



Journal homepage:
<http://www.bsu.edu.eg/bsujournals/JVMR.aspx>
Online ISSN: 2357-0520 Print ISSN: 2357-0512



Original Research Article

Characterization of autofluorescence in normal and necrosed muscles in chickens

Mohamed Kamal; EL-Shaymaa EL-Nahass*; Khalid A. El-Nesr; Adel A. Shalaby

Department of Pathology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt.

ABSTRACT

The histopathological diagnosis of muscle necrosis and hyalinosis frequently poses considerable difficulty and has a contradictory diagnosis. The present study described the morphologic features of nine clinically affected chicken pectoral muscles and one normal muscle using fluorescence microscopy on formalin fixed-paraffin embedded tissues. Histopathological examination of samples (normal and necrosed) was routinely done using stained sections with heamatoxylin and eosin. Sections examined by fluorescent microscopy showed significant or intense autofluorescence in necrosed muscles. The subsequent image/color analysis of the fluorescent images was carried out to characterize the color intensity of autofluorescence emitted from chickens' muscles and to compare autofluorescence with the normal ones. In necrosed muscles, samples exhibited a marked increase in fluorescence intensity. Normally stained section with non-specific autofluorescent revealed 99.48% for normal specimens compared to 82.93% for necrosed ones, and that of specific autofluorescent revealed 0.62% for normal specimens compared to 17.08% for necrosed ones. The technique allows imaging of chickens muscle samples, facilitating the determination of the degree of necrosis throughout the muscle using statistical analysis, particularly in those related to comparative pathology, and avoiding the disadvantages of routine histopathological examination.

ARTICLE INFO

Article history:

Received
Accepted
Online

Keywords:

autofluorescence,
necrosed muscles,
image analysis, HE

* Corresponding author: El-Shaymaa El-Nahass, Department of Pathology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt. Tel/fax: +2 0822327982, Email: shima_k81@yahoo.com

1. Introduction

Autofluorescence is the natural emission of light by biological structures such as lysosomes and mitochondria when they have absorbed light (Monici, 2005). The most commonly observed autofluorescing molecules (endogenous fluorophores) are pyridinic (NADPH) and flavin coenzymes; the extracellular matrix could also contribute to autofluorescence as a result of the intrinsic properties of collagen and elastin (Monici, 2005). Generally, proteins containing an increased amount of some amino acids as tryptophan, tyrosine and phenylalanine show some degree of autofluorescence (Menter, 2006). In a few cases, autofluorescence may actually illuminate the structures of interest, or act as a useful diagnostic indicator (Monici, 2005). For example, cellular autofluorescence could be used as cytotoxicity indicator without using fluorescent markers (Fritzsche and Mandenius, 2010). Changes occurring in the cell and tissue state during physiological and/or pathological processes result in modifications of the amount and distribution of endogenous fluorophores and chemical-physical properties of their microenvironment. In addition, the analytical techniques based on autofluorescence monitoring can be utilized in order to obtain information about morphological and physiological state of cells and tissues (Monici, 2005).

In HE-stained sections, significant fluorescence was not only expressed in various tissues without exogenous markers; epithelial, connective tissue and/or parenchymatous organs but also the biological agents (fungi, bacteria or parasites) (Graham, 1983; Elston, 2001; Monici, 2005; Menter, 2006; Bhatta et al., 2006; Daugschies et al., 2006; Deeb et al., 2010).

The aim of the present study was to promote using of autofluorescence as a useful diagnostic indicator for the healthy status of pectoral chicken muscles.

2. Materials and methods

Pectoral muscles samples were collected from ten broiler chicken aged 15 to 35 days (one control and nine clinically affected which were avian infectious bronchitis rt-PCR +ve cases). These samples were fixed in formalin 10% for 48hrs. After that they were dehydrated, cleared, embedded in paraffin wax, blocked and underwent microtomy (4-5 µm) and

stained routinely with hematoxylin and eosin (HE) (Bancroft and Gamble, 2012). Stained sections were examined by Carlzeiss, Inc. fluorescent microscope with ARC LAMP and power supply 50W HBO AC and connected to digital camera. Quantitative analysis using image analysis to verify that degenerated or necrosed sections exhibit increased autofluorescence intensity compared to the normal one (Bankhead, 2014).

