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Sexual Dimorphism of Sheep Carotid Artery

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Abstract: The aim of the present study was to compare sex differences in the histomorphometric properties of common carotid artery in sheep. 10 adult sheep (5 male and 5 female) of Lori Bakhtyari breed were selected from Shahrekord abattoir. Tissue sections showed that at light microscopic level, there was no sex difference in microscopic appearance of the common carotid arteries. The conjunct intimal plus medial layers was significantly thicker in males as compared to females but reverse was true about lumen diameter ($p<0.05$). The adventitial layer was thicker in male than in female but the differences were not statistically significant. The mean area fraction of collagen in the tunica media of the vessel wall and the mean number of vascular smooth muscle cell nuclei per unit area of the tunica media were significantly greater in males as compared to females but reverse was true about elastin ($p<0.05$). In conclusion, despite microscopic resemblance in the structure of common carotid artery in both sexes, gender affects the lumen diameter, thickness of the mural layers, scleroprotein and smooth muscle cell content of the vessel wall in sheep differently.

Key words: Sheep · Carotid · Dimorphism

INTRODUCTION

Typically, each blood vessel is composed of two or three concentric layers or tunics: tunica intima, tunica media and tunica adventitia. The largest arteries, including the aorta and its branches, the elastic arteries, are known as conducting arteries. These vessels have extremely thick, three-layered walls and possess abundant elastin to the point that they can appear yellow in their gross morphological appearance [1]. Sex hormone receptors have been identified in the cytosol and nuclear compartments of various cell types including the endothelium and vascular smooth muscle cells of the vessel wall. The interaction of sex hormones with cytosolic/nuclear receptors has long been known to stimulate a host of genomic effects that could affect vascular cell growth and proliferation. Sex hormones may also interact with specific plasmalemmal receptors and induce additional nongenomic effects [2, 3]. Sclerotic and ageing changes in all cardiovascular system is well reflected by the status and structure of carotid artery [4]. Increased carotid intima-media thickness and the presence of carotid plaques, are strong risk factors of future stroke and other cardiovascular events including myocardial infarction [5, 6]. In available literature, there is little information characterizing sex differences in histomorphometric features of elastic arteries in ruminants. The present study was, therefore aim to analyze carotid artery sexual dimorphism in sheep.

MATERIAL AND METHODS

10 adult sheep (5 male and 5 female) of Lori Bakhtyari breed were selected before their slaughter in the abattoir of Shahrekord. After cervical and thoracic dissections, tissue samples were taken from the middle part of the left common carotid artery. The tissue samples were immediately fixed in 10% buffered formalin solution at room temperature during 24 hours and processed to embed in Paraffin. Three adjacent sections of 5 µm thick were cut from the center of each block. The first was stained with Picrosirius red to reveal collagen, the second with Miller´s elastic stain to visualize elastin and the third with Ehrlich´s hematoxylin to stain cell nuclei for counting vascular smooth muscle cells. The area fraction of collagen and elastin fibers of tunica media and the number of smooth muscle nuclei per unit area of the tunica media were measured using the methods previously described

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by Stergiopulos *et al*. [7]. Thickness of each layer and lumen diameter were measured using an ocular micrometer. The mean area fraction of collagen and elastic fibers, the mean number of smooth muscle cell nuclei per unit area, the mean lumen diameter of the vessels and the mean thickness of the tunicae were compared between the two sexes using Student's t test. $P<0.05$ was considered as significant.

RESULTS

Results obtained from morphological findings showed that at light microscopic level, there was no sex difference in microscopic appearance of the common carotid arteries. In both sexes, the innermost coat of the arteries, the tunica intima is lined by endothelial cells. The connective tissue immediately beneath the endothelium is composed of fine collagenous fibers together with some elastic fibers. The deeper portion of the intima contains a few longitudinally oriented smooth muscle cells. The tunica media contains numerous elastic sheets. The adventitia consists of connective tissue composed mostly of collagenous fibers arranged in longitudinal spirals. It contains relatively few elastic fibers.

