

ABERRANT IMMUNOHISTOCHEMICAL EXPRESSION OF P53 IN AMELOBLASTOMA, AMELOBLASTIC CARCINOMA, DENTIGEROUS CYST AND ODONTOGENIC KERATOCYST: A COMPARATIVE STUDY

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ABSTRACT

Background: odontogenic lesions comprise a diverse group exhibiting a wide range of clinical and biological behaviors, demanding a meticulous understanding for accurate diagnosis and management. In response to DNA damage, the p53 tumor suppressor protein functions as a key regulator, inducing either cell cycle arrest or apoptosis. Mutations in the p53 gene are frequently observed in human cancers; however, the role of p53 expression in odontogenic lesions remains unclear.

Material and methods: A retrospective design was employed to evaluate and compare p53 protein expression patterns, detected by immunohistochemistry, in ameloblastoma, ameloblastic carcinoma, dentigerous cyst, and odontogenic keratocyst. Formalin-fixed and paraffin-embedded archival tissue specimens from 42 patients were analyzed for p53 expression. Immunopositivity mean area fraction was determined following the removal of connective tissue. Statistical analysis to compare mean area fraction between groups was performed. Clinical records were reviewed to collect demographic data (age, gender and lesion site) for all cases included in the study.

Results: immunohistochemical p53 expression was demonstrated as cytoplasmic brown granular staining in the epithelial cells of ameloblastomas, dentigerous cyst and odontogenic keratocyst groups. Conversely, the ameloblastic carcinoma group exhibited significantly less mean values of P53 immunopositivity than the other cases. There was no significant difference between ameloblastoma, dentigerous cyst and odontogenic keratocyst groups. Meanwhile, our findings revealed an overall male predominance and prevalence to the mandible.

Conclusions: aberrant expression of p53 protein could be implicated in the pathogenesis of odontogenic lesions. Further research is needed to elucidate the precise mechanisms underlying p53-mediated progression for these lesions.

KEYWORDS: p53, odontogenic keratocyst, dentigerous cyst, ameloblastoma, ameloblastic carcinoma

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INTRODUCTION

Odontogenic cysts and tumors encompass a heterogeneous group of lesions arising from remnants of the tooth germ during development. This diverse group exhibits a wide range of clinical and biological behaviors, necessitating a thorough understanding for accurate diagnosis and management ^(1,2).

Ameloblastoma (AB) is the second most commonly encountered odontogenic tumor after odontomas. It is considered clinically significant due to its aggressive nature and high recurrence rate ⁽³⁻⁵⁾. Managing ABs requires a multidisciplinary approach involving a team of healthcare professionals with expertise in oral and maxillofacial surgery, pathology, and radiology. AB has recently been shown to harbor a BRAF (V600E) mutation which is associated with overexpression of p53. Although challenging to treat, understanding the underlying molecular mechanisms brings us closer to developing targeted therapies that may minimize the need for invasive surgery ^(3,6). Ameloblastic carcinoma (AC) is an exceptionally uncommon type of malignant tumor originating from the odontogenic epithelium, comprising only about 2% of all odontogenic tumors. It may originate from a pre-existing ameloblastoma or odontogenic cyst or appear de novo ⁽⁷⁻⁹⁾. The term “ameloblastic carcinoma” was introduced by Elazy in 1982, and in 1984, AC was identified as a distinct entity separate from malignant ameloblastoma (MA) ^(9,10).

Dentigerous cysts (DCs) are the most prevalent type of developmental cyst in the jaw. DCs arise from fluid buildup around unerupted teeth, typically molars and canines ^(1,11). While having a low recurrence rate, neglected DCs can cause bone and root resorption, and tooth displacement ^(2,11). On rare occasions, DCs have also been exceptionally linked to the development of more concerning pathologies like ameloblastoma, squamous cell carcinoma, and mucoepidermoid carcinoma in rare, neglected cases ⁽¹²⁾.

Odontogenic keratocyst (OKCs), previously considered tumors, are now classified as aggressive developmental cysts with high recurrence potential (as per the 2022 World Health Organization (WHO) classification). Unlike DCs, OKC growth appears driven by increased epithelial activity rather than fluid pressure, leading to rapid expansion and potential bone destruction ⁽¹³⁻¹⁵⁾. Studies suggest that enhanced epithelial activity may be a key driver of OKC aggressiveness ^(8,14,16). Increased mitotic activity and a higher proliferation rate within the OKC epithelial lining have been observed, potentially contributing to the rapid expansion and locally destructive nature of these lesions ⁽¹³⁾.

