A Histopathology-based Assessment of Biological Behavior in Oral Hyalinizing Odontogenic Tumors and Bone Lesions by Differential Stains

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Abstract

Aim: Odontogenic tumors (OTs) and bone lesions of the oral cavity present diverse histological features and varying clinical behavior that makes predicting their biologic behavior difficult. The research undertaken in the current study aims to predict the biological behavior of oral hyalinizing odontogenic and bone lesions (OHO-BL) for the first time by employing four differential stains with clinicopathologic correlation.

Materials and methods: The study was performed on retrospectively diagnosed formalin-fixed paraffin-embedded cases of OTs (n = 53) and bone lesions (n = 10). The severity of hyalinization (SOH) was assessed from stained tissue sections. Polarizing microscopy was used to analyze hyalinization in tissues stained with differential special stains, namely periodic acid–Schiff (PAS), Safranin-O, Alcian Blue, and Picrosirius red. SOH was also analyzed for possible correlation with recurrence and clinicopathologic correlation in OHO-BL.

Results: Intense staining was observed with PAS, Alcian Blue, and Safranin-O in OTs with increased SOH with a statistical significance. Polarizing greenish yellow color correlated significantly with the recurrence potential of the OT group. Recurrence in individual lesions of the OT group showed a statistically significant association with SOH. Such individual correlation was not observed in bone diseases.

Conclusion: PAS, Alcian Blue, Safranin-O, and Picrosirius red are reliable stains to assess hyalinization in OHO-BL. Picrosirius red–polarizing microscopy is a dependable tool for identifying recurrent odontogenic lesions.

Clinical significance: SOH can be considered a histological predictor of aggressive biologic behavior in oral hyalinizing odontogenic lesions that can enable the surgeon to arrive at an appropriate management protocol.

Keywords: Alcian Blue, Bone lesions, Hyaline, Odontogenic tumors, PAS, Picrosirius red, Polarizing microscopy, Safranin-O.

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Introduction

The process of hyalinization in pathology can be recognized as cellular to extracellular matrix interactions. Hyalinated collagen is found to be modified biochemically that influences the biologic behavior of benign and malignant neoplasms.1,2 Hyalinization is often preceded by an alteration within the cells with the deposition of hyaluronan, and its concentration defines the intensity of hyalinization as reported in neoplasms; however, this has not been investigated thoroughly.3

Odontogenic tumors (OTs) are diversified mass of lesions associated with varying clinical and histopathological characteristics. Primarily, it occurs during complex interaction between the epithelial and ectomesenchyme tissues during odontogenesis and originates from the odontogenic epithelium of soft tissues and bones of the jaws.4 The biological characteristics of OTs encompass hamartomatous proliferations, benign tumors, and malignant neoplasms with varying growth patterns.5 The vast variety of lesions is localized to the jaws, and most of them are benign. Due to their varied clinical behavior, predicting their biological behavior and prognosis will greatly aid in patient management and improve treatment outcomes.

Fibrous dysplasia (FD) is a developmental disorder, which is distinguished by the replacement of a normal-appearing bone with a poorly formed woven bone along with fibrous tissue. It presents with bony expansion and asymmetry, usually diagnosed in early childhood and/or adolescence.6,7 Ossifying fibroma (OF) is a fibro-osseous lesion, presenting as aggressive (juvenile and trabecular) and nonaggressive forms. Hyalinization has been rarely explored in FD and OF and has not been correlated with its biologic behavior to date.8

The use of differential stains in histopathology is indispensable as they are cost-effective and easy to interpret, need very few equipment to perform, and give results rapidly.9 The differential stains chosen for the current study will provide an insight into the degree of hyalinization and its content in specific oral lesions. The present study has employed four differential stains, namely periodic acid–Schiff (PAS), Alcian Blue, Safranin-O, and Picrosirius red (with
polarizing microscopy). The current study tests the hypothesis of whether a correlation exists between hyalinization and the biologic behavior in OHO-BL lesions by analyzing the intensity of staining of Picrosirius red (nature of collagen fibers) and PAS stain (glycogen content). By employing differential stains, Alcian Blue and Safranin-O, stain analysis of the proteoglycan content in OHO-BL lesions will also be performed.

