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Application of stained blood cells for the differentiation of arteries and nerves in cadavers

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SUMMARY

The demonstration of the functional arteries to first year medical students in the dissection room is very important, but after cadaver fixation with formalin and other preserving materials the arteries will be empty and the fixative material will take the place of blood. Thus, the arteries will be colorless and students will find it hard to differentiate between arteries and nerves. The aim of this study was to stain arteries and differentiate them from nerves in cadavers by means of an easy method, the use of stained blood cells.

Whole blood cells (packed cells) were initially treated with a 1% glutaraldehyde solution, washed with PBS and then stained with safranin. 10 ml of stained blood cells was injected into the internal iliac artery of the cadaver. The stained blood cells injected into the internal iliac artery differentiated the internal pudendal arteries from the pudendal nerve and the inferior rectal artery from the inferior rectal nerve. The preparation of stained blood cells and their application could be used by all medical and dental students to facilitate learning of the functional arteries.

Key words: Cadaver – Packed cells – Staining – Glutaraldehyde – Artery – Nerve

INTRODUCTION

Cadavers are the only source for medical students to learn the anatomy of the arteries. The location and blood supply of the arteries are important for both general surgeons and orthopedists. Since fixative material such as formalin, phenol, alcohol, glycerin and other materials are injected into selective arteries, the structures of these will be similar to nerves. It is therefore difficult to differentiate them. To overcome this problem, several techniques such as the application of different staining solutions and colored latex have been used to stain and demonstrate the arteries (Tubbs et al., 2002; Haerle et al., 2004). Ndiaye et al. (2004) used Congo red to demonstrate the superficial temporal artery (STA) in the black population.

MATERIAL AND METHODS

Initially, 250 ml of blood cells (packed cells) were treated with a 1% glutaraldehyde and kept stirred for 5 hours. The cells were washed with PBS (phosphate buffered saline, pH=7.2), three times with the use of centrifugation at 1500 rpm for 15 minutes. After the final centrifugation, the supernatant was removed and equal amount of Safranin staining solution (2.5% concentration) was added

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to the washed cells. The final concentration of blood cells was adjusted to 50%. The mixture was vortexed for 5 minutes. The quality of blood cells staining was checked by microscopy. The physical properties of the fixed blood cells at different temperatures, 4°C and 37°C, were studied for 72 hours. The blood cells stained with the safranin solution were studied for the period of six months. The morphology of the fixed blood cells was studied by macroscopic examination over the six months period. To differentiate the inferior rectal and pudendal arteries from their associated nerves, and to demonstrate the branches of the internal iliac arteries, 5-10 ml of the prepared stained blood cells were injected very slowly into the internal iliac artery of the cadaver. A ligature was carried out above the site of each selected artery. One week after the injections the cadaver was dissected. The observation of stained arteries was assessed over one year.

Results

No morphological changes in the RBC were observed after treatment with glutaraldehyde, No lysis of RBC was found after glutaraldehyde treatment after one year (Figs. 1 and 2). No lysis of the fixed RBC was found in the different temperature conditions, at 4°C and 37°C. No color changes were found in the stained RBC after staining with safranin. After the injection, dissection revealed that all the branches of the iliac arteries were very well stained and they were completely differentiated from their associated nerves. Figure 3 shows that stained arteries were not changed after one year of observation. After dissection, all the branches of the selected arteries were very well stained. The stained arteries showed resistance to formalin and phenol and other reagents.

