Dental Journal
Majalah Kedokteran Gigi

Dental Journal
(Majalah Kedokteran Gigi)
2022 September; 55(3): 137–141

Original article

Immunohistochemical differential expression of p16 proteins in follicular type and plexiform type ameloblastoma

Haris Budi Widodo[1], Anung Saptiwulan[2], Helmi Hirawan[3], Christiana Cahyani Prihastuti[1], Tirta Wardana[4,5]
[1]Department of Oral Biology, Dental Medicine Study Programme, Faculty of Medicine, Jenderal Soedirman University, Purwokerto, Indonesia
[2]Undergraduate Student, Dental Medicine Study Programme, Faculty of Medicine, Jenderal Soedirman University, Purwokerto, Indonesia
[3]Department of Oral Surgery, Dental Medicine Study Programme, Faculty of Medicine, Jenderal Soedirman University, Purwokerto, Indonesia
[4]Department of Biomedicine, Dental Medicine Study Programme, Faculty of Medicine, Jenderal Soedirman University, Purwokerto, Indonesia
[5]Integrative Laboratory, Faculty of Medicine, Jenderal Soedirman University, Purwokerto, Indonesia

ABSTRACT

Background: *Differences in histopathological features that describe the growth mechanism and biological behaviour of follicular and plexiform ameloblastomas are associated with benign, aggressive and destructive tumour markers. p16 has inhibitory interactions between cyclin D and CDK 4/6 to block the cell cycle and alterations related to severity.* ***Purpose:*** *This study intends to evaluate and determine differential expressions of p16 protein in follicular and plexiform ameloblastomas.* ***Methods:*** *This is a descriptive analytics study. A total of 21 specimens consisting of follicular and plexiform ameloblastomas and healthy gingiva tissues as the negative control were examined using the immunohistochemistry assay. The analysis of p16 protein expression was interpreted by immunoreactive scoring. Statistical analysis was conducted using SPSS software with the Mann–Whitney test. A p-value <0.05 shows the significance of the change in expression.* ***Results:*** *An increased expression of p16 protein was found in the follicular ameloblastoma type (2.13 ± 1.808) and the plexiform type (4.44 ± 2.506) in comparison to the negative control group (0 ± 0). The increase of p16 expression in the follicular and plexiform ameloblastomas was significant compared to the negative control group (p-value <0.05); however, there was no significant difference between either type of ameloblastoma (p-value >0.05).* ***Conclusion:*** *The highest intensity of p16 protein expression was found in the plexiform type, even though it was not significantly different from the follicular type ameloblastoma.*

Keywords: *ameloblastomas; follicular; immunohistochemistry; plexiform; p16 protein expression*

Correspondence: Tirta Wardana, Department of Biomedicine, Dental Medicine Study Programme, Faculty of Medicine, Jenderal Soedirman University, Purwokerto, Indonesia. Email: tirta.wardana@unsoed.ac.id

INTRODUCTION

The most common types of ameloblastomas are follicular and plexiform, whose clinical findings and specific clinical behaviours are associated with histopathological appearance. Given that appearance, this tumour shows signs of being a benign tumour, although clinically, it is aggressive and destructive.[1] Ameloblastoma is an odontogenic tumour in tooth-forming tissue that grows slowly and locally invasive. Usually, the patient is unaware until the inflammation enlarges. Its recurrence is high, and its spread is expansive and infiltrative, giving the impression of malignancy.[2]

Ameloblastoma is commonly found in the mandibular and maxillary areas, with swelling resulting in facial deformity. On clinical examination, ameloblastoma does not have a specific feature because the stain of the tumour tissue is the same as the surrounding tissue.[3] In addition, the consistency can be soft or hard, with no pain and paraesthesia and no ulceration of the mucosa around the tumour tissue.[4] The growth of ameloblastoma is influenced by oral infection, tooth extraction, trauma to the teeth or jaws and genetic factors, such as tumour suppressor genes (TSG) and oncogenes (c-myc gene and ras gene). The loss of function of TSG, which plays a role in controlling cell proliferation and preventing cells from becoming malignant, causes tumour formation.[5–7]

p16 is a group of TSG called MTS1 (multiple tumour suppressor 1), CDKN2 (cyclin-dependent kinase inhibitor 2) and p16INK4a, which functions as an inhibitor of

