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A microscopic and stereological study of the renal artery transitional zone of the adult male dog

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Abstract: A transitional zone in the dog renal artery was documented and its parameters were recorded: length and volumes of the luminal wall, tunica intima, tunica media, and tunica adventitia—as well as these parameters in the non-transitional zone of artery. Twelve normal adult male dogs were studied. The specimens were processed in the routine manner for light and electron microscopy. After the determination of transitional zone length by light microscope, for the stereological study each renal artery was divided into transitional and non-transitional zones, and paraffin blocks were prepared. It was established that the transitional zone contains parallel and continuous elastic fibers. In this zone the elastic fibers in the extracellular space were greater in number than collagen fibers. However, in the non-transitional zone the rows of smooth muscle cells increased in number, there were more collagen fibers than elastic fibers in the extracellular space, and the elastic fibers appeared to be fragmented. The transitional zone showed an intermediate structure—between elastic and muscular arteries—and was 15 mm in length in the right and left renal arteries. There were more dense bodies in the non-transitional zone than in the transitional zone. The volumes of the lumen, tunica intima, tunica media, and tunica adventitia in the transitional zone were greater than in the non-transitional zone on both sides; in the right renal artery they were larger than in the left one in the 2 zones.

Key words: Transitional zone, renal artery, elastic fiber, ultrastructure, stereological study

Introduction

Certain arteries irrigate only defined areas of specific organs, and obstruction of the blood supply results in necrosis. This infarct occurs commonly in the heart, kidneys, cerebrum, and certain other regions. In other regions arteries anastomose frequently, and the obstruction of one artery does not lead to tissue necrosis because the blood flow is maintained (1). Atherosclerosis of the renal artery is the most common form of renal artery stenosis and the cause of renovascular hypertension. Atherosclerosis of the human renal artery is found in 2 locations; an eccentric or concentric lesion 1 cm distal to the ostium of the renal artery and at the orifice of the renal artery at the aorta (2). In fact, a preponderance of atherosclerosis at aortic branching points and bifurcations has been well documented in humans (3). In the hypercholesterolemic rabbit, atherosclerotic lesions occur at the distal and lateral side of the orifice of dorsal intercostals arteries

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(4). To understand the mechanism involved in the formation of atherosclerotic lesions, it is important to clarify why these areas tend to be involved in atherosclerosis. Therefore, in-depth study of the renal artery—which irrigates the kidneys—can increase our knowledge of how to manage and reduce the risk of disease in this artery and the kidneys. Traditionally, arteries are classified as large or elastic arteries, medium or muscular arteries, and small arteries or arterioles. In all species, renal arteries are branches of the abdominal aorta and are classified as muscular arteries. There is a gradual transition in structure and function between these 2 types of arteries, and the transition from elastic to muscular arteries is not abrupt (5). The microscopic transitional zone is the segment of the arterial tree in which the elastic wall is replaced by a muscular type (6).

The structure of arteries has interested researchers for a long time. Van Baardwijk et al. (7) showed that the bundles of elastin appear to continue from the aorta into its branches. Light microscopic studies have shown marked differences in the pattern of elastin at aorta/branch junctions on the proximal and distal lips of the junction; they have also shown that the medial layer of the branches is transitional (8). In addition, the proximal part of the renal artery in rats shows a gradual transition from elastic to a muscular type (9).

There is little information regarding the transitional zone structure. In this study the transitional zone and its parameters in the dog renal artery were investigated: length and volume of the luminal wall, tunica intima, tunica media, and tunica adventitia as well as these parameters in the non-transitional zone of the artery.

Dogs were chosen for this study because there have been no reports about the structure of this artery in dogs. The present study will help us to understand the relationship between structural differences and vascular diseases such as atherosclerosis.

Materials and methods

The study protocol was approved by the Ethics Committee of the School of Veterinary Medicine, Islamic Azad University (Kazeroun Branch, Kazeroun, Iran).

