Original Article	**Effect of Aging on the Structure of Thoracic Aorta of Male Albino Rat and the Possible Role of vitamin E**

Shahira Youssef Mikhail, Mohamed Mostafa Fadel Sonbol, Mary Refaat Isaac, Yara Hassan Mohammed

Department of Anatomy and Embryology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

ABSTRACT

Background: Life expectancy has increased recently throughout the world leading to a growing interest in the so-called age-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 gms, and 20 senile, aging from 18 to 24 months and weighing 280-300 gms. 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) 3.6 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 3.6 ml daily for 6 weeks. Group III (senile Vitamin E Group): composed of ten senile rats that were given 300mg vitamin E dissolved in 3.6 ml sesame oil daily for 6 weeks.

Results: Histological examination of the thoracic aorta of senile rats showed areas of intimal thickening and others of hypertrophy with loss of linear arrangement of endothelial nuclei and accumulation of dark brownish granules. Tunica media showed areas of degeneration, fragmentation of elastic fibers and marked increase in collagen bundles on expense of SMCs. Localized outpouching of the vessel wall was also encountered. Connective tissue of tunica adventitia was sparse, thin and widely separated. Immunohistochemically stained 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. Elastic fibers and collagen bundles in tunica media 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. Immunohistochemically stained 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 function.

Received: 16 September 2017, **Accepted:** 05 October 2017

Key Words: Aging- aorta-vitamin E.

Corresponding Author: Mary Refaat Isaac, Department of Anatomy and Embryology, Faculty of Medicine, Ain Shams University, Cairo, Egypt, **Tel.:** +20 1001171964, **E-mail:** drmaryrefaat@yahoo.com

The Egyptian Journal of Anatomy, ISSN: 0013-2446, Vol. 41 No. 2

Personal non-commercial use only. Anatomy copyright © 2018. All rights reserved *DOI: 10.21608/EJANA.2021.171457*

INTRODUCTION

The process of aging is a complex biological phenomenon associated with the presence of chronic inflammation and oxidative stress which contribute to the development of many age-related diseases. Many theories have been suggested in attempt to explain how aging occurs. However, free radical production is one of the most important cellular theories where free radicals produced by the oxidative stress have been found to cause DNA, lipids and proteins damage thus altering cellular homeostasis and integrity. However, the exact mechanisms underlying the aging process are still not yet fully understood ***(Kumar and Clark's, 2017)***.

Regarding the cardiovascular system, early symptoms of cardiovascular disease (CVD), such as orthostatic hypotension, atherosclerosis, atrial fibrillation and angina often appear with advancing age. This may lead to serious life threatening complications, among which are myocardial infarction and stroke ***(Corella and Ordovas, 2014)***.

Although vascular aging begins in infancy, yet the changes do not become obvious till the age of forty. With advancing age, aortic intimal thickening frequently occurs with structural changes leading to loss of compliance. These changes are further reflected in gross abnormalities in the form of increased aortic diameter and length with increased its liability to become tortuous ***(Buja and Butany, 2016)***.

As for the aorta, ***Meyer et al., (2016)*** declared that human vascular aging is usually presented by increased stiffness of central arteries, particularly the thoracic aorta. The stiffer the artery, the greater the exposure of its endothelium to hemodynamic load, thus promoting endothelial activation, inflammation, and subsequent damage.

Brandes et al., (2005) also stated that, reactive oxygen species (ROS) and oxidative stress had an important role in the process of endothelial aging, affecting vascular function as well as endothelial gene expression and monolayer integrity.

Nowadays the possibility to prevent the age-related diseases with antioxidants including vitamin E has become a worldwide subject of interest ***(Mocchegiani et al., 2014)***.

Vitamin E is a fat-soluble vitamin, found in many foods such as wheat germ, cereal grains, fruits, green vegetables, meat, eggs, and fish. It represents a group of eight lipid soluble substances formed of a chromanol ring and a carbon side chain. According to the site of methyl group at the ring they are classified as (α-, β-, γ-, δ-tocopherol, and α-, β-, γ-, δ-tocotrienol) among which alpha tocopherol is the most important natural form with the greatest activity. The synthetic form of vitamin E is dl-alpha-tocopherol, which is considered as a free-radical scavenger and an antioxidant, and has recently attracted attention for its potential effect in the prevention and treatment of a wide range of diseases ***(Daroff et al., 2016)***.

Recently, it has been reported that vitamin E has a vital role in the prevention of atherosclerosis through inhibition of oxidation of LDL. It has been also found to prevent homocysteine induced aortic damage in rodents. Additionally, some epidemiological studies have associated high dietary intake or high serum concentrations of alpha-tocopherol with a reduced risk of CVD in humans ***(Galli et al., 2017)***.

Since Vitamin E is a powerful, available and cheap antioxidant, hence it became the aim of the present work to access the age related hitological and immunohistochemical changes in the thoracic aorta of senile male albino rats and access the possible effect of vitamin E supplementation on these changes.

MATERIAL AND METHODS

Animals

Thirty male albino rats 10 adults, aging from 3 to 6 months and weighing 180-220 gms, and 20 senile, aging from 18 to 24 months and weighing 280-300 gms, were obtained from the Medical Research Centre of the Faculty of Medicine, Ain Shams University. Animals were housed in conventional wire-mesh cages in a room temperature regulated at 21 ±10˚C, humidity 45-50%, and light/dark cycles every 12 hours. They were fed on standard rat diet, and allowed free water access.

Drugs

Vitamin E was purchased from (Safe pharma PHARCO pharmaceuticals) in the form of capsules 1000 mg /soft gel. Each capsule was dissolved in 12 ml sesame oil and rats were given 3.6 ml/ day orally by intra-gastric tube which is equivalent to 300mg/day of vitamin E ***(Shirpoor et al., 2009)***.

Experimental protocol

Rats were divided into three groups each containing ten rats as follows:

• Group I (control adult): was further subdivided into two subgroups with five adult rats in each as follows:

- Subgroup IA: were not subjected to any procedure.
- Subgroup IB: were given sesame oil 3.6 ml daily for 6 weeks.

• Group II (control senile): was further subdivided into two subgroups with five senile rats in each as follows:

- Subgroup IIA: were not subjected to any procedure.
- Subgroup IIB: were given sesame oil 3.6 ml daily for 6 weeks.

• Group III (Senile treated with Vitamin E): was composed of ten senile rats that were given 300mg vitamin E dissolved in 3.6 ml sesame oil daily for 6 weeks.

