Original Article

# Immunohistochemical evaluation of yes-associated protein molecule in the odontogenic epithelium of different histopathological variants of ameloblastoma and unicystic ameloblastoma

Bodhiswata Pattnaik[1], Sweta Mohanty[2], Surya Narayan Das[1], Rachna Rath[1], Archana Bhatta[1], Sourav Mishra[1]

[1]Department of Oral Pathology and Microbiology, SCB Government Dental College and Hospital, Cuttack, [2]Department of Oral Pathology and Microbiology, Institute of Dental Sciences, Siksha 'O' Anusandhan, Deemed to be University, Bhubaneswar, Odisha, India

## Abstract

**Background:** Ameloblastoma is one of the major odontogenic neoplasms with an invasive and recurrence potential. Its tumourigenesis and proliferative capacity can be attributed to the activation or inactivation of certain molecular signalling pathways. Hippo signalling pathway is known to regulate diverse physiological processes related to mitosis and organ growth and is an emerging tumour suppressor pathway, the dysfunction of which is implicated in various diseases including cancers. Yes-associated protein1 (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) are the downstream effectors in the Hippo cascade, which on nuclear activation leads to cellular proliferation in various tumours.

**Aim:** The current study was undertaken to evaluate the expression of YAP in various histopathological variants of ameloblastoma and unicystic ameloblastoma.

**Materials and Methods:** Fifty formalin-fixed paraffin-embedded tissue samples of histopathologically diagnosed cases of ameloblastoma, and 10 histopathologically diagnosed cases of unicystic ameloblastoma were obtained from the departmental archives to evaluate the immunohistochemical expression of YAP both manually and by software analysis.

**Results:** More than 90% of cases of conventional ameloblastoma and unicystic ameloblastoma elicited positive expression of YAP. No statistical difference was found among different histopathological variants of conventional ameloblastoma. Significant difference between the means of all four quantitative score groups was observed.

**Conclusion:** In view of the modulating effect of YAP in tumourigenesis and its higher expression in ameloblastoma, further exploration of this molecule appears to be a promising area of research.

**Keywords:** Ameloblastoma, Hippo signalling pathway, immunohistochemistry, yes associated protein

**Address for correspondence:** Dr. Sweta Mohanty, Department of Oral Pathology and Microbiology, Institute of Dental Sciences, Siksha 'O' Anusandhan, Deemed to be University, Sum Hospital Road, Shampur, Bhubaneswar - 751 003, Odisha, India.
E-mail: pattanayak.sweta@gmail.com

**Submitted:** 12-May-2023, **Revised:** 11-Sep-2023, **Accepted:** 09-Oct-2023, **Published:** 15-Apr-2024

Access this article online

Quick Response Code:

Website: https://journals.lww.com/JPAT/

DOI: 10.4103/jomfp.jomfp_215_23



**How to cite this article:** Pattnaik B, Mohanty S, Das SN, Rath R, Bhatta A, Mishra S. Immunohistochemical evaluation of yes-associated protein molecule in the odontogenic epithelium of different histopathological variants of ameloblastoma and unicystic ameloblastoma. J Oral Maxillofac Pathol 2024;28:49-55.

## INTRODUCTION

The tooth-forming apparatus of the maxilla and mandible gives rise to varied pathologies, of which odontogenic tumours (OTs) account for not more than 3% of all diagnosed oral and maxillofacial lesions.[1] OTs are a heterogeneous group of lesions characterized by diverse biological behaviours, ranging from hamartomatous lesions to malignancy. Ameloblastoma known for its local aggressiveness, infiltrative potential, increased frequency of recurrences and significant morbidity is the most important subtype of OTs. It is a relatively rare oral tumour accounting for less than 1% of all tumours and cysts arising in the jaw bones.[2] Studies on tumourigenesis of ameloblastoma have implicated that almost 90% of cases exhibited mutations in genes belonging to the mitogen-activated protein kinase (MAPK) pathway.[3] Recurrent activating mutations in FGFR2, BRAF and RAS have implicated dysregulation of MAPK pathway signalling as a critical step in pathogenesis of ameloblastoma.[4] Several other mutations were also identified within genes not involved in the MAPK pathway like the Hippo, Sonic hedgehog (Shh) and WNT/β-catenin signalling pathways. Recently much emphasis has been given to the role of Hippo pathway in cell proliferation and metastasis. The main effectors, YAP and TAZ, of this tumour suppressor network when active undergo phosphorylation and proteasomal degradation in the cytoplasm which inactivates the transcriptional coactivators and regulates cellular growth, but when the Hippo pathway is inactive or suppressed, the translocation of YAP and TAZ to the nucleus facilitates transcriptional activity of SMADs, TEADs, TBX5 and RUNT1,2 and p73, which are involved in cell proliferation, survival, micro-RNA processing, metastases and stem cell maintenance. Thus, Hippo effectors YAP and TAZ are tumour suppressors when located in the cytoplasm but have oncogenic potential by facilitating transcriptional activation once translocated to the nucleus.[5]

