Abstract

Introduction: Life expectancy has increased recently throughout the world leading to a growing interest in the so-called aging-associated diseases affecting multiple body systems. One of the most frequently affected systems is the cardiovascular system where aging alters its histological structure thus making it more vulnerable to pathology even in absence of traditional risk factors as hypertension, diabetes, or smoking. Some studies suggested that vitamin E may be an important factor in preventing the development and progression of atherosclerosis. However, there is still no complete consensus about its age related cardioprotective effects.

Aim of the work: to study the age related histological changes in the thoracic aorta of male albino rats, and the possible effect of vitamin E.

Materials and methods: Thirty male albino rats were used in this study, 10 adults, aging from 3 to 6 months and weighing 180-220 gm and 20 senile, aging from 18 to 24 months and weighing 280-300 gm. **Group I (Control adult Group):** composed of ten adult rats and was further subdivided into: ***Subgroup IA:*** containing five rats that were not subjected to any procedure. ***Subgroup IB***: containing five rats that were given sesame oil (the solvent used for vitamin E) 3.3 ml daily for 6 weeks. **Group II (control senile Group)**: composed of ten senile rats and was further subdivided into ***Subgroup IIA***: consisted of five rats that were not subjected to any procedure. ***Subgroup IIB:*** consisted of five rats that were given sesame oil 3.3 ml daily for 6 weeks. **Group III (Vitamin E senile Group):** composed of ten senile rats that were given 300mg vitamin E dissolved in 3.3 ml sesame oil daily for 6 weeks.

Results: Histological examination of the thoracic aorta of senile rats showed areas of intimal thickening alternating with hypertrophied irregular ones together with loss of linear arrangement of endothelial nuclei and accumulation of dark

brownish granules in the endothelial and sub endothelial layers. Tunica media showed areas of degeneration with occasional separation. Some SMCs acquired bizzare shaped nuclei and some appeared binucleated. Groups of longitudinally arranged cells with vacuolated nuclei were also seen between tunica intima and media. Elastic fibers were widely spaced and fragmented with multiple areas of break up and points of fusion. Collagen was markedly increased on expense of SMCs. Localized outpouching of the vessel wall was also encountered. Tunica adventitia consisted of sparse, thin and widely separated connective tissue. Immunostained sections revealed multiple areas of weak immune reactivity interrupting the arrangement of the SMCs. On the other hand, thoracic aorta of vitamin E treated rat revealed relatively regular tunica intima with mostly flattened endothelial cells apart from few irregular ones. Tunica media showed localized areas of degeneration but without areas of separation and some SMCs still exhibited bizarre shaped nuclei. However, the groups of longitudinally placed cells between tunica intima and media were still seen but with basophilic cytoplasm. Elastic fibers and collagen bundles revealed obvious improvement and appeared almost regular apart from few vacuolation seen among collagen bundles. Tunica adventitia consisted of wavy, dense connective tissue with some areas of separation. Immunostained sections showed highly actin immune positive SMCs with very scanty areas of interruption.

Conclusion: vitamin E supplementation to senile rats obviously improved their vessel wall histology. Therefore, vitamin E supplementation is strongly advised to the senile for better vascular structure and consequently functions.

Keywords:
Aging- Aorta-Vitamin E.
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Effect of Aging on the Structure of Thoracic Aorta of Male Albino Rat and The Possible Role of vitamin E

Thesis

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2017

Acknowledgment

*First of all, I would like to thank **Allah** "the most merciful" for allowing me to perform this study.*

*I would like to sincerely express my deep gratitude and appreciation to my dear professor **Prof. Dr. Shahira Youssef Mikhail**, Head of Anatomy Department, Faculty of Medicine, Ain Shams University who gave me the golden opportunity to do this work under her kind supervision. Without her valuable assistance and great ideas this work wouldn't have been completed. I am very grateful to her for encouraging me to finish this work and also for her unwavering support, expert advice and patience to teach me and find a time for helping in spite of her very busy schedule. She had always been a constant source of motivation I can ever repay the dept I owe her.*