At the end of the experiment, rats were anaesthetized with ether according to the protocol of the Animal Care of Ain Shams University. The thoracic cage was opened; thoracic aorta was carefully dissected out, fixed in 10% formole saline. After fixation, tissues were dehydrated in ascending grades of ethanol, cleared in xylol and embedded in paraffin blocks. Sections of 5 µm in thickness were cut and stained with Haematoxylin and Eosin stain. Additionally; Orcein stain was done for demonstration of elastic laminae and Masson's Trichrome stain for collagen fibers ***(Drury and Walington, 1980)***.

Immunohistochemical staining of sections of thoracic aorta of both adult and senile rats was done using anti-actin antibody to examine the distribution of SMCs in tunica media.

Morphometric analysis was also carried out on immunohistochemically stained slides using an image analyzer Leica Q win V.3 program installed on a computer in the Histology Department, Faculty of Medicine, Ain Shams University. The computer was connected to a Leica DM2500 microscope (Wetzlar, Germany). Five specimens from five different rats of each group were examined. For each specimen, five different captured non-overlapping high-power fields (×20) were taken to measure the mean area percentage of anti actin immune reactivity.

Data analysis was performed using MedCalc® Version 11.1.1.0 for Windows (MedCalc Software, Belgium) and Microsoft Office Excel 2010 (Microsoft, USA) Mean, standard deviation and Student T test were done to compare both the pubertal and adult mobile groups with their control ones. *P values* were obtained and interpreted as follows; $p>0.05$ were considered statistically insignificant, $p<0.05$ were considered statistically significant and $p<0.001$ were considered to be highly significant.

RESULTS

Group I (Control adult group)

Histological examination of thoracic aorta of the two subgroups IA & IB were found to be almost similar. Sections stained with Hx. & E. showed that the aorta consisted of three distinct layers, namely tunica intima, media and tunica adventitia (Figure 1). Tunica intima appeared as a thin continuous layer of squamous endothelial cells with flattened nuclei (Figure 2). Tunica media was relatively thick and consisted of SMCs, elastic and collagen fibers. The SMCs had single oval nuclei (Figures 2,3). Intermingled among these SMCs, were elastic fibers which appeared as regularly distributed, parallel lamella regularly distributed (Figure 4). Lamellae were separated by narrow interlamellar space occupied by dense collagen fibers (Figure 5). Tunica adventitia appeared as the outermost layer and consisted of packed wavy connective tissue (Figures 2,4,5).

Immunohistochemically stained sections showed homogenously packed layer of highly actin immune positive SMCs in tunica media (Figure 6) The mean area percentage for immune reaction was 61.47% ±0.842% (Table1- Chart1).

Group II (Control senile group)

Histological examination of thoracic aorta revealed almost no difference between the two subgroups IIA & IIB. Sections stained with Hx. & E. showed that the aorta had its characteristic three layers (Figure 7). Tunica intima revealed areas of atrophy alternating with relatively thickened, hypertrophied and irregular ones (Figure 8). The nuclei were variable in shape and lost their linear arrangement (Figures 8,9). Dark brownish granules were dispersed in the endothelial as well as the subendothelial layers (Figure 9). Many nuclei of SMCs were bizarre shaped; few were small in size (Figure 8) or less frequently binucleated (Figures 8,9). Few oval

shaped vacuoles were frequently noticed with groups of longitudinally arranged vacuolated cells with fusiform nuclei were observed between tunica intima and media (Figure 9). Elastic fibers in tunica media were widely spaced fragmented in addition to multiple areas of break up and points of fusion (Figure 10). Occasionally the aorta of senile rats showed localized areas of outpouching that extended towards tunica adventitia (Figure 11). The outpouching had extremely irregular elastic fibers or interrupted ones (Figure 12). Moreover, abundant collagen fibers in tunica media were seen encroaching on muscle layer with occasional groups of multiple rounded vacuoles seen interrupting the collagen fibers (Figure 13). Tunica adventitia consisted of sparse, widely separated connective tissue (Figures 10,13).

Immunohistochemically stained sections showed weak immune reactivity in multiple areas, interrupting the arrangement of the SMCs (Figure 14). The mean area percentage for immune reaction was 58.44% ±0.452% (Tables1,2 - Chart 1).

Group III (Senile Group treated with vitamin E)

Histological examination of thoracic aorta sections of senile rats treated with vitamin E showed that the aorta had its characteristic three layers (Figure 15). Tunica intima was relatively regular in thickness. Endothelial nuclei were mostly flattened (Figure 16), however few nuclei were irregular in shape and appeared protruding into the lumen (Figures 17,18). Tunica media still exhibited localized areas of degeneration but without areas of separation (Figure 17). Some binucleated SMCs (Figure 18) were encountered and groups of longitudinally placed cells with basophilic nuclei were still observed between tunica intima and media (Figure 17). Elastic fibers appeared as almost parallel and regularly distributed lamellae. However, few irregular elastic fibers still had areas of break up and points of fusion (Figure 19). Moreover, bundles of collagen fibers with few vacuoles were seen in between muscle fibers (Figure 20). Tunica adventitia consisted of wavy dense connective tissue but with areas of separation (Figures 19,20).

Immunohistochemically stained sections showed highly actin immune positive SMCs with very scanty areas of interruption (Figure 21). The mean area percentage for immune reaction was 59.99% ±0.231% (Table2 - Chart1).

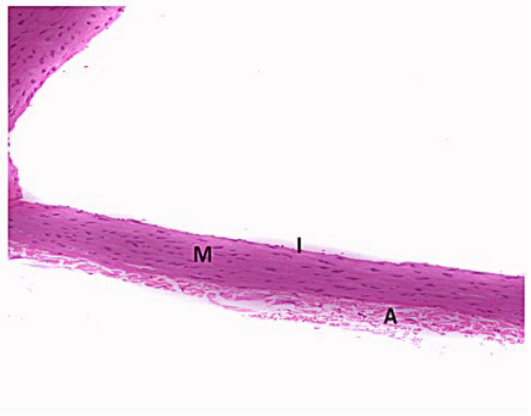

Fig. 1: A photomicrograph of a section of thoracic aorta of group I (control adult rats) showing the three layers, intima (I), media (M) and adventitia (A). (Hx. &E., x100)

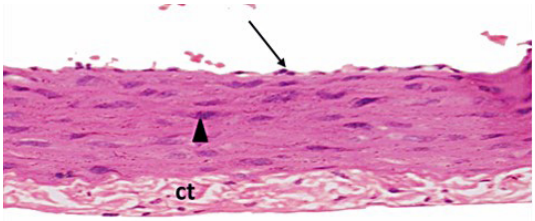

Fig. 2: A photomicrograph of a section of thoracic aorta of group I (control adult rats) showing tunica intima with squamous cells having flattened nuclei (↑) and tunica media with smooth muscles having single oval nuclei (▲). Notice the packed, wavy connective tissue (ct) in tunica adventitia. (Hx. &E., x400)