With the aforementioned enigmas about the biologically aggressive nature of ameloblastoma and tumour-augmenting aspects of YAP, this study was undertaken to evaluate the immunohistochemical expression of YAP molecules in unicystic and conventional ameloblastomas. Additionally, YAP expression was analysed in different histopathological variants of ameloblastoma, namely, follicular, plexiform, basal cell, acanthomatous and desmoplastic.

## MATERIALS AND METHODS

Fifty formalin-fixed paraffin-embedded tissue samples of histopathologically diagnosed cases of conventional ameloblastoma, and 10 histopathologically diagnosed cases of unicystic ameloblastoma were obtained from tissue archives, under prescribed norms, regulations and ethical committee approval of Institutional Ethical Committee, vide reference number: IEC/SCBDCH/050/2019, over a period of 3 years (2018–2020).

Paraffin blocks each from follicular ameloblastoma (16), plexiform ameloblasatoma (17), acanthomatous ameloblastoma (9), granular cell ameloblastoma (5), desmoplastic ameloblastoma (2), basal cell ameloblastoma (1) and unicystic ameloblastoma (10) were selected and 3-μm thickness sections were collected on poly-L-lysine-coated slides. After paraffin removal by xylene and rehydration, the slides were treated with citrate buffer to unmask the antigen. The endogenous peroxidase and protein were blocked using supplied blockers. The expression of YAP1 protein was detected using YAP 1 monoclonal antibody (Invitrogen, Thermo Fisher Scientific, USA) at dilution 1:150. After post-primary blocking the sections were incubated with novolink polymer and were then developed with DAB (3,3'-Diaminobenzidine) using supplied substrate buffer. The sections were counterstained with haematoxylin and viewed under Lawrence and Mayo trinocular microscope equipped with built-in 5 M pixel camera with ScopeImage Advance Micro-image Process Software. Light and camera settings controlled by the software resulted in average background values of 63 ± 13 ms for the red, green and blue channels. Images were captured at 10 × and 40 × magnification, and the area of interest was selected based on regions with good contrast of DAB chromogen and haematoxylin.

### Manual quantification

As per the standard protocol of scoring, five random high-power fields were selected each for all samples and were analysed independently by two observers. The following intensity scores (IS) were attributed according to degree of staining: score 0 = absence of staining; score 1 = weak staining; score 2 = moderate staining; and score 3 = strong staining. The following proportion scores (PS) were attributed as per the percentage of stained cells (0 = 0–10%; 1 = 11–25%; 2 = 26–50%; 3 ≥50%). Overall staining was obtained as a product of IS (0–3) and PS (0–3) in a range of 0–9. An average of scores of five fields was considered as the final staining score of the sample. The staining score <3 was considered as low positive, between 3 and 5 as positive and a score ≥6 considered as high positive for YAP1 expression.

Software Quantification

### Semiquantitative analysis

The captured images were analysed using Image

J software (NIH, Bethesda, MD, USA) with an immunohistochemistry (IHC) profiler plugin, which created a pixel-by-pixel analysis profile of a digital IHC image and further assigned a semiquantitative score in the four-tier system proposed by Varghese F *et al*.[6]

Quantifying the immunoreaction as a log score of high positive, positive, low positive, negative and final score with histogram was obtained. All the deconvoluted images, histogram and log score were saved as JPEG images in a separate folder. The quantified immunoscore was entered into an Excel spreadsheet.