*I would also like than **Dr. Mohamed MostafaFadel**, lecturer of Anatomy, Faculty of Medicine, Ain Shams University for his continuous guidance and his valuable remarks that helped me in the final production of this work.*

*A special thanks to my dear **Dr. Mary Refaat Isaac**, Lecturer of Anatomy, Faculty of Medicine, Ain Shams University. I am very much thankful to her. The completion of this work could not have been possible without her valuable guidance and assistance. I am highly indebted and thoroughly grateful for her extraordinary support and work. She had helped me to a very great extent to finish this study. It was always a pleasure working under her supervision.*

Finally, sincere thanks must be given to my lovely parents and beloved husband as they supported me a lot and kept me going till I reached this day.

Yara Hassan Mohammed Hassan

List of contents

List of abbreviations

CVD	**:**	Cardiovascular disease
DNA	**:**	Deoxyribonucleic acid
ECM	**:**	Extracellular matrix
eNOS	**:**	Endothelial nitric oxide synthase
GIT	**:**	Gastrointestinal tract
Hx&E	**:**	Hematoxylin and eosin
ICAM	**:**	Interstitial cell adhesion molecule
IL	**:**	Interleukin
LDL	**:**	Low density lipoprotein
MMP	**:**	Matrix metalloproteinase
NO	**:**	Nitric oxide
OH	**:**	Hydroxyl
PKC	**:**	Protein kinase c
ROS	**:**	Reactive oxygen species
SIRT1	**:**	Sirtuin1

Introduction

Aging is a very complex biological process that is influenced by many factors such as; nutrition, smoking, alcohol and environmental conditions which can strongly contribute to its anticipated appearance. A particular attention has been paid to the biological action of free radicals, especially to reactive oxygen species (ROS) that are major factors for forced aging, DNA-damage and carcinogenesis ***(Labat-Robert and Robert, 2014)***.

Generally, in normal arteries, the proteins of the extracellular matrix (ECM) (collagen, elastin, fibrillin, glycoproteins and proteoglycans) produced by smooth muscle cells (SMCs) ensure the stability, resilience and compliance of arteries thus allowing them to stretch while retaining their ability to return to their original shape when the pressure is over. Vascular aging is usually associated with structural and functional modifications of the arteries, even in healthy elderly. These modifications include accumulation and structural changes of ECM components together with disorganization of SMCs leading to intimal and medial thickening in the arterial wall ***(Zarkovic et al., 2015)***.

The age related changes particularly in the aorta are associated with thickening of tunica intima together with a decrease in the quantity of SMCs and breakdown of elastic

lamella in tunica media leading to a definite increase in the aortic diameter, decline in its elasticity and increased stiffness. This stiffness results in an increased blood pressure as well as an increased work load on the heart thus altering the hemodynamics and leading to cardiovascular pathology ***(Collins et al., 2014)***.

Alpha-tocopherol, the main component of a group of compounds known as vitamin E, is a powerful antioxidant and the main fat soluble vitamin responsible for protecting cell membranes against peroxidation. As a lipophilic compound, it accumulates in circulating lipoproteins, cell membranes and fatty deposits, thus reacting with free radicals and molecular oxygen and protecting polyunsaturated fatty acids and lipoproteins from peroxidation ***(Lima et al., 2014)***.

Several studies suggested that vitamin E might be an important factor in preventing the development and progression of atherosclerosis. The effects of vitamin E could be attributed to specific properties of this molecule in regulating a number of cell properties, including signal transduction and gene expression. However, there is still no complete consensus about its cardioprotective effects ***(Kirac et al., 2013)***.

Since Vitamin E is a powerful, available and cheap antioxidant, therefore it would be of great benefit if it had the ability to improve the age related histological changes in the aorta.