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 158/E/KPT/2021.
 Available at https://e-journal.unair.ac.id/MKG/index
DOI: 10.20473/j.djmkg.v55.i3.p137–141

the interaction between cyclin D and cyclin-dependent kinase (CDK) 4 and 6; it blocks the cell division cycle in G1 phase-control points.[8,9] High throughput technology analyses using microarray gene expression profiling offer a technology to classify the tumour subtypes, such as breast cancer,[10] bladder cancer[11] and pancreatic adenocarcinoma.[12] However, because of the high cost and complexity of laboratory procedures, alternate immunochemistry (IHC) assay is used to identify subtypes of tumour classification. The identification of the subtype and the clinical impact of the tumour can be used to determine the success of treatment due to tumour biological properties and behaviour.[13]

Based on its role, p16 can be used as a marker of the cell-cycle phase to study pathophysiological conditions, such as abnormal cell differentiation and tumour prognosis.[14,15] This study aims to determine the differential expression of p16 mutant proteins in the formation of follicular and plexiform ameloblastomas. This may inform future investigations into the molecular mechanism and increase potential therapeutics for Indonesian ameloblastoma.

MATERIALS AND METHODS

This descriptive analytics study uses 21 paraffin block-stained specimens from the Department of Anatomical Pathology Laboratory of Prof. Dr. Margono Soekarjo Hospital, Purwokerto and the Asri Medical Centre (AMC), Yogyakarta. The paraffin blocks were cut with a microtome, deparaffinised, rehydrated and then subjected to IHC staining to observe the p16 expression using the anti-CDKN2A/p16INK4a antibody (Abcam ab108349, USA) with a 30-minute incubation using 1:100 dilution. Counterstaining using Mayer's hemalum was performed to determine the differences in three groups: follicular and plexiform types of ameloblastomas as well as healthy gingiva tissue as a negative control.[16,17] All procedures followed the manufacturer's recommendations.

Observation of the p16 protein expression was performed by 400x magnification in five viewpoints using a light microscope camera with Optilab® (Motic® B2-series, USA) and software Raster Image (US National Institutes of Health, USA). Observation with a grading picture of cells recorded as positive and the reaction intensity were as follows:[13,18] grade 0 (no cells recorded) and negative reaction intensity; grade 1 (>10%–50% of recorded cells) and weak staining intensity; grade 2 (>10%–50% of recorded cells) and medium staining intensity (2); grade 3 (>50%–80% of recorded cells) and strong staining intensity; and grade 4 (>80% of recorded cells) and powerful staining intensity.

Based on a previous study, immunohistochemistry p16 expression analysis was carried out based on grading status and intensity scores, with expression scores ranging from 0 to 12.[18] Immunoreactive scores are categorised as 1–4 positive scores (+), weak definition; 5–8 positive scores (++), moderate definition; and 8–12 positive scores (+++), strong definition. Data analysis was carried out using SPSS software version 22 (IBM Corp Version 23, Chicago, IL). Cohen's kappa coefficient was used to test the validity of the examination by two observers. The analysis of differences in p16 expression was carried out using the Kruskal–Wallis non-parametric test. The Mann–Whitney test was performed to determine significant differences between groups; a p-value <0.05 indicates a significant difference.

RESULTS

In this study, the specimen sample consisted of follicular and plexiform ameloblastomas (Table 1). Immunohistochemical staining was conducted to evaluate the expression of p16 by discolouration. The brown and dark brown discolouration in follicular and plexiform ameloblastoma specimens showed mutant p16 expressions (Figure 1). The differential in colour intensity indicated weak positive,

Table 1. Mean of p16 mutant protein expression

Tissue specimens	Total sample	Average	Std. deviation	Std. error mean
Plexiform ameloblastoma	9	4.44	2.506	0.835
Follicular ameloblastoma	8	2.13	1.808	0.639
Healthy gingiva epithelial tissue control group	4	0	0	0