3. Results

Gross examination

Macroscopically, pectoral muscle showed no alterations except paleness in some cases.

Histopathological examination

Normal pectoral muscle fibers appeared normal. The sarcoplasm had ovoid or elongated nuclei located near the sarcolemma. Some nuclei were also located centrally within the muscle fibers. The myofibrils are striated and consisted of numerous myofilaments. Numerous muscle fibers grouped in fascicles. Each fascicle is surrounded by perimysium of loose connective tissue (Fig. 1A).

Affected pectoral muscles were characterized by coagulative necrosis and hyalinosis with fragmentation of some muscle fibers. The necrosed muscles fibers revealed condensation of their nuclei with structureless, homogenous and eosinophilic cytoplasm (Fig. 2A).

Fluorescent microscopy

The control sample exhibited a faint dull green fluorescence (Fig. 1B), while the necrosed one showed stronger and significant bright green autofluorescence. Meanwhile, enhanced autofluorescence was significantly observed in muscle fibers showing severe hyalinosis (Fig. 2B). Quite differences between the color intensity of the nine degenerated pectoral muscle samples were observed.

Quantitative analysis

Necrosed sections exhibited marked increase in autofluorescence intensity in which percent of non-specific autofluorescent 99.48 for normal sectioned stained muscle compared to 82.925 for degenerated one and percent of specific autofluorescent 0.62 for normal compared to 17.075 for degenerated one (Fig. 3).

