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Demonstration of Muscle Fibre Types Using Masson Trichrome Stain from Deltoideus Muscles of One-humped Camel (*Camelus Dromedarius*)

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Abstract

This study involved the use of 25 male camels (*Camelus dromedarius*) each within the ages of 6 months, 1 year, 3 years, 5 years and 7 years, which were purchased from Sokoto Municipal Modern abattoir. 1 cm^2 of each deltoideus muscle sample was taken from the middle part of the muscle belly and fixed in Bouin's solution for histological preparation using Masson trichrome according to standard procedure; the obtained slides were viewed using a miscroscope using different magnifications. The Masson trichrome stain revealed some collagen connective tissue fibres, and varying proportions and distribution patterns of muscle fibre types I, IIA and IIB. This study could help in advancing the course of knowledge in this study field and as well guide in clinical practice more especially during evaluation of muscular procedures and wound healing.

Keywords: Demonstration; Muscle fibre; Masson trichrome; Deltoideus; Camel

Introduction

The trichrome stain is one of the most utilized special stains in the histology and histopathology Laboratory. Most of the common uses for requesting a trichrome stain are liver biopsies, renal biopsies, dermatopathology, cardiac biopsies and muscle and nerve biopsies, and widely utilized techniques are the Masson, Gomori One Step, Martius Scarlet Blue and Mallory [1].

The purpose of the trichrome stain is primarily to demonstrate collagen and muscle in normal tissue or to differentiate collagen and muscle in tumors. It is also used to identify an increase in collagenous tissue or indicate fibrotic change in cirrhosis of the liver or in a renal disease such as pyelonephritis. The trichrome stain is also used to distinguish tumors that have arisen from muscle cells and fibroblasts. Gomori's trichrome is the trichrome stain of choice for distinguishing histological changes that occur in neuromuscular diseases [1].

Deltoideus is a muscle that lies partly on the triceps brachii in the angle between the scapula and the humerus, and partly on the infraspinatus and teres minor. It is named after the Greek letter *delta*, which is shaped like an equilateral triangle, and it is divided into the scapular part and the acromial part [2]. The *Pars scapularis* arises aponeurotically from the scapular spine, the vertebral border of the infraspinous fossa and the surface of the infraspinatus muscle, becoming fleshy over the caudal border of the infraspinatus. It partly covers the origin of the long and lateral heads of the triceps brachii. Some of its fibres attach to the fascia covering the lateral head, while a strong fascial band on its deep face inserts on the deltoid tuberosity and on an area proximal to it [2]. The *pars acromialis* arises from the acromion and inserts in common with the scapular part of the deltoideus [2,3]. The main action of the muscle is to flex the shoulder joint and abduct the arm [4].

Camel is used as a beast of burden more especially with loads or strain being placed on them, the animal's limbs and their musculatures are the structures that bear the strain for locomotion, traction and support. Due to the function of the deltoideus muscles in flexing the shoulder joint and abducting the arm; it was pertinent to study the anatomy of the muscle in the forelimb of this animal species. This will help in advancing the course of knowledge in this study area which could find application even in clinical practices as Masson trichrome stain could be used to diagnose or detect some muscular diseases.

Materials and Methods

Forelimbs from 25 male camels (*Camelus dromedarius*) and those of 25 male cattle (Zebu type) each within the ages of 6 months, 1 year, 3 years, 5 years and 7 years were purchased from Sokoto Municipal Modern abattoir. The age of each animal was determined, prior to slaughter, using the methods of Dyce KM et al. [3] and Wilson RT [5], while evaluation to exclude any animal with musculoskeletal deformity or diseases was done through physical examination. The live body weights of the animals were estimated using linear body measurement based on the formula of Yagil R [6].

The limbs obtained were wrapped in clean polythene bags and transported in a clean cool box containing ice cubes to the Laboratory of the Department of Veterinary Anatomy, Usmanu Danfodiyo University, Sokoto-Nigeria, where the triceps brachii muscles were all carefully dissected out using the methods of Chibuzo GA [7] as slightly modified by Sonfada ML [8] after most of the connective tissues ensheathing the muscle were trimmed off.

1 cm^2 of each muscle sample was taken from the middle part of the muscle belly and fixed in Bouin's solution for histological

preparation according to the manner of Bancroft J et al. [9]. In this procedure the samples were Mordanted in Bouin's solution, 60°C for 1 hour and washed in running tap water to remove the picric acid, this was done for 5 minutes and immersed in Weigert's working hematoxylin for 10 minutes. The sample was then blued in running tap water for 5 minutes, rinsed in distilled water and subjected to Biebrich scarlet for 5 minutes, then rinsed in distilled water. Following this step, Phosphotungstic/phosphomolybdic acid was used for 10 minutes, and the solution discarded, after which it was transferred directly into Aniline blue for 5 minutes, then rinsed in distilled water. After this stage 1% acetic acid was added for 1 minute, and rinsed in distilled water. Finally, the samples were dehydrated, cleared, and covered with coverslip before viewing.

