Immunohistochemical expression and evaluation of cyclin D1 and minichromosome maintenance 2 in oral squamous cell carcinoma and verrucous carcinoma

T. R. Menaka¹, S. Shamala Ravikumar², K. Dhivya³, N. Thilagavathi¹, J. Dinakaran², Vinoth Kalaichelvan¹

¹Consultant Oral Pathologist and Private Dental Practitioner, R.J.Dental Clinic, Kanchipuram, ²Department of Oral and Maxillofacial Pathology and ³Oral Medicine, Adhiparasakthi Dental College and Hospital, Melmaruvathur, Tamil Nadu, India

Abstract

Background: The study of cell proliferation is important for assessing the tumor behavior, prognosis and patient survival of oral carcinomas. As literature search did not reveal sufficient studies of immunohistochemical expression of cyclin D1 and minichromosome maintenance 2 (MCM2) in oral squamous cell carcinoma (OSCC) and verrucous carcinoma (VC), the present study was undertaken.

Materials and Methods: The study group included 20 cases of histopathologically diagnosed OSCC, 10 cases of VC and 10 cases of normal mucosa (NM). All samples were evaluated for the expression of cyclin D1 and MCM2 using standard Immunohistochemistry (IHC) procedure. The present study involved both qualitative and quantitative analyses. Qualitative analysis was done by evaluation of intensity and area of staining. Quantitative analysis was done by calculating the percentage of positively stained cells and assessing the labeling index (LI). Data obtained were subjected to statistical analysis using SPSS statistical package (version 23.0).

Results: On evaluating and comparing the intensity of staining and area of staining of cyclin D1 and MCM2 between the study groups, statistically significant values ($P < 0.05$) were obtained using Kruskal–Wallis ANOVA. Comparison of LI of cyclin D1 and MCM2 in NM, OSCC and VC statistically significant results ($P < 0.05$) was obtained using Mann–Whitney $U$-test. Mean LI of MCM2 was found to be significantly higher than mean LI of cyclin D1 in all the study groups.

Conclusion: From the present study, we conclude that MCM2 has the potential to serve as a novel cell proliferation biomarker in OSCC and VC when compared to cyclin D1.

Keywords: Cell proliferation, cyclin D1, minichromosome maintenance 2, oral squamous cell carcinoma, verrucous carcinoma

Address for correspondence: Dr. T. R. Menaka, Consultant Oral Pathologist and Private Dental Practitioner, R.J. Dental Clinic, No. 44 West Raja Street, Kanchipuram- 631502, Tamil Nadu, India.
E-mail: drmenakatr@gmail.com

Submitted: 21-Dec-2021, Revised: 09-Mar-2022, Accepted: 10-Jan-2022, Published: 31-Mar-2022
INTRODUCTION

Oral squamous cell carcinomas (OSCCs) belonging to a larger subgroup of tumors termed head-and-neck squamous cell carcinomas represent over 90% of malignant oral neoplasms.[1] According to the International Agency for Research on Cancer, the incidence rate of oral cancer in India is 12.6/100,000 people.[2] The high incidence of OSCC in India has been attributed to a variety of etiological factors such as tobacco smoking, tobacco chewing, alcohol consumption and human papillomavirus infections.[3] These factors may act individually or synergistically in oral carcinogenesis.[4]

Verrucous carcinoma (VC), a rare tumor first described by Ackerman[5] in the year 1948, is a low-grade variant of OSCC and is being considered as a separate clinicopathologic entity distinct from OSCC because of its unique biologic behavior and slow-growing nature. VC has a limited propensity to metastasize, hence with a better prognosis than OSCC.[6] Few studies reveal that some foci of OSCC may be observed in 20% of VC cases, making it a hybrid tumor, thus conferring it a metastatic potential.[6]

The prognosis of the patients decreases with increasing tumor stage, hence it is of great importance to detect the tumor as early as possible. If OSCC is diagnosed at an early-stage (T1N0) survival rate of up to 80% is noted, but in the later stages (T3–T4), it falls to about 20%–30%.[7] Studies have supported that oral carcinogenesis emerges from the accumulation of genetic alterations and epigenetic abnormalities in the expression of genes involved in cell proliferation.[8] Hence, the study of cell proliferation is important for assessing the tumor behavior, prognosis and patient survival.[9]