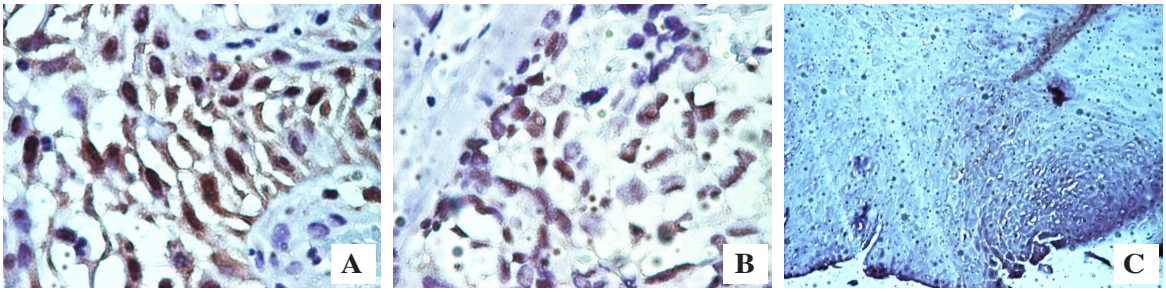

Figure 1. Positive p16 mutant protein expression by immunohistochemical staining in study groups (A) plexiform type ameloblastoma (x400); (B) follicular type Ameloblastoma (x400); and (C) healthy gingival epithelium tissue (x40).

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 158/E/KPT/2021.
 Available at https://e-journal.unair.ac.id/MKG/index
DOI: 10.20473/j.djmkg.v55.i3.p137–141

moderately positive and strong positive differences in immunosuppression scores (Figure 1). The mean value of the difference in the expression of mutant p16 from the results of immunohistochemical examinations in the sample group are shown in Table 1. Two observers analysed mutant p16 expression by multiplying the positive cell grading and the reaction intensity from five fields of view observations.

The kappa coefficient test analysis showed a significance value of p = 0.000 with an ideal value of 0.598. In our study, the mean p16 expression for the follicular ameloblastoma group was 2.13 ± 1.808, for the plexiform ameloblastoma group 4.44 ± 2.506 and for the healthy gingival epithelium control group 0 ± 0. The analysis of differences in the expression of p16 showed a significant difference (p-value <0.05) in the follicular ameloblastoma, the plexiform ameloblastoma and the control groups (can be seen in Table 2). The expression of p16 mutant proteins showed differences between the control group compared with the follicular type ameloblastoma (p-value <0.05) and the control group compared with the plexiform type ameloblastoma (p-value <0.05), whereas the follicular type ameloblastoma compared with the plexiform type ameloblastoma group showed no significant difference (p-value = 0.071; p-value >0.05) (Figure 2).

Table 2. Different expressions of p16 mutant proteins

Variable I	Variable II	Significance
Control	Follicular Ameloblastoma	0.028
Control	Plexiform Ameloblastoma	0.005
Follicular Ameloblastoma	Plexiform Ameloblastoma	0.071

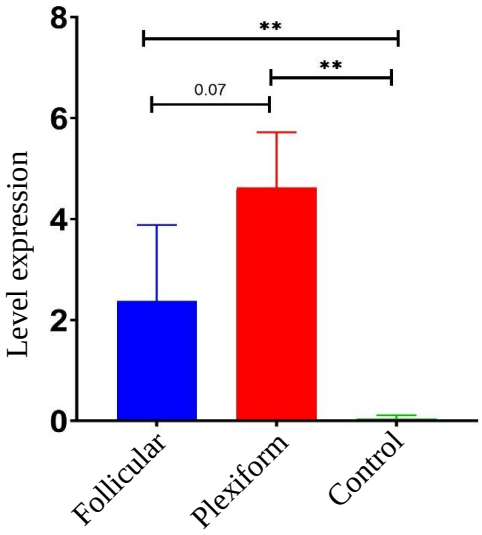

Figure 2. Immunohistochemical expressions of p16 mutant proteins on follicular and plexiform ameloblastomas and healthy gingiva control group.

DISCUSSION

Ameloblastoma is a tumour with a high incidence, unrelated to age and gender and with no specific clinical symptoms. Histopathological and radiographic examinations are the gold standard for diagnosis in the incidence of ameloblastoma, with several types often found, namely follicular, plexiform and adenomatous. Different types of ameloblastomas can represent characteristics such as aggressiveness, recurrence and severity. Increased p16 expression in the incidence of ameloblastoma can provide an overview of the severity of its role as a tumour-suppressor gene in inhibiting the uncontrolled proliferation process.[19,20] This study found a significant increase of p16 expression in both types of ameloblastomas compared to the healthy gingival tissue, with the highest expression of p16 shown in the plexiform type ameloblastoma.