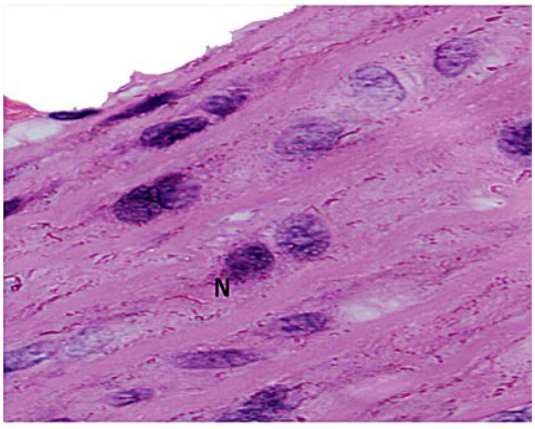

Fig. 3: A photomicrograph of a section of thoracic aorta of group I (control adult rats) showing oval nuclei (N) of smooth muscle cells. (Hx. &E., x1000)

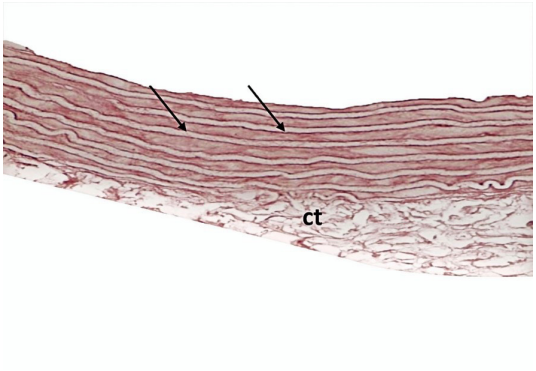

Fig. 4: A photomicrograph of a section of thoracic aorta of group I (control adult rats) showing parallel lamellae of elastic fibers (↑) in tunica media. Notice the packed connective tissue (ct) in tunica adventitia. (Orcein, x400)

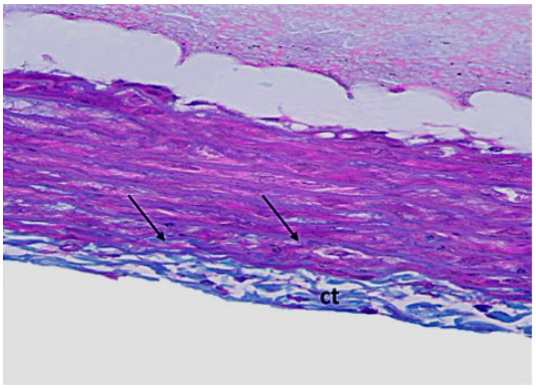

Fig. 5: A photomicrograph of a section of thoracic aorta of group I (control adult rats) showing dense collagen fibers (↑) in interlamellar space. Notice the packed wavy connective tissue (ct) in tunica adventitia. (Masson's trichrome, x400)

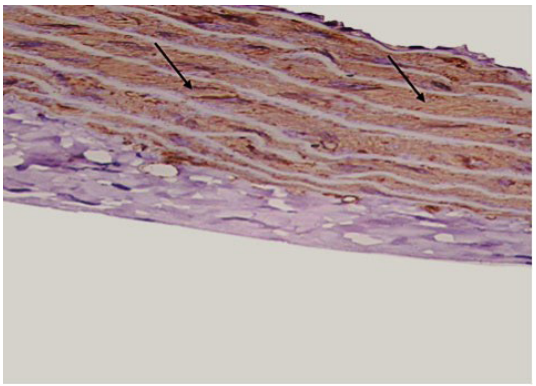

Fig. 6: A photomicrograph of a section of the thoracic aorta of group I (control adult rats) male albino rat showing homogenous packed layer of highly actin positive smooth muscle fibers (↑). (Immune staining with anti-actin antibody, x400)

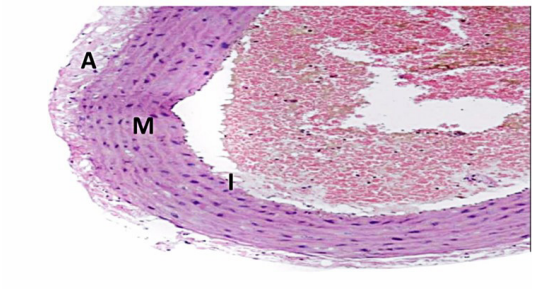

Fig. 7: A photomicrograph of a section of thoracic aorta of group II (control senile rats) showing the three layers, intima (I), media (M) and adventitia (A). (Hx. &E., x100)

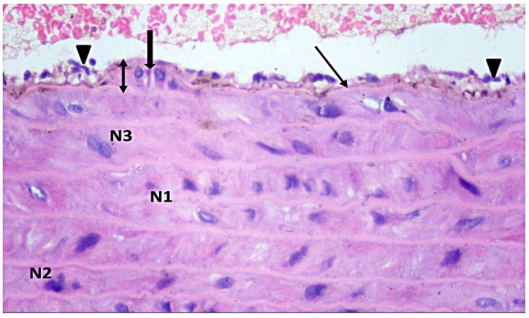

Fig. 8: A photomicrograph of a section of thoracic aorta of group II (control senile rats) showing tunica intima with alternating areas of atrophy (↑) and hypertrophied irregular ones (↕) with loss of linear arrangement of endothelial cells and variable shaped nuclei (▲). Notice the small (N1) and bizarre shaped (N2) nuclei of the smooth muscles in tunica media. Note also the binucleated (N3) smooth muscle cells and the longitudinally placed cells (thick ↑) between tunica intima and media. (Hx. &E., x400)

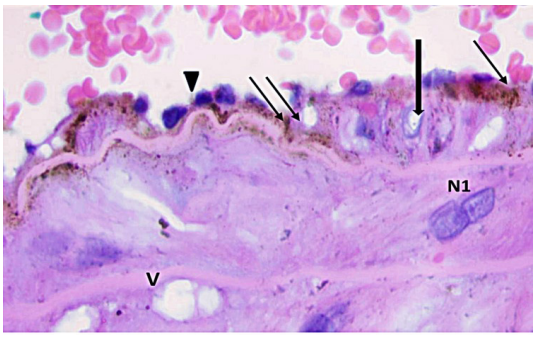

Fig. 9: A photomicrograph of a section of thoracic aorta of group II (control senile rats) showing loss of linear arrangement of endothelial cells, variable shaped nuclei (▲) and dark brownish granules in the endothelial (↑) and subendothelial layers (↑↑). Notice the oval shaped vacuoles (V) and the binucleated (N1) smooth muscle cells in tunica media. Note also the vacuolated longitudinal cells (thick ↑) between tunica intima and media. (Hx. &E., x1000)