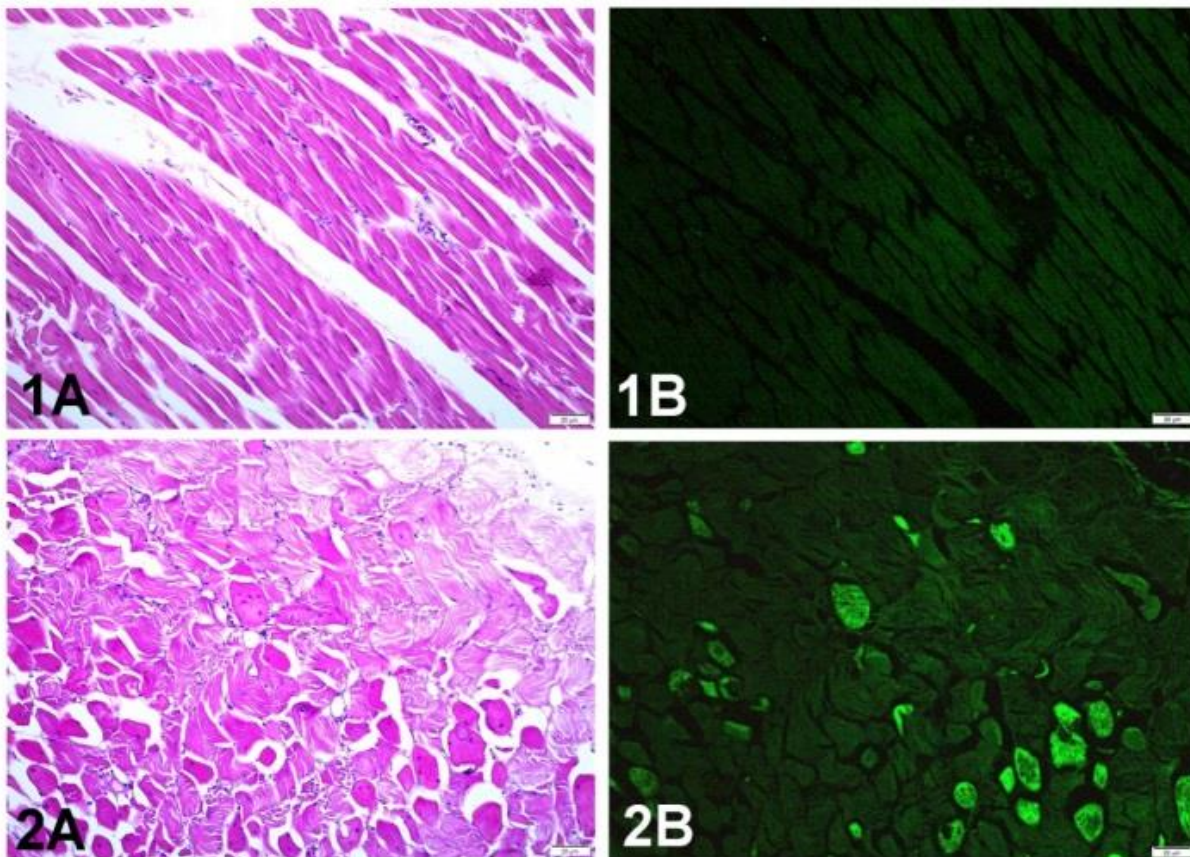


Fig.1. A) Light microscopic image of normal pectoral muscle of broiler chicken showing striation and peripheral nuclei of muscle fibers (HE; X200). **1B)** Fluorescent microscopic image of normal pectoral muscle of broiler chicken showing faint olive green autofluorescent of muscle fibers (HE; X200).

Fig.2. A) Light microscopic image of pectoral muscle of broiler chicken showing degeneration and hyalinosis of muscle fibers (HE; X200). **B)** Fluorescent microscopic image of pectoral muscle of broiler chicken showing bright green autofluorescent of necrosed muscle fibers (HE; X200).

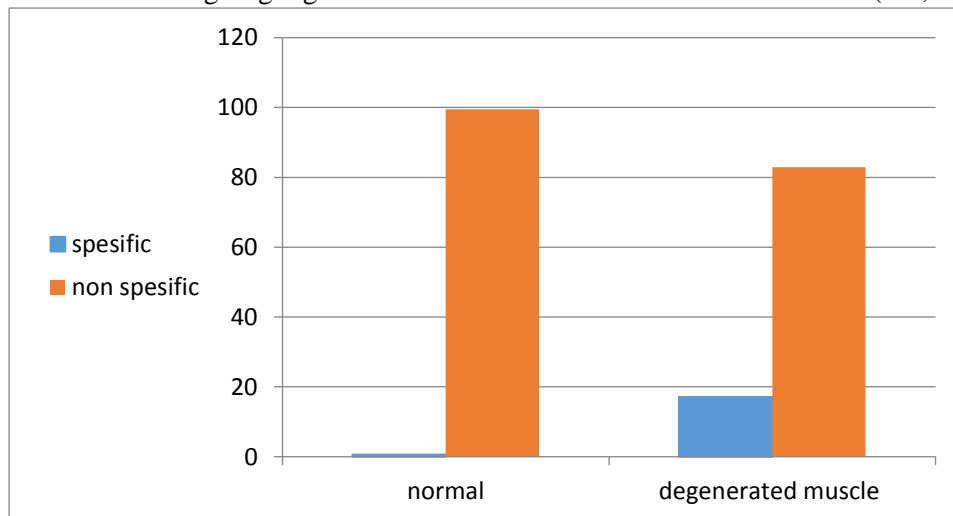


Fig.3. Percentage of specific and non-specific auto-fluorescent of normal and necrosed muscles.

4. Discussion

Using of autofluorescence could be very helpful in obtaining information about morphological and pathological states of tissues and cells especially if it is based on analytical morphological techniques. In H&E stained sections, significant fluorescence was not only expressed in various tissues; epithelial, connective tissue and or parenchymatous organs but also the biological agents (fungi, bacteria or parasites (Elston, 2001; Deeb et al., 2010). Normally stratum corneum expressed strong fluorescence more than the other epidermal layers (Elston, 2001). This technique was more helpful for identifying fungal infection in the skin and parenchymal organs (Graham, 1983; Elston, 2001). Several factors may influence the fluorescence pattern of fungi as the wavelength of light, fixation method and mounting media (Graf, 1996).

The dominant fluorophores (NADH and FAD) are the sources of the fluorescence and they are increased with the higher metabolic rate of tissues. For example, fluorescence intensity was most prominent in cases of dysplastic epithelial tissue relative to the normal samples (Chance, 1989; Drezek et al., 2001). This leads to a decreased concentration in the oxidized electron carrier FAD and increased concentration in the reduced electron carrier NADH.

