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Histological Studies on the Arterial Walls of Main Arteries Supplying the Mammary Glands of Dogs *(Canis familiaris)* in Bangladesh

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Abstract: Histological studies on the arterial wall of main arteries from the ascending aorta to the pectoral and abdomino-inguinal mammary glands of indigenous dogs in different reproductive stages were observed using a light microscope. Based on the histological characteristics and organization of connective tissue fibers and smooth muscle cells in the tunica media, the arterial segments were clearly identified into elastic, transitional and muscular types. Every artery belonging to the three types had different morphological structures at different levels. The artery distant from the heart gradually decreased its elastic lamellae in the tunica media as well as its elasticity and at the periphery of the arterial tree the elastic lamellae were replaced by the smooth muscle cells. The type of the arteries does not depend on the size or diameter of the arteries but depend on the relative distance from the heart. It is assumed that the histological arrangements of tissues in the arterial walls are certainly closely related to the functional demand of the mammary glands.

Key words: Histology, arterial wall, mammary glands, dog (*Canis familiaris*)

Introduction

The histological studies on the arterial walls of dog (Bunce, 1974 and Awal *et al.*, 1998); swine (Tanigawa *et al.*, 1985 and Awal *et al.*, 1997); rat (Awal *et al.*, 1995 and Awal *et al.*, 1997); large domestic animals (Dellmann and Eurell, 1998); Japanese dog (Awal *et al.*, 1998) and Black Bengal goats (Awal *et al.*, 1999) are available in the literature. The review of available literature reveals no information regarding the histology and types of arterial walls of main arteries supplying the mammary glands of dogs (*Canis familiaris*) in Bangladesh.

Therefore, the present study was carried out to investigate the general histology of arterial walls and special attention was given to which supplying the mammary glands of dogs in Bangladesh in order to clarify whether various reproductive stages and local breed differences affect the histological features of the arteries supplying the mammary glands or not.

Materials and Methods

The work was carried out in the Department of Anatomy and Histology, Bangladesh Agricultural University, Mymensingh, Bangladesh. A total of 16 adult females, apparently healthy dogs (*Canis familiaris*) were divided into 4 groups and each group consisted of 4 animals - at 260 days old virgin, at 40 days of pregnancy, 45 days of lactation and 50 days post weaning. The animals were collected from the local area of Bangladesh Agricultural University campus (Fig. 1).

Fig. 1: A dog (*Canis familiaris*) in Bangladesh

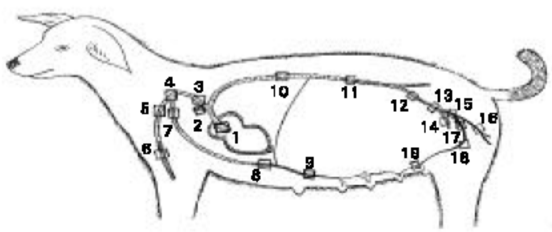

Fig. 2: Diagram showing the location of the arterial segments removed from Dog and typing of the arterial segments,□; Sites of sampling,;▨ Elastic type,⁙, Transitional type,■; Muscular type. 1: Ascending aorta 2: Brachiocephalic trunk 3: Subclavian artery (proximal part) 4: Subclavian artery (distal part) 5: Axillary artery 6: Brachial artery 7: Internal thoracic artery (proximal part) 8: Internal thoracic artery (distal part) 9: Superficial cranial epigastric artery, 10: Thoracic aorta 11: Abdominal aorta 12: External iliac artery (proximal part) 13: External iliac artery (distal part), 14: Femoral artery, 15: Deep femoral artery (proximal part), 16: Deep femoral artery (distal part), 17: Pudendoepigastric trunk, 18: External pudendal artery, 19: Superficial caudal epigastric artery.

