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Full Length Research Paper

Autofluorescence of routinely hematoxylin and eosin-stained sections without exogenous markers

Deeb S.*, Nesr K. H., Mahdy E., Badawey M. and Badei M.

Department of Pathology, Faculty of Veterinary Medicine, University of Beni Suef, Egypt.

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Hematoxylin and eosin stained paraffin section was examined by fluorescence microscopy to study the pattern and distribution of fluorescence. Autofluorescence was sensitive and specific for detection of elastic and collagen fibers. It was concluded that analytical morphological techniques based on autofluorescence can obtain information about morphological and pathological state of tissue and cells.

Key words: Histology, pathology, fluorescence microscopy.

INTRODUCTION

Study of tissue components of the body is done by many methods such as histological methods as well as histochemical methods. Hematoxylin and eosin staining method (H and E) has long been used as a routine histological method, and staining by this dying method is dependant partly upon osmosis and adsorption and partly upon chemical affinities (Carleton, 1938). For histochemistry, the substance in tissue must be identified by a procedure that is specific for it, or for the chemical group to which it belongs (DeRobertis et al., 1975). This identification can be made by: (1) chemical reactions, (2) reactions that are specific for certain groups or substances, and (3) physical methods; fluorescence microscopy is one of the latter. The objective of the present work was to study the reaction of tissue stained with H and E when exposed to ultraviolet light by fluorescence microscopy and the pattern of distribution of autofluorescence.

In the present study, the intensity and distribution of intrinsic autofluorescence in stained sections were studied in different tissue and organs without the use of any exogenous fluorophores.

MATERIAL AND METHODS

The materials (Table 1) originated from the archive of our "Veterinary Tissue Bank" (www.veterinary-tissue-bank-egypt.com). All tissues were fixed in 10% formalin solution for at least 48 h, dehydrated in alcohol, cleared in xylene and embedded in paraffin. Sections (5 - 6 µ) were stained routinely with hematoxylin and eosin (Haematoxylin: Fluka, AG, Switzerland, Buchs SG – Eosin Y: alcohol and water soluble, Winlap, UK), mounted in fluorescence-free D-P-X mountant (LOBA Chemie, India). Moreover, slides stained with van Giesson's stain, Masson's trichrome, Weigert's, Gomori's, vonKossa, Southgate's carmine, Periodic acid Schiff, turnbull's blue and Fouchet stain were also examined.

The slides were examined under vertical fluorescence microscope (American Optics, model 2071, Max Watts 100) using green/blue filter (AO 2073) and yellow filter (AO 2074). Fields were photographed optically using Yaschica camera 2000 (FX-3 super) with an exposure time 90 s. Scoring of the intensity of fluorescence ranged from 0 for non-reactive tissue to 4+ to highly intense tissue.

RESULTS AND DISCUSSION

In the present study, it has been shown that many tissue components have an intrinsic fluorescence of various intensities in sections fixed in formalin, embedded in paraffin, and stained with H and E. The results are shown in Table 1 and Figure 1.

Hematoxylin was found to have no effect on the intensity of fluorescence. In contrast, eosin appeared to intensify intrinsic fluorescence. In general, nuclear eu- and heterochromatin of epithelial, muscular and lymphoid tissue did no react while the cytoplasm showed pale fluorescence. The most intense fluorescence was imparted by elastic fibers, especially the arterial elastic layer; this layer is thrown into corrugations of intensely fluorescent elastic bundles with a clear contrast between them and the intervening collagen tissue.

*Corresponding author. E-mail: salahdeeb40@yahoo.com.

Table 1. Tissue/organ autofluorescence intensity.

Tissue/Organ	Fl.-intensity
Connective tissue fibers:	
- Elastic fibers (aorta, organ blood vessels)	+++
- Reticular fibers (adventitia of blood vessels, septa of spleen, lymph nodes, basement membrane, Bowmann Capsule)	++
- Collagen (dermis, interstitial tissue of different organs)	++
Epithelium:	
(epidermis, gastrointestinal tract, hepatocytes, epithelium of gall and urinary bladder, renal epithelium, semineferous tubules, sperms, serous membranes)	+
Muscular tissue: -Skeletal muscles	++
-Cardiac	++
-Plain muscles	++
Hyaline cartilage (bronchial)	+
Skin: -Epidermis (keratin layer)	+
-Dermis (collagen)	++
Hair	
- Hair pulp	-
- Inner root sheath	++
- Outer root sheath	+
Lymphoreticular tissue: -Lymphocyte series	-
-Reticulocytes	+
Haemopoietic elements:	
- Neutrophils, eosinophils, basophils	-
Macrophages	+
Giant cells	-
Bile pigment	-
Caseous material	-
Calcareous deposits	-
Bacterial colonies	-
Sarcosporidia	-
Fasciola eggs	-

Scores: - =non-reactive, ++++: very intense, +++: intense, ++: mildly intense, +: intense.

Autofluorescence of interstitial tissue makes structures such as glomeruli and renal tubules in the kidney, and hepatic cords to become visible (Figure 1).

The results of the present study revealed that examination of H and E-stained tissue sections with fluorescence microscopy was sensitive enough to specifically localize elastic fibers and collagen. The method was comparable to differential staining methods, such as Weigert's stain and Masson's trichrome stain, for these tissue components and can replace them.

In fluorescence microscopy, tissue sections examined under ultraviolet light, near the visible spectrum, the components are recognized by the fluorescence they emit in the visible spectrum. Two types of fluorescence may be studied: natural fluorescence (autofluorescence) which is produced by substances normally present in the tissue, and secondary fluorescence, which is produced by staining with fluorescent dyes called fluorochromes (DeRobertis et al., 1975).