In the present study, the staining characteristics of OHO-BL lesions will be correlated for the first time with clinicopathological parameters to evaluate the link between hyalinization and aggressive biologic behavior. The clinical features of OHO-BL lesions will also be correlated with recurrence to ensure that the study is comprehensive from the viewpoint of assessing biological behavior. The influence of stroma in hyalinizing lesions on the biologic behavior suffices the existence of extracellular matrix to cellular interaction, which is yet to be explored.

**METHODOLOGY**

**Study Design**

Ethical clearance to undertake the study was obtained from the MS Ramaiah University of Applied Sciences (RUAS) ethics committee. The study was performed on retrospectively diagnosed formalin-fixed paraffin-embedded archival cases of OTs (n = 53) and bone lesions (n = 10) from the Department of Oral Pathology and Microbiology, Faculty of Dental Sciences, RUAS.

The OT group is comprised of solid multicystic ameloblastoma (SMA) (n = 10), odontogenic myxoma (OM) (n = 8), unicystic ameloblastoma-luminal/intraluminal (UA-L/IL) (n = 17), and unicystic ameloblastoma-mural (UA-M) (n = 18). The intraosseous oral lesions are comprised of bone diseases, FD (n = 6), and central OF (n = 4). Descriptive features and clinical parameters of the above cases were retrieved from archival files and tabulated.

The retrieved tissues were deparaffinized by heating it at 57°C for 10 minutes, and any remaining paraffin was removed using xylene. The sections were first routinely stained with hematoxylin and eosin (H & E) followed by four histochemical differential stains—PAS, Safranin-O, Alcian Blue, and Picrosirius red using the following protocols.

**Special Staining**

**PAS Staining**

The tissues were stained using modified McManus method. The tissue sections were rehydrated and oxidized using periodic acid 1% w/v for 5 minutes, following which the sections were rinsed with distilled water and subsequently, they were covered with Schiff reagent for 15 minutes and rinsed again in tap water for 5 to 10 minutes. Lastly, the nuclei were stained using hematoxylin counterstain, and the tissue sections were dehydrated and mounted on the slide.

**Safranin-O Staining**

The tissue sections were covered with hematoxylin QS solution for 5 minutes, and subsequently, they were washed under running water. Two to three drops of ethanol were added to it followed by fast green solution for 5 minutes, rinsed thoroughly with 1% acetic acid solution for 10 to 15 seconds, and stained with Safranin-O at a concentration of 0.1% for 5 minutes. The sections were then dehydrated and cleared using 95% alcohol, absolute alcohol, and xylene for 2 minutes each.

**Alcian Blue Staining**

The tissues were stained with Alcian Blue by modified Mowry’s technique. Rehydrated tissues were stained with Alcian Blue for 30 minutes and washed under running tap water for 5 minutes. Counterstaining was done with nuclear fast red stain for 10 minutes and the sections were washed under running tap water for 1 minute. Next, the tissues were dehydrated using graded ethanol, cleared in xylene, and mounted onto a slide.

**Picrosirius Red Staining/Polarizing Microscopy**

Weigert’s hematoxylin was used to stain the rehydrated tissue for 8 minutes and washed under running water followed by Picrosirius red for 1 hour followed by washing with acidified water. Tissue sections were then dehydrated in subsequently increasing concentrations of absolute alcohol for 30 seconds each, cleared in xylene, and mounted with dibutylphthalate polystyrene xylene medium. The sections were observed under a polarizing microscope.

**Histopathological and Clinicopathologic Correlation of OHO-BL with Recurrence**

The clinical parameters of the site, radiographic features (well-defined, multilocular, diffuse, and unilocular), cortical expansion, and root resorption were analyzed for statistical correlation with severity of hyalinization (SOH) and recurrence. Finally, the histological parameters of special staining features (intensity of staining and distribution pattern) were assessed for possible correlation with SOH and recurrence of OHO-BL. A subgroup correlation of OTs and bone diseases with SOH and recurrence was also performed to specifically evaluate the relationship of individual lesions with SOH and recurrence.