After histological preparation of the slides, the prepared slides were viewed using a microscope (Olympus® CH 23, Germany) at different magnifications (X40, X100, X400) thereafter photomicrographs were obtained using a Digital Camera (Samsung® ES10, 8.1 Mega Pixels). The photomicrographs obtained were further transfered into a computer (Compac® Laptop, HDM, Presario CQ60) for further evaluation and detailed histological studies.

Results

Photomicrographs of the deltoideus muscles from the camel studied using Masson trichrome staining procedure revealed some muscle fibres and collagen connective tissue fibres which were stained bluish-green to dark blue colouration, depending on the uptake of the stain and muscle fibre types available (Figure 1). It was observed that across the different deltoideus muscles sampled, varying proportions and distribution patterns of different muscle fibre types (types I, IIA and IIB) were differentiated (Figures 2-5).

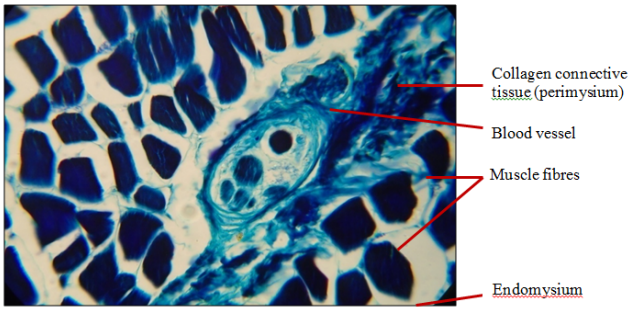

Figure 1: Photomicrograph of a cross section of camel's deltoideus muscle demonstrating deeply stained muscle fibres (dark blue) and collagen connective tissue (perimysium) (MT x400).

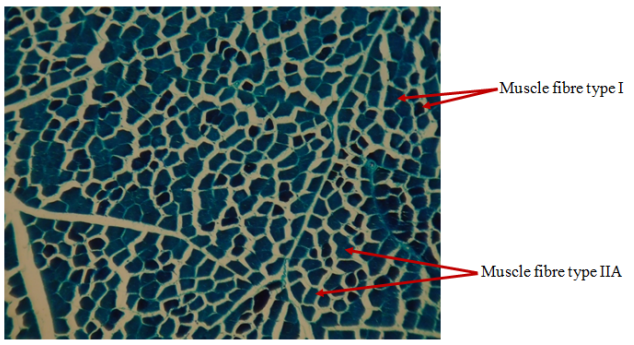

Figure 2: Photomicrograph of a cross section of camel's deltoideus muscle demonstrating types I and IIA muscle fibres types (MT x400).

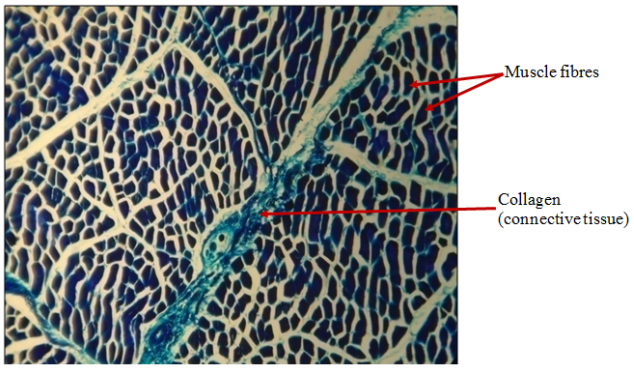

Figure 3: Photomicrograph of a cross section of camel's deltoideus muscle demonstrating muscle fibres (dark blue) and collagen connective tissue (perimysium) stained light blue (MT x100).

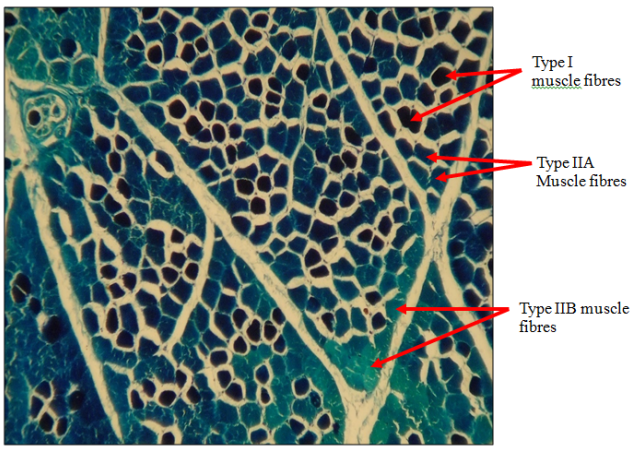

Figure 4: Photomicrograph of a cross section of camel's deltoideus muscle demonstrating types I, IIA and IIB muscle fibre types (MT x400).

