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ORIGINAL ARTICLE

The Potential Use of Elastic Tissue Autofluorescence in Formalin-fixed Paraffin-embedded Skin Biopsies

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ABSTRACT

Autofluorescence (AF) or naïve-florescence is the natural emission of light by biomolecules. During florescence microscope examination, we realized that elastic tissue is brighter or more autoflourescent than collagen and other biomolecules/cells in the skin. Consequently, we decided to review elastic tissue-related pathologies under a florescence microscope and to report the possible benefits of this technique from selected cases from the paraffin-block archive, by using the protease digestion immunofluorescence method. Selected and clinic-pathologically confirmed 3 elastofibroma dorsi, 3 pseudoxanthoma elasticum, 3 anetoderma, 3 arteriovenous malformations, 3 temporal arteritis, 3 scar tissue and 3 highly solar-damaged samples of skin from 2014-2019 were retrieved. Under the fluorescent microscope, coarse, thick and globularly-fragmented elastic fibers of elastofibroma dorsi, shortened, irregular and convoluted elastic fibers of pseudoxanthoma elasticum, internal elastic membranes of arteries and their integrity was visualized. None of the anetoderma cases had any signal representing elastic tissue. It was shown that elastic tissue can be observed easily under fluorescence microscope in the case of FFPE tissues. The resulting autofluorescence can be useful in recognizing elastic tissue-related pathologies, and it may be used as an ancillary or an alternative method to routine histochemical techniques.

Keywords: Autofluorescence, elastic, elastic membrane, skin

INTRODUCTION

Direct immunofluorescence involving frozen sections is the gold standard method to determine immune deposits in skin and renal biopsies. Antigen retrieval with proteinase on formalin-fixed and paraffin-embedded tissue has been used in renal pathology as a salvage technique when no glomeruli or frozen sections are available [1,2]. Recently, the value of direct immunofluorescence on proteinase-digested formalin fixed paraffin-embedded skin biopsies was investigated, and it was found out to be a less sensitive but still valuable technique for the identification of immune deposits on skin [3]. In addition, H&E stained sections have been evaluated under a florescent microscope in the case of alopecia, melanoma and fibrous proliferations [4-8].

Autofluorescence (AF) or naïve-florescence is the natural emission of light by biomolecules. After the invention of florescent optical technology and AF spectroscopy, AF principles have been used as a tool, not only in medical practice [9], but also in a variety of research fields [10-13].

Under a fluorescent microscope, AF is usually a redundant situation due to a reduction in immunofluorescence evaluation quality since it generates confounding signals that hamper or mimic a florescent dye. Different methodologies have been used to reduce such signals and to increase the quality of both immunofluorescence and florescent in-situ hybridization evaluation [14-16]. Autofluorescent biomolecules (fluorophores)

that can be seen under a florescence microscope are consist of elastic, collagen and sweat gland secretions.

During a routine florescence microscope examination, we realized that elastic tissue is far brighter or more autoflourescent than collagen and other biomolecules/cells in the skin. That is to say elastic tissue can easily be identified from background tissues in that it offers a sharp contrast. Additionally, the elastic tissue contrast facilitates the observation of elastic fiber microanatomy. On this basis we decided to review elastic fiber-related pathologies under a florescence microscope and to report the possible benefits of this technique from selected cases from our institution's paraffin block archive, by using the protease digestion immunofluorescence method.

MATERIALS AND METHODS

The study has been performed according to the Declaration of Helsinki. 3 elastofibroma dorsi, 3 pseudoxanthoma elasticum, 3 anetoderma cases which were selected and clinic-pathologically confirmed from the period 2014-2019 were retrieved from the files of our department. In addition, 3 arteriovenous malformations and 3 temporal arteritis cases were included to present the existence and integrity of the elastic membrane respectively, with the help of the elastin's naïve florescence. 3 scar tissue and 3 highly solar-damaged samples of skin were also included.