**Interpretation and Analysis**

The sections were mounted for histological analysis and scored by two examiners (DA and LS), in case of ambiguity a third examiner RSR scored the slide. The SOH was recorded in the H & E-stained slide and scored as 0 = absent, 1 = mild, 2 = moderate, and 3 = intense. The following scoring criteria for hyalinization were used for PAS, Safranin-O, and Alcian Blue: the intensity of staining was scored as 1 = mild, 2 = moderate, and 3 = intense. The distribution pattern of the stain was scored as 0 = not significant, 1 = focal, and 2 = diffuse. The same criteria were employed for Picrosirius red stain supplemented by polarizing microscopy; the polarizing birefringent colors were scored as 1 = greenish, 2 = greenish yellow, 3 = reddish yellow, and 4 = reddish. Photomicrographs of the slides were captured at ×100 magnification using a Jenoptik ProgRes Gryphax Arktur USB 3.0 microscope camera, Jena, Germany, mounted on an Olympus Optical Microscope BX53F2, Tokyo, Japan. The histopathological and clinicopathologic parameter scores were tabulated and subjected to statistical analysis by Chi-square test, keeping the value of significance p <0.05 using Statistical Package for the Social Sciences software version 20.0 (International Business Machines Corporation, New York, New York, USA).

**RESULTS**

**Correlation of Clinical Parameters with SOH**

The descriptive features of OTs (n = 53) and bone lesions (n = 10) are shown in Figs 1A to D. When the clinical parameters of site, radiographic features, cortical expansion, and root resorption were
analyzed for statistical correlation with SOH, all parameters were found to be statistically nonsignificant.

**Correlation of Clinical Parameters and Recurrence**
When the clinical parameters of site, radiographic features, cortical expansion, and root resorption were analyzed for statistical correlation with OTs, the presence of multilocular radiolucency ($p = 0.012$) and cortical expansion ($p = 0.035$) showed statistically significant correlation (Table 1). However, recurrence did not correlate with any of the clinical parameters of bone diseases and did not exhibit statistical significance (Table 2).

**Correlation of Histological Parameter with SOH**
*PAS, Alcian Blue, and Safranin-O Special Stain Analysis*
When the intensity and pattern of distribution of special stains were correlated with SOH of OTs, it was observed that the intensity of staining of PAS ($p = 0.000$), Alcian Blue ($p = 0.000$), and Safranin-O ($p = 0.001$) showed intense staining with significant statistical correlation with SOH (Table 3 and Fig. 2). However, bone diseases did not show the correlation of histological parameters with SOH (Fig. 3). The pattern of distribution was found to be statistically insignificant for both OTs and bone diseases.

*Picrosirius Red–Polarizing Microscopy Special Stain Analysis*
When the intensity, pattern of distribution, and polarizing colors following Picrosirius red stain were observed under conventional and polarizing microscopy, it was noted that the intensity of Picrosirius red stain significantly correlated with SOH ($p = 0.001$) (Table 3 and Fig. 4). Correlation of Picrosirius red polarizing colors did show a link with SOH in bone diseases; however, the value was not statistically significant ($p = 0.07$) (Fig. 5).

| Table 1: Comparison of correlation of clinical parameters with recurrence of odontogenic tumors |
|---------------------------------------------------|
| **Clinical parameters** | **Recurrence** | **χ²** | **p value** |
|                      | Absent (%) | Present (%) |    |            |
| Site                  | Maxilla    | Mandible   | 100 | 0 | 0.981 | 0.322 |
| Radiographic features | Well-defined radiolucency | 96.4 | 3.6 | 10.979 | 0.012* |
| Cortical expansion | Absent | Present | 100 | 0 | 4.449 | 0.035* |
| Root resorption | Absent | Present | 80 | 20 | 0.889 | 0.346 |

Chi-square test, $p$ value <0.05—statistically *significant

**Correlation of Histological Parameters with Recurrence**
*PAS, Alcian Blue, and Safranin-O Special Stain Analysis*
When the intensity and pattern of distribution of special stains were correlated with the recurrence of OTs and bone diseases, it was observed that the intensity of staining did not show a significant statistical correlation with recurrence (Table 4). The pattern of...
distribution was found to be statistically insignificant for both OTs and bone diseases.

**Picrosirius Red–Polarizing Microscopy Special Stain Analysis**

When the intensity, pattern of distribution, and polarizing colors following Picrosirius red stain were observed under conventional and polarizing microscopy, it was noted that the intensity of Picrosirius red stain and pattern of distribution did not correlate with recurrence; however, the polarizing greenish yellow color correlated significantly with the OT group only (\( p = 0.000 \)) (Table 4 and Fig. 4).