P53 protein is a crucial tumor suppressor protein that can detect cellular stress and has a key role in regulating the cell cycle and repairing DNA ^(17,18). Under typical physiological conditions, p53 is expressed at a minimal level. In case of DNA damage, p53 is induced and mediates cell cycle arrest allowing cells to repair. On the other hand, when DNA damage cannot be repaired, p53 applies its pro-apoptotic function to eliminate these cells to prevent the transfer of damaged DNA to daughter cells. ^(8,16,19). Accordingly, p53 “the guardian of the genome” has the ability to maintain the integrity of the genomic profile ^(18,19).

To sustain low levels of the p53 protein under normal conditions, it's continuously marked for degradation by the E3 ubiquitin ligase protein, mouse double minute2 (MDM2). There is a negative feedback loop between MDM2 and p53 whereby MDM2 protein regulates p53's activity at the transcription level, and in turn, p53 regulates the activity of MDM2. MDM2 is believed to decrease p53 in non-stressed cells via at least three mechanisms ^(17,20). First, MDM2 binds to the same area of p53 as the transcription factor TF_{II}D, reducing p53's capacity to transactivate target genes. Second, the MDM2 protein may drive the p53/MDM2 complex's nuclear export. Third,

MDM2 can operate as a ubiquitin ligase, directing p53 to be degraded by the proteasome^(8,20,21).

Cellular stress triggers this process by inhibiting p53 degradation and potentially increasing its mRNA translation, leading to its rapid build-up within the nucleus⁽¹⁷⁾. When cells experience stress, dysregulated p53 expression is activated. Initially, the half-life of p53 (normally 5–20 minutes in most cell types) rapidly increases, this may be coupled with increased mRNA translation, leading to its rapid accumulation. Secondly, a conformational shift occurs that activates p53's function as a transcription regulator within these cells^(17,22).

Protein kinases that target the activation region of the p53 transcriptional pathway can be broadly classified into two categories. The first category, mitogen activated protein kinase family (MAPK) which responds to an array of stressors. The second category is comprised of protein kinases, such as ataxia-telangiectasia mutated (ATM), which are associated with genome integrity checkpoints^(17,23).

In light of the aforementioned facts, it stands to reason that p53 overexpression would inhibit tumor development and serve as a compensating mechanism in the event of DNA damage. Nonetheless, numerous studies have demonstrated that increased p53 expression is typically linked to increased tumorigenesis. While the role of p53 in various human malignancies is well-established, its function in odontogenic lesions remains less clear⁽²⁴⁾. The current study aims to evaluate and contrast the immunohistochemical expression of p53 protein in AB, AC, DC and OKC. This study tests the null hypothesis of no variation in p53 expression across odontogenic lesions (AB, AC, DC and OKC).

MATERIALS AND METHODS

Study design and case selection

The current study was a retrospective observational study employing immunohistochemistry

(IHC). Forty-two paraffin-embedded tissue blocks were retrieved retrospectively from the archives of our Oral Pathology Department, following ethical approval from the Faculty's Ethics Committee. The timeframe for sample collection spanned from 2013 to 2023. Inclusion criteria comprised non-recurrent lesions. The included lesions were as follows: 11 ABs (of which five were follicular- FAB, three plexiform-PABs, and three unicystic ABs -UAB), 6 ACs, 12 DCs and 13 OKCs.

Hematoxylin and eosin (H&E)-stained slides from these specimens were re-evaluated by two independent oral pathologists to confirm diagnoses according to the WHO Classification of Head and Neck Tumors, 5th edition (2022).

Immunohistochemical staining

On positively- charged slides, 4µm thickness paraffin- sections were mounted. Immunohistochemical staining was performed using the avidin/ biotin/ peroxidase complex (ABC) technique. Sections from each group were briefly incubated with a p53 rabbit anti-human polyclonal antibody (Ab-clonal, Woburn, MA, USA, Catalog No.: A3185) at a dilution of 1:100 for 60 minutes at room temperature. Application of biotinylated/ secondary antibody and avidin-conjugated- horseradish peroxidase (Vectastain- ABC-HRP kit, Vector Laboratories) was performed following the manufacturer's instructions. Marker expression was visualized using diaminobenzidine (DAB, Sigma) as the chromogen, resulting in a brown precipitate at the site of antigen-antibody complex formation. Negative control sections were processed identically, except that non-immune serum was substituted for the primary or secondary antibodies to assess for non-specific staining. Immunohistochemically stained sections were examined using an Olympus BX-53 microscope.