Numerous proliferation markers have been developed to detect and quantify the proliferation of cells in oral carcinoma.[9] Indeed, the strongest connection between cyclins and oncogenesis has been reported in studies conducted in OSCC.[10] Among the cyclins, cyclin D1 appears to be important in the G1 phase which is the only phase where the extracellular stimuli like growth factors can have an effect on the cell cycle.[11] Amplification and overexpression of cyclin D1 have been reported in head-and-neck, oral, laryngeal and nasopharyngeal carcinoma.[12] Similarly, because of its expression in the early G1 phase, few studies have demonstrated that minichromosome maintenance (MCM) proteins can be used as proliferation markers for determining the tumor behavior.[13] MCM2 protein can be used to estimate the proliferative index and also as a prognostic factor to determine the survival rate of patients with OSCC.[9]

Although few studies have been carried out to detect the expression of cyclin D1 and MCM2 in different grades of OSCC, literature search reveals very few studies on the expression of these markers in VC. With this background, the present study has been undertaken to evaluate the immunohistochemical expression of MCM2 and cyclin D1 in OSCC and VC.

MATERIALS AND METHODS

Study design and patient selection
A retrospective cross-sectional immunohistochemical analysis was carried out on 40 archival retrieved formalin-fixed paraffin-embedded tissue blocks. The study comprised 20 histopathologically diagnosed cases of OSCC (10 cases of well-differentiated squamous cell carcinoma [WDSCC] and 10 cases of moderately differentiated squamous cell carcinoma [MDSCC]), 10 cases of VC and 10 cases of normal mucosa (NM), and the immunohistochemical expression of cyclin D1 and MCM2 was analyzed in all the three groups.

Immunohistochemistry
The expression of cyclin D1 and MCM2 was evaluated using standard IHC procedure with anti-cyclin D1 (rabbit monoclonal antibody – EP12 [PathnSitu Biotechnologies Private Limited]) and anti-MCM2 (rabbit monoclonal antibody – EP40 [PathnSitu Biotechnologies Private Limited]). Positive control sections included tonsil for cyclin D1 and MCM2 and were treated in the same manner as the test groups.

Immunohistochemical analysis
The presence of brown-colored end product at the site of target antigen indicated positive staining. All the cases showed variable intensities of nuclear staining. To know the extent of stain uptake, intensity of staining was analyzed. Ten random fields were selected at ×40 magnifications in each slide. Sections were scored for staining intensity and scaled as follows:[14–16] 0 – no stain, 1 – mild stain, 2 – moderate stain and 3 – intense stain.

To know the expression pattern and also to determine the levels of protein expression in the epithelial layers, area of staining was determined by scanning the entire section of the epithelium and area of stained epithelial cells was recorded as:[17] 0 – 0%, 1 – <25%, 2 – 25%–49%, 3 – 50%–74% and 4 – 75%–100%.

To determine the labeling index (LI), the slides were examined under a light microscope (Olympus CX21) at ×40 magnification and representative photomicrographs were taken in five hotspot areas for each slide. The
photomicrographs were then analyzed using image processing program (ImageJ, http://imagej.nih.gov/ij/). Percentage of IHC-positive tumor cells per hot spot (A) was calculated and total number of tumor cells in each slide was calculated till a minimum of 400 cells were reached, i.e., the sum of the denominators (x). LI was calculated using the formula: \[ LI\% = \frac{A \times 100}{\text{Total no. of tum or cells}(x)} \]

Data obtained were subjected to statistical analysis using SPSS Version 17.0 (SPSS, Inc, Chicago, IL, USA). Kruskal–Wallis ANOVA and Mann–Whitney U-test were performed, and \( P < 0.05 \) was considered statistically significant.

**RESULTS**

Cyclin D1 and MCM2 positivity was seen in all cases. On comparison of staining intensity of cyclin D1 and MCM2 among the study groups, a statistically significant value (\( P < 0.05 \)) was obtained using Kruskal–Wallis ANOVA [Table 1]. Similarly, on comparing the area of staining of MCM2 among the study groups, a statistically significant value (\( P < 0.05 \)) was obtained using Kruskal–Wallis ANOVA. However, comparison of area of staining of cyclin D1 among the study groups was statistically not significant (\( P > 0.05 \)) [Table 1].