**Table 2:** Comparison of correlation of clinical parameters with recurrence of bone diseases

| Clinical parameters | Recurrence | \( \chi^2 \) | \( p \) value |
|---------------------|------------|-------------|--------------|
| Site                |            |             |              |
| Maxilla             | 88.9       | 11.1        | 0.123        | 0.900 |
| Mandible            | 100.0      | 0.0         |              |       |
| 1–2 cm              | 50.0       | 50.0        |              |       |
| Size                |            |             |              |
| 3–4 cm              | 100.0      | 0.0         | 4.444        | 0.108 |
| >4 cm               | 100.0      | 0.0         |              |       |
| Radiographic features |         |             |              |
| Radiolucent lesion  | 100.0      | 0.0         | 4.444        | 0.200 |
| Mixed radiolucency  | 50.0       | 50.0        |              |       |
| Cortical expansion  |            |             |              |
| Present             | 87.5       | 12.5        | 4.138        | 0.126 |

Chi-square test, \( p \) value <0.05—statistically significant

**Correlation of SOH with Recurrence in Individual Lesions**

When the SOH was correlated with recurrence in OT subgroups, it was observed that SOH was intense and significantly correlated with recurrence in all three subgroups (SMA and OM, \( p = 0.014 \), (UA-IL and L, \( p = 0.011 \)), and (UA-M, \( p = 0.033 \)) (Table 5, Figs 6 and 7). Such individual correlation was not observed in bone diseases (Table 6 and Fig. 8).

**Discussion**

OTs are a heterogeneous group of lesions with variable clinical and histopathological features. OTs are capable of inductive interactions between odontogenic ectomesenchyme and epithelium, even the classification of OTs is essentially based on this interaction. SMA and OM, UA-L and IL, and UA-M were considered under the OT group in the present study.

Ameloblastomas, even though classified as benign tumors, exhibit aggressive growth and are locally destructive. A recent multivariate analysis study by Yang et al. has determined the recurrence rate of solid ameloblastomas to be 9.78%. OM is known for a high recurrence rate of up to 25% after curettage. UA-M is a subtype of UA characterized by the expansion or infiltration of tumor nodules into the fibrous wall of the cyst. The behavior of this subtype is highly aggressive, with a risk of recurrence, comparing with that of conventional ameloblastoma. However, UA-L and UA-IL are nonaggressive histologic types of UA. The functional and aesthetic preservation of the craniofacial region is important for an

**Table 3:** Comparison of correlation of histological parameters with SOH of odontogenic tumors

| Histological parameters | Mild (%) | Moderate (%) | Intense (%) | \( \chi^2 \) | \( p \) value |
|-------------------------|----------|--------------|-------------|-------------|--------------|
| PR color                |          |              |             | 1.675       | 0.433        |
| Reddish                 | 0.0      | 0.0          | 100         |             |              |
| Yellowish red           | 0.0      | 0.0          | 100         |             |              |
| Greenish yellow         | 6.7      | 11.1         | 82.2        |             |              |
| Mild                    | 17.6     | 29.4         | 52.9        |             |              |
| PR intensity            |          |              |             | 19.953      | 0.001*       |
| Moderate                | 0.0      | 0.0          | 100.0       |             |              |
| Intense                 | 0.0      | 0.0          | 100.0       |             |              |
| Mild                    | 17.6     | 29.4         | 52.9        |             |              |
| AB intensity            |          |              |             | 19.953      | 0.000*       |
| Moderate                | 0.0      | 0.0          | 100.0       |             |              |
| Intense                 | 0.0      | 0.0          | 100.0       |             |              |
| NS                      | 0.0      | 0.0          |             |             |              |
| AB pattern              |          |              |             | —           | —            |
| F                       | 0.0      | 0.0          |             | —           | —            |
| D                       | 5.7      | 9.4          | 84.9        |             |              |
| Mild                    | 17.6     | 29.4         | 52.9        |             |              |
| SNO intensity           |          |              |             | 19.953      | 0.001*       |
| Moderate                | 0.0      | 0.0          | 100.0       |             |              |
| Intense                 | 0.0      | 0.0          | 100.0       |             |              |
| NS                      | 0.0      | 0.0          |             |             |              |
| SNO pattern             |          |              |             | —           | —            |
| F                       | 0.0      | 0.0          |             | —           | —            |
| D                       | 5.7      | 9.4          | 84.9        |             |              |
| PAS intensity           |          |              |             | 19.953      | 0.000*       |
| Moderate                | 0.0      | 0.0          | 100.0       |             |              |
| Intense                 | 0.0      | 0.0          | 100.0       |             |              |
| NS                      | 0.0      | 0.0          |             |             |              |
| PAS pattern             |          |              |             | —           | —            |
| F                       | 0.0      | 0.0          |             | —           | —            |
| D                       | 5.7      | 9.4          | 84.9        |             |              |