The proteinase digestion protocol applicable for formalin-fixed paraffin embedded tissues was carried out as it has been previously [3]. 5µ-thick sections were cut from representative blocks and placed on charged slides. Following deparaffinization and rehydration, the slides were incubated in phosphate buffer saline (PBS) for 10 minutes. Antigen was retrieved by protease induction at 37°C with a 0.05% proteinase (Sigma cat # P8038) in PBS for 5 minutes. Tissue sections were incubated for 45 minutes in the dark with FITC (fluorescein isothiocyanate) - conjugated IgG (Dako; dilution,1:20; cat. no. #F0315) and a C3 (Dako; dilution,1:200; cat. no. #F0201) antibody. The sections were then rinsed twice in PBS. The stained slides were cover-slipped with a mounting medium (Dako; cat. #S3023). The slides were evaluated under a florescence microscope.

RESULTS

Similar results were obtained with both a FITC-conjugated IgG, and C3 antibodies. None of the cases had a background collagen autofluorescence signal that would hamper elastic fiber morphology.

Elastofibroma Dorsi: Coarse, thick and globularly-fragmented elastic fibers were easily seen in all cases. At a higher power, serrated edges were demonstrated. No background collagen florescence that could hamper elastic evaluation was identified (Figure 1).

Pseudoxanthoma Elasticum: Under a florescence microscope, all 3 cases had granular, shortened, irregular and convoluted elastic fibers, haphazardly oriented at the mid-dermis (Figure 2).

Anetoderma: All the selected cases were clinically and pathologically correlated with advanced stage anetoderma cases. On hematoxylin and eosin stained slides, each case showed abundant sclerodermoid collagen. Staining was repeated 3 times, but all cases were devoid of an elastic fiber autofluorescence signal (Figure 3).

Temporal Arteritis: Luminal-oriented sections demonstrate a linear autofluorescence of the internal elastic membrane. Discontinuity and fragmentation could easily be identified (Figure 4a-d).

Arteriovenous Malformation

Arteries can be distinguished from surrounding veins in the lesion by noting the autofluorescence of the elastic membrane (Figure 4e-h).

Equivocal and variable results are obtained from the scarred and solar-damaged skin tissues. Both solar elastosis and scarring can hinder or aggravate the autofluorescence signal.

DISCUSSION

Autofluorescence (AF) or naïve-florescence is a natural emission of light by biomolecules. After the invention of florescent optical technology, AF began to be used in research and in the medical field in various ways. For instance, with regard to the determination of dysplastic or cancerous epithelium, AF characteristics were proposed as a