Immunohistochemical Evaluation

Five microscopic fields exhibiting the strongest p53 immunoreactivity were selected

from each slide for photomicrography. Images were captured at a magnification of x20 using a digital video camera (Olympus, C5060, Japan). The immunohistochemical reaction product was quantified using image-analysis software (ImageJ 1.53t, National Institutes of Health, USA). Briefly, the software calculated the total area (area percentage %) of immunopositive reaction. The immunopositivity mean area fraction (MAF) was determined following the removal of connective tissue (in order to assess immunoreactivity specifically within the epithelium) as follows: images were transferred to the computer system for analysis where brightness and contrast were corrected manually. Areas of interest were selected and the excluded areas were covered by white pixels. Following that, the images were converted into 8-bit monochrome type then color thresholding was performed and area fraction was automatically measured. The employed method represents a reproducible and time-efficient approach for quantifying immunoreactivity⁽²⁵⁾.

Statistical analysis

Data were investigated for normality using Kolmogorov-Smirnov test of normality. Data were presented as mean \pm standard deviation (SD). The results of the Kolmogorov-Smirnov test showed that data recorded values were normally distributed. The comparison between groups was performed using one-way analysis of variance (ANOVA) test. Subsequently, pairwise comparisons were conducted using Tukey's post hoc test. The significance level was established at $p \leq 0.05$. Statistical analysis was done using SPSS 23 (Statistical Package for Scientific Studies (SPSS), Inc., Chicago, IL, USA) for Windows.

RESULTS

Patient Demographics and Lesion Distribution

Clinical records were reviewed to collect demographic information (age, gender and lesion site) for all patients. Table 1 summarizes these findings.

TABLE (1) Clinical-demographic characteristics of the cases involved in the study.

Variable	Total	FAB	UAB	PAB	AC	DC	OKC
Cases (n)	42	5	3	3	6	12	13
Age /years							
Range		20-35	10-25	23-40	14-25	12-39	15-76
Gender							
Females		0	1	2	3	2	6
Males		5	2	1	3	10	7
Anatomical Location							
Maxilla						2	4
Anterior						2	2
Posterior						0	2
Mandible		5	3	3	6	10	9
Anterior						0	0
posterior		5	3	3	6	10	9

Gender Distribution: A distinct gender predominance was observed for the cases included in this study. All included FAB cases were exclusively male (n=5), while UAB exhibited a male bias (2:1 male-to-female ratio). Conversely, a female predilection was evident for included PAB cases with a 2:1 female-to-male ratio. DC and OKC, also exhibited male predominance (5:1 and 7:6 male-to-female ratios, respectively). Whereas AC displayed no significant gender preference.

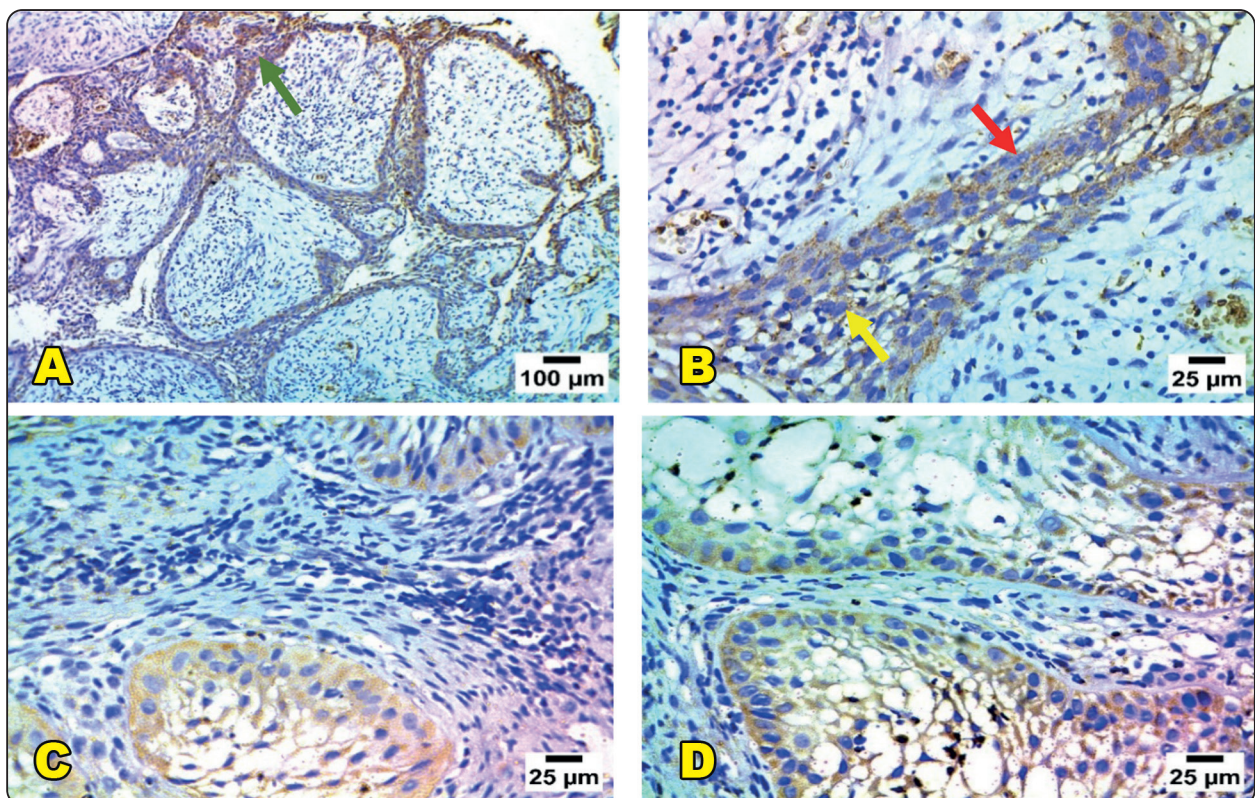
Lesion Site: The posterior mandible emerged as the most prevalent site for all lesion types included in the study (100% for ameloblastoma subtypes, 80.33% for DC, and 69.2% for OKC). A smaller portion of DC cases (16.6%) and OKC cases (30.7%) were located in the maxilla. Notably, all maxillary DC cases were positioned anteriorly, while half (50%) of maxillary OKC cases were anterior.