As has been reported by Monici (2005), changes occurring in the cell and tissue state during physiological and/or pathological processes result in modification of the amount and distribution of endogenous fluorophores and chemical-physical properties of their microenvironment. This was clear in our study through the case of three-day sickness included in our material. In this case, filtration of plasma from injured blood vessels and permeation or soaking of skeletal muscle bundles in the area led to intense fluorescence emission. Our result is in consistency with many authors (Rubio and Slezak, 2002; Goujon et al., 2003; Rotomskis and Streckyte, 2004; Monici, 2005) that the extra-cellular matrix contributes to the autofluorescence emission more than the cellular components, because collagen and elasin have, among

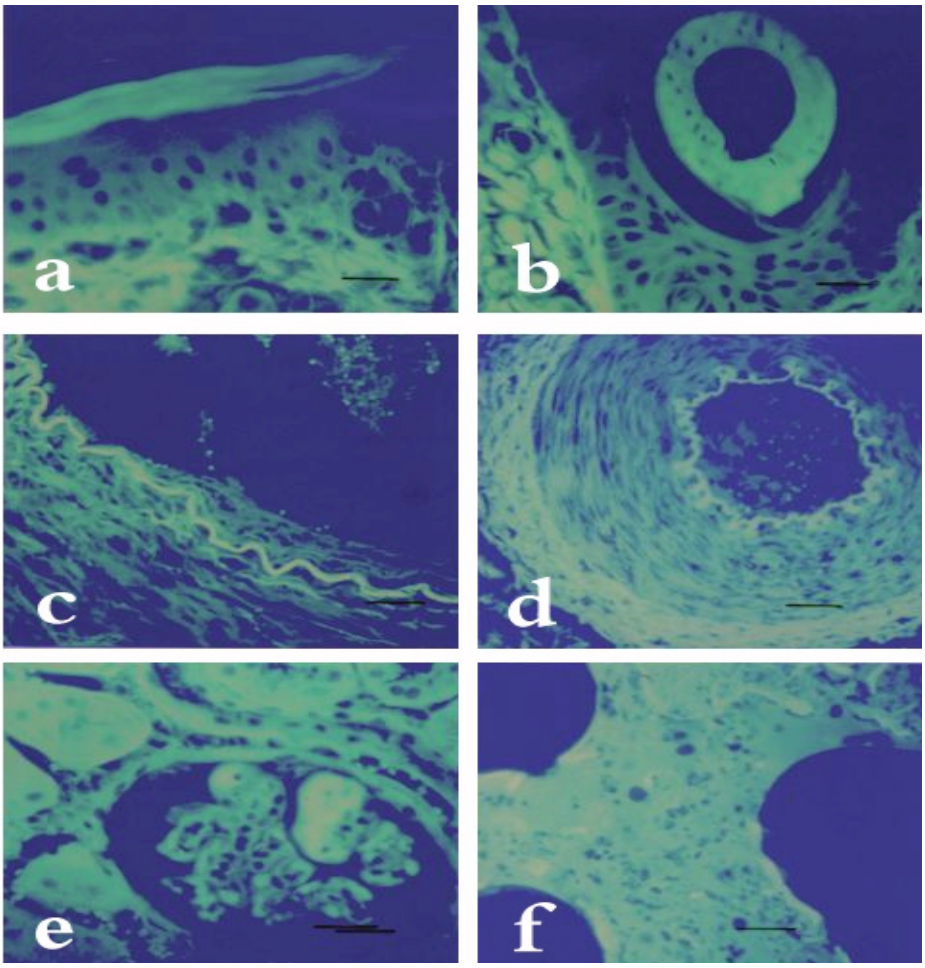

Figure 1. a: Skin (epidermis), **b:** skin (hair follicle), **c:** Elastic bundle in the wall of a blood vessel, **d:** wall of a blood vessel (uterus), **e:** Kidney (glomerulus), and **f:** Lung (pneumonic).

endogenous fluorophores, a relatively high quantum yield. In their studies, Sikder et al. (2005) observed that the distribution of mitochondria in a cell can varies with environmental influence, degree of differentiation, and disease. The authors suggested that these differences in the distribution of mitochondrial autofluorescence may be used to distinguish different cellular states.

It is known that a major obstacle in application of clinical diagnosis of immunofluorescence staining is the high fluorescent background of formalin-fixed, paraffin-embedded tissue sections (Niki et al., 2004); hematoxylin and eosin counterstaining was found to be ineffective in quenching of this fluorescent background. All other stains used in the present study were sufficient to inhibit or reduce autofluorescence in tissue and organs; the reaction of these stains with fluorophore-bound immuno-globulins must be studied. To reduce background immunofluorescence and autofluorescence of hemoglo-bin, Casella et al. (2004) pre-treated the tissue sections with 0.1% sodium borohydride in phosphate buffered saline for 30 min, followed by 1 to 5 min incubation in 0.5% Sudan black in 70% ethanol. Viegas et al. (2007) have found that the combination of short-duration, high intensity ultra-violet irradiation and Sudan black was the best approach to reduce autofluorescence in highly vascularized, high lipofucins' content tissue, such as murine liver and kidney, and poorly vascularized, low lipofuscins' content tissues such as the pancreas.

Autofluorescence optical imaging, especially depth-resolved fluorescence spectroscopy, is rapidly becoming a widely used clinical tool. Generally, it can be concluded that analytical morphological techniques based on auto-fluorescence can obtain information about morphological and pathological state of the tissue and cells.

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