A total of 24 renal arteries were harvested from 12 clinically healthy, mixed breed, native adult male dogs (3 for histology, 2 for ultrastructure, and 7 for stereology). The chosen dogs were normal size and between 3 and 4 years old. The dogs were euthanized with an overdose of thiopental sodium (30 mg/kg). The abdominal cavity was opened, the renal arteries exposed, and the length of left and right renal arteries between the aortic origin and the ramification into the segmental arteries was measured with a ruler.

For histology, 6 left and right renal arteries were freed from their aortic origin to the renal hilus and transected from the aortic origin to the ramification into the segmental arteries. After removal, arteries were fixed in 10% formalin. After 24 h of fixation, each artery was divided into 3 samples: proximal part, middle part, and distal part. Each sample was processed for routine paraffin processing. The paraffin blocked tissue was cut into 6 μm serial sections. Each 10th section was mounted and stained with orcein for elastic fibers. Microscopic structure was viewed to determine and record the presence of transitional zone structures. The length of the transitional zone was determined by totaling the number of transitional sections and multiplying by section thickness.

After the determination of renal artery transitional zone by light microscope, renal arteries were removed from 2 more dogs, following the same harvesting procedure. Immediately after euthanasia and removal of the renal arteries, the samples for ultrastructural study were taken from the proximal part of the renal artery as transitional zone and the distal part as muscular artery. Samples were rinsed with normal saline and fixed in 2.5% glutaraldehyde for 8 h. The specimens were rinsed with cold buffer twice (15 min each time) and postfixed in 1% osmium tetroxide for 2 h. Next, specimens were dehydrated in a graded series of alcohols and cleared with the addition of propilenoxide. After this, the specimens were infiltrated in a mixture of propilenoxide and Epon resin. Gradually the Epon:propilenoxide ratio was increased until only pure Epon resin was used. The samples were embedded in Epon 812 resin, transverse sections were made and stained with uranyl acetate, and the structure of the transitional zone was studied.

Stereological analysis of serial sections was carried out using a Bausch and Lomb microscope. After determination of the length of the transitional zone, each renal artery (7 right renal arteries and 7 left renal arteries) was divided into transitional and non-transitional zones. From each zone 1 cm was separated out, and paraffin blocks were prepared in the same manner as those used for light microscopy. The volumes of the lumen, tunica intima, tunica media, and tunica adventitia were determined using the point counting methods of Cavalier (10). For this study, each specimen was serially sectioned at a distance T (T is $\frac{\text{length of specimen}}{10}$). From each specimen, 10 sections 5 μm thick were taken and stained with orcein. The volumes of the lumen and the different layers were calculated using the equation:

$$V = \frac{\sum P.a(P).T}{m^2} \text{ and } a(P) = \triangle X. \triangle Y$$

∑p: A number of points falling within the lumen and the different layers.

T: Distance between sections.

M: Magnification.

a(P): Area associated with each point.

ΔX: Distance between points (10).

All data were analyzed, using the paired Student's t-test and SPSS software (SPSS Inc., Version 12, Chicago IL, USA), and then compared. Differences were considered significant at the level of $P \leq 0.05$.

Results

The structure of the renal artery of the adult male dog is similar to that of other mammalian arteries with tunica intima, tunica media, and tunica adventitia. Light microscopy confirmed the structural change of the tunica media in the canine renal artery from the origin to the renal hilus of the kidney. The length mean of the right renal artery in an adult male dog was 3.4 ± 0.3; in the left renal artery it was 2.7 ± 0.1. The proximal part of the renal artery showed a structure between elastic and muscular artery types. After 15 mm the renal artery showed the morphologic features typical of a muscular artery. The length of the transitional zone in both the left and the right renal arteries was 15 ± 0.06 mm.

