Original Article

Collagen during odontogenesis and in ameloblastoma: A polarizing microscopic study

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Abstract

Background: Odontogenesis is a highly coordinated and complex process which depends on cell–cell interactions that result in initiation and generation of tooth. Tissue remnants of developing tooth can form odontogenic tumors possibly, reflecting different developmental stages in tooth formation. In both odontogenesis and odontogenic tumors, stroma plays a prominent role in maintaining epithelial tissues with continuous molecular interactions. As the collagen forms an integral part of connective tissue stroma, in the present study, polarization colors and thickness of the collagen fibers were assessed in both tooth germ papillae and ameloblastoma using picrosirius red (PSR) stain.

Materials and Methods: Collagen fibers in 20 cases of ameloblastoma and 10 tooth germs from the human fetus were evaluated with PSR stain and examined under polarizing microscopy.

Results: Polarization colors of red-colored collagen fibers with greater diameter were more in ameloblastoma when compared to tooth germ papillae in which green-colored collagen fibers with smaller diameter being more.

Conclusion: The absence of hard tissue formation in ameloblastoma might be due to the presence of significantly more number and greater thickness of red-colored collagen fibers. Thus, the nature of collagen fibers can predict the nature in terms of biologic behavior and prognosis.

Keywords: Ameloblastoma, collagen, picrosirius red

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INTRODUCTION

Tooth morphogenesis is a systematic, scrupulous process regulated by sequential and reciprocal interactions between the epithelial and mesenchymal tissues.[1] These interactions play an important role in health by maintaining homeostasis; however, on the contrary, an imbalance is envisaged in disease state with the same cells interacting differently and culminating in varied histopathological appearances of odontogenic tumors. Ameloblastoma is a tumor of odontogenic epithelial origin in which no calcified dental tissues are formed. The inductive potential of proliferating odontogenic epithelium acts effectively on ectomesenchymal cells during odontogenesis but perhaps does not act appropriately on differentiated fibrous connective tissue as in ameloblastoma.[2] The integral part of connective tissue stroma is collagen that makes up 34% of the total extracellular matrix (ECM) proteins and plays

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a crucial role in maintaining the structural integrity and in determining tissue function.[3,4] Picrosirius red (PSR) stain is considered a highly specific and selective stain for collagen due to its ability in differentiating different types of collagen fibers in various conditions. PSR in conjunction with a polarized light microscope can serve as a valuable tool in differentiating procollagens and intermediate and pathological collagen from normal collagen fibers.[5]

The present study assessed the collagen bundles in tooth germs and ameloblastoma by comparing the polarization colors and thickness of the collagen fibers using PSR stain and to clear the ambiguity of why hard tissue formation is absent in ameloblastoma.

MATERIALS AND METHODS

The present study was carried out in the Department of Oral and Maxillofacial Pathology, St. Joseph Dental College and Hospital, Eluru. Twenty paraffin blocks of histologically proven ameloblastoma were retrieved from archives of the department, and ten tooth germs from the human fetus in cases of terminated pregnancies were collected with parent consent from ASRAM Medical College and Hospital, Eluru.

The human fetus collected between 12 and 36 weeks were carefully dissected for maxilla and mandible [Figure 1], and the excised specimens were rinsed. This was followed by fixation in 10% formalin and dissection into right and left halves. With proper labeling, the specimens were processed and embedded in paraffin wax.

From all the tissue blocks, two sections of 5-μm thickness were taken of which one was stained with hematoxylin and eosin for routine histological examination [Figures 2 and 3]. Another section, after deparaffinization and hydration, was placed in PSR dye solution for 1 h at room temperature. The PSR stained section was rinsed in distilled water and stained with Weigert's heamtoxylin for 15 min which was followed differentiation in 1% acid alcohol and blueing was done. The section was dehydrated in alcohol and cleared in xyelene and mounted with dibutyl phthalate xylene.

Olympus Trinocular research microscope model Bx 53 ProgRes CT with an attached polarizer was used for the study. At the outset, under higher magnification of ×40, five fields of each section were selected and viewed for different polarized colors of the collagen fibers such as green, greenish-yellow, reddish-yellow and red and their thickness was also measured, respectively. The criteria to measure the collagen fibers were followed from the study

