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Original Article

Correlation of Adventitial Vasa Vasora Density with Intima-Media Thickness in Aorta of Cholesterol-Fed Rabbits

Uzma Shahid*, Shadab Ahmed Butt*, Zubia Athar*, Asma Hafeez* and Rehmah Sarfraz**
Wah Medical College, Wah Cantt, Army Medical College, Wah Medical College, Wah Cantt
. **Islamabad Medical and Dental College, Islamabad

Abstract

Background: Atherosclerosis has been considered as the disease of intima with the role of tunica adventitia so far neglected. Current evidence shows that adventitia, and particularly the vasa vasora (VV) reacts to the process of atherogenesis.

Objective: The study was aimed to calculate the VV density in adventitia and to correlate it with intima-media thickness in aorta of cholesterol-fed rabbits.

Materials & Methods: This experimental study was conducted in Army Medical College Rawalpindi. Samples of vessels were obtained from twenty adult NZW rabbits fed normal (group A) or 2% high-cholesterol (group B) diet for 6 weeks (n=10/group). Aortic sections were taken from each part (ascending, arch, descending thoracic and abdominal) of every aorta. Light microscopic cross sectional analysis was performed in H&E stained slides. Intima-media thickness (IMT) and adventitial VV density were calculated and their correlation was investigated in each part of aorta.

Results: A total of 80 cross sections were analyzed. Mean±SE IMT and adventitial VV density was significantly increased in ascending, arch, and descending thoracic parts of group B versus their equivalent aortic parts in group A. But, there was an insignificant difference of means regarding VV density of abdominal aorta between both groups, despite a significant increase of IMT in group B. A very strong positive correlation was observed between the adventitial VV density and IMT in each aortic part of cholesterol-fed rabbits.

Conclusion: The present study showed that increased IMT is strongly associated with enhanced adventitial VV density in each aortic part of cholesterol-fed rabbits.

Key words: ***Aorta, Cholesterol*** **intima-media thickness, adventitia, vasa vasorum density**

Introduction

Despite several decades of overwhelmingly targeted interventions, atherosclerosis remains the leading cause of morbidity and mortality all over the world[1]. To investigate the pathogenesis of the disease, cholesterol-fed New Zealand white (NZW) rabbits are the most common and well accepted models on account of their susceptibility and sensitivity to present atheromatous lesions, quite similar to humans[2]. The aorta, beginning in the thorax and ending in the abdomen, represents a complex organ system[3]. Its diameter also decreases in a tapering fashion. Its subdivision into different parts (ascending, arch, descending thoracic and abdominal) has been proved to be clinically important as each aortic part experiences diverse local hemodynamics and different pattern of lesion distribution[4,5].

Intima-media thickness (IMT) is considered to be the earliest and most widely accepted predictor of atherogenesis. Moreover, it is strongly associated with high cholesterol diet[6]. Traditionally atherosclerosis is characterized as the disease of intima with the involvement of media in later stages of disease progression[7].

The role of the adventitia in atheroclerosis is so far neglected although several studies have addressed its

Correspondence: Dr. Uzma Shahid, Associate Professor of Anatomy, Wah Medical College, Wah Cantt
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potential importance[8,9,10]. Human and animal blood vessels normally harbor a fine network of microvasculature called vasa vasorum (VV) in the adventitial layer, presumably to nourish the tunica media of large vessels. Current evidence shows that adventitia, and particularly the VV react to the process of atherogenesis[9]. Adventitial inflammation also contributes in intimal disease[11]. The question arises whether there is any link between adventitia and other tunics of the vessel during the process of atherogenesis or not. The present study was, therefore, designed to investigate the adventitial VV density in each part of aorta and to correlate it with aortic IMT in rabbits fed on high cholesterol diet.

Methodology

The present experimental study was conducted in the department of Anatomy at Army Medical College Rawalpindi in collaboration with National Institute of Health (NIH) Islamabad; from June 2009 to May 2010. The experimental protocol was approved by the institutional animal ethical committee.