Increased expression of the p16 mutant ameloblastoma indicated the incidence of a malignancy. On the other hand, the wild-type p16 protein is difficult to detect in normal conditions because it has a short half-life.[21] Increased expression of mutant p16 causes failure of cell proliferation in the G1 phase so that it is often found in follicular and plexiform types of ameloblastomas.[22,23] The imbalance of cell cycle regulatory pathways involving p16-RB can impair cell proliferation, ultimately leading to unrestricted proliferation and tumourigenesis.[22,24,25] Mutant p16 expression was not found in the regular gingival epithelial control group in normal cell proliferation, implying there were no malignant changes in cells.

The p16 protein expressed in the G1 phase is a product of the CDKN2A gene, a tumour suppressor gene (anti-oncogene) that can prevent the overgrowth of cells in the G1 phase.[26] The p16 protein acts as a negative regulator of cell proliferation. In normal cells, wild-type p16 is expressed and binds to CDK4 and CDK6 so that free cyclin D and protein kinase complexes are inactive.[27] Decrease or inactivation of p16 causes CDK4/6 to bind to cyclin D, causing an active protein kinase complex. The protein kinase complex triggers the phosphorylation of pRb so that pRb is inactive. Inactivation of pRb causes the release of the transcription factor E2F so that the cell enters the S phase. Continuous E2F transcription will cause normal cells to become ameloblastoma.[28–30]

The results showed an increase in mutant p16 expression in follicular and plexiform ameloblastoma types. These results are supported by the research of Kumamoto et al.(2001), who demonstrated over-expression of p16 in most neoplastic cells from ameloblastoma so that odontogenic epithelium would be found to be under the control of this oncoprotein.[31] Another study also showed the immunohistochemical expression of p16 in odontogenic tumours, including ameloblastomas, finding a particularly positive trend in tumour cell nuclei for tumours with low recurrence risk and a similar reaction for the nucleus and cytoplasm of tumours with high recurrence rates.[32]

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 158/E/KPT/2021.

DOI: 10.20473/j.djmkg.v55.i3.p137–141

The difference in p16 expression in follicular type ameloblastoma was not significantly different from the plexiform type ameloblastoma group. This shows that the two groups have similar characteristics of mutant p16 expression. The results of this study are supported by a previous study that showed that there was no statistically significant difference in the expression of positive p16 in the central cells of low-risk and high-risk odontogenic tumours; in both groups, the results were equally high.[32] Another study showed that the expression of the tumour suppressor p16 was not significantly different in odontogenic keratoses and unicystic ameloblastomas.[13] This suggests that the invasive growth of odontogenic keratosis and the cystic behaviour of unicystic ameloblastoma are closely related to the state of p16 expression in the lesional epithelium. A candidate tumour marker can be used to analyse mutant p16 protein expression changes in follicular and plexiform ameloblastomas. However, it cannot be used as a progression marker between follicular and plexiform ameloblastoma groups.

This study has several limitations: the limited number of specimens involved and the clinical data that may have risk factors associated with p16 protein expression. In addition, we believe that our findings impact the understanding of p16 protein expression in different types of ameloblastomas. There are significant differences in p16 protein expression using immunohistochemical analysis between the follicular and plexiform types of ameloblastomas compared to healthy tissue. In addition, the highest increase in the expression of the p16 protein is shown in the plexiform type's ameloblastoma.

ACKNOWLEDGMENTS

The authors would like to thank the educators and teaching staff of the Faculty of Medicine, Jenderal Soedirman University; the Anatomical Pathology Laboratory of the Regional General Hospital Prof. Dr Margono Soekarjo Purwokerto; the Anatomical Pathology Laboratory of Faculty of Medicine, Gadjah Mada University Yogyakarta; and the Anatomical Pathology Laboratory of Dr Sardjito General Hospital Yogyakarta for their help. All authors declare there is no conflict of interest in this study.