The animals were anaesthetized with Nembutal® (Sodium Pentobarbital 50 μg/kg body weight) by intramuscular injection and bled to death by giving incision through left common carotid artery. The pectoral and pelvic cavity was opened and 19 arterial segments were carefully dissected soon after the death of the animals (Fig. 2), and were immediately fixed in 10% buffered neutral formalin. Aseptic measures were taken during collection and processing of the samples in order to avoid inadvertent contamination. All the tissues were dehydrated with a series of graded ethanols, routinely embedded in paraffin wax and sectioned at 5 μm thickness. Four stains were used- Mayer's hematoxylin and eosin for general histological studies, Weigert's elastica Van Gieson for elastic fibers, Weigert's resorcin fuchsin for smooth muscle cells and Azan stain for collagen fibers (Gridley, 1960 and Awal *et al.*, 1995). All the tissues were observed with a light microscope (Olympus, Japan). Elastic lamellae were counted (30 slides for each arterial segment) using higher magnification and from the enlarged microphotographs (Awal *et al.*, 1995). The diagram and photographs were taken from selected areas to illustrate the results.

Results and Discussion

The arterial segments of dogs were classified into elastic, transitional and muscular types. This classification was based on the histological characteristics and organization of connective tissue fibers and smooth muscle cells in the three distinct tunics- tunica intima, tunica media and tunica externa as reported by Arthur (1969), Adam *et al.* (1970), Bunce (1974), Bloom and Fawcett (1975), Brown (1976), Tanigawa *et al.* (1985) and Awal *et al.* (1995). Our present observations regarding identification and classification of arterial walls were made on the basis of Tanigawa *et al.* (1985).

The ascending aorta (Fig. 2, NO. 1), brachiocephalic trunk (Fig. 2, NO. 2), subclavian artery (Fig. 2, NO. 3 and 4), axillary artery (Fig. 2, NO. 5), proximal part of the internal thoracic (Fig. 2, NO. 7), and thoracic aorta (Fig. 2, NO. 10) were of elastic type. The tunica intima (TI) was consisted of a single layer of flattened endothelial cells resting on the internal elastic lamina. The subendothelial layer was clearly observed in the ascending aorta, aortic arch, brachiocephalic trunk, and in the thoracic aorta and was composed of predominantly branched elastic fibers, collagen fibers and reticular fibers (Fig. 3a and Table 1). These observations were similar to Awal *et al.* (1998) in Japanese dog and Awal *et al.* (1998) in Black Bengal goat. On the other hand, Dellmann and Eurell (1998) stated that the tunica intima of large domestic animals was comparatively thicker than the tunica intima of laboratory animals. The arterial segments from the ascending aorta to the thoracic aorta were of elastic type in miniature swine (Tanigawa *et al.*, 1985) and was in complete accord with our present study. Awal *et al.* (1995) in wistar rats reported that the arterial segments from the ascending aorta to the abdominal aorta were of elastic type. The tunica media (TM) of elastic arteries was the thickest among three tunics and was consisted of predominantly concentric layers of well defined elastic lamellae (Fig. 3a). The number of elastic lamellae in the tunica media were 60-70, in the ascending aorta, 50-55, in brachiocephalic trunk, 18-20, in subclavian artery, 8-10, in axillary artery, 5-7, in proximal part of internal thoracic artery and 35-40, in thoracic aorta. The number of elastic lamellae in the tunica media depends on the relative distance of the arteries from the heart (Awal *et al.*, 1995), and also varies among domestic and laboratory animals (Awal *et al.*, 1997 and Awal *et al.*, 1998). It has been reported that the lamellar unit is a basic system of structure and function in the wall of the elastic arteries (Berry *et al.*, 1972). The lamellar unit of the tunica media with aligned collagen fibrils bear the high tensile forces of blood pressure, whereas the network of elastic lamellae and their branches distributes the stress uniformly throughout the arterial wall (Wolinsky and Glagov, 1967). The spaces between the elastic lamellae were occupied with connective tissue fibers and smooth muscle cells arranged circumferentially. The tunica externa (TE) was comparatively thin and composed of connective tissue fibers, vasa vasorum, smaller blood vessels and nerves (Fig. 3a). Similar histological findings were observed in miniature swine (Tanigawa *et al.*, 1985), wistar rats (Awal *et al.*, 1995), Japanese dog (Awal *et al.*, 1998), and Black Bengal goats (Awal *et al.*, 1999).

