

## Short Paper

# Structure of the orifice of the renal artery in the abdominal aorta in adult male dog

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## Summary

One of the locations of renal artery atherosclerosis is at the orifice of the renal artery, therefore the structure of this orifice was assessed in 6 normal, adult male dogs by light microscopy (LM) and transmission electron microscopy (TEM). For the LM study, processed tissues were embedded in paraffin and sectioned serially into 6- $\mu\text{m}$  thickness. Sections were stained with orcein. For the TEM study, after fixation and dehydration, samples were embedded in resin, and ultrathin sections stained with uranyl acetate. In the LM study, two edges (proximal and distal) at the orifice of the renal artery were observed. Extra formation of elastin was clearly seen at the tip of the distal edge compared with the proximal edge. The curve from the aorta wall into the renal artery at the distal edge of the orifice was sharper than that of the proximal edge. In the TEM study, the tunica intima in the distal and lateral edges was thicker than that in the proximal edge. At the proximal edge of the orifice, there was only one layer of endothelial cells, and the internal elastic membrane was situated directly beneath it. In the distal edge, besides the endothelial cells, smooth muscle cells (SMCs) were found in the tunica intima that were separated from each other with elastic and collagen fibers. In the tunica intima of the lateral edge, two layers of SMCs were observed, between which were collagen fibers. This distance between the internal elastic membrane and endothelial cells at the distal and lateral edges can be important in diseases such as atherosclerosis. These data indicate that there are differences between the proximal edge and the distal and lateral edges of the orifice, and the involvement of special structures in atherosclerosis is suggested.

**Key word:** Orifice, Renal artery, Proximal edge, Distal edge, Lateral edge

## Introduction

Vessel bifurcations are prone to accumulation of atherosclerosis plaques (Badak *et al.*, 2003). Fatty streaking has been found to be localized initially along the posterior wall of bifurcations (Meyer *et al.*, 1980). Studies of age-related changes in the elastic properties of the aortic tree showed that, at the aortic bifurcation, mechanical aging seems to proceed faster than at non-bifurcated arterial segments (Gillessen *et al.*, 1995).

MacKinnon *et al.* (2004) found that atherosclerosis tends to occur at the carotid bifurcation and internal carotid artery. In studies of the distribution of atheromas in

the aorta, they were observed to be most marked around the origins of the intercostal arteries and lumbar arteries (Bell, 1935).

Valenta *et al.* (1999) studied the opening angles of human coronary arteries with different degrees of atherosclerosis. They demonstrated that the opening angle decreases with increasing age, and that the opening angle can be important in atherosclerotic disease.

Atherosclerotic disease of the renal artery is the commonest form of renal artery stenosis and the commonest cause of renovascular hypertension. Atherosclerosis of the renal artery is present in two locations: 1) an eccentric or concentric lesion 1 cm distal to the ostium of the renal

artery, and 2) at the orifice of the renal artery (Schrier, 2007). To understand the mechanism involved in the formation of atherosclerotic lesions, clarifying why these areas tend to be involved in atherosclerosis is important. It has been stated that, at the origin of renal artery in humans there is an elastic-type structure, and at the distal 10-mm section there is a muscular-type structure (Janzen, 2004). In cats, renal arteries have a structure that is intermediate between the muscular and elastic type at their origin with several elastic layers in the tunica media which gradually change to a muscular type at the distal section 7 mm from the origin of the aorta (Ramezani Norouzani *et al.*, 2008). The normal structure of the renal artery in the proximal part is different from other parts of the renal artery, and suggests the involvement of a special structure at the proximal part of the renal artery in atherosclerosis. It was decided to assess the structure of the orifice of the normal renal artery in dogs because it has been guessed that there is a specific structure in this area that can be important in certain diseases such as atherosclerosis. Such reports are lacking in the literature.

## Materials and Methods

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

Six healthy mixed breed native male dogs from the Animal House of the School of Veterinary Medicine, Islamic Azad University, eating a normal diet were used in this study. The dogs were killed with an overdose of thiopental sodium. Specimens of renal arteries were then obtained by dissection.