### Quantitative analysis

The optical density score was calculated using the following algebraic formula:

Optical density score = (Percentage contribution of high positive × 4+ Percentage contribution of positive × 3+ Percentage contribution of low positive × 2+ Percentage contribution of negative × 1)/100 as recommended by Jafari SM *et al*.[7]

### Statistical analysis

The validation of immunoscores of both the observers was done using SPSS software version 22 (Standard statistical analysis software) by implementing Kohen's kappa statistics (slight agreement: 0–0.2, fare agreement: 0.21–0.4, moderate agreement: 0.41–0.6, substantial agreement: 0.61–0.8 and almost perfect agreement: 0.81-1); $P < 0.005$ was considered statistically significant.

## RESULTS

The demographic variables are summarised in Table 1. Out of the 50 patients with conventional ameloblastoma, 60% were male and 40% female. The age of patients ranged from 13 to 68 years with a mean age of occurrence of 38.74 ± 10.89 years. A greater percentage (88%) of cases showed predilection for the mandible than the maxilla, and the angle of the mandible was the most commonly affected site. The mean tumour size was noted to be 5.02 ± 2.81 cm. Unicystic ameloblastoma showed similar results about gender and site predilection and showed an average tumour size of 4.2±0.0.89 cm.

Among the histopathological variants, the representation of follicular variant (16/50) and plexiform variant (17/50) were nearly equal. Positive YAP expression was found in 96% (48/50) of conventional ameloblastoma cases with negative expression in one case of follicular variant and granular cell variant. YAP was positively expressed in both ameloblast-like cells and stellate reticulum-like cells in ameloblastic follicles [Figure 1]. In total, 90% (9/10) cases of unicystic ameloblastoma also showed positive expression for YAP. Fisher's exact test was applied to observations of observer 1 and observer 2, and software method showed insignificant statistical difference ($P > 0.05$) in the expression of YAP1 among different variants of ameloblastoma. Histogram profiling of the DAB stained nuclear stained images of follicular, plexiform and unicystic ameloblastoma [Figures 2-4]. The scoring assigned by IHC profiler was almost in perfect agreement with manual scoring by pathologist1/observer 1 (kappa – 0.872) and pathologist2/observer 2 (kappa – 0.803). Interobserver agreement of score was also in substantial agreement (kappa – 0.802). Results obtained by software method [Table 2] were graphically represented [Figure 5] and were utilized for further statistical comparisons as it was reproducible and reduced the risk of intra- or interobserver bias.

Analysis of the scores of four zones of software assessment and the IOD revealed a significant difference with an increase in the mean optical density score from

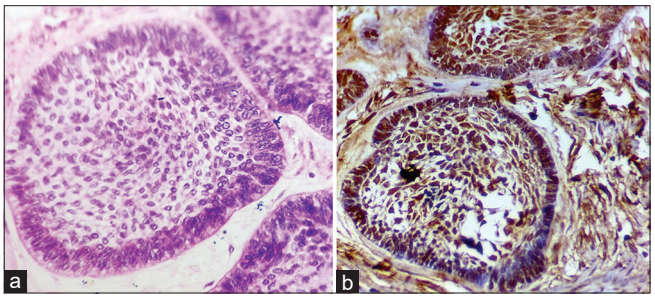


**Figure 1:** Photomicrograph showing (a) ameloblastic follicle delineated by peripheral columnar cells displaying reverse polarization and central stellate reticulum-like cells (x40×) (original). (b) Positive Yes-associated protein expression in peripheral ameloblast-like and central stellate reticulum-like cells within the ameloblastic follicles (x40×) (original)

**Table 1: Demographic distribution of YAP1 expression among study samples (original)**

| Group | *n* (%) | Age (Mean±SD) | Male *n* (%) | Female *n* (%) | Maxilla *n* (%) | Mandible *n* (%) | Tumour size (cm) (Mean±SD) |
|---|---|---|---|---|---|---|---|
| Follicular | 16 (26.7) | 40.25±10.36 | 9 (56.3) | 7 (43.8) | 1 (6.3) | 15 (93.8) | 5.12±2.68 |
| Plexiform | 17 (28.3) | 37.71±12.28 | 11 (64.7) | 6 (35.3) | 1 (5.9) | 16 (94.1) | 5.49±3.50 |
| Acanthomatous | 9 (15) | 43.78±10.67 | 6 (66.7) | 3 (33.3) | 2 (22.2) | 7 (77.8) | 4.55±2.26 |
| Granular cell | 5 (8.3) | 29.40±3.87 | 2 (40) | 3 (60) | 1 (20) | 4 (80) | 5.64±1.49 |
| Desmoplastic | 2 (3.3) | 34±5.65 | 2 (100) | 0 | 1 (50) | 1 (50) | 2.5±2.12 |
| Basal cell | 1 (1.7) | 43 | 0 | 1 (100) | 0 | 1 (100) | 2 |
| Unicystic | 10 (16.7) | 38.40±8.73 | 5 | 5 | 1 | 9 | 4.2±0.0.89 |