The abdominal aorta (Fig. 2, NO. 11), proximal and distal part of external iliac, (Fig. 2, NO. 12 and 13), brachial artery (Fig. 2, NO. 6), distal part of external thoracic, (Fig. 2, NO. 8), and femoral arteries (Fig. 2, NO. 14), were of transitional type. The transitional type was between elastic and muscular types and possessed a mixture of characteristic features common to both of them. The subendothelial layer was ill-defined in the abdominal aorta, but in other cases this layer was indistinct or absent (Fig. 3b and Table 1). The subendothelial layer gradually becomes thinner and eventually disappears with decreasing vessel size (Dellmann and Eurell, 1998). This was confirmed in present study. The internal elastic lamina was present but external lamina was indistinct (Fig. 3b). Tunica media consisted of thin and irregular elastic lamellae. The number of elastic lamellae in the tunica media was 12-15, in abdominal aorta, 5-7, in external iliac, 3-4, in distal part of internal thoracic and 2-3, in the femoral and brachial arteries. The interlaminal spaces were broad and occupied by bundles of smooth muscle cells (Fig. 3b). The island of smooth muscle fibers in the interlaminal spaces interrupted the continuity of the normal coarseness of the elastic lamellae in the tunica media as reported by Tanigawa *et al.* (1985) in miniature swine; Awal *et al.* (1995) in wistar rats ; Awal *et al.* (1998) in Japanese dog and Awal *et al.* (1999) in Black Bengal goats. The tunica externa was well developed and almost equal in thickness with that of tunica media. This observation was dissimilar to Dellmann and Eurell (1998) in large domestic animals. The tunica externa was made up of the bundles of collagen fibers, together with some elastic and smooth muscle fibers. Cross sections of blood vessels and nerves were also observed. Coarse elastic fibers in the tunica externa formed a circular arrangement around the tunica media (Fig. 3b). Similar histologic characteristics were observed in dog (Bunce, 1974) and Japanese swine (Awal *et al.*, 1997), but was not absent in Black Bengal goat (Awal *et al.*, 1999).

The proximal and distal part of the deep femoral (Fig. 2, NO. 15 and 16), pudendoepigastric trunk (Fig. 2, NO. 17), external pudendal (Fig. 2, NO. 18), superficial cranial and caudal epigastric arteries (Fig. 2, NO. 9 and 19), were of muscular type. The tunica intima consisted of a single layer of flattened endothelium resting on the internal elastic membrane. The tunica media completely devoid of elastic lamellae and consisted mainly of smooth muscle cells with a mixture of few elastic fibers (Fig. 3c). The subendothelial layer was absent (Fig. 3c and Table 1). Usually the smaller arteries, the peripheral branches of the arterial tree were identified as muscular type and the media was composed of circularly arranged smooth muscle cells with a few fine elastic fibers (Gross *et al.*, 1933; Tanigawa *et al.*, 1985; Awal *et al.*, 1995 and Awal *et al.*, 1997). Similar histological findings were observed in present study and accord well with their descriptions. The internal elastic lamina was present and well developed, but the external elastic lamina was either indistinct or absent. This observation was similar to Awal *et al.*, (1999) in Black Bengal goats. Both internal and external elastic lamina were present in the muscular arteries of wistar rats (Awal *et al.*, 1995). The tunica externa was well developed and consisted of mainly collagen fibers with coarse elastic fibers and smooth muscle cells. Cross sections of small blood vessel and nerve bundles were also observed . A similar histological feature was reported in dogs (Bunce, 1974), Japanese swine (Awal *et al.*, 1997) and Black Bengal goats (Awal *et al.*, 1999). In present study, the tunica media of muscular artery was the thickest of the three tunics whereas the tunica media of muscular arteries of miniature swine, wistar rats and Black Bengal goats were thinner than that of the tunica externa (Tanigawa *et al.*, 1985; Awal *et al.*, 1995 and Awal *et al.*, 1999 respectively). The reproductive stages and local breed