P53 Expression Results:

Immunohistochemical analysis revealed cytoplasmic p53 protein expression as brown granular staining in the epithelial cells of ABs, DCs, and OKCs. Conversely, ACs appeared to be mostly immune-negative for p53.

Ameloblast-like cells and stellate reticulum-like cells in AB cases displayed cytoplasmic immunopositivity p53. Notably, the expression appeared to be more intense in PAB and UAB compared to FAB (*Figure 1*). ACs appeared to be mostly immune-negative (*Figure 2*).

In OKC cases, positive brown cytoplasmic p53 immunostaining was present throughout all layers of the epithelial lining. A similar pattern of expression was also noted in the DC cases. P53 expression was absent in the stroma except for inflammatory cells within the wall of inflamed dentigerous cysts (*Figure 3*).



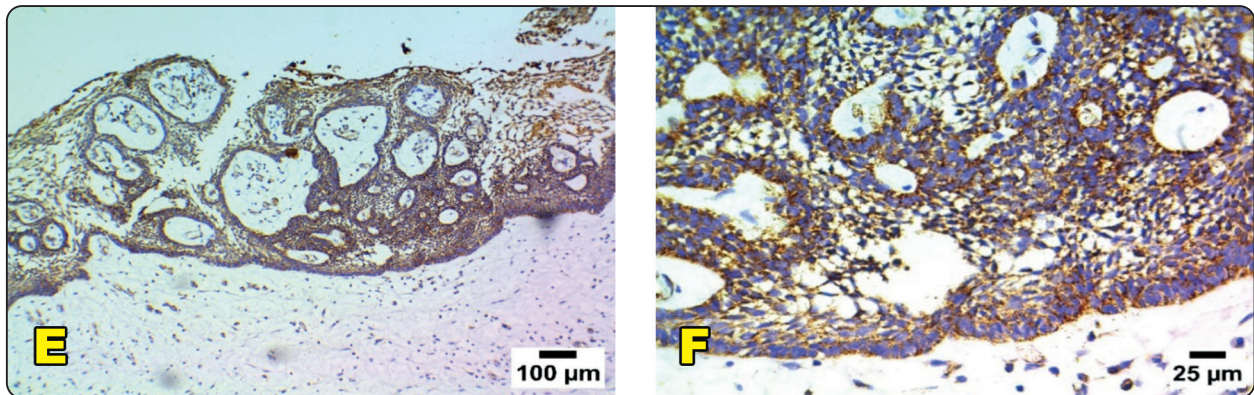


Fig. (1) Photomicrograph of: (A) PA exhibiting cytoplasmic immunopositivity in the majority of the ameloblast-like cells and also the stellate-reticulum like cells, few cells were immunonegative (green arrow) (p53 X10), (B) PA exhibiting cytoplasmic granular immunopositivity in the ameloblast-like cells (red arrow) and also stellate-reticulum like cells (yellow arrow). Note the nuclei were immunonegative in both cell types. (p53 X40), (C), (D) FA exhibiting cytoplasmic immunopositivity in the majority of the ameloblast-like cells as well as the stellate-reticulum like cells, few cells were immunonegative (p53 X40), (E) UA exhibiting immunopositivity in the plexiform lining (p53 X10), (F) UA demonstrating granular cytoplasmic immunopositivity in the ameloblast like cells as well as the stellate reticulum like cells (p53 X40).