Twenty adult male NZW rabbits acquired from NIH were used in this study. The rabbits were housed individually in cages & each animal was given 100g/day routine NIH diet. Water was available *ad libitum*. After one week of acclimatization to experimental environment, rabbits were randomly divided into two groups: group A (n= 10) and group B (n= 10). Each rabbit in group A continued 100g/day routine NIH diet while those of group B were fed 2% high cholesterol diet {2g cholesterol powder (Applichem, Germany) mixed with 100g routine NIH diet/ animal/day}. Following six weeks of experiment[12], each rabbit was euthanized with ether and aorta from every animal was dissected out and preserved in 10% formol calcium. Following 48 hours of fixation, aortic sections (each 3-4 mm in width) were taken from all four; ascending, arch, descending thoracic and abdominal parts of aorta. Each aortic section was processed for light microscopic examination and H&E staining was

done for histomorphological examination. At the point of maximum atherosclerotic lesions, under 40X objective, intima-media thickness (IMT) was measured from the lumen–intima interface to the media–

counting the number of microvessels per visual field[14,15]. Correlation between adventitial VV density and IMT was investigated.adventitia interface[13]. VV density was quantified by

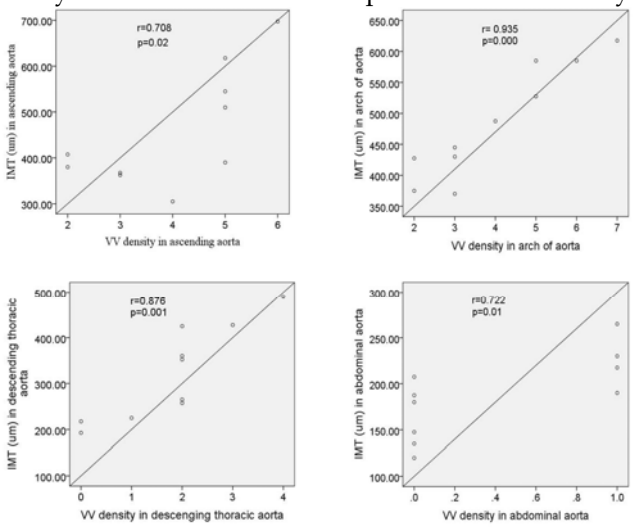

Figure 2: Correlation of adventitial Vasa Vasora (VV) density with Intima Media Thickness (IMT) in each part of aorta of cholesterol-fed rabbits (n=10)

Statistical analysis:

All data were analyzed using SPSS (Statistical package for social sciences) windows version 20. The results were expressed as Mean ± SE (Standard error of mean). For each variable, group differences were compared by independent-samples T test. Correlation between IMT and VV density was assessed by Pearson's rank correlation coefficient. All the results were considered statistically significant at a p-value equal to or less than 0.05.

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All data were analyzed using SPSS (Statistical package for social sciences) windows version 20. The results were expressed as Mean ± SE (Standard error of mean). For each variable, group differences were compared by independent-samples T test. Correlation between IMT and VV density was assessed by Pearson's rank correlation coefficient. All the results were considered statistically significant at a p-value equal to or less than 0.05.

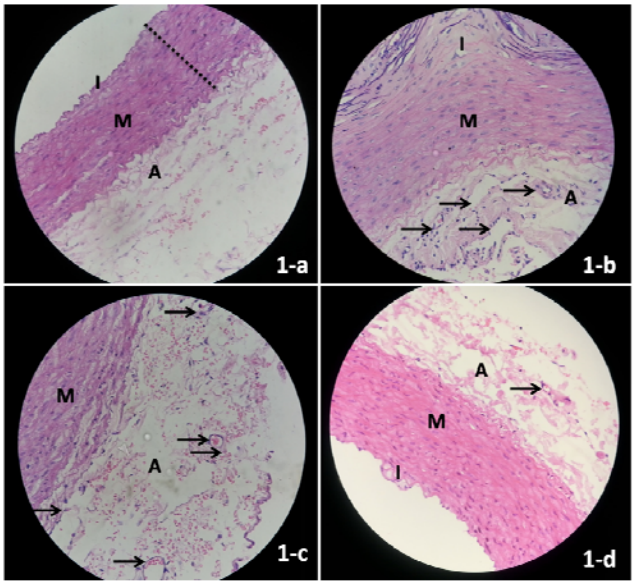

Figure 1: Aortic cross sections of rabbit fed normal diet {1-a (ascending aorta)} and 2% high cholesterol diet {1-b (ascending aorta), 1-c (arch of aorta), 1-d (abdominal aorta)} for 6 weeks. Arrows indicate vasa vasora while dotted line represents intima-media thickness. I=Intima, M=Media, A=Adventitia. H&E stain. Magnification X400.