**Table 2: Expression of YAP 1 among conventional and unicystic ameloblastoma (original)**

| Group | *n* (%) | Negative *n* (%) | Low positive *n* (%) | Positive *n* (%) | High positive *n* (%) | *P* |
|---|---|---|---|---|---|---|
| Follicular | 16 (26.6) | 1 (6.3) | 4 (25) | 9 (56.3) | 2 (12.5) | 0.461 |
| Plexiform | 17 (28.3) | 0 | 4 (23.5) | 8 (47.1) | 5 (29.4) | |
| Acanthomatous | 9 (15) | 0 | 5 (55.6) | 4 (44.4) | 0 | |
| Granular | 5 (8) | 1 (20) | 2 (40) | 2 (40) | 0 | |
| Desmoplastic | 2 (3) | 0 | 0 | 2 (100) | 0 | |
| Basal cell | 1 (0.1) | 0 | 0 | 1 (100) | 0 | |
| Unicystic | 10 (16.6) | 1 (10) | 5 (50) | 3 (30) | 1 (10) | |
| Total | 60 | 3 | 20 | 29 | 8 | |

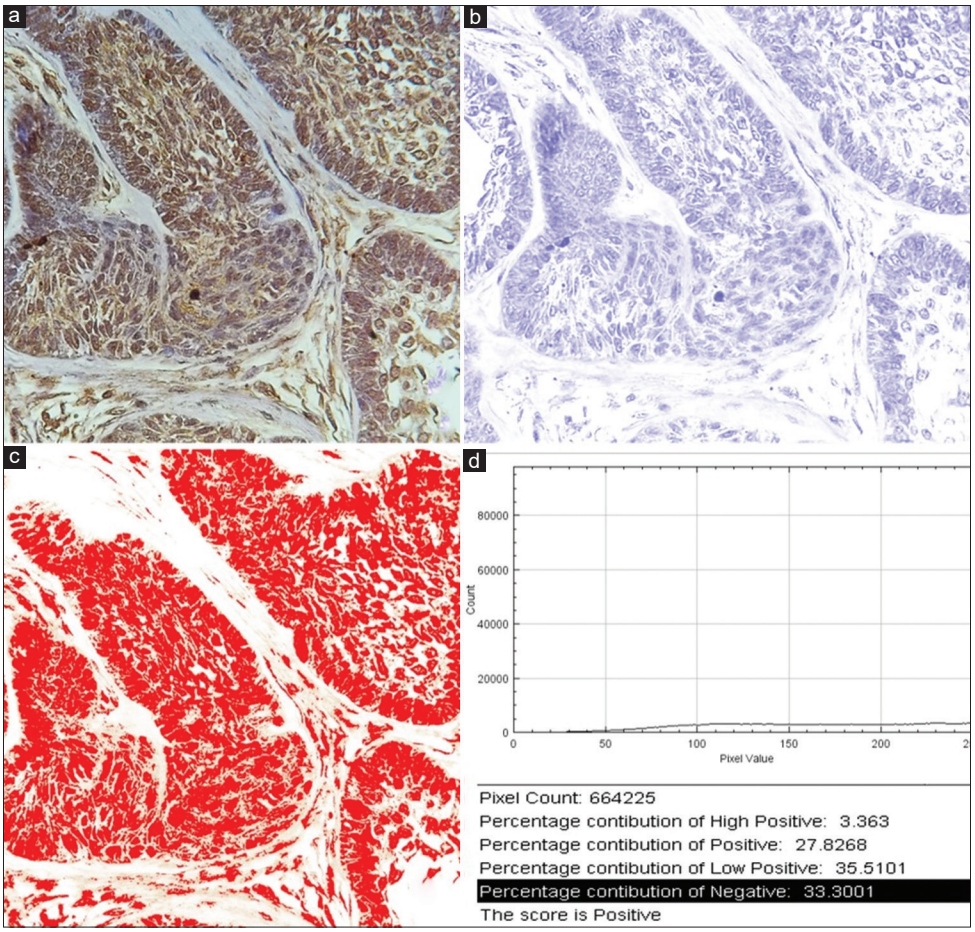


**Figure 2:** Representative histogram profile and a score of the nuclear stained image of follicular ameloblastoma using IHC profiler. (a) Photomicrograph of immunohistochemistry image of YAP. (b and c) Deconvoluted images highlighted and selected immunoexpression. (d) Histogram and log of quantified immunoexpression of YAP (original)

negative to low positive, low positive to positive and positive to high positive. Hence, it can be projected as a method for quantitatively scoring an image to facilitate statistical comparison.