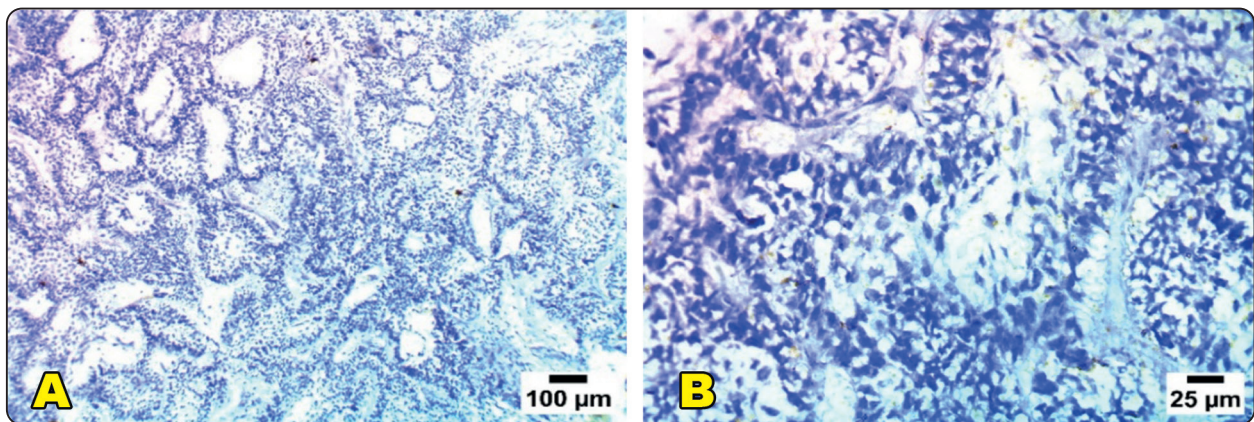


Fig. (2) Photomicrograph of: (A) AC showing immunonegativity in all cells (p53 X10), (B) AC showing abnormal N/C ratio, cellular and nuclear pleomorphism (p53 X40).

Statistical Results:

The highest value of p53 MAF was recorded in DC group (11.63±3.80); followed by AB group (9.20±2.62); then OKC group (8.93±2.12) and the lowest value in AC group (1.40±0.61). One-way ANOVA identified a statistically significant difference in p53 expression among different groups (p = 0.018). However, post hoc analysis using Tukey’s test did not reveal any significant pairwise differences between groups AB, DC, and OKC. (p>0.05) (Table 2, Figure 4).

TABLE (2) Showing the mean ± SD values, results of ANOVA and post hoc tests for the comparison between different groups regarding Area Fraction% for P53.

Groups	Mean	±SD
I: Ameloblastoma	9.20A	2.62
II: Dentigerous Cyst	11.63A	3.80
III: Odontogenic Keratocyst	8.93A	2.12
IV: Ameloblastic Carcinoma	1.40B	0.61

SD: Standard deviation; SE: Standard Error
*Significance level p≤0.05, *significant*
Tukey’s post hoc: Means sharing the same superscript letter are not significantly different