The histological study demonstrated that there is a structure between elastic and muscular artery types at the origin of the renal artery, with parallel elastic fibers between smooth muscle cells in the tunica media. In this section, the internal elastic membrane was present, but there was no external elastic membrane between the tunica media and the tunica adventitia (Figure 1). Nearer to the hilus of the kidney in the distal region 7 mm from the aorta, more rows of smooth muscle cells and fewer layers of elastic fibers were seen; however, the external elastic membrane was not observed (Figure 2). After 15 mm, the typical morphologic features of muscular arteries were found. In this area smooth muscle cells predominated in the media, and there were rare remnants of elastic fibers between the smooth muscle cells. In this section an internal elastic membrane separated the tunica intima from the tunica media, and there was an external elastic membrane at the junction of the tunica media and tunica adventitia (Figure 3).

The ultrastructural studies confirmed these differences in the tunica media. At the origin of the renal artery more elastic fibers were present in the tunica media extracellular space in a continuous arrangement, in comparison with the collagen fibers. Myofilaments were found in the cytoplasm of most of the smooth muscle cells, and dense bodies were seen to lie under the cell surface and between

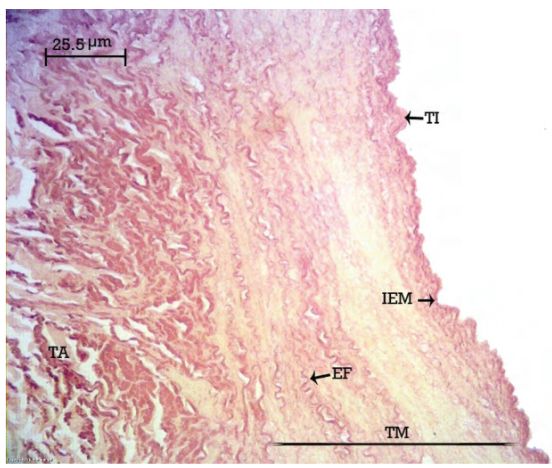

Figure 1. Photomicrograph—canine renal artery transitional zone. TI, tunica intima; TM, tunica media; TA, tunica adventitia; IEM, internal elastic membrane; EF, elastic fibers; orcein stain, ×700.

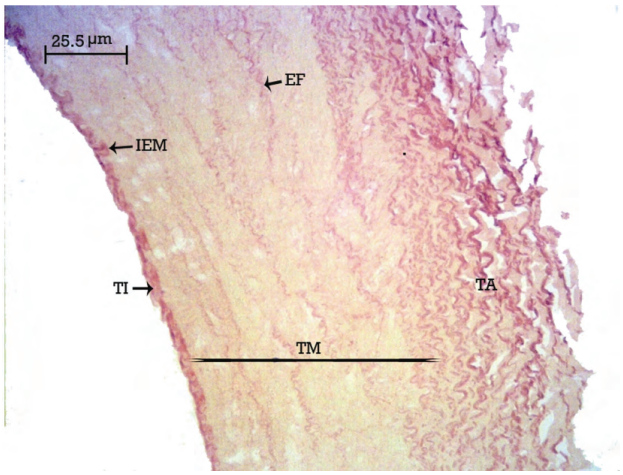

Figure 2. Photomicrograph—transitional zone in the distal 7 mm region from aorta of right canine renal artery. TI, tunica intima; TM, tunica media; TA, tunica adventitia; IEM, internal elastic membrane; EF, elastic fibers; orcein stain, ×700.