## DISCUSSION

Ameloblastoma though considered locally aggressive has a slow pattern of growth pertaining to which it is diagnosed late in its clinical course and causes substantial tissue damage and high morbidity. The tumourigenesis of ameloblastoma reflects dysregulation at the molecular level of multiple genes associated with MAPK, Hippo, Shh and WNT/β-catenin signalling pathways. The Hippo signalling pathway has been recognized now as an important player in both organ size control and tumourigenesis.[8] YAP is one of the major downstream effectors of the Hippo pathway, and its overexpression in the nucleus of hepatocellular carcinoma cells in the mouse model demonstrated its association with hepatocarcinogenesis.[9] Overexpression of the YAP molecule was also noted in other bodily cancers like oesophageal, colon, prostate, ovarian and breast cancers.[10-14]

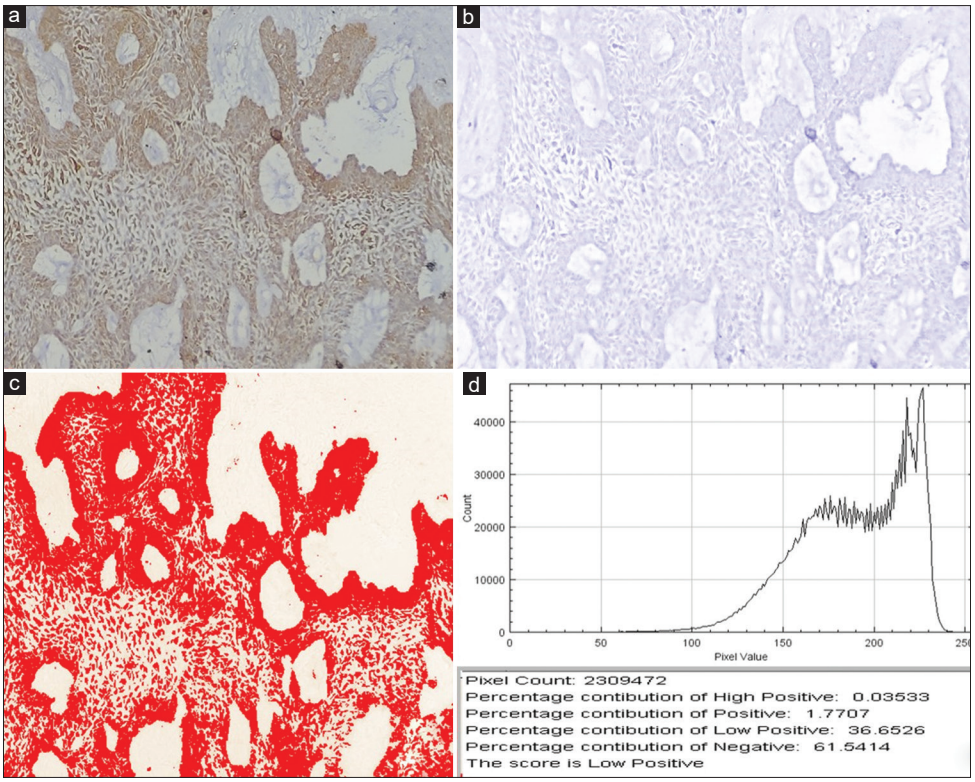


**Figure 3:** Representative histogram profile and a score of the nuclear stained image of plexiform ameloblastoma using IHC profiler. (a) Photomicrograph of immunohistochemistry image of YAP. (b and c) Deconvoluted images highlighted and selected immunoexpression. (d) Histogram and log of quantified immunoexpression of YAP (original)