On comparing the intensities of staining and area of staining between cyclin D1 and MCM2 in OSCC, a statistically significant value (\( P < 0.05 \)) was obtained by using Mann–Whitney U-test. This could be because both markers predominantly showed intense staining (score 3) with 74%–100% positive cells (score 4) in OSCC [Table 2].

Although cyclin D1 predominantly showed moderate-to-intense staining (score 2 and 3) and MCM2 predominantly showed intense staining (score 3), on comparing the intensities of expression of staining between cyclin D1 and MCM2 in VC, a statistically insignificant value (\( P > 0.05 \)) was obtained by using Mann–Whitney U-test [Table 2].

Similarly, on comparing the area of staining between cyclin D1 and MCM2 in VC, a statistically insignificant value (\( P > 0.05 \)) was obtained by using Mann–Whitney U-test. This can be because, in VC, cyclin D1 showed mostly <24% positive cells (score 1), whereas MCM2 showed both <24% and 74%–100% positive cells (score 1 and 4) [Table 2].

On comparison of labeling index (LI) of cyclin D1 and MCM2 between the study groups, a statistically significant value (\( P < 0.05 \)) was obtained [Table 3] using Kruskal–Wallis ANOVA.

On comparing the LI between cyclin D1 and MCM2 in NM, OSCC and VC, a statistically significant value was obtained (\( P < 0.05 \)) using Mann–Whitney U-test [Table 4].

**DISCUSSION**

Cell cycle progression is regulated by factors such as cyclins, cyclin-dependent kinases (CDKs), inhibitory enzymes, the retinoblastoma protein, p21, p27 and p53. Among the cyclins, cyclin D1, a 45 kDa, 295 amino acid protein, is encoded by CCND1 gene located at chromosome 11q13. Overexpression of cyclin D1 is thought to provide the tumor cells with a selective growth advantage. MCM proteins were first reported by Maine in 1984 in an attempt to identify factors that originate DNA replication. MCM2–7 are imported into the nucleus when CDK activity is low in early G1 and exported from the nucleus during S phase when CDK activity is high.

According to molecular studies, MCM2 proteins identify both cycling cells and noncycling cells with proliferative potential. Therefore, detection of cyclin D1 and MCM2 can be used to distinguish cells that exhibit aberrant cell proliferation activity.

In the present study, in NM, 60% of the cases showed mild staining intensity of cyclin D1 in the nucleus of basal cells and few cells in the parabasal layer which is similar to
the study results of Swaminathan et al.\cite{24} and Angadi and Krishnapillai.\cite{15}

However, in the present study, 80% of cases showed intense staining for cyclin D1 in WDSCC [Figure 1] which is similar to the results of Ohnishi et al.\cite{25} This is in contrast to the study of Patel et al.,\cite{26} Angadi and Krishnapillai\cite{15} and Goto et al.\cite{27} where mild-to-moderate intensity of staining was observed in WDSCC.

Similarly, 50% of cases showed intense staining and 50% of cases showed moderate staining for cyclin D1 in MDSCC [Figure 2] which is nearly similar to the observations of Swaminathan et al.\cite{24}

Forty percent of cases showed intense staining for cyclin D1 in cases of VC and 30% of cases showed moderate and mild staining [Figure 3] in contrast to Angadi and Krishnapillai\cite{15} where predominantly mild staining was observed.

The immunoreactivity for area of staining of cyclin D1 in NM showed <25% of positivity in the nucleus of basal and parabasal cells. Whereas, in WDSCC, 80% of samples showed 50%–100% of positivity [Figure 4]. On analyzing the immunoreactivity for area of staining of cyclin D1 in MDSCC [Figure 5] and VC [Figure 6], 60% of cases showed <25% of positivity.

MCM2 expression in NM shows that controlled cell division and proliferation ability occur only in basal and parabasal compartments while the superficial cells do not possess proliferative ability. This result was similar to that of Chatrath et al.\cite{29} and Feng et al.\cite{31} In contrast, Torres-Rendon et al.\cite{30} investigated MCM2 expression in NM and found that MCM2 was mainly expressed at the suprabasal compartment only.