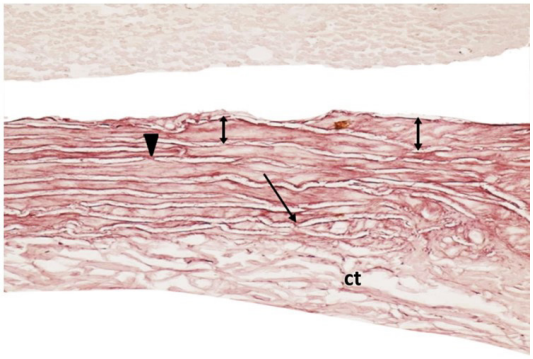

Fig. 10: A photomicrograph of a section of thoracic aorta of group II (control senile rats) showing the elastic fibers widely spaced (↕), fragmented (▲) and with areas of break up and fusion (↑). Notice the widely separated connective tissue (ct) in tunica adventitia. (Orcein, x400)

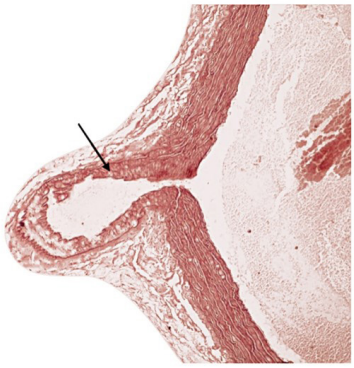

Fig. 11: A photomicrograph of a section of thoracic aorta of group II (control senile rats) showing outpouching of the vessel wall (↑) extending toward tunica adventitia. (Orcein, x100)

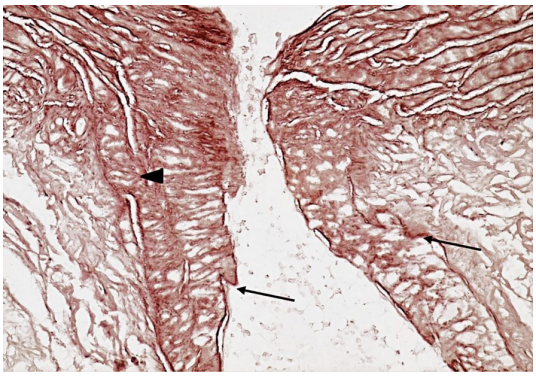

Fig. 12: A photomicrograph of a section of thoracic aorta of group II (control senile rats) showing the beginning of the outpouching revealing irregular (↑) and interrupted (▲) elastic fibers. (Orcein, x400)

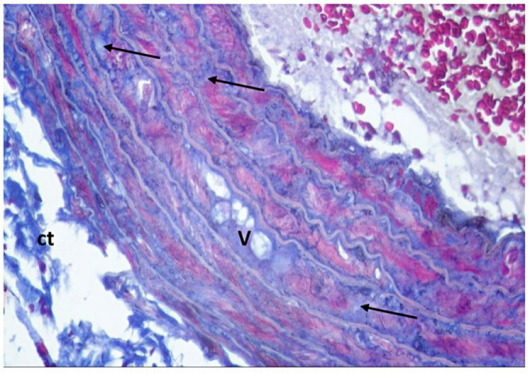

Fig. 13: A photomicrograph of a section of thoracic aorta of group II (control senile rats) showing abundant collagen fibers (↑) encroaching on the muscle layer in tunica media and rounded vacuoles (V) occupying interlamellar space. Notice the widely separated connective tissue (ct) in tunica adventitia. (Masson's trichrome, x400)

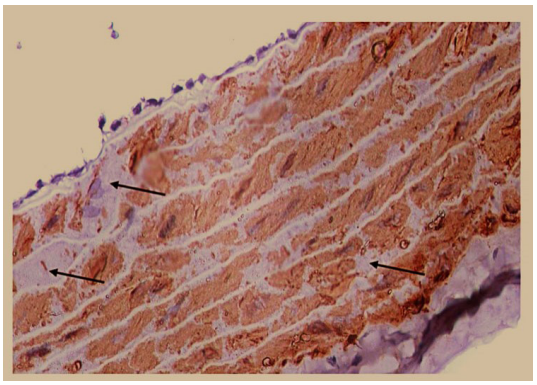

Fig. 14: A photomicrograph of a section of thoracic aorta of group II (control senile rats) showing areas of weak immune reactivity (↑) interrupting arrangement of smooth muscles. (Immune staining with anti-actin antibody, x400)

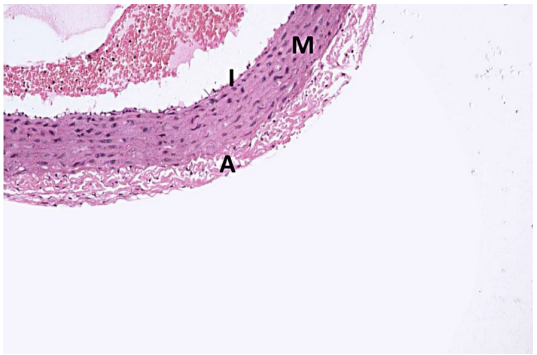

Fig. 15: A photomicrograph of a section of thoracic aorta of group III (Senile rats treated with vitamin E) showing the three layers, intima (I), media (M) and adventitia (A). (Hx. &E., x100)

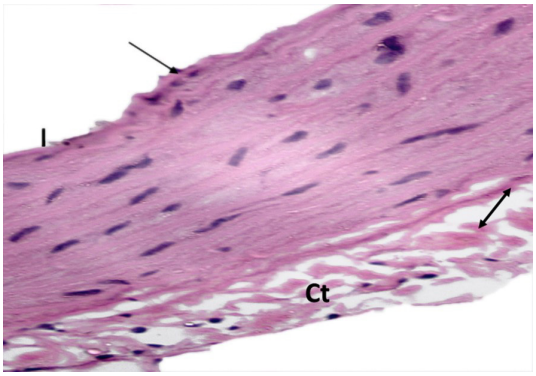

Fig. 16: A photomicrograph of a section of thoracic aorta of group III (Senile rats treated with vitamin E) showing relatively regular tunica intima (I) with flattened endothelial nuclei (↑). Notice wavy dense connective tissue (ct) with areas of separation (↕) in tunica adventitia. (Hx. &E., x400)