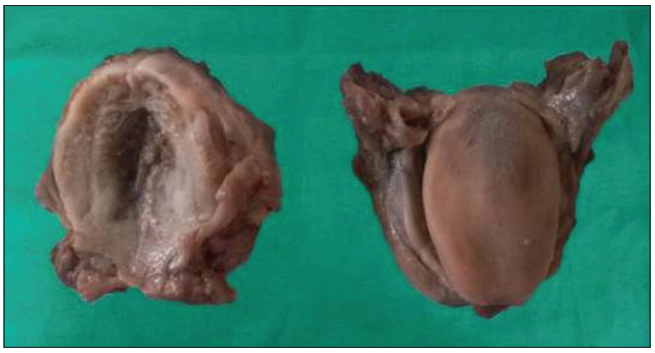


Figure 1: Maxilla and mandible from the human fetus of 36 weeks of age

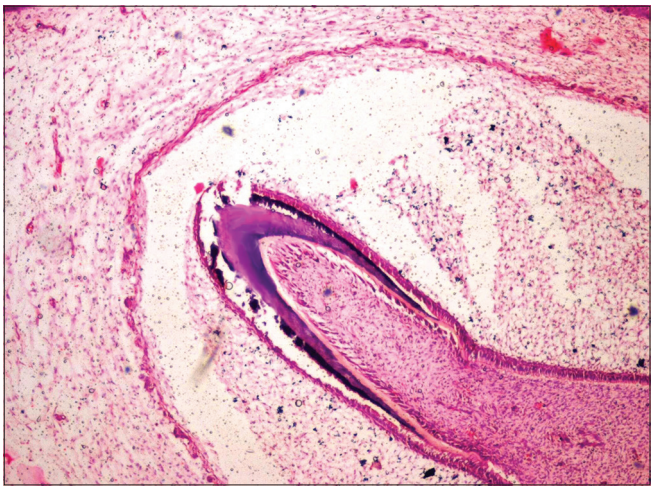


Figure 2: Photomicrography of H&E-stained section of tooth germ papillae of 36 weeks of age showing collagen fibers ×4

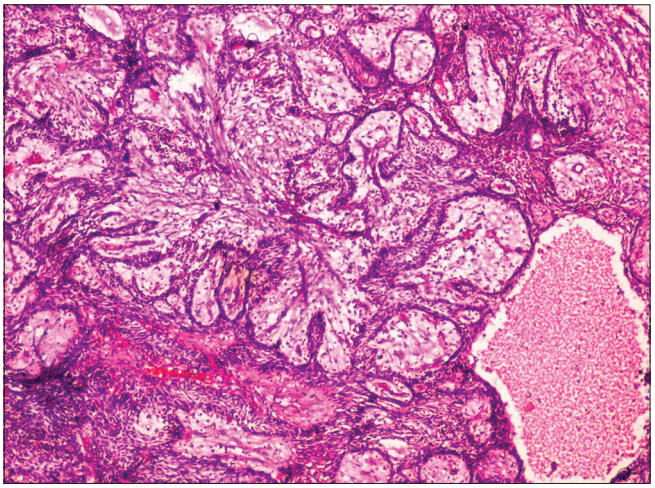


Figure 3: Photomicrography of H&E-stained section of plexiform ameloblastoma showing collagen fibers (×4)

done by Vaidhehi *et al*.[6] where the range for thin fibers is 0.8 μm or less and for thick fibers is 1.6–2.4 μm.

For statistical analysis, ANOVA was used to determine whether the polarization colors of the collagen fibers and

their thickness differed significantly within the single-study group. Unpaired *t*-test was used to determine whether the polarization colors of the collagen fibers and their thickness differed significantly between the two study groups.

RESULTS

In the present study, comparison of the polarization colors of collagen fibers showed that the mean number of green-polarized color of collagen fibers was more in tooth germs when compared with red, greenish-yellow and reddish-yellow collagen fibers ($P < 0.001$) [Figure 4a, b and Graph 1], whereas in ameloblastoma, the mean number of red-polarized color of collagen fibers was more when compared to green, greenish–yellow and reddish-yellow collagen fibers ($P < 0.001$) [Figure 5a, b and Graph 2]. However, when thickness was compared, red collagen fibers showed greater thickness in both tooth germs and ameloblastoma [Graph 3].

DISCUSSION

Epithelial–mesenchymal interactions (EMIs) are described as a series of programmed, sequential and reciprocal communications between the epithelium and the mesenchyme with its heterotypic cell population that results in differentiation of one or both cell populations. These interactions play a key role in odontogenesis[7] as well as diverse histopathological features in odontogenic tumors, varying from hyalinization around the islands as in ameloblastoma to a complete structured tooth as in odontoma.[2] Majority of the studies have focused on the evaluation of proliferative activity in the epithelium of odontogenic lesions, with fewer studies highlighting the role of EMIs in the progression of these odontogenic entities. It has been suggested that stroma is essential for maintaining the epithelial tissues, and both these make up an ecosystem with continuous molecular interactions in odontogenesis.[8]