In skeletal muscle, several distinct fiber types are found; oxidative and glycolytic fibers. Oxidative fibers are distinguished from glycolytic fibers by their high level of NADH dehydrogenase activity (Allen et al., 2001). The autofluorescence is associated with the fibers containing the highest levels of NADH dehydrogenase activity; this indicates that the autofluorescent fibers are oxidative. Alkaline or acidic pH may influence autofluorescence illumination; alkaline pH increase autofluorescence relative to acid pH (Jackson et al., 2004) and this come with our result in which the more prominent autofluorescence intensity indicate the more tissue necrosis.

Autofluorescence emitted from structural proteins, particularly collagen and elastin, which can be considered the most important fluorophores in the extracellular matrix (Fujimoto, 1977; Blomfield et al., 1969), so, there was faint autofluorescence emitted from muscles in which collagen and elastin act as the main component of myofibers protein. Several emissions have been observed for collagen and

elastin within which the molecular origin has not been completely explored (Richards and Seavick, 1996). The fluorescence emission of these proteins is mainly due to the cross-links (Fujimoto, 1977; Deyl et al., 1980). Changes of collagen and elastin contents in tissues have been found in several pathological conditions (Campo et al., 1992). Thus, monitoring of autofluorescence emitted by muscle structural protein have diagnostic value and indication for healthy state of muscles, as our result showed that intensity of fluorescence was high in necrosed muscles with special brightness to those muscle fibers showed severe hyalinosis relative to normal muscle fibers.

The biochemical properties of tissues may be changed significantly *in vitro* and *in vivo*. The ratio of NAD/ NADH may also be changed, as well as blood content and oxidation state. These changes can influence UV-visible autofluorescence (Bigio et al., 1980). Consequently, fluorescence patterns of tissues *in vitro* differ from those *in vivo*. Moreover, *in vitro* the fluorescence from NADH appears to decay exponentially with time, while the fluorescence from collagen and FAD remains relatively constant (Schomacker, 1992). And this may explore our result in relation to collagen and oxidative state from normal to degenerated and necrosed muscles.

The anaerobic component of energy metabolism increases while the aerobic component decreases (Warburg, 1956). The decreased efficiency of the aerobic component increase the NADH (Obi-Tabot, 1993) of tissue, from normal to abnormal state, is accompanied with a decrease in the absolute fluorescence related to collagen, and an increase in the relative contribution of NADH. Thus, differentiation of the various tissues state is determined by the extent of each of these factors (Ramanujam, 1994), therefore, our results could differentiate between normal and necrosed muscle. Such finding came in accordance with that given by Salinas and Sotelo (1986) in which bright yellow auto fluorescence was observed in the necrotic tubules stained with HE. Finally, this method identifies strongly autofluorescence intensity in necrosed muscle fibers than normal ones and also facilitates determining the healthy state and quality of muscles.

5. Conclusion

Autofluorescence provides a reliable determination of the degree of necrosis in muscles, and can overcome the disadvantages of the routine histopathology.

