Short Communication

The Utility of Azan Trichrome Staining in Ameloblastoma

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Abstract

Background: It is occasionally difficult to distinguish the stellate reticulum-like region of ameloblastoma from the fibrous connective tissue stroma. This difficulty is further pronounced in the plexiform variant of ameloblastoma that has very sparse fibrous connective tissue.

Aim: To test the utility of Azan trichrome stain in marking tumour regions and the peri-tumour environment of ameloblastoma.

Materials and Methods: Sections were prepared for 18 formalin fixed paraffin-embedded blocks of ameloblastoma cases and stained with Azan trichrome stain according to the manufacturer's specification.

Results and Conclusions: The tumour areas were stained mostly brown, with the ameloblasts mainly marked as deep brown while the stellate reticulum-like region was light brown. The structures in the peri-tumour region were marked with different shades of blue. Azan trichrome staining was able to distinguish between the fibrous connective tissue and the stellate reticulum-like areas in 100% of the cases.

Key Words: *Ameloblastoma, Azan trichrome stain, connective tissue, stellate reticulum*

Introduction

It is occasionally difficult to distinguish the stellate reticulum-like region of ameloblastoma from the fibrous connective tissue stroma area. This difficulty is further pronounced in the plexiform variant of ameloblastoma that has intricate interlacing islands, strands and cords of the tumour and connective tissue, such that in some cases it is virtually impossible to distinguish the fibrous connective tissue from the stellate reticulum-like region. It has been shown that the estimated monthly growth rate of desmoplastic ameloblastoma, which has abundant dense fibrous connective tissue stroma, is significantly lower than other conventional variants of ameloblastoma.[1] Hence, it can be projected that the less the fibrous connective tissue stroma, the faster the rate of monthly growth of ameloblastoma. Compared to other conventional variants, the fibrous connective tissue stroma of plexiform ameloblastoma is very sparse indeed. It may thus be useful to be able to estimate the area covered by connective tissue in this variant as a possible predictor of growth rate. Plexiform ameloblastoma is reported to be the second most recurrent variant after the follicular variant.[2]

Trichrome staining is a method in which three anionic dyes are used to mark a varied number of structures histologically.[3] Heidenhain Azan trichrome staining is an improvement over the traditional Mallory's method.[3] It is commonly used to distinguish cells from extracellular components and stains muscle fibres red, cartilage and bone matrix blue.[3]

Our study tested the utility of Azan trichrome stain in marking tumour regions and the peri-tumour environment of ameloblastoma, especially to distinguish the stellate reticulum-like region from the fibrous connective tissue stroma area.

Materials and Methods

Eighteen formalin fixed paraffin-embedded blocks of ameloblastoma cases from the Oral Pathology Department of University College Hospital, Ibadan, Nigeria, were sectioned and stained with haematoxylin and eosin for re-evaluation and inclusion in the study. At the REPAIR Laboratory, Institute of Pathology, School of Medicine,

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University of Mainz Germany, sections were prepared for Azan trichrome staining using the following procedure: The sections were de-paraffinised using xylene and hydrated with descending alcohol series (100%, 90%, 70% and 60%) each for 15 min. Thereafter, they were rinsed in distilled water for 3 min and then immersed in the nuclear fast red solution for 30 min, rinsed two times under running water and once in distilled water. The sections were differentiated in 0.1% aniline alcohol solution for 3 min; this was followed by one change of acetic alcohol solution to terminate the differentiation. They were immersed in 5% aqueous phosphotungstic acid for 10 min and rinsed in distilled water, after which they were then immersed for 5 min in 1 part aniline blue-orange mixture to three parts distilled water and briefly rinsed again in distilled water. The section was then taken through an ascending series of alcohol solution (60%, 70%, 80%, 90% and 100%) to dehydrate the sections through two changes of each solution for 2 min each. The sections were finally cleared in xylene three times for 3 min each and then mounted with distyrene, plasticiser and xylene, then cover slips were placed. The staining pattern was varied but consisted of brown, blue and reddish colours. Two of the authors examined the slides together and resolved the controversy by a third review.

Results

Eighteen cases of ameloblastoma comprising seven plexiform, three follicular, six cystic and two haemangiomatous variants were selected for inclusion in the study. There was no relationship between histological variant and azan staining colouration/pattern. The tumour areas were stained mostly brown [Figures 1 and 2], with the ameloblasts mainly marked as deep brown (61.1%) while the stellate reticulum-like

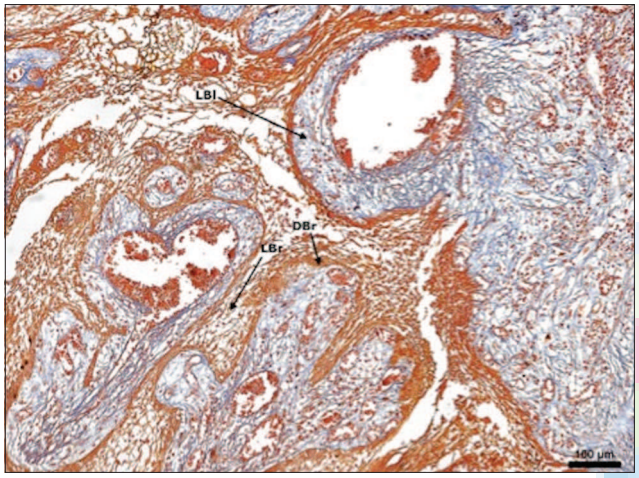

Figure 1: Plexiform ameloblastoma showing light blue fibrous connective tissue, light brown stellate reticulum-like region and palisaded deep brown ameloblasts (×100). Note the relative absence of blood vessel in the stellate reticulum compared to the fibrous connective tissue area. LBl: Light blue, DBr: Deep brown, LBr: Light brown