Aim of the Work

1- To observe the age related histological and immunohisto-chemical changes in thoracic aorta.

2- To observe the histological and immunohistochemical changes in aged thoracic aorta following vitamin E supplementation.

Review of Literature

Structure of Aorta

Anatomy and Histology of Human Aorta

The aorta was called "the greatest artery" by the ancients. It is the largest blood vessel in the human body and absorbs the impact of 2.3-3 billion heart beats a year while delivering roughly 200 million liters of blood to the various parts of the body. Its wall absorbs the impact of systole and diastole by expanding and recoiling, respectively thus helping to propel the blood distally. It is classically divided into two major anatomic segments, the thoracic and abdominal aorta ***(Buja and Butany, 2016)***.

The thoracic aorta arises from the left ventricle at the level of the third sternocostal joint, and then ascends upwards and to the right till the level of the second sternocostal joint where it arches obliquely to the left and descend to reach the lower border of the fourth thoracic vertebra. It descends within posterior mediastinum to the left side of the vertebrae from T5 toT12 at this level cross through the aortic hiatus in the diaphragm to become abdominal. Considering its 3 different directions, the thoracic aorta is divided into three parts: the ascending aorta, the aortic arch and the descending aorta ***(Dagenias, 2011)***.

The ascending aorta ranges from 5 to 7 cm in length and its width varies from 2.5 to 3.0 cm. It is divided into two parts: the aortic root and the tubular ascending aorta. It lies within the fibrous pericardium, where it enclosed in a tube of serosal pericardium together with the main pulmonary trunk ***(Anderson, 2000)***.

Considering its relations to the surroundings the pulmonary infundibulum and the right auricle lie anterior to its lower part with only the pericardium and thymus remnants separating it from the sternum. Posteriorly, the dome of the left atrium and the right pulmonary artery are present and form the posterior wall of the transverse sinus, while the superior vena cava lies to the right and main pulmonary artery to the left. Inferior to the serous pericardium between the aorta and the main pulmonary artery lie lymphatic vessels and the cardiac plexus ***(Dagenias, 2011)***.

The aortic arch is that part in between the ascending and the descending aorta and gives rise to the brachiocephalic trunk, the left common carotid and the left subclavian arteries. Its normal diameter varies from 2.2to 2.7according to body size. The arch crosses over the right pulmonary artery, the left main stem bronchus, the left recurrent laryngeal nerve, and the roof of the left atrium. The esophagus lies to the left posterior aspect of the arch, and the trachea lies to its right posterior aspect ***(Hutchison, 2009)***.

The short portion of the aorta between the left subclavian artery and the ductus arteriosus is called the isthmus. The descending aorta is the part of the aorta extending from the isthmus to the aortic hiatus in diaphragm. It gives rise to intercostal arteries, spinal arteries including the artery of Adamkiewicz, and bronchial arteries. At its beginning, it is located on the left side of the thoracic vertebra, but at the level of the 7th vertebra, it becomes slightly to the right and anterior. The left pulmonary hilum, the pericardium covering the left atrium, the esophagus, and the diaphragm are anterior to the descending thoracic aorta while the vertebral column and the hemiazygos vein are posterior. The esophagus spirals around the aorta as it is located in a right anterolateral position superiorly, then becomes anterior and goes to the left anterolateral position at the level of the diaphragm ***(Dagenias, 2011)***.

The abdominal aorta extends from the diaphragm down to the bifurcation of the aorta. Its branches include inferior phrenic arteries, celiac artery, renal arteries, superior mesenteric artery, inferior mesenteric artery, lumbar and spinal arteries, and iliac arteries. The normal diameter of the suprarenal segment is 2.0 cm and that of the infrarenal is <2.0 cm (mean ± 2 SD) ***(Hutchison, 2009)***

The diameters of the three aortic segments are variable being widest in the ascending thoracic aorta with a progressive

decrease towards the abdominal segment ***(Mello et al., 2004)***.