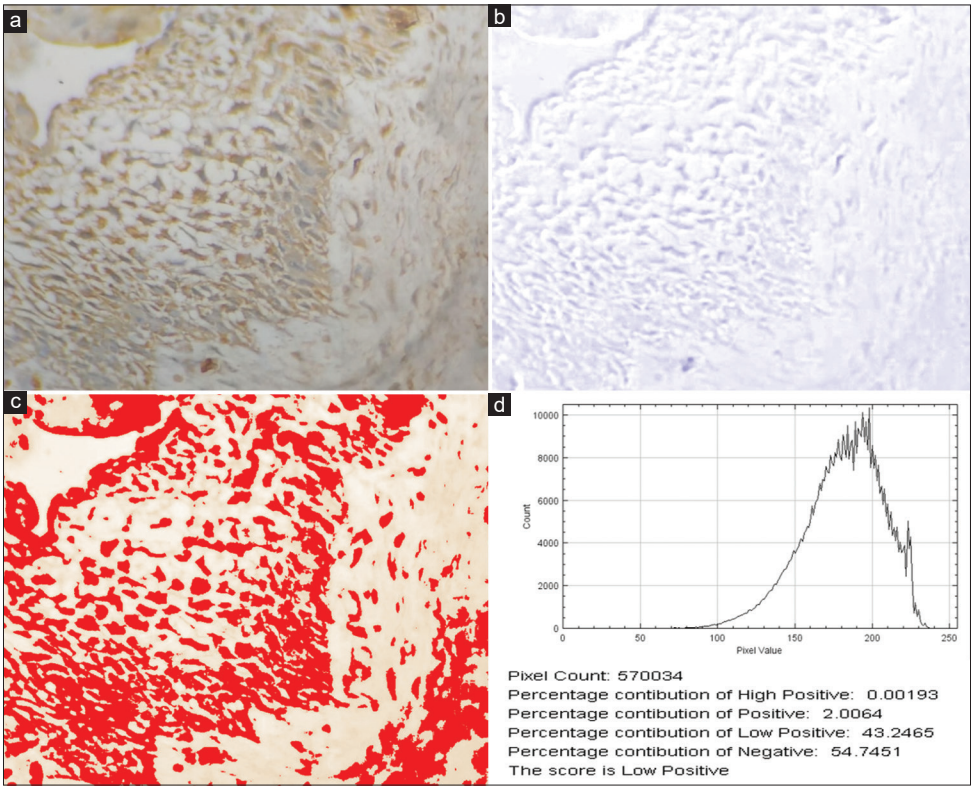


**Figure 4:** Representative histogram profile and a score of a nuclear stained image of unicystic ameloblastoma using IHC profiler. (a) Photomicrograph of immunohistochemistry image of YAP. (b and c) Deconvoluted images highlighted and selected immunoexpression. (d) Histogram and log of quantified immunoexpression of YAP (original)