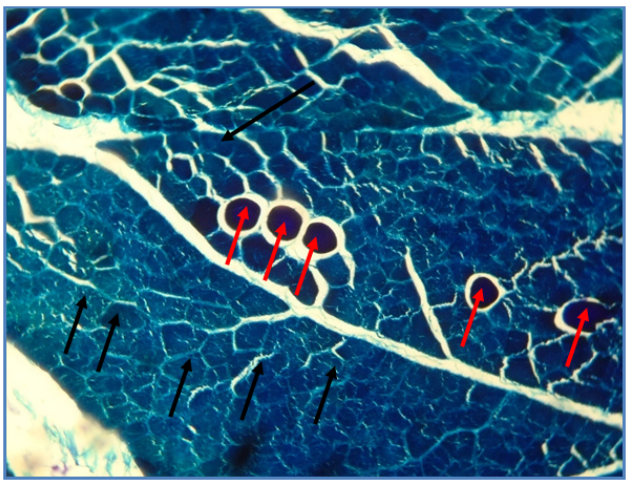

Figure 5: Photomicrograph of a cross section of camel's deltoideus muscle differentiating type I (red arrows) and type IIA (black arrows) muscle fibres (MT x100).

Discussion

In this work, the relative amount and distribution of collagen fibres in the different muscles were demonstrated using the Masson trichrome stain, although the distribution and nature of the collagen fibres may depend on the species, sex, age, muscle group, and as well as on the individual animal itself. They also depend on the level of physical activity, disuse, nutritional status, denervation, and chronic physiological stress. Although in this work no special procedure was done further to classify the collagen fibres rather than just marking their presence by their uptake of the trichrome stain and noting their distribution. The stained collagen fibres demonstrated is consistent with the reports of [1] and that of [10] who reported that on Masson trichrome stain collagen fibres appears green/blue.

Although literatures have reported that muscle fibres could be demonstrated on Masson trichrome but no detail record of the different fibre types being differentiated on this stain, but in the present work the different fibre types (types I, IIA and IIB) have been identified with Masson trichrome stain. In mammals, skeletal muscles are composed of a mixed population of red, intermediate and white muscle fibres [11]. The phenotypic differences among skeletal muscle cells, termed fiber types, their potential for adaptation and underlying mechanisms have been a topic of study for several decades. Most mammalian skeletal muscles are composed of varying proportions of the three major types (I, IIA and IIB) of fibers, while certain muscles such as the rat soleus consist predominantly of type I fibers [12]. The distinctive physiological properties of different skeletal muscle fibre types allow the muscle to respond to various mechanical (speed and endurance) and metabolic (anaerobic or aerobic) demands [13].

Postnatally, the phenotypic profiles of muscle fibres can change by responding to altered functional demands. Previously, Ashmore et al. [14] reported in their work on postnatal development of muscle fibre types that the increasing degree of muscularity in domestic animals is achieved by practices (i.e., domestication and selection) that favour transformation of intermediate fibres to fast twitch fibres. The profiles of muscle fibres can also change by a variety of signals such as cross-innervation, exercise, immobilization, and electro-stimulation [15]. Muscle fibre type distribution was evaluated in the present study, but in view of the conflicting results reported in the literature, variations in the muscle fibre type composition must be considered as a possible explanation for the variation in muscle strength per unit cross-sectional area. Skeletal muscle is uniquely sensitive to mechanical load, with individual muscle fibres able to increase and decrease in size. Due to this robust adaptive property, muscle biologists have long understood the importance of accurately quantifying muscle fibre size [16].

Conclusion

The trichrome stain utilized was able to show distinguishing histologic changes in the connective tissue and specifically showing clear differentiating features of type I, IIA and IIB muscle fibres from the camel's deltoideus muscle. This however, to the best understanding and literature searches by the researchers, has not been reported anywhere that Masson trichrome stain could differentiate the three muscle fibre types (types I, IIA and IIB). With the utilization of immunohistochemistry expressions, the trichrome techniques still offer a great deal of diagnostic results. The researchers however wish to recommend further research on same or other muscles in same or other animal models using the same Masson trichrome stain so as to validate the findings in the present work.

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