Discussion

With our protocol, the preparation and staining of blood cells are very inexpensive and readily carried out at the laboratory. Its application is excellent for the easy learning of the functional arteries and to differentiate arteries from their associated nerves in cadavers especially for medical students. To date, different materials have been used to demonstrate the functional arteries. The use of resin was shown for demonstration of the arteries. Pavkov et al. (2004) described the anatomy of the utero-ovarian venous system in the adult post-menopausal female cadaver using casts and the von Hagen's plastination technique. To qualitatively and quantitatively evaluate the uterine vein, the utero-ovarian arcade, the ovarian vein and the ramus communicates between left and right parametrium, Pavkov et al. (2004) injected epoxy resin into the vein and artery of internal iliac and

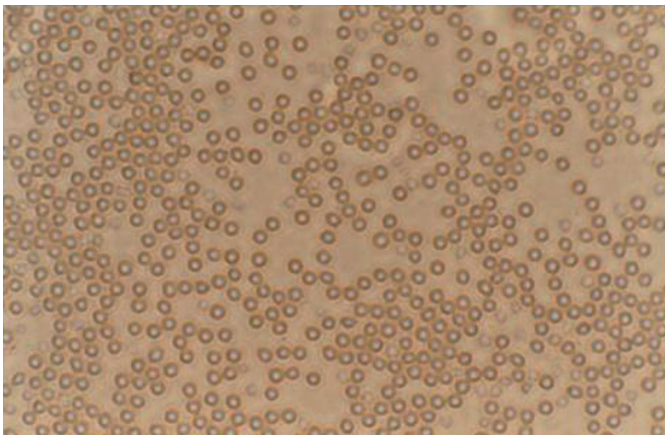

Figure 1. Fixed RBC without staining

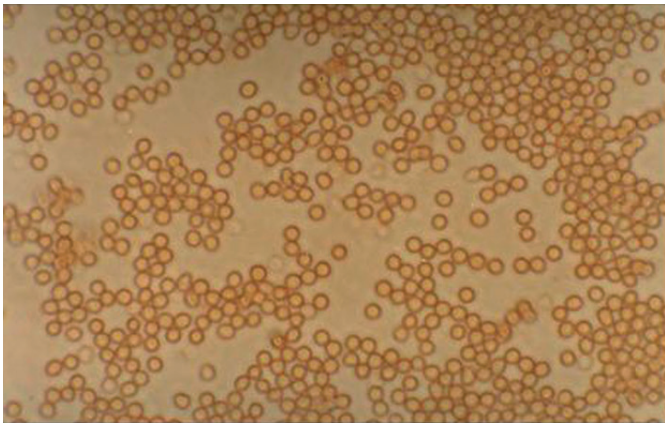

Figure 2. Fixed RBC stained with safranin

Figure 3. The branches of the internal iliac artery

ovary in six cadavers. The internal genital organs of two of the cadavers were excised before injection and in the other four after the injection and polymerization of the resin, the veins were completely filled out and examined in the four cases where in situ injection was performed. Pavkov et al. (2004) reported that the utero-ovarian arcade is an important vein with a caliber similar to that of the ovarian and uterine veins, that there is an impressive network of venous anastomoses between the left and right parametrium and that the fallopian tubes were drained by three separate veins: internal, median, and external tubal veins. Pinar et al. (2005) reported the anatomy of the perioral branches of the facial artery (FA), confirming the consistent presence of the septal and alar branches in the upper lip and a labiomental branch in the lower lip. Mucosal flaps from the upper lip based on the deep septal branch or the alar branch of the FA can be used to restore lower lip defects. A composite flap from the lower lip supplied by the labiomental branch of the FA can be used to restore combined defects of the upper lip and nose or partial defects of the lower lip. Pinar et al. (2005) studied the vascular anatomy of the perioral region in 25 cadaver dissections. Fixation was carried out with 10% formaldehyde solution followed by injection of Red latex into the common carotid arteries before dissection. Pinar et al. (2005) revealed that the labiomental arteries, which formed anastomoses between the FA, inferior labial artery (ILA), and submental artery, showed variations in their course in the labiomental region. Different materials and staining solutions such as Indian ink was used in the vascular supply. Ling et al. (1990) studied the vascular supply of the rotator- cuff in 22 adult shoulders by a mixture of infusion of gelatin and Indian ink and vascular cast, in combination with Scanning Electronic Microscopy of the vascular pattern in the supraspinatus tendon. It was found that the vessels of the supraspinatus tendon mainly derive from the anterior circumflex humeral and suprascapular arteries. Kim and Mastic (2005) reported the arterial blood supply and innervation of the rectus muscles of the eyeball from human orbital dissections. One hundred human orbits were dissected with the use of a superior approach after arterial injection with colored latex. The different arterial pedicles for each muscle were noted and the nervous supply was studied. Rivero et al. (2005) provided a new reference