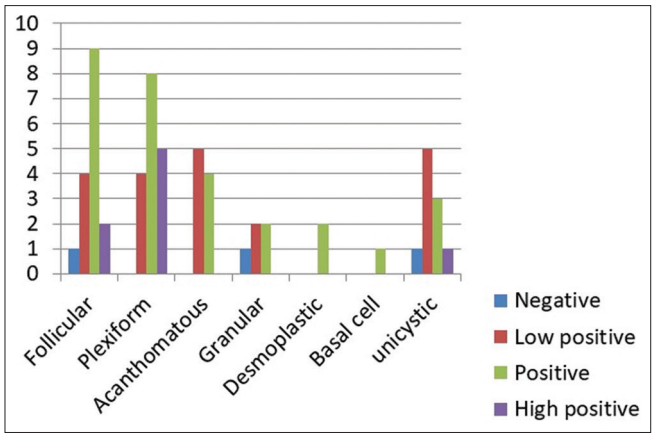


**Figure 5:** Graphical representation of YAP expression in different ameloblastoma variants (original)

In head and neck squamous cell carcinoma (SCC), overexpression and nuclear localization have shown significant association with tumour size, grade and prognosis.[15] Furthermore, YAP was more frequently expressed in the invasive tumour margin as compared to the tumour interior in a pilot study by Chen L *et al*.[16] The main mechanism towards the progression of epithelial carcinoma involves disruption of apical-basal polarisation, invasive migration and trauma/inflammation, which can induce YAP/TAZ nuclear localisation.[17] Apical-basal polarity regulates YAP/TAZ subcellular localization and activity through interactions with cell-polarity proteins (scribble and crumbs) or cell-junction molecules (angiomotin and α-catenin). Cumulative evidence indicates that YAP/TAZ act as sensors of mechanical forces and modulate the fibrotic response as well as the behaviour of cancer cells.[18] Oral submucous fibrosis (OSF), a potentially malignant disorder with progressive fibrosis of the submucosal connective tissue, leads to an increase in the expression of YAP in the overlying epithelium which could be a plausible mechanism for carcinogenesis in OSF.[19] Although many studies have evaluated the expression of YAP in cancers, there are limited studies on OTs. Odontogenesis is a complex process that results from sequential and reciprocal interactions between oral epithelium and the underlying neural-crest-derived mesenchyme. When the role of Hippo signalling was analysed during tooth development in transgenic mice, overexpression of YAP in oral and dental epithelium led to the formation of the aberrant enamel organ and widened dental lamina, thus affecting tooth morphogenesis.[20] Anand R *et al*. evaluated the expression of YAP in odontogenic epithelial islands in dental follicle and ameloblastic tissue. YAP expression when compared in stellate cells of normal dental follicles, which represent the mature and quiescent component and often remain dormant in the ectomesenchymal tissue, and ameloblastic follicles revealed a statistically significant difference with strong positive expression in both stellate cells and basal/peripheral cells of ameloblastoma.[21] Another study by Man QW *et al*. on the expression of YAP/TAZ in keratocystic odontogenic tumours (KCOT) and its association with proliferative behaviour found upregulation of YAP/TAZ and its downstream proteins (Cyr61, CTGF) in KCOT. In addition, double-labelling immunofluorescence revealed a synchronous distribution for YAP/TAZ with Ki-67 in KCOT samples, thus suggesting the involvement of YAP/TAZ in the proliferative behaviour of KCOT.[22] In our study, YAP expression was found to be positive in 96% of ameloblastoma cases with both peripheral ameloblast-like cells and central stellate reticulum-like cells showing positive staining with YAP antibody. Statistically significant differences were not elicited among different histopathological variants probably due to the uneven distribution of histopathological variants in the study sample owing to the rarity of certain variants like desmoplastic and basal cell ameloblastoma. The peripheral cells expressed YAP in a similar manner to other proliferative markers.[23]

An automated IHC scoring system with quantitation by optical density score of IHC images was used to have a detailed and uniform IHC data quality. The expression of YAP as assessed by IHC profiler and pathologist 1/observer 1 (kappa – 0.872) and pathologist 2/observer 2 (kappa – 0.803) were almost in perfect agreement with good strength of agreement (kappa value 0.802) between the observers. The mean value of IHC optical density increased from negative to low positive, from low positive to positive and from positive to high positive. Thus, quantitative scoring of an image can be proposed as a relevant method to facilitate statistical comparison. Although no significant difference was found in the expression of the YAP molecule among different variants of amelobastoma and among the peripheral ameloblast-like cells and centrally placed stellate reticulum cells, elevated expression suggests its invasive and aggressive potential. Thus, YAP could be a plausible aspect for research and targeted therapeutics in ameloblastoma.

## Future research

YAP expression was positive in both the cases of desmoplastic ameloblastoma (DA) in the present study. With a distinctive histopathological feature of stromal desmoplasia in DA and fibrosis and stiff ECM facilitating the YAP/TAZ localization to the nucleus, elevating their transcriptional activity, the role of YAP/TAZ in the aggressiveness DA can be further investigated in a big sample size.

## CONCLUSIONS

Despite the discovery of a multitude of molecular markers of pathways involved in the pathogenesis of odontogenic cysts and tumours, exact pathways responsible for aggressive behaviour in odontogenic cysts and tumours remain elusive. In light of recent discoveries relating to the modulating role of YAP on various signalling pathways during tumourigenesis, further exploration of this molecule appears to be a promising area of research. Future studies with a larger sample size and equal representation of histopathological variants are recommended to substantiate the findings and expand our knowledge of the functioning of YAP in different variants of ameloblastoma.