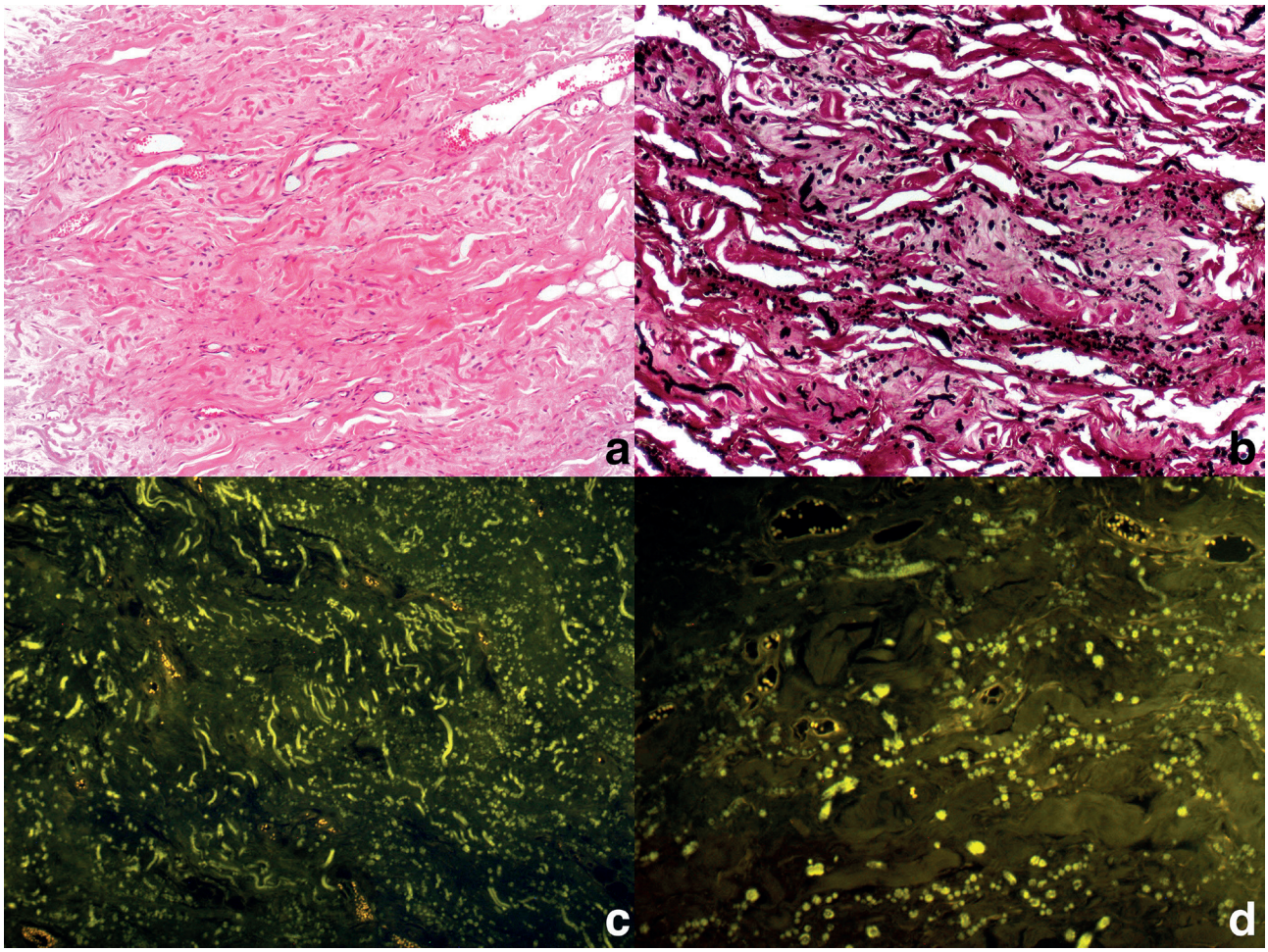

Figure 1. Elastofibroma dorsi a: Thick, globular, elastic fiber fragments are scattered throughout the collagenous stroma (100x) b: Elastica Van Giesson stain (100x) c-d: The autofluorescence of thick, globular elastic fiber fragments can be seen under fluorescence microscope with a minimal collagenous background signal (100x, 200x).