In our study, the intensity of staining expression for MCM2 in NM was found to be moderate, whereas 100% of WDSCC [Figure 7], 80% of MDSCC [Figure 8] and 70% of VC [Figure 9] showed intense staining. These findings are in accordance with Kodani et al.\cite{28} Chatrath et al.,\cite{29} Shalash\cite{21} and Torres-Rendon et al.\cite{30} with regard to OSCC.

Table 2: Comparison between cyclin D1 and minichromosome maintenance 2 staining intensity and area of staining in normal mucosa, oral squamous cell carcinoma and verrucous carcinoma

| Comparison | n | NM | Mean | SD | P | OSCC | Mean | SD | P | VC | Mean | SD | P |
|------------|---|----|------|----|---|------|------|----|---|----|------|----|---|
| Cyclin D1 - I | 10 | 1.5 | 0.707 | 0.079 | 2.45 | 0.887 | 0.155 | 2.1 | 0.876 | 0.173 |
| MCM2 - I | 10 | 2.1 | 0.738 | | 2.8 | 0.616 | | 2.6 | 0.699 |
| Cyclin D1 - A | 10 | 1.5 | 0.707 | 0.525 | 2.350 | 1.309 | 0.022* | 1.6 | 0.843 | 0.097 |
| MCM2 - A | 10 | 1.7 | 0.675 | | 3.25 | 1.070 | | 2.4 | 1.174 |

*Statistically significant using Mann-Whitney U-test. MCM2: Minichromosome maintenance 2, SD: Standard deviation, NM: Normal mucosa, OSCC: Oral squamous cell carcinoma, VC: Verrucous carcinoma

Table 3: Comparison of labeling index of cyclin D1 and minichromosome maintenance 2 between the groups

| n | Mean | SD | Mean rank | \( \chi^2 \) | P |
|---|------|----|-----------|--------------|---|
| Cyclin D1 LI | NM | 10 | 8.41 | 5.10 | 8.30 | 14.663 | 0.002* |
| OSCC | 20 | 21.05 | 5.44 | 25.70 |
| VC | 10 | 21.68 | 10.90 | 24.10 |
| MCM2 LI | NM | 10 | 18.33 | 3.99 | 8.40 | 14.869 | 0.002* |
| OSCC | 20 | 43.53 | 11.69 | 25.50 |
| VC | 10 | 40.67 | 19.84 | 23.30 |

*Statistically significant using Kruskal-Wallis ANOVA. MCM2: Minichromosome maintenance 2, SD: Standard deviation, NM: Normal mucosa, OSCC: Oral squamous cell carcinoma, VC: Verrucous carcinoma, LI: Labeling index

Figure 1: Cyclin D1 expression in well-differentiated squamous cell carcinoma with intense staining (score 3) at x40 magnification

In our study, the intensity of staining expression for MCM2 in NM was found to be moderate, whereas 100% of WDSCC [Figure 7], 80% of MDSCC [Figure 8] and 70% of VC [Figure 9] showed intense staining. These findings are in accordance with Kodani et al.,\cite{28} Chatrath et al.,\cite{29} Shalash\cite{21} and Torres-Rendon et al.\cite{30} with regard to OSCC. The difference between the mean scores of intensity of staining of MCM2 between the study groups was found to be statistically significant.

MCM2 expression in NM shows that controlled cell division and proliferation ability occur only in basal and parabasal compartments while the superficial cells do not possess proliferative ability. This result was similar to that of Chatrath et al.\cite{29} and Feng et al.\cite{31} In contrast, Torres-Rendon et al.\cite{30} investigated MCM2 expression in NM and found that MCM2 was mainly expressed at the suprabasal compartment only.