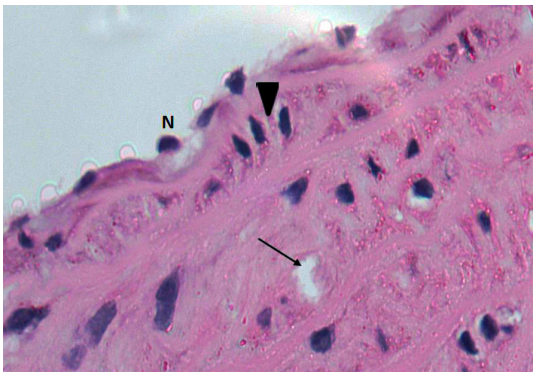

Fig. 17: A photomicrograph of a section of thoracic aorta of group III (Senile rats treated with vitamin E) showing endothelial nuclei (N) protruding into the lumen. Notice areas of degeneration (↑) in tunica media. Note also longitudinally placed cells (▲) with basophilic nuclei between intima and media. (Hx. &E., x1000)

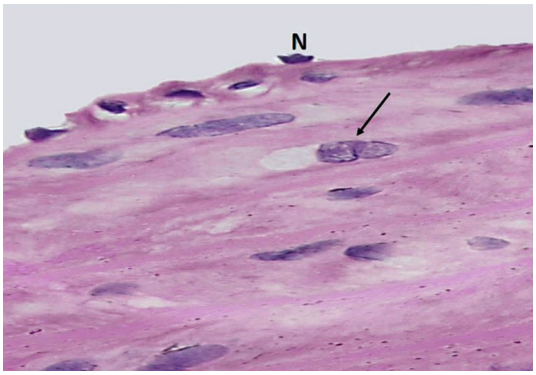

Fig. 18: A photomicrograph of a section of thoracic aorta of group III (Senile rats treated with vitamin E) showing endothelial nuclei (N) protruding into the lumen. Notice the binucleated SMC (↑). (Hx. &E., x1000)

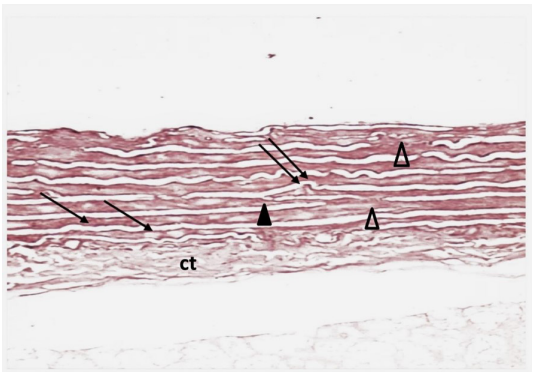

Fig. 19: A photomicrograph of a section of thoracic aorta of group III (Senile rats treated with vitamin E) showing areas with parallel lamellae of elastic fibers (↑), others with irregular fibers (↑↑) and points of break up (Δ) and fusion (▲). Notice the wavy dense connective tissue (ct) in tunica adventitia. (Orcein, x400)

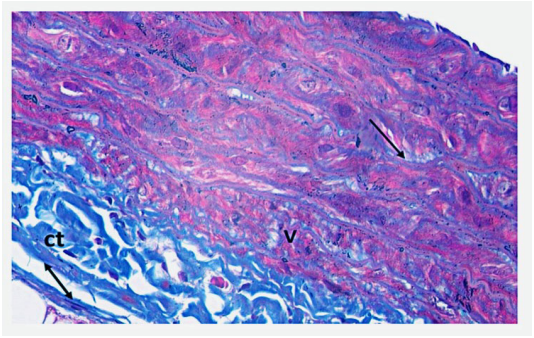

Fig. 20: A photomicrograph of a section of thoracic aorta of group III (Senile rats treated with vitamin E) showing bundles of collagen fibers (↑) with few vacuoles (V) in tunica media. Notice the wavy, dense connective tissue (ct) with area of separation (↕) in tunica adventitia. (Masson's trichrome, x400)

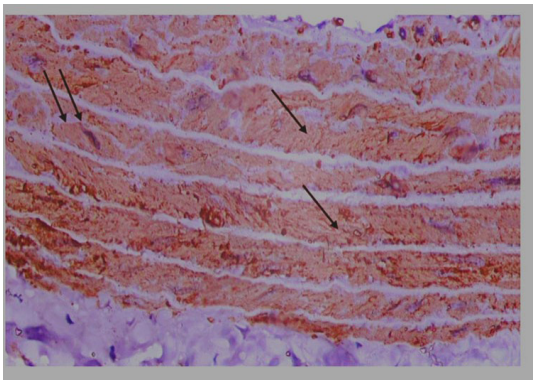

Fig. 21: A photomicrograph of a section of thoracic aorta of group III (Senile rats treated with vitamin E) showing actin positive (↑) smooth muscle fibers with scanty areas of interruption (↑↑). (Immune staining with anti-actin antibody, x400)

Table 1: Comparison between groups I & II as regards mean area percentages of anti actin immune reactivity

	Group I (Control Adult)	Group II (Control Senile)
Mean	61.47%	58.44%
Standard Deviation	0.842%	0.452%
T test	0.000011773 $P < 0.001$ Highly significant	

Table 2: Comparison between groups II & III as regards mean area percentages of anti actin immune reactivity

	Group I (Control Adult)	Group II (Control Senile)
Mean	58.44%	59.99%
Standard Deviation	0.452%	0.231%
T test	0.0000177503 $P < 0.001$ Highly significant	

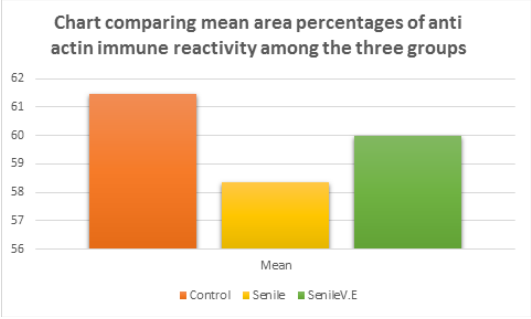

Chart 1: The mean area percentage for immune reaction

DISCCUSSION

The present work revealed that aging had remarkable effects on the structure of the three layers of thoracic aorta. Tunica intima showed areas of atrophy alternating with thickened hypertrophied and irregular ones.

Orlandi et al., (2006) stated that, intimal thickening was one of the most common age associated changes which typically occur in large human arteries as the aorta. ***Eskurza et al., (2004)*** added that, aging has been also found to induce endothelial dysfunction even in absence of any CVD or traditional cardiovascular risk factors. This endothelial dysfunction was broadly defined by ***Brandes et al., (2005)*** as an imbalance between vascular protective and deteriorating factors generated by the endothelium.