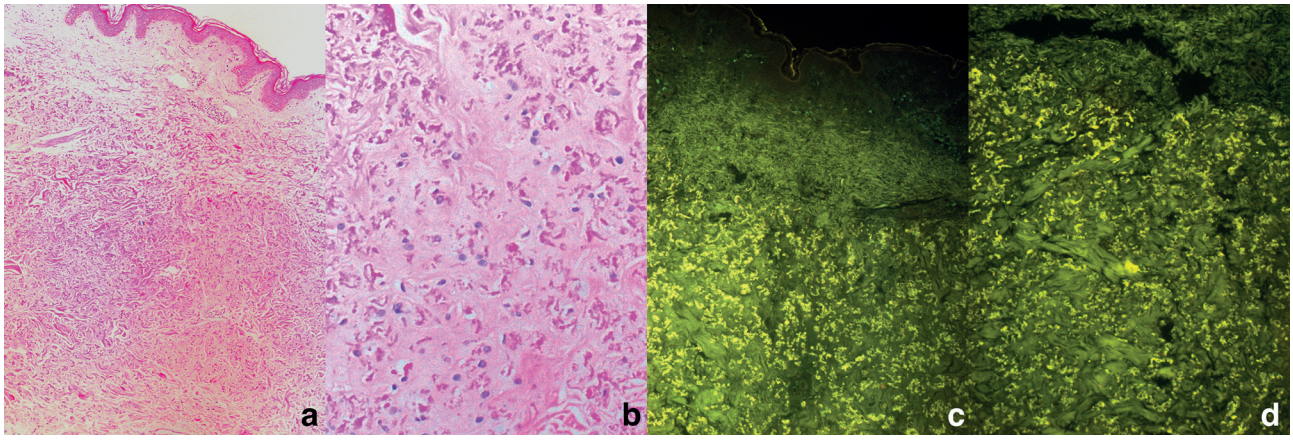

Figure 2. Pseudoxantoma elasticum a: Short, curled and irregular elastic fibers in the reticular dermis. Papillary dermis is spared (100x). b: İrregular fragmentation of the elastic fibers (200x) c-d: Autofluorescence of haphazardly oriented curled elastic fibers in the reticular dermis (100x).

tool for detecting the biopsy site or with regard to deciding on the most appropriate form of clinical management (10-12). It has also been considered for identifying the lung tumor histologic type based on the color textures of AF bronchoscopic images (13). Regarding the skin, AF has been suggested as a useful parameter for detecting skin cancer recurrence [14] and for increasing the reproducibility of the patch test [15]. Moreover, autofluorescence principles have been used in-vivo multiphoton microscopy to provide information with regard to age-related delays in healing, an

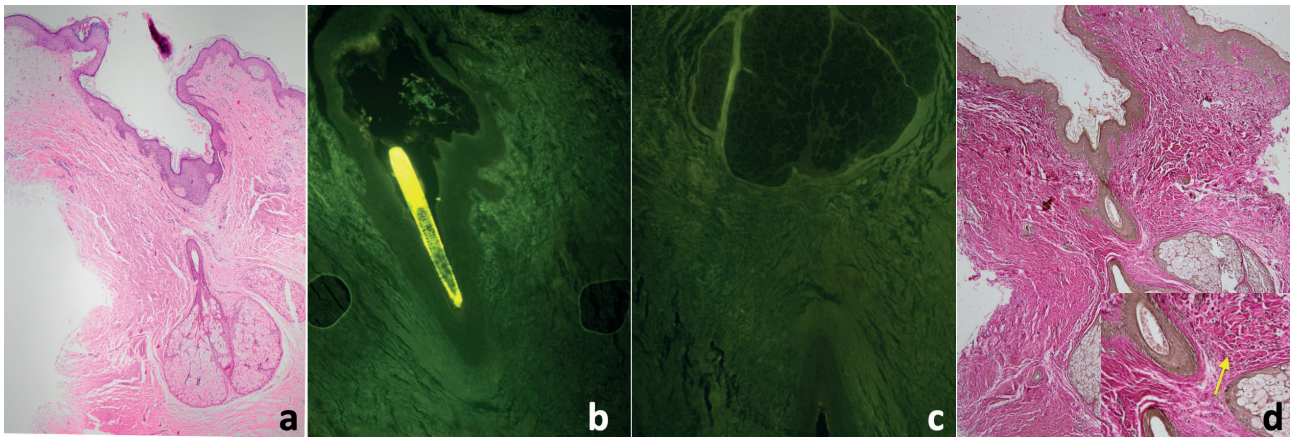

Figure 3. Anetoderma a: Established lesion has increased collagenous activity (40x) b-c: No elastic tissue autofluorescence is identified under fluorescent microscope (100x) d: Elastica Van Giesson stain, elastic fibers are diminished (40x). Residual focal and fragmented black elastic fibers are inner positive control of the stain (inlet-200x).