### Financial support and sponsorship
Nil.

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

## REFERENCES

1. Kokubun K, Yamamoto K, Nakajima K, Akashi Y, Chujo T, Takano M, *et al*. Frequency of odontogenic tumors: A single center study of 1089 cases in Japan and literature review. Head Neck Pathol 2022;16:494-502.
2. Fregnani ER, Perez DE, Paes de Almeida O, Fonseca FP, Soares FA, Castro-Junior G, *et al*. BRAF-V600E expression correlates with ameloblastoma aggressiveness. Histopathology 2017;70:473-84.
3. Brown NA, Betz BL. Ameloblastoma: A Review of recent molecular pathogenetic discoveries. Biomark Cancer 2015;7(Suppl 2):19-24.
4. Harvey K, Tapon N. The Salvador-Warts-Hippo pathway-an emerging tumour-suppressor network. Nat Rev Cancer 2007;7:182-91.
5. Sebio A, Lenz HJ. Molecular pathways: Hippo signaling, a critical tumor suppressor. Clin Cancer Res 2015;21:5002-7.
6. Varghese F, Bukhari AB, Malhotra R, De A. IHC Profiler: An open source plugin for the quantitative evaluation and automated scoring of immunohistochemistry images of human tissue samples. PLoS One 2014;9:e96801.
7. Jafari SM, Hunger RE. IHC optical density score: A new practical method for quantitative immunohistochemistry image analysis. Applied immunohistochemistry & molecular morphology 2017;25:e12-13.
8. Zhu C, Li L, Zhao B. The regulation and function of YAP transcription co-activator. Acta Biochim Biophys Sin (Shanghai) 2015;47:16-28.
9. Xu MZ, Yao TJ, Lee NP, Ng IO, Chan YT, Zender L, *et al*. Yes-associated protein is an independent prognostic marker in hepatocellular carcinoma. Cancer 2009;115:4576-85.
10. Steinhardt AA, Gayyed MF, Klein AP, Dong J, Maitra A, Pan D, *et al*. Expression of Yes-associated protein in common solid tumors. Hum Pathol 2008;39:1582-9.
11. Muramatsu T, Imoto I, Matsui T, Kozaki K, Haruki S, Sudol M, *et al*. YAP is a candidate oncogene for esophageal squamous cell carcinoma. Carcinogenesis 2011;32:389-98.
12. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012;487:330-7.
13. Hall CA, Wang R, Miao J, Oliva E, Shen X, Wheeler T, *et al*. Hippo pathway effector yap is an ovarian cancer oncogene. Cancer Res 2010;70:8517-25.
14. Cordenonsi M, Zanconato F, Azzolin L, Forcato M, Rosato A, Frasson C, *et al*. The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. Cell 2011;147:759-72.
15. Ge L, Smail M, Meng W, Shyr Y, Ye F, Fan KH, *et al*. Yes-associated protein expression in head and neck squamous cell carcinoma nodal metastasis. PLoS One 2011;6:e27529.
16. Chen L, Chan SW, Zhang X, Walsh M, Lim CJ, Hong W, *et al*. Structural basis of YAP recognition by TEAD4 in the hippo pathway. Genes Dev 2010;24:290-300.
17. Elbediwy A, Vincent-Mistiaen ZI, Thompson BJ. YAP and TAZ in epithelial stem cells: A sensor for cell polarity, mechanical forces and tissue damage. Bioessays 2016;38:644-53.
18. Noguchi S, Saito A, Nagase T. YAP/TAZ Signaling as a molecular link between fibrosis and cancer. Int J Mol Sci 2018;19:3674.
19. Sharma M, Hunter KD, Fonseca FP, Shetty SS, Radhakrishnan R. Role of yes-associated protein and transcriptional coactivator with PDZ-binding motif in the malignant transformation of oral submucous fibrosis. Arch Oral Biol 2021;128:105164.
20. Liu M, Zhao S, Wang XP. YAP overexpression affects tooth morphogenesis and enamel knot patterning. J Dent Res 2014;93:469-74.
21. Anand R, Sarode SC, Sarode GS, Rajpurohit LS, Patil S. Yes-associated protein (YAP) molecule expression in odontogenic epithelium of ameloblastoma. Clin Oral Investig 2019;23:2389-94.
22. Man QW, Ma YQ, Liu JY, Zhao Y, Liu B, Zhao YF. Expression of YAP/TAZ in keratocystic odontogenic tumors and its possible association with proliferative behavior. Biomed Res Int 2017;2017:4624890.
23. Gupta K, Chaturvedi TP, Gupta J, Agrawal R. Cell proliferation proteins and aggressiveness of histological variants of ameloblastoma and keratocystic odontogenic tumor. Biotech Histochem 2019;94:348-51.