In the current study, 70% of the WDSCC [Figure 10] and 50% of MDSCC [Figure 11] showed more than 75% positivity of area of staining of MCM2, with expression along the periphery of the invaded epithelial islands, and at the invasive fronts. On the other hand, the central cores of the cell nests mostly showed negative MCM2 reaction.
The observations in WDSCC are in accordance with Shalash,\textsuperscript{[21]} Szalachowska \textit{et al}.,\textsuperscript{[35]} Scott \textit{et al}.,\textsuperscript{[33]} and Gouvê\textsuperscript{a}

\textit{et al}.,\textsuperscript{[34]} Whereas, the findings in MDSCC are in accordance with Kodani \textit{et al}.,\textsuperscript{[28]} Chatrath \textit{et al}.,\textsuperscript{[29]} Shalash\textsuperscript{[21]} and
The increase in MCM2 expression in the peripheral tumor cells and at the invasive fronts suggests a high rate of cellular proliferation and subsequent invasion into the surrounding structures.\(^{[35]}\) In our study, VC cases showed an average of 50% positivity for area of staining of MCM2 \(\text{[Figure 12]}\). However, the results could not be compared due to the lack of published studies. VC is a tumor characterized by a differentiation of a high order in which the epithelium shows little mitotic activity.\(^{[36]}\) This could be the reason for the cells taking up lesser MCM2 in our study.

In our study, the mean LI of cyclin D1 in NM was nearly similar to that of Moharil \textit{et al.}\(^{[37]}\), while the mean LI of cyclin D1 for OSCC was nearly similar to the findings of Swaminathan \textit{et al.}\(^{[24]}\). The mean LI of cyclin D1 in VC in our study could not be compared directly due to lack of published reports.

Table 4: Comparison of labeling index between cyclin D1 and minichromosome maintenance 2 in normal mucosa, oral squamous cell carcinoma and verrucous carcinoma

| Comparison    | n   | NM       | Mean | SD       | P       | OSCC  | Mean | SD       | P       | VC     | Mean | SD       | P       |
|---------------|-----|----------|------|----------|---------|-------|------|----------|---------|--------|------|----------|---------|
| Cyclin D1 LI  | 10  | NM       | 8.41 | 5.098    | 0.007*  | OSCC  | 21.048| 5.436    | 0.005*  | VC     | 21.681| 10.902   | 0.017*  |
| MCM2 LI       | 10  | NM       | 18.33| 3.993    |         | OSCC  | 43.532| 11.689   |         | VC     | 40.667| 19.836   |         |

*Statistically significant using Mann-Whitney U-test. MCM2: Minichromosome maintenance 2, SD: Standard deviation, NM: Normal mucosa, OSCC: Oral squamous cell carcinoma, VC: Verrucous carcinoma, LI: Labeling index.
In the present study, the mean value of LI of MCM2 in NM and OSCC was similar to observations by Kodani et al. but lesser than the values published by Niranjan et al., Torres-Rendon et al. and Razavi et al. The mean LI of MCM2 for VC in the present study was lower than the value reported by Niranjan et al. On comparing the mean LI of cyclin D1 and MCM2 between the NM, OSCC and VC, a statistically significant value was obtained ($P = 0.001^*$ and $P = 0.002^*$, respectively).

In the present study, the mean LI of MCM2 in the study groups was found to be higher than the mean LI of cyclin D1. This could be because MCM2 proteins identify both cycling cells and noncycling cells with proliferative potential throughout the cell cycle and expressed in the cell nucleus from early G1 phase. Hence, MCM2 can serve as a more potential biomarker for cell proliferation in OSCC and VC when compared to cyclin D1. Further studies need to be performed with larger sample size to validate the present findings.

**CONCLUSION**

The present study is probably an early initiative to evaluate the immunohistochemical expression of cyclin D1 and MCM2 in OSCC and VC. There was a substantial increase in the immunoreactivity and mean LI of MCM2 and cyclin D1 from NM to OSCC. A similar progressive increase in the immunoreactivity and mean LI of MCM2 and cyclin D1 was observed from NM to VC. A thorough literature search was done to find out the expression of cyclin D1 and MCM2 in VC-like lesions. However, only a very few articles were obtained for reference. Hence, the present study can have a place as one of the early studies attempted in expression of MCM2 and cyclin D1 in VC.

The interpretation of the present study also showed the highest expression of MCM2 in OSCC followed by VC, again showing the ability of the biomarker to be correlated with higher grade and establishing MCM2 as a better prognostic marker.