References

- Allen DL, Harrison BC, Maass A, Bell ML, Byrnes WC, Leinwand LA (2001). Cardiac and skeletal muscle adaptations to voluntary wheel running in the mouse. *J. Appl. Physiol.*, 90:1900–1908.
- Bancroft J, Gamble M (2012). In: *Theory and Practice of Histological Techniques*. Philadelphia, Churchill, Livingstone, Elsevier.
- Bankhead P (2014). Analyzing fluorescence microscopy images with Image J. The Nikon Imaging Center @ Heidelberg University.
- Bhatta H, Goldys EM, Learmonth RP (2006). Use of fluorescence spectroscopy to differentiate yeast and bacterial cells. *Appl. Microbiol. Biotechnol.*, 71(1):121–126.
- Bigio IJ, Mourant JR (1997). Ultraviolet and visible spectroscopies for tissue diagnostics: fluorescence spectroscopy and elastic-scattering spectroscopy. *Phys. Med. Biol.*, 42: 803–814.
- Blomfield J, Farrar JF (1969). The fluorescent properties of maturing arterial elastin. *Cardiovasc. Res.*, (3):161–170.
- Campo E, Perez M, Charonis AA (1992). Patterns of basement membrane laminin distribution in nonneoplastic and neoplastic thyroid tissue. *Mod. Pathol.*, 5:540–546.
- Chance B (1989). Metabolic heterogeneities in rapidly metabolizing tissues. *J. Appl. Cardiol.* 4, 207–221.
- Dauguschies A, Bialek R, Joachim A, Mundt HC (2006). Autofluorescence microscopy for the detection of nematode eggs and protozoa, in particular *Isospora suis*, in swine faeces. *Parasitol. Res.*, 87(5): 409–512.
- Deeb S, Nesr KH, Mahdy E, Badawey M, Badei M (2008). Autofluorescence of routinely hematoxylin and eosin stained sections without exogenous markers. *Afr. J. Biotech.*, 7(5): 504–507.
- Deyl Z, Macek K, Adam M, Van-Cikova O (1980). Studies on the chemical nature of elastin fluorescence. *Biochim. Biophys. Acta* 625: 248–254.
- Drezek R, Brookner C, Pavlova I, Boiko I, Malpica A, Lotan R, Follen M, Richards-Kortum R (2001). Autofluorescence microscopy of fresh cervical-tissue sections reveals alterations in tissue biochemistry with Dysplasia. *Photochem. Photobiol.*, 73(6): 636–641.
- Elston DM (2001). Fluorescence of fungi in superficial and deep fungal infections. *BMC Microbiol.*, 1:21.
- Fritzsche M, Mandenius CF (2010). Fluorescent cell-based sensing approaches for toxicity testing. *Anal. Bioanal. Chem.*, 398 (1): 181–191.
- Fujimoto D (1977). Isolation and characterization of a fluorescent material in bovine achilles tendon collagen. *Biochem. Biophys. Res. Commun.* (76):1124–1129.
- Graf B, Gobel UB, Adam T (1998). Qualitative and quantitative Untersuchungen zur Autofluoreszenz bei Pilzen. *Mycoses* 41:39–46.
- Graham AR (1983). Fungal autofluorescence with ultraviolet illumination. *Am. J. Clin. Pathol.*, 79: 231–234.
- Jackson KA, Snyder DS, Goodell MA (2004). Skeletal muscle fiber-specific green Autofluorescence: Potential for stem cell engraftment artifacts. *Stem Cells*; 22:180–187.
- Menter JM (2006). Temperature dependence of collagen fluorescence. *Photochem. Photobiol. Sci.*, 5 (4): 403–410.
- Monici M (2005). Cell and tissue autofluorescence research and diagnostic applications. *Biotechnol. Annu. Rev.*, 11: 227–256.
- Obi-Tabot ET, Hanrahan LM, Cachecho R, Beer ER, Opkins SR, Chan JCK, Shapiro JM, La Morte WW (1993). Changes in hepatocyte NADH fluorescence during prolonged hypoxia. *J. Surg. Res.*, 55:575–580.
- Ramanujam N, Mitchell MF, Mahadevan A, Warren S, Thomsen S, Silva E, Richards-Kortum R (1994). *In vivo* diagnosis of cervical intra-epithelial neoplasia using 337 nm-excited-laser induced fluorescence. *Proc. Natl. Acad. Sci. USA* 91:10193–10197.
- Richards-Kortum R, Sevick-Muraca E (1996). Quantitative optical spectroscopy for tissue diagnosis. *Ann. Rev. Phys. Chem.*, (47):555–606.
- Salinas-Madrigal L, Sotelo-Avila C (1986). Morphologic diagnosis of acute tubular necrosis (ATN) by autofluorescence. *Am. J. Kidney. Dis.*, 7(1):84–87.

- Schomacker KT, Frisoli JK, Compton CC, Flotte TJ, Richter JM, Nishioka NS, Deutsch TF (1992). Ultraviolet laser-induced fluorescence of colonic tissue: basic biology and diagnostic potential. *Lasers Surg. Med.*, 12: 63–78.
- Stetler-Stevenson WG, Liotta LA, Kleiner DE (1993). Extracellular matrix 6: role of matrix metalloproteinases in tumor invasion and metastasis. *FASEB J.*, 7(15):1434–1441.
- Warburg O (1956). On the origin of cancer cells. *Science* 123:309–311.
- Wikipedia (2016). Autofluorescence. <https://es.wikipedia.org/wiki/Autoflorecientes>.