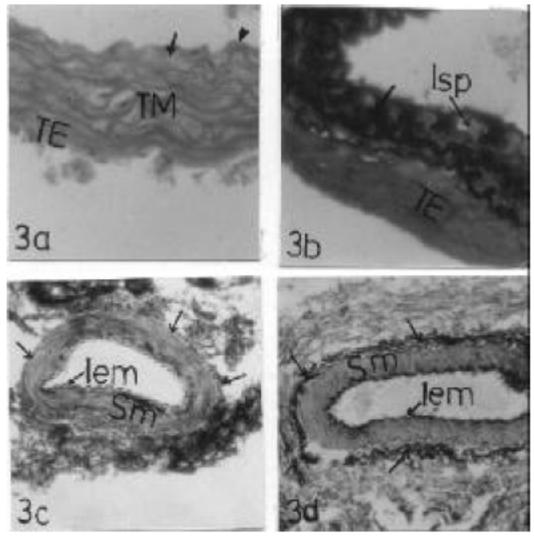

Fig. 3: a: Ascending aorta. Elastic type. The tunica intima (TI) is thin (arrow head). Subendothelial layer is scanty (arrow). The tunica media (TM) is thick and consists of well defined elastic lamellae. The tunica externa (TE) is thin and consists of connective tissue fibers. Van Gieson stain, x 87; scale bar = 100 μm.

b: Abdominal aorta. Transitional type. The interlaminal spaces (Isp) are wide. Bundles of collagen fibers are seen in the interlaminal spaces (arrow). The tunica externa (TE) is well-developed. Van Gieson stain; x 87; scale bar = 100 μm.

c: External pudendal artery (Virgin). Muscular type. Internal elastic membrane (Iem) is present and wavy. The tunica media consists of smooth muscle cells (Sm). The tunica externa (TE) contains a high number of coarse elastic tissues (arrows). Weigert's resorcin fuscin stain; x 87; scale bar = 100 μm.

d: External pudendal artery (Lactation). Muscular type. Internal elastic membrane (Iem) is straight. The tunica media consists of smooth muscle cells (Sm). The tunica externa (TE) contains a high number of coarse elastic tissues and formed circular arrangement around the tunica media (arrows). Weigert's resorcin fuscin stain; x 87; scale bar = 100 μm.

Table 1: Histological classification of arterial walls.

Type	Tunica intima		Tunica media		Tunica externa	
	Subendo-thelial layer	Internal elastic lamina	Elastic tissue	Smooth muscle	External elastic lamina	Collagenous & elastic fibre
Elastic type	present	not distinct	stratified laminae	circular helical longitudinal	not distinct	absent or present (less)
Transitional type	absent	prominent	stratified laminae (relatively thin)	"	"	abundant
Muscular type	absent	prominent	elastic fibre		present or absent	abundant

differences did not affect on the general histological features of the arterial walls. The internal elastic membrane of external pudendal artery was found wavy in nature during virgin stage (Fig. 3c), but during lactation period, increased volume and forced flow of blood gives pressure to the internal elastic membrane resulting the straightening the internal elastic membrane (Fig. 3d).

The histological classification of the arterial walls of dog (*Canis familiaris*) in Bangladesh was similar to that of large domestic and laboratory animals. It may be stated that every artery belonging to the three types had some differential characters at different segments of the arterial tree. The type of arterial wall does not depend on the size or diameters of the arteries but is related to relative distances from the heart. The vessels away from the heart gradually loose their elastic lamellae in the tunica media by increasing the smooth muscle cells. Given the presence of elastic lamellae in the elastic arteries, it is assumed that the vessels nearest to the heart might resist comparatively high arterial pressure which might not be the case with peripheral muscular arteries. Therefore the tunica media of the arteries nearest to the heart consisted of a comparatively higher amount of elastic lamellae. Similarly, the media of muscular arteries are composed of smooth muscle cells for efficient response to the functional physiological demands or state of the body.

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