Chi-square test, \( p \) value <0.05—statistically *significant
Figs 2A to R: (A to F) SMA: Follicular type showing intense staining for stains (PAS, Alcian Blue, and Safranin-O—×100). (G to I) Odontogenic myxoma showing intense staining for stains (PAS, Alcian Blue, and Safranin-O—×100). (J to L) UA—luminal type showing mild staining for stains (PAS, Alcian Blue, and Safranin-O—×100). (M to R) UA—mural type showing intense staining for stains (PAS, Alcian Blue, and Safranin-O—×100).
adequate quality of life in these patients. Thus, the need of the hour is to predict the aggressive behavior and recurrence potential of OTs to deliver enhanced treatment and to better the quality of life postoperatively.

FD is a developmental anomaly that is idiopathic and nonhereditary in origin; it is characterized by proliferation and replacement of the bone by fibrous tissues. Patients usually present with swelling, pain, or numbness on the involved side. The incidence of monostotic FD (MFD) is four times greater than that of polyostotic FD. The maxilla is more commonly involved than the mandible. The clinical behavior and rapid progression of FD render the treatment challenging. The malignant potential is
Figs 4 A to L: Picrosirius red stain/polarizing microscopy. (A to D) SMA: Follicular type showing intense staining and yellowish red collagen fibers with patches of green (×100). (E and F) Odontogenic myxoma showing intense staining and greenish yellow collagen fibers (×100). (G and H) UA—luminal type showing mild staining and reddish collagen fibers (×100). (I and J) UA—mural type showing intense staining and yellowish red collagen fibers (×100). (K and L) UA—mural type showing intense staining and greenish yellow collagen fibers (×100)
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The clinicopathologic parameters of OTs and bone diseases, except with cortical expansion ($p=0.035$) and multilocular radiolucency ($p=0.012$) in OTs. Cortical expansion indicates an aggressive lesion proliferating rapidly, eventually leading to thinning of the cortical plates. A multilocular lesion represents an entity with multiple growth centers proliferating at varying rates.

In a study conducted by Syed et al., the authors analyzed the clinicopathologic features of OTs based on a 20-year institutional study and stated that the posterior aspect of the mandible was the most favored site of presentation (77.2%). However, no correlation to recurrence was made by the authors.

Another study by Carvalho et al. analyzed 52 ameloblastomas in a 16-year clinicopathological study in the Goan population; the authors concluded that a male preponderance was noted. Age predisposition was seen to favor the third–fourth decade, and the most common site was the posterior aspect of the lower jaw for new and recurrent cases. A significant finding was that the solid/multicystic variant predominated (60% of all recurrences).

PAS is used to stain glycogen and mucosubstances, such as glycoproteins in the stromal tissue. Alcian Blue stains glycosaminoglycans (GAGs) and hyaluronic acid in the stroma while Safranin-O stains the proteoglycans present. In the hyalinized connective tissues, the intensity of PAS, Alcian Blue, and Safranin-O stain is expected to correlate with the SOH as the hyalinized tissue is rich in glycoproteins, proteoglycans, GAGs, and hyaluronic acid. The intensity of Picrosirius red stain directly correlates with the thickness of untreated cases of FD.$^{18}$ Juvenile ossifying fibroma (JOF) is a fibro-osseous lesion that is encountered in the facial bones, predominantly the mandible. It is an aggressive lesion that often causes bone lysis with a strong tendency to recur. It is histologically classified as trabecular and psammomatomoid JOF. Surgical resection is the chosen line of treatment.$^{19}$ Based on the above-mentioned behavioral characteristics of FD and JOF, we must develop a simple yet accurate method to evaluate its recurrence potential to ensure the best management protocol.

Recently, hyalinization as a prognostic risk factor variable has gained significant importance in predicting aggressive behavior and recurrence in odontogenic keratocysts.$^{20}$ The origin of hyaline in odontogenic lesions and bone diseases is a debatable concept. Therefore, analysis of hyalinization and its correlation with biologic behavior and prognosis is an interesting prospect to investigate to establish its role as a recurrent predictive histological marker in OTs and bone diseases.