Morphometric Findings of the Present Investigation Are as Follows:

- The conjunct intimal plus medial layers was significantly ($p<0.05$). thicker in males (465±34.44 µ) as compared to females (370±38.24 µ)
- The lumen diameter was significantly ($p<0.05$) more in females (3.12±0.32 µ) as compared to males (2.32±0.22 µ).
- The adventitial layer was thicker in male (250±32.38) than in female (210±28.48) but the differences were not statistically significant.
- The mean area fraction of collagen in the tunica media of the vessel wall was significantly ($p<0.05$).greater in males (0.458±0.004) as compared to females (0.428±0.002).
- The mean area fraction of elastin in the tunica media of the vessel wall is significantly ($p<0.05$) greater in females (0.498±0.004) as compared to males (0.474±0.003) .
- The mean number of vascular smooth muscle cell nuclei per unit area of the tunica media is significantly ($p<0.05$).greater in males (1884±188) as compared to females (1428±166).

DISCUSSION

The present study aimed to analyze gender effect on histomorphometric features of common carotid artery in sheep. Results of this study confirmed that gender differently affects the carotid structure of sheep. The conjunct intimal plus medial layers was significantly thicker in males as compared to females, but reverse was true about the lumen diameter of the vessels. These findings may be due to the different effects of sex steroid hormones (androgens and estrogens) on the arterial wall. The main structural components of the arterial media are elastin, collagen, vascular smooth muscle cells and ground substance in the form of a mucopolysaccharide gel. Receptors for estrogen, progesterone and testosterone are expressed in varying numbers in both the endothelium and vascular smooth muscle cells of multiple vascular systems [8, 9]. A sexually dimorphic pattern in the development of atherosclerotic vascular diseases in humans has been observed in epidemiological and clinical studies [10]. Estrogens exert an inhibitory effect on the development of cardiovascular disease in women [11]. There are many possible estrogen-induced beneficial effects such as: modification of circulating lipoproteins, inhibition of lipoprotein oxidation [12], attenuation of atherosclerotic lesions, favorable modulation of homocysteine [13], changes in blood coagulation [14] and inhibition of intravascular accumulation of collagen [15]. The androgen receptor has been found to be expressed in endothelial cells, smooth muscle cells, macrophages, platelets and cardiomyocytes, all of which are relevant to atherosclerosis and heart failure [16, 17]. In several vascular cells androgen receptor expression was higher if they were derived from male rather than female donors [18-20].

Results obtained from the present study also indicated that the mean area fraction of collagen in the tunica media of the vessel wall was significantly greater in males, but reverse is true about elastin which is significantly greater in female carotid media as compared to males. The sex steroid hormones oestradiol and testosterone alter collagen and elastin metabolism in the aorta in a different manner [21]. Oestradiol decreases and testosterone increases the ratio of collagen to elastin. Estrogen replacement therapy significantly inhibits coronary artery atherosclerosis in ovariectomized monkeys and in cholesterol-fed chicks [22, 23]. Androgen has been shown to be associated with increases in the content of skin collagen in hirsute women [24].

Parchami and Fatahian Dehkordi [25] stated that androgens have a stimulatory effect on scleroprotein metabolism in the male rabbit arterial media. Cembrano *et al*. [26] stated that collagen and elastin content of the aorta were significantly higher in males than in females. Gonadectomy in male chickens decreased significantly the content of collagen and elastin, so that the values became similar to those observed in females. They also stated that the treatment of females with testosterone increased significantly the collagen and elastin content of the aorta to levels similar to those observed in males.

Our results also showed that the mean number of vascular smooth muscle cell nuclei per unit area of the tunica media is significantly greater in males as compared to females. Some studies have suggested that androgens accelerate vascular growth by stimulating the proliferation of vascular smooth muscle cells, whereas other studies show androgen-induced inhibition of growth and proliferation [27, 28]. Smooth muscle cells of the arterial wall play an important role in atherosclerosis by proliferation, migration and matrix production [29-31]. Whereas estradiol can inhibit proliferation and migration of smooth muscle cells, testosterone had no effect [32, 33]. Estradiol appears to inhibit cell growth and to induce antiproliferative effects in vascular smooth muscle cells [34]. The rate of growth in vascular smooth muscle cells of female aorta is slower than that in male aorta [35]. Progesterone also inhibits vascular smooth muscle cell proliferation and migration and may facilitate the inhibitory effects of estrogen [36].

In conclusion, despite microscopic resemblance in the structure of common carotid artery in both sexes, gender affects the lumen diameter, thickness of the mural layers, scleroprotein and smooth muscle cell content of the vessel wall in sheep differently.

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