Aged vascular endothelial cells usually show impairment of intracellular signal conducting system with subsequent decrease in the production of (NO) and endothelial derived NO synthase (eNOS) ***(Smith et al., 2006)***. Nitrous oxide is considered as an important vasoprotective and cardioprotective agent ***(Wildansky et al., 2003)***. It has been found to play a pivotal role in the regulation of vascular wall integrity through its characteristic anti-sclerotic ***(Cernadas et al., 1998)***, anti-inflammatory, anti- thrombotic and antioxidant actions ***(Wildansky et al., 2003)***.

Additionally, vascular aging is usually associated with a chronic low grade inflammatory process described as "inflamm-aging" provoked by a constant antigenic load and stress ***(Franceschi et al., 2000)***.

The aging process also alters the structure of endothelial cells leading to an increase in their size and irregularity of their shape ***(Fillit et al., 2017)*** in addition to an increase in endothelial cellular apoptosis leading to endothelial dysfunction ***(Shalini et al., 2015)***. This could explain the variability in endothelial cells shape and loss of their linear arrangement found in the present study.

Moreover, the present work revealed the presence of dark brownish granules in the endothelial and sub endothelial layers. ***Procop and Pritt, (2014)*** stated that, among the endogenously produced pigments are hemosiderin and lipofuscin which could be confused together as both of them appear brown in color. Lipofuscin is produced from oxidation of unsaturated fatty acids and normally accumulates in tissues with aging due to damage of lysosomes, cellular membranes and mitochondria.

On the other hand, hemosiderin is considered as an iron storage protein on which 25% of body iron is normally bound ***(Cheng and Li, 2007)***. It has been reported that superoxide radical formation with iron mediation usually occurs during the development of heart disease and that this can be inhibited by iron chelators ***(Bolger et al., 2006)***. In addition, atherosclerotic plaque has been accompanied by the accumulation of iron, oxidized lipids and fibrous elements in arteries. Moreover, a correlation has been found between iron status and atherosclerosis where free or poorly ligated iron can participate in lipid peroxidation ***(Jomova and Valko, 2011)***. Therefore, these intimal dark brown granules encountered in the present study could be explained as age

related accumulation of lipofuscin pigment or hemosiderin pigment deposition accompanied by the inflammatory wear and tear intimal state.

Regarding tunica media, the present study revealed marked disorganization of its normal constituents. Smooth muscle cells appeared separated from each other and their nuclei were bizarre shaped, small or less frequently binucleated. Elastic fibers were widely spaced and fragmented with multiple areas of break up and points of fusion. Regarding collagen fibers, Masson's trichrome stain revealed abundancy of collagen content on the expense of SMCs which were seen encroaching on them.

Aging was associated with a decrease in the quantity of SMCs and a concomitant decrease in elastic fibers since SMCs were responsible for synthesizing elastin within the aorta ***(Fritze et al., 2012)***. The elastic lamella become further apart and filled with proteoglycans and altered collagen which changes from being thin and wavy to become thicker and more linear in arrangement with advancing age ***(Martin et al., 2013)***. Thus aging is usually associated with progressive decline in the elastic properties of the aortic wall ***(Aquaro et al., 2013)***.

The present study also revealed an outpouched part of the vessel wall that extended toward tunica adventitia. The elastic fibers in this portion were extremely irregular and fragmented. ***Rodella et al., (2016)*** added that, the change in the nature of the connective tissue in the aortic wall can play a crucial role in the aneurysm development. Moreover, the fragmented elastic fibers and the decrease in the elastin concentration are among the most significant structural changes that occur in the aneurysmal tissue ***(Swaminathan et al., 2014). Barker et al., (1995)*** also reported that, endothelial cells, SMCs and macrophages which are stimulated by inflammatory signals, produce MMPs which in turn degrade the connective tissue matrix and weaken arterial wall leading to aneurysm formation.

Similarly Moon et al., (2001) stated that, SMCs from the arteries of aged mice showed accumulation of oxidative mitochondrial DNA damage with higher levels of ROS which may promote SMCs apoptosis with subsequent oxidative damage in the arterial wall.

These data could explain the histopathological changes encountered in SMCs in the present study including the bizarre shaped and small sized nuclei.

Moreover, immunohistochemical stained sections of senile rats in this study revealed weak immune reactivity to anti-actin antibody in multiple areas of tunica media interrupting the arrangement of the SMCs with highly significant decrease in its mean area percentage when compared with control adult group. Similarly, ***Ferlosio et al., (2012)*** reported that SMCs of senile rats have a decreased quantity of both α-smooth muscle actin and myosin when compared to young ones. This means that the aged SMCs have a less contractile ability which affects arterial compliance.

Therefore, this weak immune reactivity might be attributed to the age related oxidative damage promoting SMCs apoptosis or to the accumulation of collagen fibers that were seen encroaching on the SMCs.

Another important pathology noted in aged SMCs, is the alteration of its migration site where SMCs were found to migrate to the thickened intima with advancing age ***(Sata et al., 2002)***. In addition, chronic metabolic or mechanical injury induces SMCs migration and proliferation into the neointima to form hyperplastic intimal cells population ***(Ross, 1993)***.

These finding could explain the appearance of the longitudinally arranged cell groups with fusiform vacuolated nuclei encountered in the present study between tunica intima and media of aged rats. ***Collins et al., (2014)*** further clarified that, SMCs migration plays a crucial role in the thickening of endothelium and added that this migration usually happens in response to injury and atherosclerosis.

Regarding tunica adventitia, the present study revealed that its connective tissue was sparse, thin and widely separated. However, unlike our finding, ***Ungvari et al., (2010)*** reported that aged tunica adventitia of rat aorta shows marked fibrosis. In addition, ***Falk et al., (2009)*** clarified that the thickening of adventitia was attributed to the increased activity of fibroblasts which produced more ECM.

On the other hand, examination of sections of thoracic aorta of rats treated with vitamin E, revealed obvious improvement in the histological architecture of the vessel wall. Tunica intima appeared relatively regular in thickness. Endothelial cells were mostly flattened apart from some irregularly shaped nuclei and others seen protruding into the lumen.

Nanyakkara et al., (2009) reported that, vitamin E supplementation had beneficial effects on the endothelium. It prevented endothelial cell damage by reducing plasma levels of vascular cell adhesion and increasing NO concentration. ***Van der loo et al., (2002)*** also stated that, the anti-aging effects of vitamin E depend mainly on its ability to balance the oxidative stress that usually accompanies the aging process.