Regarding the histological structure of the aorta. It is considered as a large elastic artery composed of three distinct layers: the intima, media, and adventitia**.** The intima is made up of an endothelial monolayer and a subendothelial space. Then, an internal elastic lamina separating the intima from the media. The role of the intima is to provide a smooth nonthrombogenic surface for blood flow **(*Hutchison, 2009)***.

The media which is the thickest layer of the three layers of the aortic wall is made up of SMCs arranged circularly along the long-axis of the aorta and is separated by multiple layers of elastic lamellae joined by elastin fibrils and collagen sheets. In proximal aorta,the ratio between elastin: collagen is 70: 30 (hence, it is the most elastic segment of the aorta), while the ratio becomes 50: 50 in distal aorta, and 30: 70 in peripheral arteries ***(Collins et al., 2014)***.

The adventitia is the outermost layer and consists of collagen fibers and fibroblasts that provide support to the aortic wall. Small blood vessels called vasa vasorum lie in the adventitia and in the outer medial wall. These vasa vasorum supply oxygen to the outer one-third to one-half of the thoracic aorta while the inner half is supplied from the lumen ***(Buja and Butany, 2016)***.

Vasa vasorum resides mainly in the adventitia; their branches also penetrate into the media of the larger vessels. There is an inner zone that contains no vasa vasorum as it is supplied by

diffusion from the vascular lumen. This avascular zone has been described to be approximately 0.5mm thick on average in adults. The avascular or less vascularized regions of major arteries, and especially the aorta, have proved to be prone to atherosclerosis. In contrast, vasa vasorum proliferation within the intima and media is considered to be a part of the inflammatory response during atherosclerotic plaque development ***(Tonara et al., 2016)***.

Anatomy and Histology of Rat Aorta

The anatomy of the cardiovascular systems of the human and rat are more or less similar**.** The structure of the aortic wall has typically been defined as being an elastic artery in both man and rat ***(Suckow et al., 2006)***.

The general histological structure of the rat aorta is composed of the three coats of the vessel wall; tunica intima, media and adventitia ***(Berry et al., 1972)***.

The intima is made up of a thin endothelium, subendothelial connective tissue and an elastic core containing the inner elastic lamina**.** The flattened endothelial cell is the only one cell type present in the tunica intima. In the adult rats the endothelial layer lies directly on the internal elastic lamina, while in the newborn it lies on a subendothelial layer or space which can only be seen with electron microscopy. This subendothelial space is largely filled by amorphous extracellular material, some coarse fibrillary strands, fragments of elastin and collagen fibrils ***(Mello et al., 2004)***. The

intimal endothelial cells can produce elastin *in vitro*, so they could play a role in the formation of the internal elastic lamina***(Cantor et al., 1980)***.

The media which lies immediately adjacent to the intimal layer is the thickest of the aortic layers and is formed of interrelated elastic lamellae, collagen fibers and SMCs. The elastic lamellae and fibers are arranged concentrically with circular and oblique orientations in the different aortic regions. The elastic lamellae are interconnected by bridges of intertwined elastic fibers and are intercalated with collagen bundles and SMCs. The quantity of elastic lamellae and fibers in the medial layer varies in the different aortic regions. Thus, the interstitium among the elastic lamellae of the aortic medial layers contain fusiform SMCs and collagen bundles ***(Mello et al., 2004).***

There are no blood vessels or nerves in the media of the rat, and the SMC is the only type of cell found. The number of elastic lamellae in the medial layer of the abdominal segment is lower than that in thoracic segments and showed mainly a circular orientation ***(Mello et al., 2004)***.

The tunica adventitia is made up of bundles of collagen which run longitudinally or encircle the artery. Numerous blood vessels, nerves, fibroblasts, mast cells, few elastic fibers and occasional SMCs are also found ***(Berry et al., 1972)***.