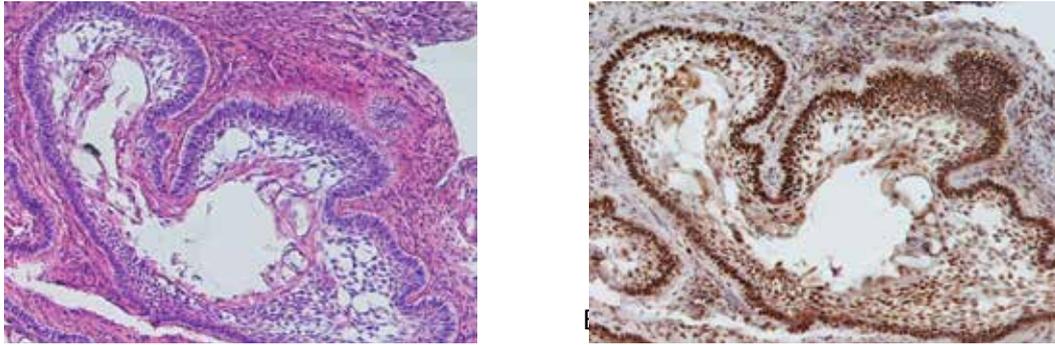


Figure 7. Mixed type of SMA focusing on follicular pattern

(A) H&E staining $\times 200$ (B) Immunohistochemical expression of PCNA $\times 200$

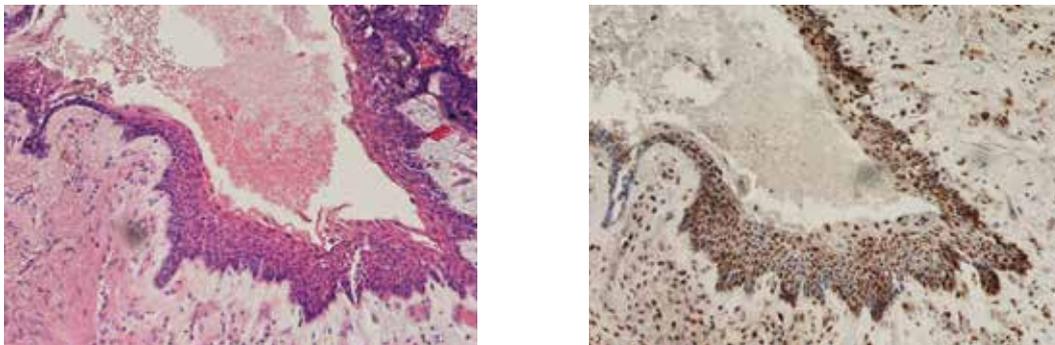


Figure 8. Luminal and intramural type of UA focusing of luminal area

(A) H&E staining $\times 200$, (B) Immunohistochemical expression of PCNA $\times 200$

In this study, labeling index of PCNA ranged from 65.50% to 83.52% in SMA and from 46.20% to 96.45% in UA (Table 5). When the mean labeling indices of the solid multicystic ameloblastoma (mean = 74.63 %) and unicystic groups (mean = 67.30 %) were compared, a statistically significant difference was obtained ($p=0.034$), with the higher PCNA labeling indices in the solid and multicystic ameloblastomas compared to the UA respectively. (Figure 6, Figure 7.B) and Figure 8.B)

Our results were similar to results of Li et al. (1995) who showed that the solid ameloblastomas exhibited higher mean PCNA labeling index than all areas of unicystic ameloblastomas. It was also supported by results of Sandra et al. (2001), unicystic ameloblastoma showed lower values than plexiform and follicular ameloblastomas. Again, Maya et al. (2012) reported that the cystic type showed a low positive PCNA labeling index. By comparing SMA and UA, higher PCNA positivity may indicate higher cellular proliferation rate, which would explain the more aggressive nature of the SMA compared to the UA.