Additionally, ***Patel et al., (2016)*** recorded that, owing to its anti-oxidant properties, vitamin E helps to reduce ROS formation which inhibits the formation of atherosclerotic plaque. Thus, it reduces the incidence of atherosclerosis and subsequent endothelial damage. ***Azzi et al., (2004)*** also attributed this beneficial effect to its ability to regulate signal transduction and gene expression. Moreover, vitamin E has the ability to maintain the integrity of endothelium and inhibit platelet adhesion, cytokines release and monocyte ROS production ***(Azzi, 2007)***.

Regarding tunica media of this group, examination revealed localized areas of degeneration but without the areas of separation observed in the senile control group. Few bizarre shaped nuclei of SMCs were seen. Additionally, groups of longitudinally placed cells were still encountered between tunica intima and media as seen in group II. Elastic fibers showed obvious improvement and were almost parallel apart from few areas of break up and points of fusion. Moreover, collagen bundles appeared with much less vacuolation than that observed in the senile group.

Similarly, ***Norouzi et al., (2015)*** reported that vitamin E exerted an effective role in improving the ethanol induced histopathological action on aortic media. It alleviated the increased medial width and fiber thickness. It also decreased the proliferating cell nuclear antigen positive indices which are indicators of SMCs proliferation, nearly back to normal level.

Moreover, some studies also attributed this vitamin E induced inhibition of SMCs proliferation to its inhibition to PKC ***(Boscobinik et al., 1995)***.

Similarly, ***Kirac et al., (2013)*** reported that vitamin E is protective against cholesterol induced aortic wall damage as it inhibits medial thickening, elastic fibers fragmentation and increased collagen deposition. Moreover, vitamin E has an effective role in blocking different stages of the pathological process in vascular diseases by modulating cell signaling and regulating gene expression.

CONCLUSION

Aging had deleterious effect on the structure of thoracic aorta of male albino rats. Age related changes greatly affected tunica intima and media and to a less extent, tunica adventitia. On the other hand, vitamin E supplementation to senile rats obviously improved their vessel wall histology. Therefore, vitamin E supplementation is recommended.

CONFLICT OF INTERESTS

There are no Conflicts of Interest.

REFERENCES

Aquaro G.D, Cagnolo A., Tiwari K.K., Todiere G., Bevilacqua S., Bella G.D, Ait-Ali L., Festa P., Glauber M. and Lombardi M. (2013): Age-dependent changes in elastic properties of thoracic aorta evaluated by magnetic resonance in normal subjects. Interact. Cardiovasc. Thor. Surg.; 17(4):674-679.

Azzi A., Gysin R., Kempná P., Munteanu A., Negis Y., Villacorta L., Visarius T. and Zingg J.M. (2004): Vitamin E mediates cell signaling and regulation of gene expression. Ann N Y Acad Sci; 1031:86-95.

Azzi A. (2007): Molecular mechanism of alpha-tocopherol action.Free Radic. Biol. Med.; 43(1):16-21.

Buja L.M., and Butany J. (2016): cardiovascular pathology, Chapter 5: Aneurysms of the Aorta: Ascending, Thoracic and Abdominal and Their Management; 169-211.

Boscoboinik D., Ozer N.K., Moser U. and Azzi A. (1995): Tocopherols and 6-hydroxy-chroman-2-carbonitrile derivatives inhibit vascular smooth muscle cell proliferation by a nonantioxidant mechanism. Arch Biochem Biophys; 318(1):241-6.

Bolger A.P., Bartlett F.R., Penston H.S., O'Leary J., Pollock N., Kaprielian R. and Chapman C.M. (2006): Intravenous iron alone for the treatment of anemia in patients with chronic heart failure. J Am Coll Cardiol; 48(6):1225–1227.

Brandes R.P., Fleming I. and Busse R. (2005): Endothelial aging. Cardiovascular Res.; 66(2): 286- 294.

Cernadas M.R., Sanchez de Miguel L., Garcia-Duran M., Gonzalez-Fernandez F., Millas I., Monton M., Rodrigo J., Rico L., Fernández P., Frutos T., Rodríguez-Feo J.A., Guerra J., Caramelo C., Casado S. and López-Farré. (1998): Expression of constitutive and inducible nitric oxide synthases in the vascular wall of young and aging rats. Circ Res; 83: 279-286.

Cheng Z. and Li Y. (2007): What is responsible for the initiating chemistry of iron mediated lipid peroxidation: an update. Chem. Rev; (3):748–766.

Collins J.A., Munoz J-V., Patel T.R., Loukas M. and Tubbs R.S. (2014): The anatomy of the aging aorta. Clin Anat; (3):463-466.

Corella D. and Ordovás J.M. (2014): Aging and cardiovascular diseases: the role of gene-diet interactions. Ageing Res Rev; 18:53-73.

Daroff R.B., Jankovick J., Mazziotta J.C. and Pomeroy S.L. (2016): Bradley´s neurology in clinical practice seventh edition. Chapter 85: Deficiency disease of the nervous system; 1226-1236.

Drury R.A.B. and Wallington E.A. (1980): Carleton's histology technique. Fifth edition. Oxford University Press; 241-242.

Eskurza I., Monahan K.D., Robinson J.A. and Seals D.R. (2004): Effect of acute and chronic ascorbic acid on flow-mediated dilatation with sedentary and physically active human ageing. J. Physiol.; 556(1): 315-324.

Falk E., Thim T. and Kristensen I.B. (2009): Atherosclerotic Plaque, Adventitia, Perivascular Fat, and Carotid Imaging. JACC Cardiovascular Imaging; 2(2):183-186.

Ferlosio A., Arcuri G., Doldo E., Scioli M.G., De Falco S., Spagnoli L.G. and Orlandi A. (2012): Age related increase of stem marker expression influences vascular smooth muscle cell properties. Atherosclerosis; 224(1): 51-57.

Fillit H.M., Rockwood K. and Young J. (2017): Brocklehurst's Textbook of Geriatric Medicine and Gerontology eighth edition. Chapter 16: Effects of Aging on the Cardiovascular System; 96-100.

Franceschi C., Bonafe M., Valensin S., Olivieri F., De Luca M., Ottaviani E. and De Benedictis G. (2000): Inflamm-aging. An evolutionary perspective on immunosenescence. Ann. N. Y. Acad. Sci.; 908:244-254.

Fritze O., Romero B., Schleicher M., Jacob M.P., Oh D-Y., Starcher B., Schenke-Layland K., Bujan J. and Stock U.A. (2012): Age-related changes in the elastic tissue of the human aorta. J Vasc Res; 49(1):77-86.