for the interpretation of the normal anatomy of the canine thorax as imaged using computed tomography (CT). Three mature dogs, all mixed breed males, were used. The dogs were sedated, anaesthetized, and maintained in sternal recumbency. A CT study from the first to the thirteenth thoracic vertebrae was performed with a TOSHIBA 600 HQ scanner (third generation equipment). The dogs were killed and the vascular injection technique was performed: red latex and blue latex were used to fill the vascular system. The injected dogs were frozen and sectioned with an electric band saw, cuts matching to the CT images as closely as possible. The CT images from this study are intended to be a reference for clinical CT imaging studies of the thoracic cavity of the dog and for interpreting lesions of the thorax and associated structures. Ozgel et al. (2004) defined the arteries that supply the cardiac muscle in donkeys with regard to their course and possible variations. Six hearts belonging to donkeys of different age and sex were used. After exposure of the arteries by injection of latex coloured with Rotring ink to the a. coronaria sinistra and the a. coronaria dextra, dissection was performed. Tubbs et al. (2002) injected red and blue latex into the arteries and veins of twelve human cadavers (8 males and 4 females). That author found the primary arterial supply to the sympathetic chain and ganglia were from superior to inferior the ascending pharyngeal, ascending cervical, thyrocervical trunk, and supreme intercostal arteries. The primary venous drainage of these structures was mainly through the direct posterior branches into the internal jugular vein. Tubbs et al. (2002) revealed that the current data should be useful to the surgeon who operates in the cervical region so as to avoid potential complications from disruption of the primary blood supply of the cervical sympathetic chain and ganglia.

Owing to the scarcity of cadavers in Iran and also due to some religious restrictions in Islamic rules and regulations it is impossible to obtain cadaver easily. Accordingly our study was only carried out in two cadavers.

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References

Haerle M, Tonagel F and Schaller HE (2004). Collateral arterial pathways in the forearm. *Surg Radiol Anat*, 26: 208-211.

Kim S and Mastic DB (2005). The anatomy of temporal hollowing: the superficial temporal fat pad. *J Craniofac Surg*, 16: 651-654.

Ling Sc, Chen CF and Wan RX (1990). A study on the vascular supply of the supraspinatus tendon. *Surg Radiol Anat*, 12: 161-165.

Ndiaye A, Toure S, Diop FM, Ndiaye AS, Diallo BK and Sow ML (2004). The superficial temporal artery: anatomical type and clinical application to the flap of the fascia temporal superficialis. *Morphologie*, 88: 155-159.

Ozgel O, Haligur AC, Dursun N and Karakurum E (2004). The macroanatomy of coronary arteries in donkeys (*Equus asinus L.*). *Anat Histol Embryol*, 33: 278-283.

Pinar YA, Bilge O and Govsa F (2005). Anatomic study of the blood supply of perioral region. *Clin Anat*, 18: 330-339.

Pavkov ML, Koebke J, Notermans HP and Brokelmann J (2004). Quantitative of the utero-ovarian venous pattern in the adult human cadaver with plastination. *World J Surg*, 28: 201-205.

Rivero MA, Ramirez JA, Vazquez JM, Gil F and Arencibia A (2005). Normal anatomical imaging of the thorax in three dogs: computed tomography and macroscopic cross sections with vascular injection. *Anat Histol Embryol*, 34: 215-219.

Tubbs RS, Salter G, Wellons JC and Oakes WJ (2002). Blood supply of the human cervical sympathetic chain and ganglia. *Eur J Morphol*, 40: 283-288.