Table 6. Distribution of Ki-67 Labeling index in different types of SMA

| Type of SMA | Number of cases | Ki-67 labeling index | |
|-------------|-----------------|----------------------|-------------|
| | | Mean% LI±SD | Range % |
| Follicular | 6 | 12.74±6.50 | 3.27-20.62 |
| Plexiform | 7 | 10.81±6.61 | 4.49-22.97 |
| Mixed | 5 | 11.22±6.12 | 5.72-19.71 |
| Granular | 2 | 14.59±5.51 | 10.69-18.49 |

Mean labeling indices of granular type was highest and lowest in plexiform type of SMA

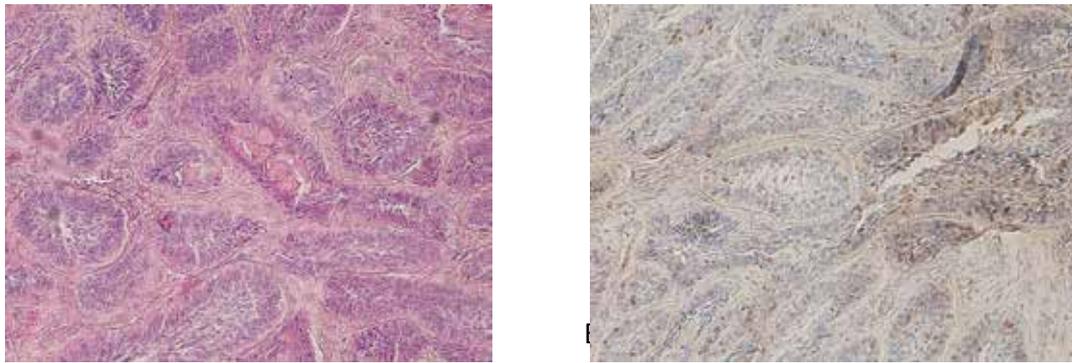


Figure 9. Granular type of SMA (A) H&E staining × 200 (B) Immunohistochemical expression of Ki-67 × 200

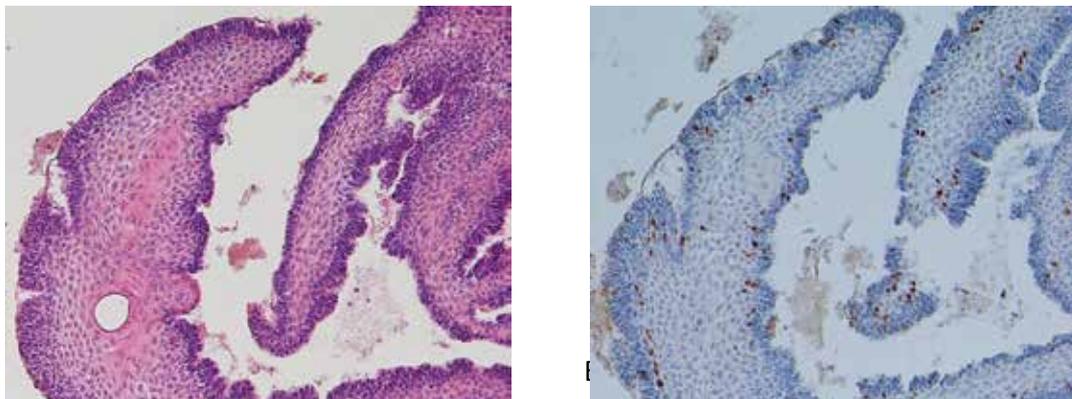


Figure 10. Plexiform type of SMA (A) H&E staining × 200 (B) Immunohistochemical expression of Ki-67 × 200

Expression of Ki-67 Antigen in SMA and UA

Among the different types of SMA, mean labeling index of follicular type was higher than plexiform and mixed type in present study (Table 6). Proliferative activities between the different histologic types of the SMA were not statistically significant. The granular type of ameloblastoma presented the strongest Ki-67 labeling index while the plexiform type of SMA showed the weakest labeling index. (Figure 9. B, Figure 13. B)

Sandra et al. (2001) revealed that Ki-67 labeling indices of follicular ameloblastoma were higher than

that of plexiform ameloblastoma as suggested by previous researchers (Nakamura et al., 1994 and Li et al., 1995). The findings of Hirayama et al. (2004); and Piattelli et al. (1998) are not similar to above studies, as well as the present study. In the study of Koizumi et al. (2004); no statistically significant difference was shown in Ki-67 labeling index between follicular and plexiform patterns of ameloblastoma.