Tumor stroma has a very important role to play in the progression of a neoplasm by acting as a physical barrier to ward off the host immunological reactions, supplying the essential nutrients to the tumor via blood, and also serves to remove waste products.[9] However, the amount of stroma differs from one tumor to another. Although immunohistochemistry and molecular techniques have a wide application in diagnostic purposes, they have minuscule impact in demonstrating pathological collagen fibers which are integral part of connective tissue stroma. These tribulations have initiated in further search for various investigative procedures.[10]

As the collagen is an anisotropic structure, it exhibits the phenomenon of birefringence that can be selectively visualized using polarized light microscopy. Weak birefringence in the biological specimens is enhanced by the addition of dyes or impregnating metals in an orderly linear arrangement. Collagen molecules being

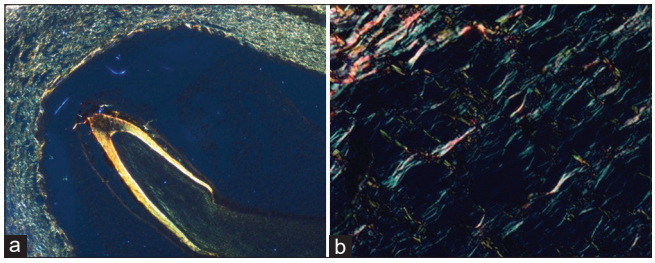
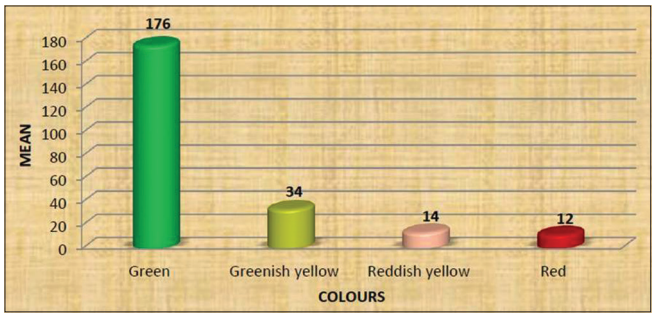


Figure 4: (a) Photomicrography of picrosirius red-stained section of tooth germ papillae (×4). (b) Tooth germ papillae showing collagen fibers predominantly greenish birefringence (×40)



Graph 1: Number of polarization colors of collagen fibers in tooth germs

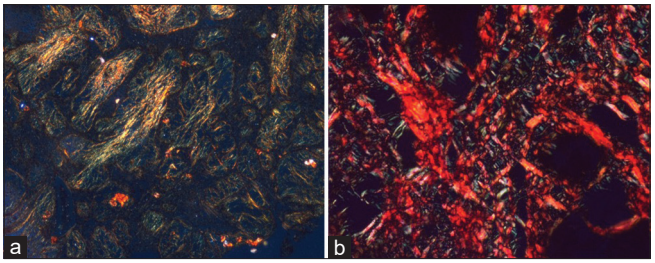
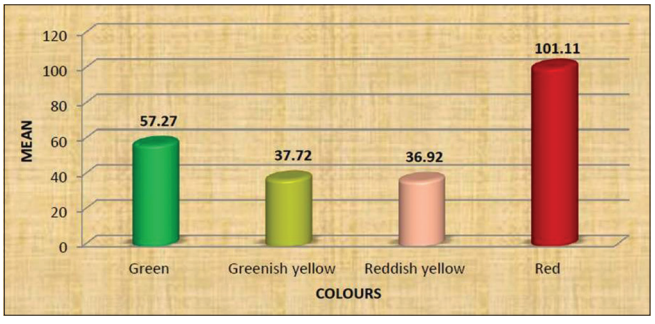
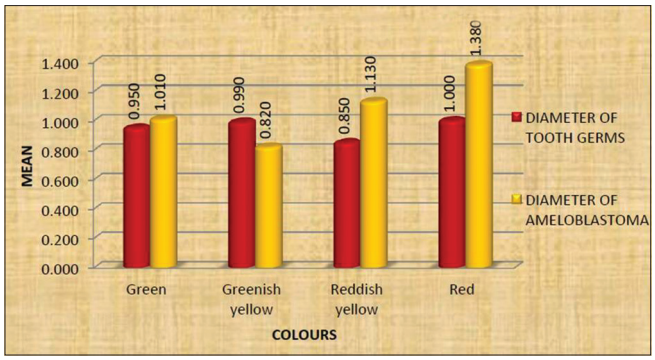


Figure 5: (a) Photomicrography of Picrosirius red-stained section showing plexiform pattern with collagen fibers (×4). (b) Picrosirius red-stained section showing plexiform pattern with collagen fibers predominantly reddish birefringence (×40)