REFERENCES

1. Gomes CC, de Sousa SF, Gomez RS. Craniopharyngiomas and odontogenic tumors mimic normal odontogenesis and share genetic mutations, histopathologic features, and molecular pathways activation. Oral Surg Oral Med Oral Pathol Oral Radiol. 2019; 127(3): 231–6.
2. Staines KS, Crighton A. Benign oral and dental disease. In: Watkinson JC, Clarke RW, editors. Scott-Brown's Otorhinolaryngology and Head and Neck Surgery. 8th ed. Boca Raton: CRC Press; 2018. p. 699–718.
3. Speight PM, Takata T. New tumour entities in the 4th edition of the World Health Organization classification of head and neck tumours: odontogenic and maxillofacial bone tumours. Virchows Arch. 2018; 472(3): 331–9.
4. Doroy GAR, Gelbolingo N. Primary intraosseous carcinoma of the mandible: a case report. Philipp J Otolaryngol Head Neck Surg. 2020; 35(1): 56–9.
5. Dean KE. A radiologist's guide to teeth: An imaging review of dental anatomy, nomenclature, trauma, infection, and tumors. Neurographics. 2020; 10(5): 302–18.
6. Schwartz JL, Sroussi H. Genomic foundation for medical and oral disease translation to clinical assessment. In: Sonis ST, Villa A, editors. Translational Systems Medicine and Oral Disease. St. Louis: Elsevier; 2020. p. 17–92.
7. Du Y, Cheng Y, Su G. The essential role of tumor suppressor gene ING4 in various human cancers and non-neoplastic disorders. Biosci Rep. 2019; 39(1): BSR20180773.
8. Sánchez-Martínez C, Gelbert LM, Lallena MJ, de Dios A. Cyclin dependent kinase (CDK) inhibitors as anticancer drugs. Bioorg Med Chem Lett. 2015; 25(17): 3420–35.
9. Tripathy D, Bardia A, Sellers WR. Ribociclib (LEE011): Mechanism of action and clinical impact of this selective cyclin-dependent kinase 4/6 inhibitor in various solid tumors. Clin Cancer Res. 2017; 23(13): 3251–62.
10. Shan M, Zhang X, Liu X, Qin Y, Liu T, Liu Y, Wang J, Zhong Z, Zhang Y, Geng J, Pang D. P16 and p53 play distinct roles in different subtypes of breast cancer. PLoS One. 2013; 8(10): e76408.
11. Dadhania V, Zhang M, Zhang L, Bondaruk J, Majewski T, Siefker-Radtke A, Guo CC, Dinney C, Cogdell DE, Zhang S, Lee S, Lee JG, Weinstein JN, Baggerly K, McConkey D, Czerniak B. Meta-analysis of the luminal and basal subtypes of bladder cancer and the identification of signature immunohistochemical markers for clinical use. EBioMedicine. 2016; 12: 105–17.
12. Moffitt RA, Marayati R, Flate EL, Volmar KE, Loeza SGH, Hoadley KA, Rashid NU, Williams LA, Eaton SC, Chung AH, Smyla JK, Anderson JM, Kim HJ, Bentrem DJ, Talamonti MS, Iacobuzio-Donahue CA, Hollingsworth MA, Yeh JJ. Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. Nat Genet. 2015; 47(10): 1168–78.
13. Razavi SM, Poursadeghi H, Aminzadeh A. Immunohistochemical comparison of cyclin D1 and P16 in odontogenic keratocyst and unicystic ameloblastoma. Dent Res J (Isfahan). 2013; 10(2): 180–3.
14. Ogrodnik M, Salmonowicz H, Jurk D, Passos JF. Expansion and cell-cycle arrest: common denominators of cellular senescence. Trends Biochem Sci. 2019; 44(12): 996–1008.
15. Canaud G, Bonventre J V. Cell cycle arrest and the evolution of chronic kidney disease from acute kidney injury. Nephrol Dial Transplant. 2015; 30(4): 575–83.
16. Cadavid AMH, Araujo JP, Coutinho-Camillo CM, Bologna S, Junior CAL, Lourenço SV. Ameloblastomas: current aspects of the new WHO classification in an analysis of 136 cases. Surg Exp Pathol. 2019; 2(1): 17.
17. Verneuil A, Sapp P, Huang C, Abemayor E. Malignant ameloblastoma: classification, diagnostic, and therapeutic challenges. Am J Otolaryngol. 2002; 23(1): 44–8.
18. Surowiak P, Materna V, Maciejczyk A, Pudelko M, Suchocki S, Kedzia W, Nowak-Markwitz E, Dumanska M, Spaczynski M, Zabel M, Dietel M, Lage H. Decreased expression of p16 in ovarian cancers represents an unfavourable prognostic factor. Histol Histopathol. 2008; 23(5): 531–8.
19. Wang Y, Chen S, Yan Z, Pei M. A prospect of cell immortalization combined with matrix microenvironmental optimization strategy for tissue engineering and regeneration. Cell Biosci. 2019; 9(1): 7.
20. Lazăr CS, Şovrea AS, Georgiu C, Crişan D, Mirescu ŞC, Cosgarea M. Different patterns of p16INK4a immunohistochemical expression and their biological implications in laryngeal squamous cell carcinoma. Rom J Morphol Embryol. 2020; 61(3): 697–706.
21. Li M, Yang J, Liu K, Yang J, Zhan X, Wang L, Shen X, Chen J, Mao Z. p16 promotes proliferation in cervical carcinoma cells through CDK6-HuR-IL1A axis. J Cancer. 2020; 11(6): 1457–67.