Table 7. Ki-67 labeling index in peripheral cell layer and central portion of SMA

| Cytological pattern | Number of cases | Ki-67 labeling index | |
|---------------------|-----------------|----------------------|------------|
| | | Mean% LI±SD | Range % |
| Peripheral cell | 20 | 12.12±6.88 | 3.53-24.16 |
| Central cell | 20 | 11.79±5.54 | 2.18-23.62 |

Higher expression of Ki-67 expression observed in peripheral ameloblast like cells than central area of SMA

Paired sample t test(p = 0.674)

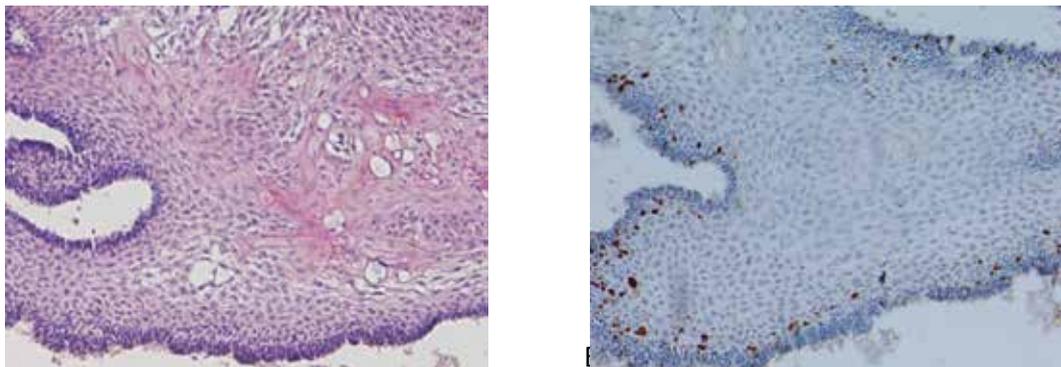


Figure 11. Follicular type of SMA (A) H&E staining $\times 200$ (B) Immunohistochemical expression of Ki-67 $\times 200$

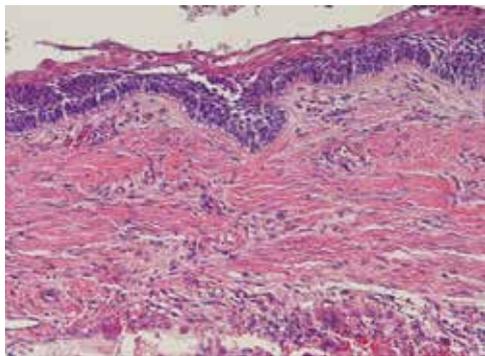
In this study, there was higher expression of Ki-67 expression in peripheral ameloblast like cells than in stellate reticulum like cells located in central area of SMA but no significant difference was found. (Table 7, Figure 11.B) Similarly, Bologna-Molina et al. (2009) and Gomes et al. (2010) showed that cellular proliferation index of Ki-67 positive nuclei are mainly located in peripheral ameloblast like cells in the follicular as well as in the plexiform areas of the solid ameloblastoma. Otero et al. (2012) also showed that there was significantly higher expression of Ki-67 in peripheral ameloblast like cells than in stellate reticulum like cells. Stellate reticulum like cells in ameloblastoma and in stellate reticulum cells in developing teeth tend to be negative to this marker.