**Financial support and sponsorship**
Nil.

**Conflicts of interest**
There are no conflicts of interest.

**REFERENCES**

1. Vigneswaran N, Williams MD. Epidemiologic trends in head and neck cancer and aids in diagnosis. Oral Maxillofac Surg Clin North Am 2014;26:123-41.
2. Khan ZU. An overview of oral cancer in Indian subcontinent and recommendations to decrease its incidence. Webmed Central Cancer 2012;3:WMC003626.
3. Rivera C, Venegas B. Histological and molecular aspects of oral squamous cell carcinoma (Review). OncoL Lett 2014;8:7-11.
4. Sathawane RS, Agrawal N, Deoghare A, Patel D. Aggressive variant of oral verrucous carcinoma with extensive mandibular involvement: A rare. Int J 2017;2:41.
5. Zanini M, Wulkan C, Paschoal FM, Maciel MH, Machado Filho CD, Apparecidia S. Verrucous carcinoma: A clinical-histopathologic variant of squamous cell carcinoma. An Bras Dermatol 2004;79:619-21.
6. Hosseinpoor S, Mashhadiabbas F, Ahsaie MG. Diagnostic biomarkers in oral verrucous carcinoma: A systematic review. Pathol OncoL Res 2017;23:19-32.
7. Dionne KR, Warnakulasuriya S, Zain RB, Cheong SC. Potentially malignant disorders of the oral cavity: Current practice and future directions in the clinic and laboratory. Int J Cancer 2015;136:503-15.
8. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis 2010;31:27-36.
9. De Moraes M, Monteiro Maia CA, de Almeida Freitas R, Galvao HC. Cell proliferation markers in oral squamous cell carcinoma. J Mol Biomark Diagn 2012;8:206.
10. Dhangra V, Verma J, Misra Y, Srivastav S, Hasan F. Evaluation of cyclin D1 expression in head and neck squamous cell carcinoma. J Clin Diagn Res 2017;11:C01-4.
11. Saawarn S, Saawarn N, Astekar M, Jain M, Gupta A. Cyclin D1: An insight into its physio-pathological role in oral squamous cell carcinoma. J Mol Biomark Diagn 2015;6:1.
12. Das SN, Khare P, Singh MK, Sharma SC. Correlation of cyclin D1 expression with aggressive DNA pattern in patients with tobacco-related intraoral squamous cell carcinoma. Indian J Med Res 2011;133:381-6.
13. Bochman ML, Schwacha A. Differences in the single-stranded DNA binding activities of MCM2-7 and MCM467: MCM2 and MCM5 define a slow ATP-dependent step. J Biol Chem 2007;282:33795-804.
14. Angadi PV, Krishnapillai R. Cyclin D1 expression in oral squamous cell carcinoma and verrucous carcinoma: Correlation with histological differentiation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103:103-5.
15. Castle JT, Cardinall M, Kratochvil FJ, Abbondanzo SL, Kessler HP, Auclair PL et al. P53 and cyclin D1 staining patterns of malignant and premalignant oral lesions in age-dependent populations. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;88:326-32.
16. Choudhary A, Kesarwani P, Gaikwad P, Hiremath SS, Gupta R, Koppara S. Expression of cyclin D1 in oral squamous cell carcinoma and its correlation with histological differentiation: An immunohistochemical study, J Indian Acad Oral Med Radiol 2016;28:140-4.
17. Iatropoulos MJ, Williams GM. Proliferation markers. Exp Toxicol Pathol 1996;48:175-81.
18. van Diest PJ, Brugal G, Baak JP. Proliferation markers in tumours: Interpretation and clinical value. J Clin Pathol 1998;51:716-24.
19. Todd R, Hinds PW, Munger K, Rustgi AK, Opitz OG, Suliman Y, et al. Cell cycle dysregulation in oral cancer. Crit Rev Oral Biol Med 2002;13:51-61.
20. Mehdi MZ, Nagi AH, Naseem N. MCM–2 and Ki–67 as proliferation markers in renal cell carcinoma: A quantitative and semi–Quantitative analysis. Int Braz J Urol 2016;42:1121-8.