Table 1: Comparison of Mean ±SE intima-media thickness (IMT) and adventitial vasa vasora (VV) density between rabbits fed normal diet (Group A) and rabbits fed 2% high cholesterol diet (Group B) for 6 weeks

Part of aorta	PARAMETERS					
	IMT (um)		p-value	VV density		p-value
	Group A	Group B		Group A	Group B	
Ascending	211.500± 8.426	458.250± 40.421	0.000	0.60± 0.221	4.00± 0.447	0.014
Arch	218.500± 6.435	485.00± 28.406	0.000	0.40± 0.163	4.00± 0.537	0.000
Descending thoracic	183±6.879	321.25± 32.802	0.001	0.20± 0.20	1.80± 0.389	0.002
Abdominal	128.00± 6.018	188.00± 14.175	0.001	0.10± 0.10	0.40± 0.163	0.135

Discussion

The major finding of the present study was that increased IMT is strongly associated with enhanced adventitial VV density in each aortic part of cholesterol-fed rabbits. These adventitial VV were especially more pronounced beneath the intimal atherosclerotic lesions. Ye *et al*[16] induced atherosclerotic lesions in carotid artery of NZW rabbits and concluded that extensive adventitial VV neovascularization is associated with intimal hyperplasia. Experimental and clinical evidence

suggests that arterial wall thickening may restrain nutrient diffusion from the vessel lumen to deeper layers of vessels, potentially increasing regional hypoxia[10]. In an experimental study on mice, increase in plaque volume was found in close association with the increase in quantity and volume of VV. It was also demonstrated that VV influence plaque progression and stability as they supply oxygen and nutrients to the growing lesion and serves as conduit for inflammatory cells[15]. Kwon *et al*[17] suggested that adventitial neovascularization of VV in experimental hypercholesterolemic porcine coronary arteries might be a part of the early atherosclerotic remodeling process. Contrary to our findings, VV density inversely correlated with intima/media ratios in six cholesterol-fed pigs. The difference can be accredited to different animal model and small sample size[18].

VV proliferation in response to hypoxia occurs when viable cells within an arterial wall are situated 250 to 500 um away from the vessel lumen[19]. In the present study, mean IMT in cholesterol-fed rabbits was >250um in each part of aorta except abdominal aorta. Of particular interest, VV density significantly increased in all parts except abdominal aorta. Thus it seems probable that although IMT increased in abdominal aorta compared with their equivalent part in control rabbits, yet it was <200µm, showing no need of VV to proliferate. We concluded from our previous study[4] in accordance with similar studies[20,21] that proximal thoracic aorta is more prone to atherosclerosis than abdominal aorta in NZW rabbits fed on high cholesterol diet. Therefore, we selected all four parts of aorta in the present study so as to present a unique method for examining the density of adventitial VV in atherosclerosis-prone as well as in atherosclerosis-resistant parts of aorta. More pronounced density of VV in ascending and arch than descending thoracic and abdominal aorta validated the findings of Galili *et al*[22] who found low VV density in atherosclerosis-resistant internal thoracic artery in contrast with atherosclerosis-prone coronary artery.

Worthy to consider, abdominal aorta in humans is considered more atherosclerosis-prone compared with thoracic aorta[5] differing from laboratory mammals . It is virtually possible as humans walk bipedally while other mammals including NZW rabbit, highly needed for research purposes, are quadrupedal and the effect of hemodynamics and other mechanical forces including gravity can affect various vascular beds differently[23].

Enhanced density of adventitial VV beneath the atherosclerotic lesions reflects their strong role in the process of atherogenesis. Thus adventitial VV might be a new surrogate target aimed at atherosclerosis prevention and treatment

Conclusion

We concluded that increased IMT is strongly associated with enhanced adventitial VV density in each aortic part of cholesterol-fed rabbits.

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Contribution of authors

Uzma Shahid, Shadab Ahmed Butt , Zubia Athar, Asma Hafee and Rehmah Sarfraz

All authors participated in conception, designing,, planning experimentation manuscript writing