The current study attempted to analyze the SOH in OTs and bone diseases and correlated it with the clinicopathologic parameters and recurrence to determine whether hyalinization can be employed as a histopathologic marker for predicting prognosis.

The descriptive features of the 53 OT cases considered showed 66% male predisposition with 43.4% of cases in the age range between 21 and 40 years; over 90% of lesions occurred in the mandible (Fig. 1). Through the results obtained in the current study, it was clear that SOH and recurrence did not correlate with the clinicopathologic parameters of OTs and bone diseases, except with cortical expansion ($p=0.035$) and multilocular radiolucency ($p=0.012$) in OTs. Cortical expansion indicates an aggressive lesion proliferating rapidly, eventually leading to thinning of the cortical plates. A multilocular lesion represents an entity with multiple growth centers proliferating at varying rates.

In a study conducted by Syed et al., the authors analyzed the clinicopathologic features of OTs based on a 20-year institutional study and stated that the posterior aspect of the mandible was the most favored site of presentation (77.2%). However, no correlation to recurrence was made by the authors.$^{21}$ Another study by Carvalho et al. analyzed 52 ameloblastomas in a 16-year clinicopathological study in the Goan population; the authors concluded that a male preponderance was noted. Age predisposition was seen to favor the third–fourth decade, and the most common site was the posterior aspect of the lower jaw for new and recurrent cases. A significant finding was that the solid/multicystic variant predominated (60% of all recurrences).$^{22}$

PAS is used to stain glycogen and mucosubstances, such as glycoproteins in the stromal tissue.$^{23}$ Alcian Blue stains glycosaminoglycans (GAGs) and hyaluronic acid in the stroma while Safranin-O stains the proteoglycans present.$^{24}$ In the hyalinized connective tissues, the intensity of PAS, Alcian Blue, and Safranin-O stain is expected to correlate with the SOH as the hyalinized tissue is rich in glycoproteins, proteoglycans, GAGs, and hyaluronic acid. The intensity of Picrosirius red stain directly correlates with the thickness...
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This study indicates that an increase in the components of hyalinized stroma correlates with aggressive biologic behavior.

Results obtained in the present study demonstrated that there is no statistical correlation between special staining characteristics and recurrence in both OTs and bone diseases. However, the polarizing color correlated significantly with the recurrence of OTs \( (p = 0.000) \). The reddish color indicates closely packed thick fibers of mature collagen fibers, whereas greenish yellow indicates loosely packed fibers or immature collagen fibers. It has been shown in studies related to OSCC that change of polarizing colors from reddish to greenish yellow is indicative of aggressive change and poor prognosis.

Kulkarni et al. analyzed 56 odontogenic lesions, comprising odontogenic cysts and tumors by Picrosirius red–polarizing microscopy: greenish yellow birefringence was seen in SMA

of the collagen fibers. When the special staining characteristics (intensity and distribution of PAS, Alcian Blue, and Safranin-O) and polarizing colors of Picrosirius red–polarizing microscopy were correlated with the SOH in OTs, it was noted that the intensity of staining of PAS \( (p = 0.000) \), Alcian Blue \( (p = 0.000) \), and Safranin-O \( (p = 0.001) \) showed a significant statistical correlation with SOH. Also, the intensity of Picrosirius red stain significantly correlated with SOH \( (p = 0.001) \).

Safranin-O has not been employed to stain hyalinized tissue components in oral lesions to date. Recently Picrosirius red has been used to stain the connective tissues of odontogenic cysts and tumors to demonstrate collagen packing. Only one study in the literature has employed Alcian Blue with PAS to determine mucin intensity in oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC). The group of Sahni et al. stated that mucins are glycoproteins and showed a gradual increase in its intensity in grades of OED and OSCC, with no predominant pattern. This indicates that an increase in the components of hyalinized stroma correlates with aggressive biologic behavior.