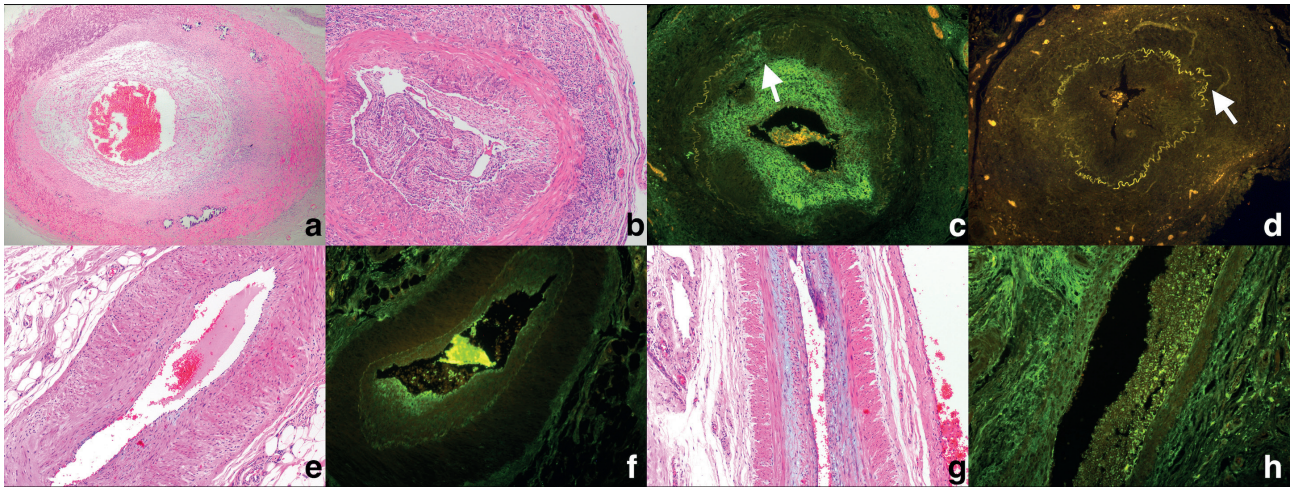

Figure 4. a-b: Clinical-pathological correlated giant cell (temporal) arteritis cases, luminal oriented sections (100x). c-d: Autofluorescence of the elastic membrane helps to examine the integrity or fragmentation of the elastic lamina. The white arrow indicates fragmented lamina (100x). e-f: Luminal orientation of a vessel from arteriovenous malformation case. Autofluorescence of the elastic membrane helps to identify arterial component (100x). g-h: Venous component of arteriovenous malformation, devoid of arterial elastic membrane autofluorescence (100x).

aspect which can be useful for future patient-specific wound care [20].

AF could be redundant since it results in a confounding signal which restricts effective immunofluorescence evaluation. This is especially true in the case of myeloproliferative disease resulting from due to the high level of collagen and elastic production. Hence, the AF characteristics of the elastic tissue under a fluorescence microscope are very well-known.

It is known to be used in nephropathology practice when a frozen section is not available [1,2]. Recently, this technique has been applied to formalin-fixed paraffin-embedded skin tissues [3]. Although in this research the aim was not to detect immune accumulation, the protease digestion protocol was preferred. This is because the authors think that protease digestion will contribute to the brightness of elastic tissue in FFPE tissues. Moreover, an antibody was not needed for generating an AF signal. Because FITC (fluorescein isothiocyanate)-conjugated IgG and C3 antibody consumables were available and ready for use, conjugated antibodies were applied to the FFPE tissues. FITC (fluorescein isothiocanate) or DAPI (4′,6-diamidino-2-phenylindole) could be suitable for detecting elastic AF signals. Consequently, it is expected that both antibodies will give the same results.