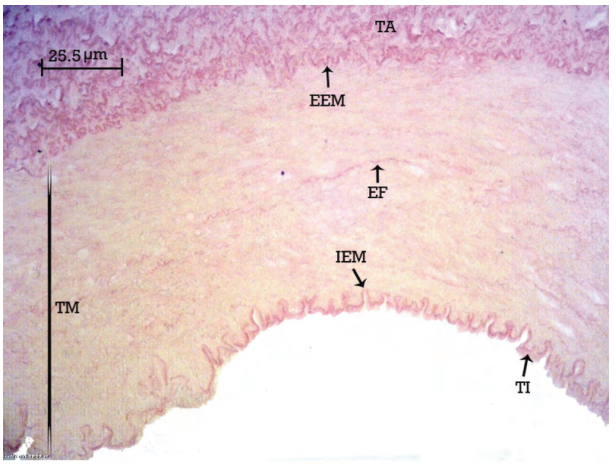

Figure 3. Photomicrograph—non-transitional zone after 15 mm from aorta of right canine renal artery. TI, tunica intima; TM, tunica media; TA, tunica adventitia; IEM, internal elastic membrane; EEM, external elastic membrane; EF, elastic fibers; orcein stain, ×700.

myofilaments (Figure 4). However, in the section near the hilus of the kidney—in the distal 20 mm region from the aorta—elastic fibers became rare and fragmented; here collagen fibers were more numerous than elastic fibers in the extracellular spaces between smooth muscle cells. Myofilaments were found in the cytoplasm of most of the smooth muscle cells, and dense bodies were seen to lie under the cell surface and between myofilaments. Dense bodies were more numerous in the distal 20 mm region from the aorta than at the origin of the renal artery (Figure 5).

Stereological observations of the transitional and non-transitional zones demonstrated that the volume of the lumen, tunica intima, tunica media, and tunica adventitia decreased from the transitional zone towards the non-transitional zone

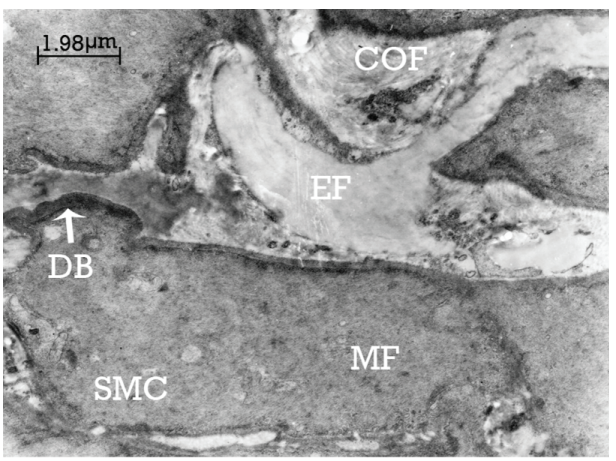

Figure 4. TEM micrograph of transitional zone of right canine renal artery. EF, elastic fibers; COF, collagen fibers; SMC, smooth muscle cell; MF, myofilaments; DB, dense bodies; ×8900.

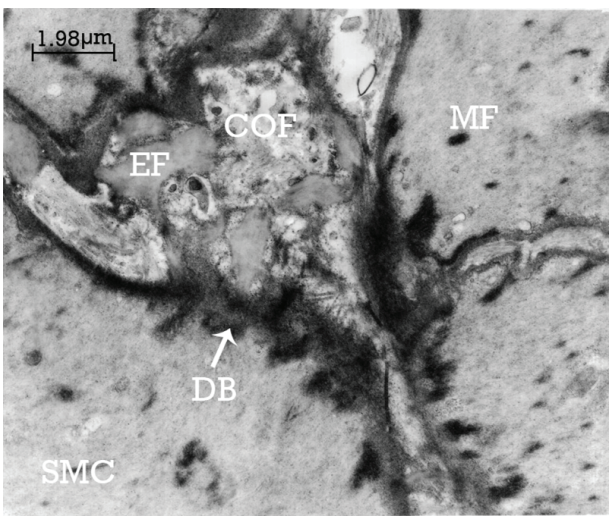

Figure 5. TEM micrograph of non-transitional zone of right canine renal artery. EF, elastic fibers; COF, collagen fibers; SMC, smooth muscle cell; MF, myofilaments; DB, dense bodies; ×8900.

in the right and left renal arteries (Table 1). There was a significant difference in the volume of the lumen, tunica media, and tunica adventitia between the transitional and non-transitional zones ($P \leq 0.05$). The volumes of the lumen and the different layers were greater in the right renal artery than in the left, and a significant difference was observed in the volumes of the lumen, tunica media, and tunica adventitia between the 2 sides at $P \leq 0.05$ (Table 2). In all comparisons the coefficient of error was between 0.01 and 0.03, demonstrating a small amount of error.