Thus, it was suggested that the growth of ameloblastomas is produced by peripheral expression of the follicles (Ong' uti et al., 1997; Gomes et al., 2010). In this study, although Ki-67 staining was higher in the basal cells than the central stellate reticulum-like cells, due to the paucity of these neoplasms, no significant differences were found.

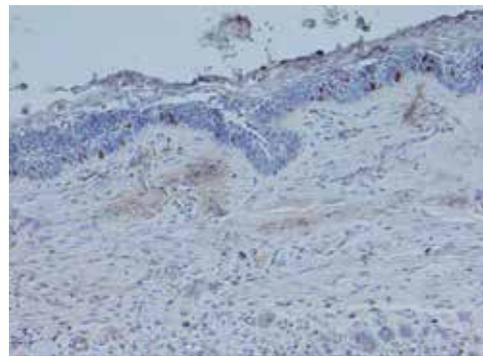
Table 8. Distribution of Ki-67 labeling index in different types of UA

| Type of UA | Number of cases | Ki-67 labeling index | |
|-----------------------------------|-----------------|----------------------|------------|
| | | Mean% LI±SD | Range % |
| Luminal | 3 | 11.24±4.42 | 6.13-13.93 |
| Luminal & intraluminal | 2 | 7.00±2.45 | 5.27-8.74 |
| Luminal, intraluminal & intramual | 3 | 10.17±1.55 | 8.77-11.85 |
| Luminal & intramual | 12 | 10.34±4.79 | 4.84-22.12 |

The highest value was found in luminal type and lowest in luminal & intraluminal type of UA



A



B

Figure 12. Luminal type of UA (A) H&E staining × 200 (B) Immunohistochemical expression of Ki-67 × 200

When the various subtypes of UA were considered, the highest value was found in the luminal type of UA (11.24 %). Intramural type is the second highest labeling indices. The lowest indices were found in luminal and intraluminal type. (Table 8, Figure 12.B)

Proliferative activities between the different histologic types of the UA were not statistically significant. This finding was also similar to Bologna-Molina et al. (2008) and could be explained by the fact that luminal type of UA contains less stellate reticulum like cells, as compared to the other subtypes of UA and SMA.

Table 9. Ki-67 labeling index in basal cell layer and luminal portion of UA

| Cytological pattern | Number of cases | Ki-67 labeling index | |
|---------------------|-----------------|----------------------|---------|
| | | Mean% LI±SD | Range % |
| Basal cell | 20 | 10.26±5.58 | |
| Luminal cell | 20 | 10.35±4.40 | |

Mean labeling index of the immunostaining in basal cell layer and that of luminal area in UA Paired t test ($p = 0.946$).

No significant difference were found in the distribution of Ki-67 immuno expression between basal cells and luminal cells of UA (Table 9)

Table 10. Ki-67 labeling index in SMA and UA

| Type of ameloblastoma | Number of cases | Ki-67 labeling index | |
|-----------------------|-----------------|----------------------|------------|
| | | Mean % LI±SD | Range % |
| SMA | 20 | 11.87±5.99 | 3.27-22.96 |
| UA | 20 | 10.12±4.16 | 4.84-22.12 |

Higher Ki-67 expression observed in SMA than UA
Independent sample t test ($p= 0.289$).