Six left and right renal arteries were taken from 3 dogs for observation under a light microscope. The abdominal aorta and the adjoining right and left renal arteries were dissected out and stored in 4% formalin solution. The specimens were transferred to a 10% formalin solution after 48 h. After dehydration through a graded series of alcohol solutions, the samples were

cleared in xylol and embedded in paraffin so that serial longitudinal sections could be cut at 6  $\mu$ m. Slides were then stained with orcein, and the proximal and distal portions of the orifice of the renal artery investigated.

The materials for study using a transmission electron microscope (Philips CM10 electron microscope, Eindhoven, the Netherlands) were taken immediately after killing the 3 dogs. The abdominal aorta and the adjoining right and left renal arteries were dissected out. The proximal, distal and lateral parts of the orifice of the renal artery in the abdominal aorta were separated. Specimens were fixed in 4% glutaraldehyde and post-fixed in 1% osmium tetroxide. The latter treatment was carried out through bathing dehydration, adding propylene oxide and embedding in Epon 812 resin. Semi-thin sections were mounted on glass slides and stained with toluidine blue. Thin sections were stained with lead citrate and examined under transmission electron microscopy (TEM).

## Results

By viewing the orifice of the renal artery in longitudinal sections of the aorta, two edges of the opening were distinguished: the proximal edge (which formed a gradual arc from the aorta wall into the lumen of the renal artery) and the distal edge (where there was a sharp arc). Zone 1 on the tip of the distal edge was sharper in comparison with the proximal edge. More compact elastic fibers compared with the proximal edge were observed in this zone (Figs. 1 and 2). The proximal edge showed a gradual arc with compact elastic fibers in zone 1 (Fig. 1). In Fig. 2 the tunica media of the aorta had parallel elastic fibers, but smooth muscle cells (SMCs) with fragmented elastic fibers were observed in the tunica media of the renal artery (Fig. 2). Zone 1 on the tip of the distal edge was sharper in comparison with the proximal edge. More compact elastic fibers compared with the proximal edge were observed in this zone.

TEM observation of the orifice of the renal artery revealed more differences in the proximal, distal and lateral edges.

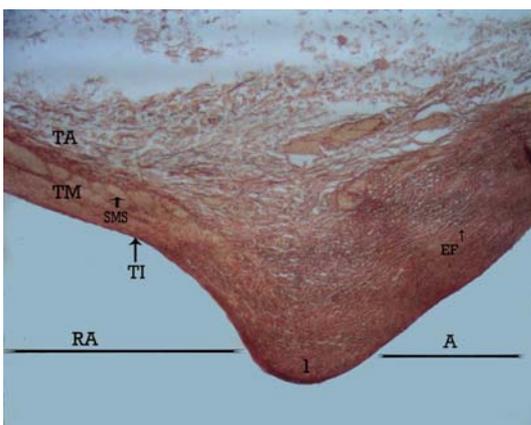
In the proximal edge of the orifice, the

tunica intima contained endothelial cells adjacent to the internal elastic membrane. SMCs were observed in the tunica media of the proximal edge. The extracellular space between SMCs were comprised of collagen and elastic fibers (Fig. 3) In contrast, the tunica intima in the distal edge was thicker. Two cell types were found in the tunica intima in this edge: innermost endothelial cells and outermost SMCs. Elastic and collagen fibers were observed in the extracellular space around SMCs in the tunica intima (Fig. 4).