"Is it an artery or a vein?" Under a light microscope the answer to this question is sometimes difficult, but if elastic membrane is detected, the answer

has already been given, in that the vessel is most probably an artery. Routine histomorphology practice involving the differential diagnosis of malformative vascular anomalies is challenging. Differential diagnoses include arteriovenous malformation and venous malformation. This distinction can be easily made by identifying the elastic membrane involved. Consequently, this particular question can be answered with the use of a florescence microscope with the help of the elastic membrane's AF characteristics (Figure 4e-h). In the literature we can also find evidence of autoflorescence technology's success in terms of identifying vessel type in dorsal skin, the cerebral cortex, and in large vessels in the abdominal cavity [21]. Moreover, it is clear that the integrity of elastic lamina can easily be recognized with the aid of a florescence microscope. Thus, we decided to retrieve the giant cell arterititis from our institution's archive and stain it with FITC (fluorescein isothiocyanate) - conjugated IgG and C3 antibodies. The diagnostic morphologic clue of giant cell arteritis - a fragmentation of the internal elastic membrane - can readily be identified under a florescence microscope (Figure 4a-d).

Elastic tissue anomalies in the skin, such as pseudoxanthoma elasticum and anetoderma, can also be evaluated under routine florescence microscope examination. In the case of pseudoxanthoma, the elastic tissue microanatomy can easily be visualized. No elastic tissue signal was recognized in anetoderma cases (Figures 2 and 3) although it was repeated 3 times. When the issue is early anetoderma biopsy, the comparison of lesional skin with intact skin by quantifying the AF signal, might potentially be the subject of further research. It may be the case that in the future, diagnosis might be made by comparing AF signals obtained from an anetoderma lesion and non-lesional skin. Perhaps, as a result of these studies, a threshold value might be determined for a precise distinction to be made.

Equivocal and variable results are obtained in the case of scarred and solar-damaged skin tissue. The AF signal is aggravated in some cases, whereas in some cases it is attenuated. This may be due to the variability of the presence and amount of intact elastic fibers in tissues associated with the aforementioned morphology.

In routine pathology practice, elastic fiber stains such as elastica Van Giesson or Verhoeff elastic stains have been used to detect elastic fibers. However, with regard to these stains it can be technically difficult to achieve the required standard, and microscopic control is necessary between staining steps. In contrast, immunofluorescence microscopy is a technique that requires some microscope use experience. However, the fact that the AF technique is standard and does not require microscopic control is a situation that facilitates its applicability. Which type of elastic fiber is more autofluorescent, or which is superior to the routine elastic histochemistry, are questions that are waiting to be answered. Specificity and sensitivity results can be obtained by comparing the protease-mediated results with histochemical studies. Measuring and comparing quantified AF and the histochemistry signals of large series can give such a result.

Autofluorescence occur in H&E sections on IF microscope. Dr. Elston and his team searched for elastic paterns on H&E stained slides of alopecia, fibrous proliferations and melanocytic lesions under a florescent microscope to get rapid assessment of elastic pattern [4-8]. However, in our experience, elastic fibers appear more contrast and brighter than the surrounding tissues in the protease digestion method.

In summary, it was shown that elastic tissue can be observed easily under a fluorescence microscope in formalin-fixed paraffin-embedded tissues. The resulting autofluorescence can be useful in recognizing elastic tissue-related pathologies. Moreover, this technique can be used as an ancillary or an alternative method to routine histochemical techniques. In addition, we believe that the quantification of elastic tissue autofluorescence under a fluorescence microscope is a very promising research field, and a method for use in the case of the abovementioned elastic fiber-related pathologies.

Author contribution

Study conception and design: DAO; data collection: DAO and KS; analysis and interpretation of results: DAO and KS; draft manuscript preparation: DAO. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

Not applicable, anonymity of the patients' and their confidentiallity was preserved.

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Conflict of interest

The authors declare that there is no conflict of interest.

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