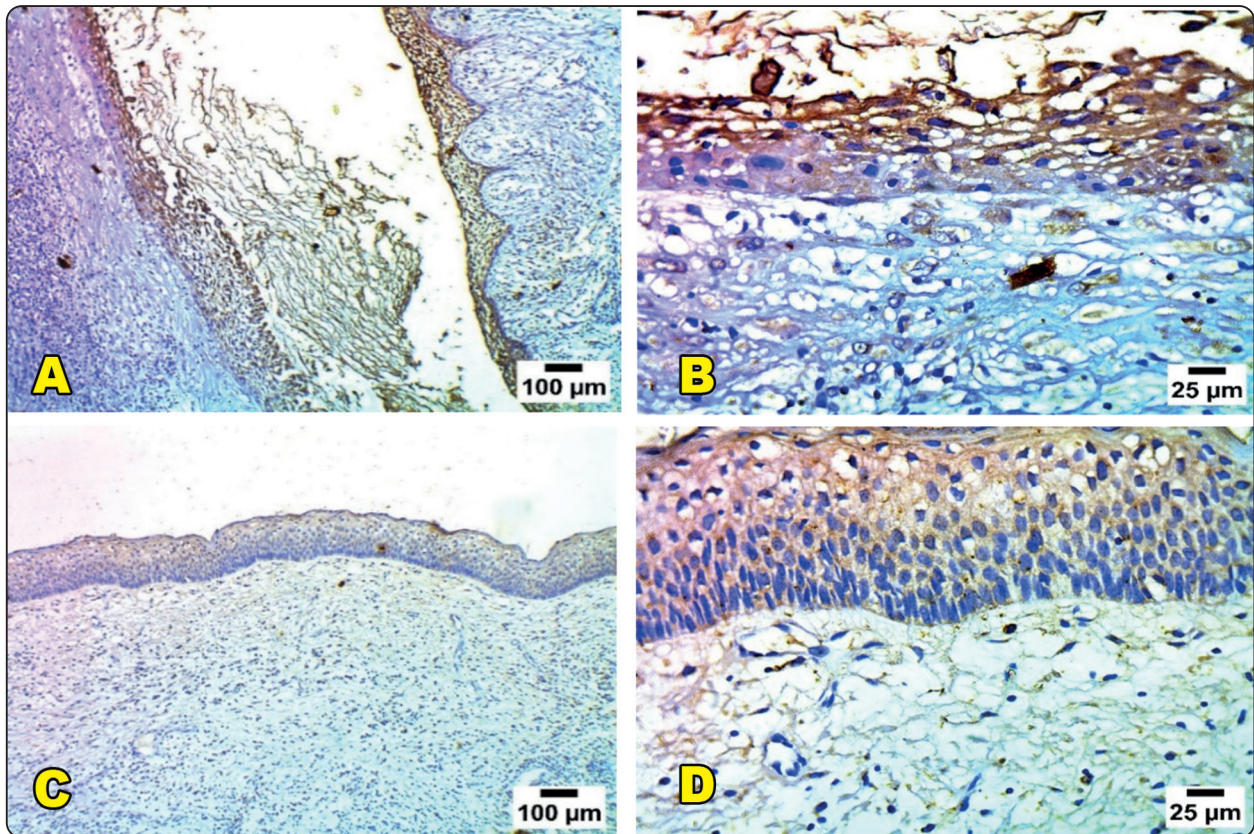


Fig. (3) Photomicrograph of: DCs lining exhibiting cytoplasmic immunopositivity (p53x10) (A) (p53x40) (B), epithelial lining of OKC demonstrating granular cytoplasmic immunopositivity (p53 X10) (C), (p53 X40) (D).

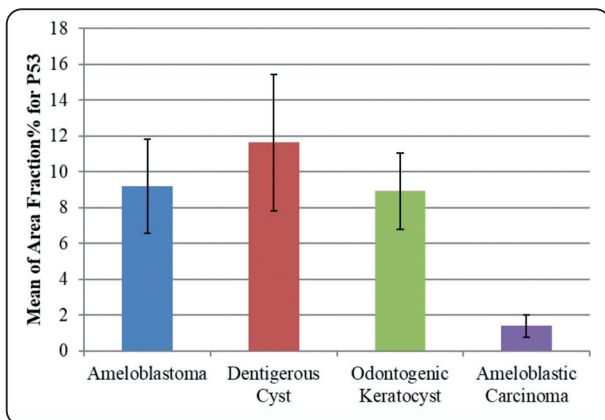


Fig. (4) Comparison between different groups regarding p53 immunopositivity MAF.

On comparing the p53 MAF among the AB subtypes, the highest mean value was recorded in PAB (13.30 ± 4.96), followed by UAB (10.24 ± 1.22); then FAB (5.39 ± 2.80), and the lowest value in AC (1.40 ± 0.61). One-way ANOVA identified a statistically significant difference in p53 expression between different groups ($P=0.001$). However, post hoc analysis using Tukey’s test did not reveal significant differences between PAB and UAB cases, with p-value ($p>0.05$) while there was a significant difference in p53 MAF in the other subtypes (Table 3, Figure 5). While, on comparing DC (11.63 ± 3.80); and UAB (10.24 ± 1.22), it was noted that there was no statistically significant difference in p53 immunopositivity MAF between them, with p-value ($p>0.05$) (Table 4, Figure 6).

TABLE (3) Showing the mean ± SD values, results of ANOVA and post hoc tests for the comparison between different sub-groups regarding Area Fraction% for P53.

Groups	Mean	±SD
I: Plexiform Ameloblastoma	13.30A	4.96
II: Follicular Ameloblastoma	5.39B	2.80
III: Unicystic Ameloblastoma	10.24A	1.22
IV: Ameloblastic carcinoma	1.40C	0.61

SD: Standard deviation; SE: Standard Error

*Significance level $p \leq 0.05$, *significant*

Tukey's post hoc: Means sharing the same superscript letter are not significantly different

TABLE (4) Showing the mean ± SD values, results of t-test for the comparison between different two groups regarding Area Fraction% for P53.