Table 1. Volume of lumen, tunica intima, tunica media, and tunica adventitia of transitional and non-transitional zones of right and left renal arteries of adult male dog.

	Right		**Left**	
	Transitional zone	Non-transitional zone	Transitional zone	Non-transitional zone
Lumen	[a] 47.84 ±317.48	[b] 39.59 ± 208.72	[a] 89.03 ± 370	[b] 42.69 ± 277.52
Coefficient of error	0.01	0.02	0.02	0.02
Tunica intima	2.31 ± 52.14	1.43 ± 40.24	8.44± 53.52	3.24 ± 43.72
Coefficient of error	0.03	0.03	0.03	0.02
Tunica media	[a] 63.79 ±448	[b] 27.08 ± 316.20	[a] 36.64 ± 564.68	[b] 53.15 ±385.52
Coefficient of error	0.01	0.01	0.01	0.02
Tunica adventitia	[a] 35.73 ± 268.36	[b] 20 ±228.92	[a] 22.22 ±344.02	[b]48.46 ± 316.16
Coefficient of error	0.01	0.01	0.01	0.01

Note: a and b show significant differences between transitional and non-transitional zones in the right and left renal arteries of adult male dogs ($P \leq 0.05$).

Table 2. Volume of lumen, tunica intima, tunica media, and tunica adventitia of transitional and non-transitional zones of right and left renal arteries of adult male dog.

	Transitional zone		**Non-transitional zone**	
	Right	Left	Right	Left
Lumen	[A] 42.69 ± 277.52	[B] 39.59 ± 208.72	[A] 89.03 ± 370	[B] 47.84 ± 317.48
Coefficient of error	0.02	0.02	0.02	0.01
Tunica intima	3.24 ± 43.72	1.43 ± 40.24	8.44± 53.52	2.31± 52.14
Coefficient of error	0.02	0.03	0.03	0.03
Tunica media	[A] 53.15 ±385.52	[B] 27.08± 316.20	[A] 36.64 ± 564.68	[B] 63.79 ± 448
Coefficient of error	0.02	0.01	0.01	0.01
Tunica adventitia	[A] 48.46 ±316.16	[B] 20 ±228.92	[A] 22.22 ±344.02	[B] 35.73 ± 268.36
Coefficient of error	0.01	0.01	0.01	0.01

Note: A and B show significant differences between right and left renal arteries in 2 zones in adult male dogs ($P \leq 0.05$).

Discussion

Several studies have been performed on arteries (11-14), but little information is available about the transitional zone in arteries (15,16). In the histological studies we were able to show a gradual transition—about 15 mm length—in the structure of the media of the renal artery in dogs, from its origin to the hilus of the kidney on both sides. In the white Carneau pigeon a transitional zone of about 1 cm was reported in the coeliac artery (17). The typical transitional zone in the renal artery was reported with a maximum length of 10 mm; in the internal and external carotid artery it ranged from 5 to 15 mm in length (18,19). It is postulated that the difference in transitional zone length stems from the different arteries involved. The origin of the renal artery showed an intermediate structure between the aorta and the distal renal artery and was therefore named the transitional zone. In this zone the rows of elastic fibers were more numerous, and the external elastic membrane was absent. This may be a result of the similarity of the external elastic membrane to the elastic fibers of the tunica media in arteries such as the aorta.