21. Shalash HN. Immunohistochemical Evaluation of the Proliferation Marker MCM-2 Oral Squamous Cell Carcinoma. CU Theses; 2012.
22. Razavi SM, Jafari M, Heidarpoor M, Khaledes S. Minichromosome maintenance-2 (MCM2) expression differentiates oral squamous cell carcinoma from pre-cancerous lesions. Malays J Pathol 2015;37:253-8.
23. Jiang WG, Sanders AJ, Katoh M, Ungefroren H, Gieseler F, Prince M, et al. Tissue invasion and metastasis: Molecular, biological and clinical perspectives. Semin Cancer Biol 2015;35 Suppl: S244‑75.
24. Swaminathan U, Joshua E, Rao UK, Ranganathan K. Expression of p53 and cyclin D1 in oral squamous cell carcinoma and normal mucosa: An immunohistochemical study. J Oral Maxillofac Pathol 2012;16:172-7.
25. Ohnishi Y, Watanabe M, Wato M, Tanaka A, Kakudo K, Nozaki M. Cyclin D1 expression is correlated with cell differentiation and cell proliferation in oral squamous cell carcinomas. Oncol Lett 2014;7:1123-7.
26. Patel SB, Manjunatha BS, Shah V, Soni N, Saturiya R. Immunohistochemical evaluation of p63 and cyclin D1 in oral squamous cell carcinoma and leukoplakia. J Korean Assoc Oral Maxillofac Surg 2017;43:324-30.
27. Goto H, Kawano K, Kobayashi I, Sakai H, Yanagisawa S. Expression of cyclin D1 and GSK-3β and their predictive value of prognosis in squamous cell carcinomas of the tongue. Oral Oncol 2002;38:549-56.
28. Kodani I, Shomori K, Osaki M, Kuratake I, Ryoke K, Ito H. Expression of minichromosome maintenance 2 (MCM2), Ki-67, and cell-cycle-related molecules, and apoptosis in the normal-dysplasia-carcinoma sequence of the oral mucosa. Pathobiology 2001;69:150-8.
29. Chatrath P, Scott IS, Morris LS, Davies RJ, Rushbrook SM, Bird K, et al. Aberrant expression of minichromosome maintenance protein-2 and Ki67 in laryngeal squamous epithelial lesions. Br J Cancer 2003;89:1048-54.
30. Torres-Rendon A, Roy S, Craig GT, Speight PM. Expression of Mcm2, geminin and Ki67 in normal oral mucosa, oral epithelial dysplasias and their corresponding squamous-cell carcinomas. Br J Cancer 2009;100:1128-34.
31. Feng CJ, Li HJ, Li JN, Lu YJ, Liao GQ. Expression of Mmc7 and Cldc in oral squamous cell carcinoma and precancerous lesions. Anticancer Res 2008;28:3763-9.
32. Szalachowska J, Dziegiel P, Jelen-Krzeszewska J, Jelen M, Markowski R, Pomiczek A, et al. Mcm-2 protein expression predicts prognosis better than Ki-67 antigen in oral cavity squamouscellular carcinoma. Anticancer Res 2006;26:2473-8.
33. Scott IS, Odell E, Chatrath P, Morris LS, Davies RJ, Vowler SL, et al. A minimally invasive immunocytochemical approach to early detection of oral squamous cell carcinoma and dysplasia. Br J Cancer 2006;94:1170-5.
34. Gouveia AF, Vargas PA, Coletta RD, Jorge J, Lopes MA. Clinico pathological features and immunohistochemical expression of p53, Ki-67, Mmc2- and Mmc-5 in proliferative verrucous leukoplakia. J Oral Pathol Med 2010;39:447-52.
35. Niranjan KC, Sarathy NA, Alrani D. MCM-2 expression differentiates potentially malignant verrucous lesions from oral carcinomas. Ann Diagn Pathol 2018;34:72-6.
36. Gimenez-Conti IB, Collet AM, Lanfranchi H, Itoiz ME, Luna M, Xu HJ, et al. p53, Rh, and cyclin D1 expression in human oral verrucous carcinomas. Cancer 1996;78:17-23.
37. Moharil RB, Khandeekar S, Dive A, Bodhade A. Cyclin D1 in oral premalignant lesions and oral squamous cell carcinoma: An immunohistochemical study. J Oral Maxillofac Pathol 2020;24:397.