Groups	Mean	±SD
Dentigerous Cyst	11.63	3.80
Unicystic Ameloblastoma	10.24	1.22

SD: Standard deviation; SE: Standard Error

Significance level $p > 0.05$ insignificant

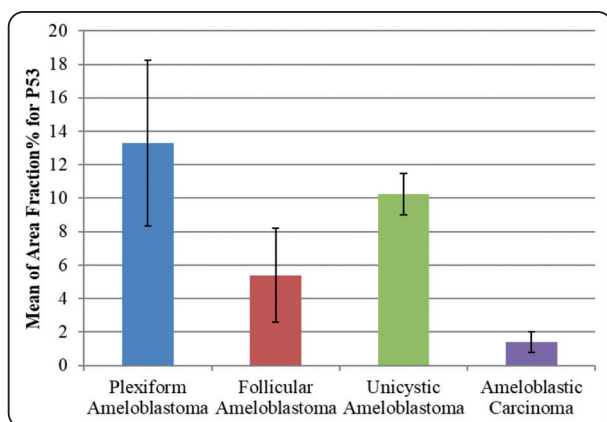


Fig. (5) Comparison between different sub-groups regarding p53 immunopositivity MAF.

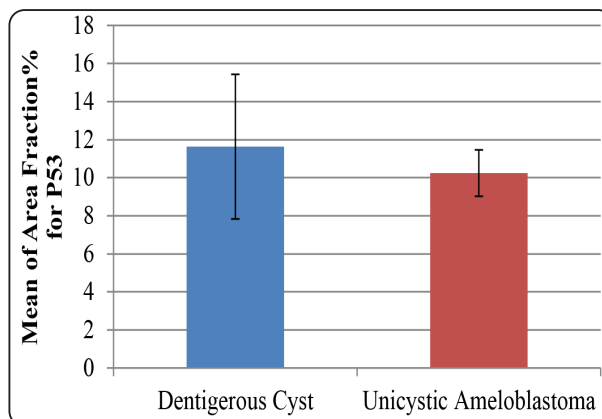


Fig. (6) Comparison between DC and UAB P53 IAF

DISCUSSION

Mutations in the p53 gene and the buildup of its protein product have been linked to heightened cell proliferation and advancement. (8,11,26). Despite the established role of p53 in oral cancers, understanding its function in odontogenic lesions, remains elusive (4,27). By analyzing p53 expression across a spectrum of odontogenic lesions, encompassing benign, potentially aggressive, locally aggressive and malignant lesions, the current study strives to illuminate the function of p53 in the development and behavior of these lesions. This knowledge can potentially guide the development of improved diagnostic and therapeutic strategies for these conditions.

Traditionally, the tumor suppressor protein p53 has been primarily recognized for its role in cellular response to DNA damage (17,19,28). However, recent research has revealed a more intricate picture, with seemingly contradictory influences on cell migration, metabolism, and differentiation (18,19,29,30). Additionally, p53 can exhibit pro-survival functions, which appear to contradict its well-established proapoptotic role (20,21,23).

Within the limitations of this study, our findings showed an overall male predominance and prevalence to the mandible in all the cases included in the study, which aligns with previous

reports^(1,3,10,26,26,31,32). Interestingly, our study observed a female predominance within the PAB subtype. Further investigation is needed to determine the potential reasons for this observed difference in the PAB subtype.

In the current study, the immunohistochemical analysis revealed distinct p53 expression patterns across the included cases. DCs displayed the strongest p53 immunostaining among the benign lesions, followed by ABs. and interestingly, OKCs exhibited the lowest levels of p53 expression. However, statistical analysis did not reveal significant differences in p53 expressions between ABs, DCs, and OKCs. Conversely, ACs demonstrated significantly lower p53 immunoreactivity compared to all other lesions.

These findings partially align with observations of Nagao et al. (2022) in colorectal cancer, where complete loss of p53 expression correlated with poorer clinical outcomes and tumor progression⁽³³⁾. This may suggest that the lack of significant p53 expression in ACs could be associated with a loss of its tumor suppressive function, potentially contributing to their malignant behavior. Moreover, since wild-type p53 protein is short-lived and hence difficult to detect, the relatively negative p53 immunostaining in the AC cells can possibly be justified according to Carvalhais et al., 1999 by either deletion of p53 gene or the mutation did not result in stabilization of the protein⁽³⁴⁾. However, conflicting results have been reported by other studies, with some showing increased p53 immunoreactivity in ACs compared to benign lesions⁽³⁵⁾. This variation highlights the complexity of p53 regulation and function. As suggested by Nodit et al. (2024), genetic or epigenetic mechanisms beyond p53 dysfunction might be involved in the malignant behavior of ameloblastic carcinomas⁽³⁶⁾. Additionally, emerging evidence suggests that p53's protein conformation may influence its paradoxical effects, leading to either tumor suppression or promotion depending

on its structure, and not merely the amount of expression^(20,21,23).