Beyond 15 mm from the origin of the renal artery in the non-transitional zone, the rows of smooth muscle cells increased in number, and rare remnants of elastic fibers were found between the smooth muscle cells. In this zone collagen fibers in extracellular space increased in number, and the external elastic membrane and a typical muscular morphology were clearly observed. This observation is similar to the findings in other studies. Osborn-Pellegrin (9) reported that the elastic lamellae in the media of the renal artery become thinner as the size of the vessel decreases, and they disappear completely in the distal renal artery. Different structures occur at other vessel sites; for instance at the origin of the renal artery and the common carotid artery in humans, a segment has been observed in which the elastic type wall architecture is replaced by the muscular type (6). A study of the vertebral artery in the giraffe has shown that the caudal segment of the vertebral artery has a largely elastic structure, while the cranial segment has a muscular structure. The transition in the arterial wall normally occurs between the seventh and fifth cervical vertebral levels and involves the diminution of elastic tissue in the luminal portion of the tunica media and a simultaneous increase in smooth muscle cell content (20). Examination of the musculophrenic and superior epigastric arteries has shown that the media of the first 1 to 2 cm of the musculophrenic and superior epigastric arteries is elastomuscular or muscular with rare elastic lamellae; more distally the media is purely muscular (21). We know that during systole the blood enters the large elastic arteries with considerable force, and these arteries distend. They are able to do so because of the large amount of elastic tissue in their walls, and during diastole the arteries return to their original size because of the elastic recoil of the walls. The flow of blood to the organ is controlled by the contraction or relaxation of the smooth muscle cells of the tunica media (1). Research has shown that the amount of elastin along the thoracic aorta is decreased compared to the abdominal aorta, and this is linearly related to the pulse pressure (22). Therefore, the existence of more elastic fibers in the transitional zone compared to the non-transitional zone—and the replacement of elastic fibers by smooth muscle cells in the non-transitional zone—can be viewed as physiological. The larger number of elastic fibers in the transitional zone is due to the proximity of this part of the renal artery to the aorta, and this helps to maintain the blood pressure. However, in the distal renal artery, which contains smaller amounts of elastic fibers, smooth muscle cells help to maintain the blood pressure and regulate the amount of blood flowing into the kidneys by their contraction.

The dense bodies were more numerous in the non-transitional zone than in the transitional zone. In a study of rat arteries Osborn-Pellegrin (9) reported that dense bodies are more numerous in the renal artery than in the aorta. Dense bodies have been observed previously in the smooth muscle cells of cats and monkeys, in varying detail (23). These bodies in the cytoplasm and the cell membrane serve as anchor sites for the myofilaments that are well developed in muscular arteries (24). One study suggests that this difference between smooth muscle cells in the 2 zones of the artery reflects an adaptation to the functional demands made on the smooth muscle cells (9).

In the stereological studies the volumes of the lumen, tunica intima, tunica media, and tunica

adventitia in the transitional zone were greater than in the non-transitional zone on both sides; and they were more numerous in the right renal artery than in the left renal artery in the 2 zones. This may be due to the fact that the size of the artery decreases from elastic to muscular types, and the transitional zone is nearer to the aorta. Therefore, the volume of the artery decreases from the transitional zone to the non-transitional zone.

The clinical implications of the transitional zone in the renal artery are not yet fully understood, but the existence of the transitional zone and its length can be important when planning percutaneous revascularization for lesions of the proximal or orifice of the renal arteries. The link between elastin and a rare occlusive vascular disease has been established. Elastin should now be seen as an important elastic component that provides extensible tissues with resilience, as well as a major developmental regulator of the life cycle of vascular smooth muscle cells and smooth muscle tissue organization (25,26). Atherosclerosis involves the proliferation of smooth muscle cells with an imbalance of the extracellular matrix elements, elastic fibers in particular. Elastin is a critical molecule that regulates the phenotypic modulation and proliferation of smooth muscle cells (27). Therefore, an imbalance between elastic fibers and smooth muscle cells in the transitional zone can predispose this area to vascular diseases such as atherosclerosis.

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