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Kulkarni et al. analyzed 56 odontogenic lesions, comprising odontogenic cysts and tumors by Picrosirius red–polarizing microscopy: greenish yellow birefringence was seen in SMA

Figs 6A to E: Hematoxylin and eosiin stain. (A and B) \( (\times 100 \text{ and } \times400) \), SMA—plexiform type showing intense SOH. (C and D) \( (\times 100 \text{ and } \times400) \), Odontogenic myxoma showing intense SOH. (E and F) \( (\times 40 \text{ and } \times40) \), UA—intraluminal type showing mild SOH.
lesions by the principle of differential staining of collagen fibers based on maturity. The SOH and recurrence correlated significantly in subgroups of OTs (SMA and OM, \(p = 0.014\)) and (UA-M, \(p = 0.033\)). From these findings, it is evident that components of hyalinized stroma favor aggressive biologic behavior and recurrence in odontogenic lesions. An interesting observation was that UA-IL and L-type showed a reduced rate of recurrence when hyalinization was present. Hyalinization in UA-IL and L-type was significantly associated with a lower rate of recurrence when compared to other subgroups in OTs (UA-IL and L, \(p = 0.011\)). The explanation for this could be that hyalinized stroma in UA-IL and L-type prevents the proliferation of the luminal cells into the connective tissue capsule, thereby preventing it from becoming more aggressive like UA-M that is biologically similar to SMA. The hyalinized component could also be a basement membrane secretion in UA. UA-M and SMA showed an increased rate of recurrence when hyalinization was present. The presence of hyalinization in these lesions is due to the inductive effect of the proliferating odontogenic epithelium on the connective tissue stroma to induce hard tissue formation.\(^{29,30}\) The ability of the odontogenic epithelium to induce an inductive change in the stroma is a direct measure of its active rather than senescent nature, making it potentially aggressive.

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Figs 7A to F: Hematoxylin and eosin stain. (A and B) (×100 and ×400), UA—luminal type showing mild SOH. (C and D) (×40 and ×40), UA—luminal type showing mild SOH. (E and F) (×100 and ×400), UA—mural type showing intense SOH

Fig. 8: Comparison of recurrence in the OT group of lesions and its correlation with hyalinization whereas complete greenish birefringence was seen in ameloblastic carcinoma.\(^{28}\) The lesions with increased immature collagen fibers were found to be aggressive. This study too suggests that Picrosirius red is a consistent stain in determining the aggressive potential of
In contrast to the findings of the current study, the research group of Kazi et al. analyzed hyalinization in six cases of ameloblastomas aided by immunohistochemistry and concluded that hyalinization has an inhibitory effect on tumor growth and stops stromal tumor cell interaction with the help of heparan sulfate proteoglycans, resulting in deficient angiogenesis. They suggested that the tumor cells adjacent to the hyalinized region undergo programmed cell death.31 However, the major drawback was the small sample size considered. Further research on a larger sample with well-defined subgroups of SMA, UA-IL and L, and UA-M correlating SOH with the biologic behavior and recurrence could validate the above-mentioned findings obtained in the present study.

The clinicopathologic parameters, SOH, and recurrence did not correlate in bone diseases (FD and OF). The closest correlation observed was that of Picrosirius red polarizing colors that showed a close correlation with SOH in bone diseases; however, the value was not statistically significant (p = 0.07). Histology of case reports of recurrent OFs with hyalinization has been reported in the literature, indicating the association of hyalinization with aggressiveness and recurrence. However, the literature review lacks original research studies to confirm the same. Hyalinization has been considered a secondary change in FDs; the association of hyalinization in recurrent FDs has not been studied to date based on the literature review.32 The current study considered only six FDs and four OFs that explains the nonsignificant statistical values; this is also a drawback of the current study. Future studies should aim to evaluate the recurrent potential correlation of these lesions on a larger sample size to arrive at definitive conclusions regarding their biological behavior.

The aggressive behavior and recurrent potential of OTs and bone diseases have been correlated with hyalinization for the first time, comprehensively aided by four special stains and clinicopathologic parameters. The key results obtained through this study indicate that PAS, Alcian Blue, and Safranin-O are reliable stains to detect hyalinization. The results also indicate that SOH correlates with the recurrent potential of OHO-BL and can be easily assessed by Picrosirius red–polarizing microscopy.

**Conclusion**

Predicting the biological behavior histologically on incisional biopsies helps clinicians to decide effective treatment plans. This permits the clinicians to categorize the individuals in need of radical or conservative treatment in OTs and bone diseases. Such strategies will prevent unwanted over- or undertreatment of lesions, leading to a better prognosis.

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