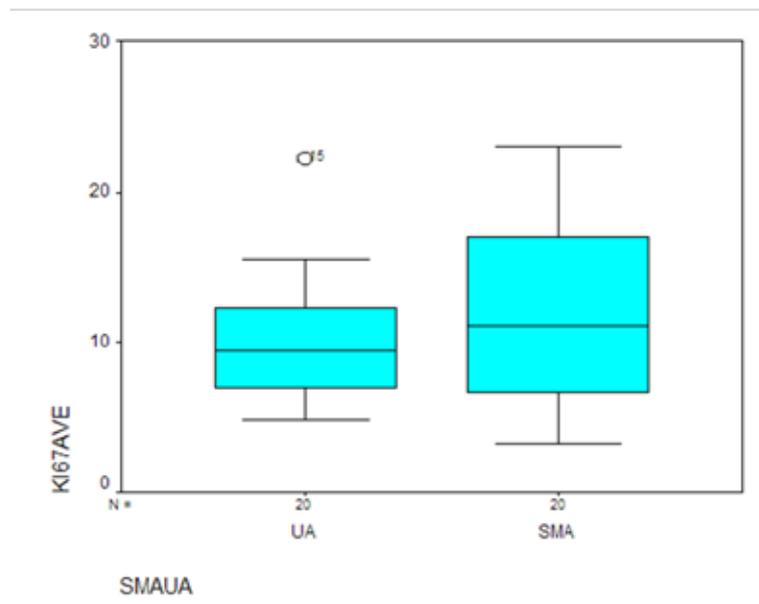


Figure 13. Comparison of Ki-67 labeling index between SMA and UA

In the present study, Higher Ki-67 expression observed in SMA than UA but not significant. (Table 10, Figure.13) Bologna-Molina et al. (2008,2013) and Nafarzadeh et al. (2013) studied on Ki-67 expression in ameloblastoma, and demonstrated that higher Ki-67 expression observed in SMA than UA but there is no significant difference. The possible reason might be the difference in the morphology of the tumours, with the solid lesions providing large follicles or plexiform sheets for analysis, whereas only a thin lining is available in the unicystic cases.

Meer et al. (2003) stated that the expression of Ki-67 markers has been studied in ameloblastoma and the expression level of this marker was significantly higher in UA compared with SMA. 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. The reason for the variable results regarding differences in mean labeling indices of Ki-67 between unicystic and solid lesions is still unclear. A possible explanation might be the difference in methodology, especially the counting protocol. The published reports were frequently failed to provide sufficient detail or explanation.

Table 11. Comparison of expression of PCNA and Ki-67 in SMA and UA

| Histological type | PCNA (%) Median (IQR) | Ki-67 (%) Median (IQR) | z statistics | P value |
|-------------------|-----------------------|------------------------|--------------|---------|
| SMA | 75.36 (9.84) | 11.03(10.32) | 3.92 | 0.0001 |
| UA | 70.92 (26.80) | 9.40 (5.24) | 3.92 | 0.0001 |

PCNA expression is significantly higher than Ki67 expression
Wilcoxon signed-rank test (p= 0.0001)

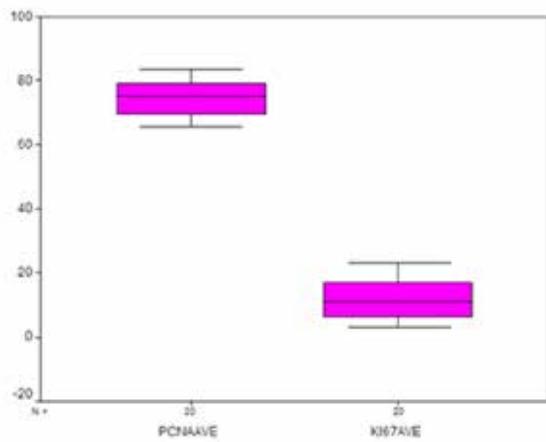


Figure 14. Comparison of PCNA labeling index and Ki-67 labeling index in SMA (higher PCNA labeling index)

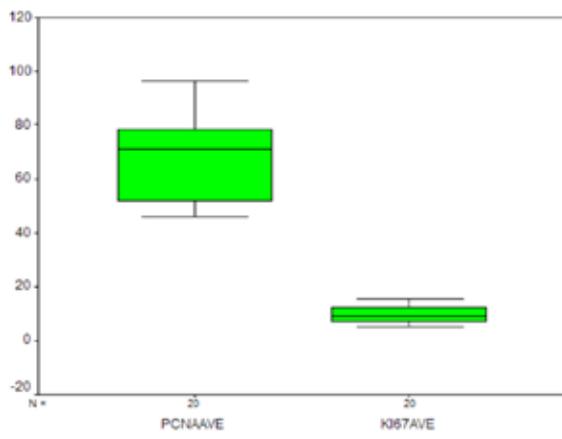


Figure 15. Comparison of PCNA labeling index and Ki-67 labeling index in UA (higher PCNA labeling index)