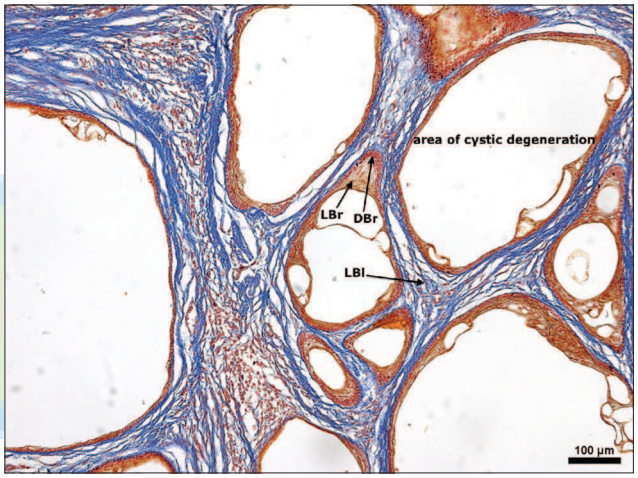

Figure 2: Plexiform ameloblastoma with cystic degeneration showing light blue fibrous connective tissue, light brown stellate reticulum-like region and palisaded deep brown ameloblasts (×100). LBl: Light blue, DBr: Deep brown, LBr: Light brown

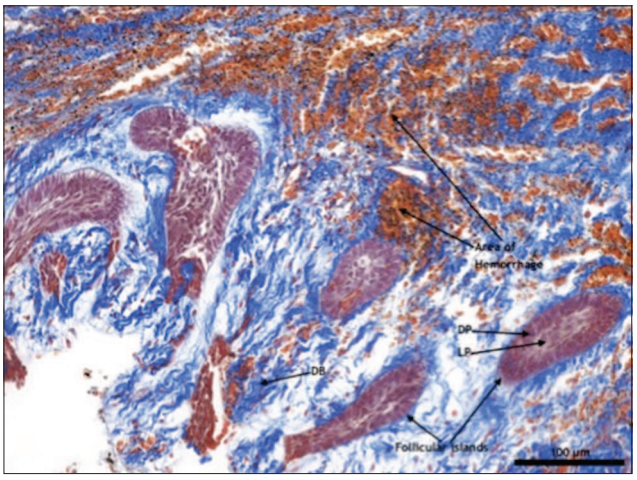

Figure 3: Follicular ameloblastoma with the ameloblasts and stellate reticulum appearing deep purple and light purple, respectively (×200). The fibrous connective tissue is deep blue and regions of haemorrhage appear light brown to orange. LP: Light purple, DP: Deep purple, DB: Deep blue

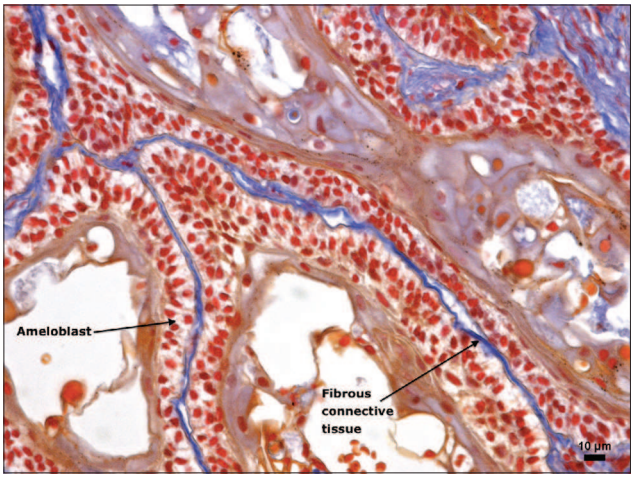

Figure 4: Plexiform ameloblastoma showing the reversed nuclei of ameloblasts taking up a bright orange colour (x400). The thin light blue line marks the fibrous connective tissue stroma, and the light brown region is the stellate reticulum

Table 1: Azan colouration of tumour and peri-tumour areas of ameloblastoma

Region	Colour (number of cases) (percentage)			
Ameloblasts	Deep brown (11) (61.1)	Orange (3) (16.7)	Purple (2) (11.1)	Mixed purple/deep brown (2) (11.1)
Stellate reticulum	Light brown (13) (72.2)	Purple (3) (16.7)	Mixed purple/light brown (2) (11.1)	
Fibrous connective tissue	Light blue (17) (94.4)	Mixed light blue/deep blue (1) (5.6)		
Blood vessels	Light blue (18) (100.0)			
Bone	Deep blue (18) (100.0)			

region was light brown (72.2%) [Table 1]. The fibrous connective tissue, blood vessels and bone in the peri-tumour region were marked with different shades of blue [Table 1 and Figures 1-4].

Discussion

In this report, we have demonstrated the staining characteristics of Azan in ameloblastoma. We did not find any other such study in English literature either describing this pattern in ameloblastoma or linking any deductions with biologic behaviour or outcome after management. This was a retrospective study and since all the cases were treated by surgical resection with an apparently normal margin of at least 1.5 cm, follow-up and outcome were not factored into the analysis. The clinical-pathological relevance, or lack thereof, of our description may be clarified by future longitudinal studies.

Conclusions

Azan trichrome staining was able to distinguish between the fibrous connective tissue and the stellate reticulum-like areas in all of the cases in the present study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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