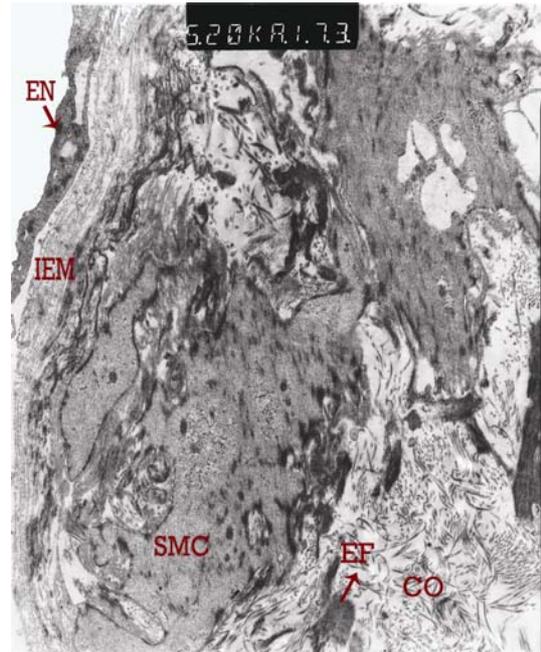
The lateral edge of the orifice of the renal artery had a thick tunica intima. In this



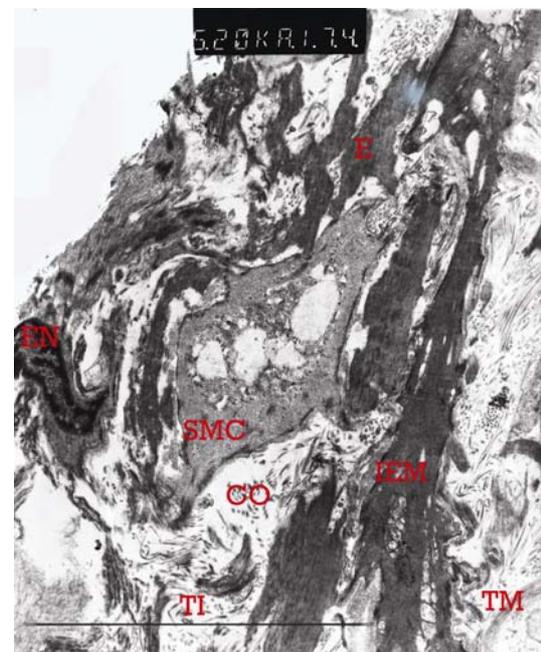
**Fig. 1:** Longitudinal section of the proximal edge of the right renal artery orifice in a healthy dog. A: aorta, RA: renal artery, and 1: zone 1, (orcein stain,  $\times 300$ )



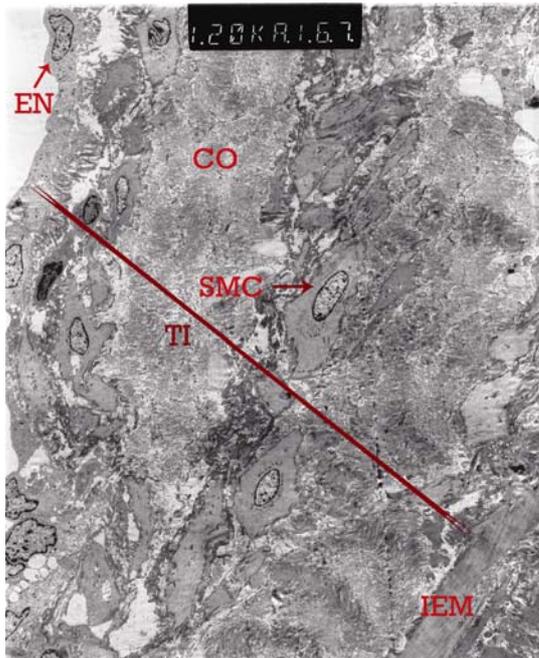
**Fig. 2:** Longitudinal section of the distal edge of the right renal artery orifice in a healthy dog. A: aorta, RA: renal artery, TI: tunica intima, TM: tunica media, TA: tunica adventitia, SMC: smooth muscle cell, EF: elastic fibers, and 1: zone 1 (orcein stain,  $\times 300$ )



**Fig. 3:** Transmission electron micrograph of a transverse section of the abdominal aorta at the proximal edge of the renal artery in a healthy dog. IEM: internal elastic membrane, EN: endothelial cell, SMC: smooth muscle cell, CO: collagen fibers, and EF: elastic fibers, ( $\times 5200$ )