DOI: 10.20473/j.djmkg.v55.i3.p137–141

22. Lee SK, Kim YS. Current concepts and occurrence of epithelial odontogenic tumors: I. Ameloblastoma and adenomatoid odontogenic tumor. Korean J Pathol. 2013; 47(3): 191–202.
23. Diniz MG, Guimarães BVA, Pereira NB, de Menezes GHF, Gomes CC, Gomez RS. DNA damage response activation and cell cycle dysregulation in infiltrative ameloblastomas: A proposed model for ameloblastoma tumor evolution. Exp Mol Pathol. 2017; 102(3): 391–5.
24. Boscolo-Rizzo P, Da Mosto MC, Rampazzo E, Giunco S, Del Mistro A, Menegaldo A, Baboci L, Mantovani M, Tirelli G, De Rossi A. Telomeres and telomerase in head and neck squamous cell carcinoma: from pathogenesis to clinical implications. Cancer Metastasis Rev. 2016; 35(3): 457–74.
25. Merlin JPJ, Rupasinghe HPV, Dellaire G, Murphy K. Role of dietary antioxidants in p53-mediated cancer chemoprevention and tumor suppression. Oxid Med Cell Longev. 2021; 2021: 9924328.
26. LaPak KM, Burd CE. The molecular balancing act of p16(INK4a) in cancer and aging. Mol Cancer Res. 2014; 12(2): 167–83.
27. Pack LR, Daigh LH, Chung M, Meyer T. Clinical CDK4/6 inhibitors induce selective and immediate dissociation of p21 from cyclin D-CDK4 to inhibit CDK2. Nat Commun. 2021; 12(1): 3356.
28. Ombiro EM, Kwena A, Melly E, Kamau T, Maiyoh GK. Genotypes and prevalence of high-risk human papillomavirus among patients diagnosed with head and neck cancer at Alexandria Cancer Centre. JCO Glob Oncol. 2020; 6(Supplement 1): 30–30.
29. Mahale S, Bharate SB, Manda S, Joshi P, Bharate SS, Jenkins PR, Vishwakarma RA, Chaudhuri B. Biphenyl-4-carboxylic acid [2-(1H-indol-3-yl)-ethyl]-methylamide (CA224), a nonplanar analogue of fascaplysin, inhibits Cdk4 and tubulin polymerization: evaluation of in vitro and in vivo anticancer activity. J Med Chem. 2014; 57(22): 9658–72.
30. Salari Fanoodi T, Motalleb G, Yegane Moghadam A, Talaee R. p21 gene expression evaluation in esophageal cancer patients. Gastrointest Tumors. 2015; 2(3): 144–64.
31. Kumamoto H, Kimi K, Ooya K. Detection of cell cycle-related factors in ameloblastomas. J Oral Pathol Med. 2001; 30(5): 309–15.
32. Artese L, Piattelli A, Rubini C, Goteri G, Perrotti V, Iezzi G, Piccirilli M, Carinci F. p16 expression in odontogenic tumors. Tumori. 2008; 94(5): 718–23.

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 158/E/KPT/2021.

DOI: 10.20473/j.djmkg.v55.i3.p137–141