Graph 2: Number of polarization colors of collagen fibers in ameloblastoma



Graph 3: Mean comparison between thickness of collagen fibers in tooth germs and ameloblastoma

rich in basic amino acids strongly react with acidic dyes.[11,12] Sirius red is an elongated dye molecule which reacts with collagen and promotes an enhancement of its normal birefringence when viewed under polarized light microscopy.[13] Moreover, the stain persists with time and serves as a valuable tool in differentiating procollagens and intermediate and pathological collagens from normal collagen fibers as well as enables us to discrete fibers of different thickness. This property is due to the fact that very thin fibril of collagen undetectable by normal microscopy becomes visible with this method as a source of light against a dark background.[11,12]

In the present study, collagen fibers displayed different interference colors from green to greenish-yellow, reddish-yellow to red birefringence when the tissue sections were stained using PSR and viewed under the polarizing microscope.[12] This property is explained by the fact that these different interstitial collagens display distinct patterns of physical aggregation.[14]

The predominant color of collagen in tooth germ papillae was green when compared to other collagen fibers, which is in accordance with the study done by Abrahão *et al*.,[15] where they also found Type III collagen as a regular component in tooth germ papillae seen as a weak birefringence of greenish color. This could be explained by the fact that collagen found in tooth germs is loosely packed immature fibers with predominance of Type III collagen. In ameloblastoma, the predominant color of collagen was red compared to other fibers, which is consistent with the study done by Vaidhehi *et al*.[6] and Singh HP *et al*.,[10] where they found the predominance of red and reddish-yellow fibers in unicystic ameloblastoma and in odontogenic cysts (dentigerous and radicular cyst), respectively. This could possibly be attributed to the tightly packed mature collagen fibers and predominance of Type I collagen in the stromal tissue. The connective tissue of odontogenic keratocysts, dentigerous cysts and ameloblastomas predominantly exhibited red collagen fibers, because of similar developmental origin and their longstanding duration may lead to closely packed mature collagen fibers.[16] Under a polarizer, thin, normal collagen fibers (0.8 μm or less) are green to greenish-yellow, whereas thick fibers (1.6–2.4 μm) range from yellowish to red birefringence.[6,10]

In the present study, when the thickness of collagen fibers was compared in tooth germ papillae, red-colored fibers were thickest, whereas reddish yellow-colored fibers were thinnest, but the difference was statistically insignificant. In case of ameloblastoma, when the thickness was compared red-colored fibers were thickest whereas greenish-yellow colored fibers were thinnest and the difference was statistically significant. These findings were similar to the study conducted by Vaidhehi *et al*.[6] and Singh HP *et al*.[10] This might be due to the fact that as the collagen fibers mature, there is change in proteoglycan content of fibers causing dehydration of fibers, resulting in increase in the diameter of collagen fibers and intensity of birefringence. Hence, there is change in polarizing color of collagen fibers from greenish-yellow to yellowish-red.[17] Densely packed collagen gives out longer wavelengths. It is also found that the difference in color patterns of collagen fibers could be due to various growth factors and cytokines that cause proliferation of fibroblasts and ECM, resulting in the formation of thick mature collagen.[18] Polarization colors also depend on the age of the lesion. Longer duration of the lesions increases the amount of collagen fibers, which exhibit yellowish-red and red polarization colors, indicating tighter packing and better alignment of the microfibrils.[16,19]

In the present study, there was predominance of tightly packed mature collagen fibers with greater diameter in ameloblastoma when compared to tooth germ papillae where loosely packed immature collagen fibers were found. Moreover, ameloblastomas are tumors arising from remnants of odontogenic epithelium, more specifically rests of dental lamina. The inductive potential of proliferating odontogenic epithelium acts effectively on ectomesenchymal cells during odontogenesis but perhaps does not act appropriately on differentiated fibrous connective tissue as in ameloblastoma. Hence, instead of activating cell proliferation in dental papilla as in early stages of tooth development, in ameloblastoma, the result of epithelial influence on the connective tissue is rather a degenerative process of present collagenous fibers. As seen in most cases of plexiform ameloblastoma, studies have found the role of markers of ECM degradation in the invasion of ameloblastoma.[20] This might be the reason for the rationale of why hard tissue formation is not seen in ameloblastoma.

CONCLUSION

Ameloblasts do not attain functional maturation in tumor cells due to the absence of ectomesenchyme. Even expression of enamel protein was not found in ameloblastoma that resulted in the absence of hard tissue. Our study suggests that in ameloblastoma, the predominance of tightly packed mature collagen fibers with greater diameter gave promising results for the absence of hard tissue formation. Thus, the collagen fibers play a pivotal role in modeling the biologic behavior of various pathological lesions, as well as vanguard its bystander epithelium during normal odontogenesis.

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Conflicts of interest

There are no conflicts of interest.

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