**Fig. 4:** Transmission electron micrograph of a transverse section of the abdominal aorta at the distal edge of the renal artery in a healthy dog. TI: tunica intima, TM: tunica media, IEM: internal elastic membrane, EN: endothelial cell, SMC: smooth muscle cells, EF: elastic fibers, and CO: collagen fibers, ( $\times 5200$ )



**Fig. 5: Transmission electron micrograph of transverse section of the abdominal aorta at the lateral edge of the renal artery in a healthy dog. TI: tunica intima, IEM: internal elastic membrane, EN: endothelial cell, CO: collagen fibers, and SMC: smooth muscle cells**

edge one row of endothelial cells was observed with two rows of SMCs underneath separated from each other by collagen fibers (Fig. 5).

## Discussion

Arterial junctions are very complex structures that are not easy to study. The present study demonstrated structural differences around the orifice of the renal artery in healthy dogs. The curve in the proximal and distal edge of the orifice of the renal artery was different: the distal edge was sharply curved but the proximal edge formed a gradual arc. On the tip of the distal edge more compact elastic fibers were observed compared with the proximal edge.

A similar structure has been described around the orifice of the intercostal arteries and renal arteries in sheep and lambs (van Baardwijk *et al.*, 1985). Siu and Roach (1979) indicated that the bifurcation region of the iliac artery is less prone to distension than the abdominal artery; they stated that this feature is due to the structural arrangement of elastic lamellae and fewer

collagen fibers in the bifurcation. In studies of the abdominal aorta at its branches in dogs and sheep, the distal junction region was found to be the most extendable compared with the proximal region (Cleave and Roach, 1983; Touw *et al.*, 1985).

The present study showed that the structure of the tunica intima at the lateral and distal edges of the orifice of the renal artery were different from that of the tunica intima at the proximal edge. In the lateral and distal edges there were SMCs in the tunica intima. These cells are probably more susceptible to atherosclerotic changes.

Atherosclerosis has a complex etiology and several cell types are involved, including monocytes, SMCs and endothelial cells (Winifred, 1995). The thickness of the tunica intima in the distal and lateral edges was more than that in the proximal edge of the orifice of the renal artery, and can probably play an important part in atherosclerosis. A relationship between atherosclerotic plaques and thickening of the carotid tunica intima-media has been reported (Bonithon-Kopp *et al.*, 1996; Zhang, 2004). It has been established that, at the distal and lateral edges of the orifice of the renal artery, the atherosclerotic lesion will initially develop if the animal is fed a high-cholesterol diet (Cornhill and Roach, 1976). In this study at the proximal edge of the orifice of the renal artery, the internal elastic membrane was situated directly beneath the endothelial cells. However, there was a distance between the internal elastic membrane and endothelial cells in the distal and lateral edges. At the distal edge between SMCs in the tunica intima, elastic and collagen fibers were observed; in the lateral edge, the tunica intima comprised collagen fibers which were sited between SMCs.

It has been reported that atherosclerotic plaques occur at discrete locations in the arterial system and involve the proliferation of SMCs together with an imbalance in the elements of the extracellular matrix (Seyama and Wachi, 2004). This distance over the internal elastic membrane at the distal and lateral edge may provide a convenient space for lipids and the development of atherosclerosis. This is because the internal elastic membrane is known to act as a barrier to macromolecules such as

fibrinogen and low-density lipoproteins (Smith and Staples, 1980). It has been reported that the condition of the internal elastic membrane may play an important role in the development of non-atherosclerotic intima thickening and continuous elasticity. Continuous elasticity could make migration of medial myocytes into the intima more difficult (Krus *et al.*, 2000).

In this study of the renal artery orifice in the abdominal aorta, some differences between the proximal edge and the distal and the lateral edges of the orifice were observed. Also, the involvement of special structures in the development of atherosclerosis was suggested.

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