Comparison of the Value of PCNA and Ki-67 as Markers of Cell Proliferation in Ameloblastoma

Median labeling index of PCNA is significantly higher than Ki-67 in both SMA and UA (Table 11). The results were in line with the published study by Bologna-Molina et al. (2013) as the percentages were higher for PCNA in all types of ameloblastomas.

This higher PCNA positivity in the nuclei of the ameloblastic tumour cells can be explained by the following factors:

PCNA is an essential molecule for the synthesis of DNA. It expressed in non-proliferating cells undergoing DNA repair. This protein has half-life of at least 20 hours within the tissues. This finding could indicate that nuclei can continue to express PCNA even after completing the cell cycle. The lower the dilution of antibody, the higher is the percentage of PCNA expressed in the nucleus. (Bologna-Molina et al., 2013). Therefore, all of the points mentioned above could explain the increased positivity of PCNA compared with other markers of cellular proliferation, such as Ki-67. Ki-67 has significantly lower cellular proliferation index than PCNA in the nuclei of the ameloblastic tumour cells.

This difference can be explained by the following factors:

The expression of the human Ki-67 protein is strictly associated with cell proliferation. The fact that the Ki-67 protein is present during all active phases of the cell cycle (G1, S, G2, and mitosis) but absent from resting cells (G0) makes

it an specific cell proliferation marker for determining the growth fraction of a given cell population (Schalzen and Gerdes., 1996). In addition, the Ki-67 protein has been shown to have an extremely short half-life of around 20 minutes throughout the cycle, with major catabolism occurring at the end of mitosis, indicating that few cells that have left the cycle will contain the antigen. (Ross and Hall., 1995) Since the procedures in immuno-histochemical studies affect the results, incomparable results of the present and the previous studies are probably due to different experimental conditions such as appropriate pH for citrate buffer, duration of slice exposure to hydrogen peroxide solution, hydrogen peroxide concentration and the type of antibody used.

CONCLUSION AND RECOMMENDATION

Present study indicated that the PCNA labeling indices were significantly higher in the peripheral cells of tumour nests and strands than in the central cells of the SMA as well as higher in basal cells than luminal portion of UA. Therefore, the cellular proliferation and consequently the ameloblastoma growth are concentrated in the peripheral areas composed by ameloblast like cells.

PCNA labeling indices was significantly higher in the SMA compared to the UA. Higher PCNA positivity indicate higher cellular proliferation rate, which would explain the more aggressive nature of the SMA. Therefore, adequate surgical management should need to consider for SMA.

When compared between PCNA and Ki-67 labeling indices, PCNA labeling indices was significantly higher in both SMA and UA than Ki-67. Moreover, no significant difference in Ki-67 expression was found in peripheral and central portion of SMA and basal cell layer and luminal portion of UA. Therefore, PCNA was found to be more liable immunohistochemical marker for evaluating cell proliferation in ameloblastic tumours.

Because of limited numbers of cases of each

subtype of both SMA and UA, proliferative activity of PCNA and Ki-67 were shown to be not statistically significant. Therefore, further studies with large sample sizes with equal subtype are required to draw definite conclusion on the proliferative activity not only among the different types of benign cases but also malignant ameloblastomas.

Further tumour behaviour studies on ameloblastoma using various types of markers such as MMPs, TIMP-2, Heparanase and CD147 for ECM degradation, expression of CD138, Cadherins for cell adhesion, WNT5A and Podoplanin for cell migration, Cyclin E, P21, p27 for cell proliferation and molecular marker involved in bone invasion including interleukin -1, interleukin-6, and tumor necrosis factor alpha (TNF- α) are warranted.

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