Within the AB, DC and OKC cases the immunopositivity with p53 was noted to be clearly cytoplasmic with absence of nuclear reaction. Aberrant p53 expressions patterns noted in this study, expression in the cytoplasm, and relative loss, have been documented to be associated with the presence of a p53 mutation. In fact, several studies have stated that cytoplasmic expression of p53 is a hallmark of tumor cells and increased proliferation. Under normal conditions, p53 is localized to the nucleus, where it functions as a tumor suppressor protein^(29,30,37).

Several theories have been proposed as to how cytoplasmic p53 expression contributes to increased proliferation, and loss of its tumor-suppressing function. Interestingly, cytoplasmic, rather than nuclear p53, appears to mediate the inhibition of autophagy. Consequently, physiological triggers that induce autophagy, such as nutrient depletion, must eliminate the cytoplasmic p53 pool for successful autophagic activation. Given the critical role of autophagy in maintaining genomic stability, its inhibition by cytoplasmic p53 suggests a potential oncogenic mechanism⁽³⁸⁻⁴⁰⁾. In addition, cytoplasmic p53 seems to impede AMP-dependent kinase (AMPK), a promoter of autophagy, while activating mammalian target of rapamycin (mTOR), a suppressor of autophagy.^(38,39,41)

This cytoplasmic expression may be attributed to a number of factors like, the overexpression of MDM2. MDM2 can bind to p53 and export it from the nucleus to the cytoplasm. Another mechanism of cytoplasmic p53 expression is the mutation of p53 itself. Certain p53 mutations can disrupt its nuclear localization signal, leading to its accumulation in the cytoplasm^(7,42). Moreover, precise p53 folding is essential for its "proper" canonical functions. Research has determined that, wild-type (WT) p53 does not inherently have a WT conformation; rather, it must attach to a number of molecular chaperones,

such as chaperonin-containing t-complex polypeptide 1 (CCT-1), in order to maintain its WT conformation, which may not always be the case⁽¹⁴⁾.

It is thus safe to say that changes in p53 levels cannot explain the entire picture of p53 function control. Both post-translational modifications and changes in p53-binding proteins appear to be important modulators of p53 activity^(7,14). It is also noteworthy, that the p53 immunopositivity in the cyst cases mostly spanned the entire epithelial layers this in contrast to the results documented by Gaballah E, et al, 2010, who stated that “p53 expression was noted in the basal and parabasal epithelial cells of cysts”. They also reported in their work that p53 expression exhibited the highest expression in the OKC cases⁽⁴³⁾. In the current study the highest expression was noted in the DC cases, however, the difference between it and expression in OKC was insignificant. The high expression noted in this study in the DC and the fact that the expression was noted throughout the epithelial lining layers may be attributed to the effect of inflammation, as documented by Gaballah E, et al, 2010 and de Oliveira M, et al, 2008^(43,44).

Moreover, Mighell A, 1995 stated that “the complicated biology of P53, the impact of histologic processing, and the immunohistochemical labeling technique” should all be taken into account when interpreting immunohistochemical investigations of P53 because they all affect how accurate the results are⁽⁴⁵⁾. Regarding the subtypes of AB included in the study, the expression was upregulated in the PAB and UAB cases than in the FAB cases. This is consistent with results of Kumamoto et al., 2004,⁽⁴⁾ who stated that reactivity for p53 was notably higher in PAB than in FAB, indicating that p53 expression may take part in tissue architecture of ABs⁽⁷⁾.

Overall, the available evidence suggests that p53 dysregulation plays a significant role in the pathogenesis of odontogenic lesions. Further research is needed to elucidate the precise

mechanisms underlying p53-mediated progression for these lesions. Understanding the interplay between p53 and signaling pathways in odontogenic tumors opens exciting avenues for future research, potentially leading to the development of more effective therapeutic approaches.

CONCLUSION

Our findings suggest that aberrant cytoplasmic p53 expression may contribute to benign odontogenic lesion development, while relative absence of expression might be associated with ameloblastic carcinoma. Further investigation is warranted to validate these observations and elucidate the underlying mechanisms.

List of abbreviations

- AB: Ameloblastoma
- AC: Ameloblastic carcinoma
- MA: Malignant ameloblastoma
- DC: Dentigerous cysts
- OKC: Odontogenic keratocyst
- MDM2: mouse double minute2
- MAPK: mitogen-activated protein kinase
- ATM: ataxia-telangiectasia mutated
- IHC: immunohistochemistry
- FAB: Follicular ameloblastoma
- PAB: Plexiform ameloblastoma
- UAB: Unicystic ameloblastoma
- H&E: Hematoxylin and eosin
- ABC: avidin-biotin-peroxidase complex
- DAB: diaminobenzidine
- MAF: mean area fraction
- AMPK: AMP-dependent kinase
- mTOR: mammalian target of rapamycin
- WT: wild-type
- CCT-1: t-complex polypeptide 1

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