Galli F., Azzi A., Birringer M., Cook-Mills J.M., Eggersdorfer M., Frank J., Cruciani G., Lorkowski S. and Ozer N.K. (2017): Vitamin E: Emerging aspects and new directions. Free Radic. Biol. Med.; 102:16-36.

Jomovaa K. and Valko M. (2011): Advances in metal-induced oxidative stress and human disease. Toxicology; 283:65-87.

Kirac D., Negis Y. and Ozer N.K. (2013): Vitamin E attenuates homocysteine and cholesterol induced damage in rat aorta. Cardiovascular pathology; 22:465-472.

Kumar P. and Clark´ M. (2017): Kumar and clark clinical medicine ninth edition. Chapter 10: nutrition; 183-218.

Martin C., Sun W., Primiano C., McKay R. and Elefteriades J. (2013): Age dependent ascending aorta mechanics assessed through multiphase CT. Ann Biomed Eng.; 41(12).

Meyer M.L., Tanaka H., Palta P.,Cheng S., Gouskova N., Aguilar D. and Heiss G. (2016): Correlates of segmental pulse wave velocity in older adults: The Atherosclerosis Risk in Communities (ARIC) Study. Am. J. Hypertens.; 29(1):114-122.

Mocchegiani E., Costarelli L., Giacconi R., Malavolta M., Basso A., Piacenza F., Ostan R., Cevenini E., Gonos E.S., Franceschi C. and Monti D. (2014): Vitamin E-gene interactions in aging and inflammatory age-related diseases: Implications for treatment. Ageing Res Rev; 14:81-101.

Moon S.K, Thompson L.J., Madamanchi N., Ballinger S., Papaconstantinou J., Horaist C., Runge M.S. and Patterson C. (2001): Aging, oxidative responses, and proliferative capacity in cultured mouse aortic smooth muscle cells. Am J Physiol Heart Circ Physiol; 280(6):H2779-2788.

Nanayakkara P.W., Kiefte-de Jong J.C., ter Wee P.M., Stehouwer C.D., van Ittersum F.J., Olthof M.R., Teerlink T., Twisk J.W.R., Guldener C.V. and Smulder Y.M. (2009): Randomized placebo-controlled trial assessing a treatment strategy consisting of pravastatin, vitamin E, and homocysteine lowering on plasma asymmetric dimethylarginine concentration in mild to moderate CKD. Am J Kidney Dis; 53(1):41–50.

Norouzi L., Shirpoor A., Ansari M-H.k. and Ilkhaizadeh B. (2015): vitamin E attenuates alcohol-induced aortic wall damage in rats. Artery Research; 10:20-26.

Orlandi A., Bochaton-Piallat M., Gabbiani G. and Spagnoli L.G., (2006): Aging, smooth muscle cells and vascular pathobiology: implications for atherosclerosis. Atherosclerosis; 188(2):221-230.

Patel N., Amin P. and Shenoy A. (2016): Is vitamin E supplementation effective in reducing mortality related to cardiovascular events in people with type 2 diabetes mellitus. I.J.C. Metabolic& Endocrine; 12:42-45.

Procop G.W. and Pritt B. (2014): Pathology of Infectious Diseases. Chapter 29: Artifacts and Pitfalls in Infectious Disease Pathology; 647-688.

Rodella L.F., Rezzani R., Bonomini F., Peroni M., Cocchi M.A., Hirtler L. and Bonardelli S. (2016): Abdominal aortic aneurysm and histological, clinical, radiological correlation. Acta Histochemica; 118: 256–262.

Ross R. (1993): The pathogenesis of atherosclerosis: a perspective for the1990s. Nature; 362(6423):801-809.

Sata M., Saiura A., Kunisato A., Tojo A., Okada S., Tokuhisa T., Hirai H., Makuuchi M., Hirata Y. and Nagai R. (2002): Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis. Nat. Med.; 8(4):403-409.

Shalini S., Dorstyn L., Dawar S. and Kumar S. (2015): Old, new and emerging functions of caspases. Cell Death Differ.; 22(4):526-539.

Shirpoor A., Salamib S., Khadem-Ansari M.H., Ilkhanizadehc B., Pakdel F.G. and Khademvatani K. (2009): Cardioprotective effect of vitamin E: rescues of diabetes-induced cardiac malfunction, oxidative stress, and apoptosis in rat. Journal of Diabetes and its Complications; 23:310-316.

Smith A.R., Visioli F., Frei B. and Hagen T.M. (2006): Age-related changes in endothelial nitric oxide synthase phosphorylation and nitric oxide dependent vasodilation: evidence for a novel mechanism involving sphingomyelinase and ceramide activated phosphatase 2A. Aging Cell; 5(5):391-400.

Swaminathan G., Gadepalli V.S., Stoilov I., Mecham R.P., Rao R.R. and Ramamurthi A. (2014): Pro-elastogenic effects of bone marrow mesenchymal stem cell-derived smooth muscle cells on cultured aneurysmal smooth muscle cells. J Tissue Eng Regen Med; 11(3):679-693.

Ungvari Z., Kaley G., de Cabo R., Sonntag WE. and Csiszar A. (2010): Mechanisms of Vascular Aging: New Perspectives (Review Article. J Gerontol A Biol Sci Med Sci; 65(10): 1028-1041.

Van der Loo B., Labugger R., Aebischer CP., Skepper J.N., Bachschmid M., Spitzer V., Kilo J., Altwegg L., Ullrich V. and Lüscher T.F. (2002): Cardiovascular Aging Is Associated with Vitamin E Increase. Circulation; 105:1635-1638.

Widlansky M.E., Gokce N., Keaney J.F., Vita J.A. (2003): The clinical implications of endothelial dysfunction. J. Am. Coll. Cardiol.; 42(7):1149-1160.

تأثير الشيحخوخة على تركيب شريان الأورطى الصدري لذكور الفئران البيضاء والدور المحتمل لفيتامين هـ

شهيرة يوسف مخائيل، محمد مصطفى فاضل، ماري رفعت إسحق، حسن محمد حسن

قسم التشريح والأجنة، كلية الطب، جامعة عين شمس، القاهرة، مصر

ملخص البحث

المقدمة :أوضحت هذه الدراسه التغيرات النسيجية الناتجه عن الشيخوخه في الشريان الأورطي الصدري للفئران البيضاء ، وفحصت التأثير المحتمل لفيتامين ه على هذه التغيرات. وقد أستخدم في هذه الدراسة ثلاثون من ذكور الفئران، خمسة عشر فأرا بالغا وخمسة عشر فأرا مسنا. تم تقسيم الحيوانات إلى أربعة مجموعات, عشرة فئران فى كل مجموعة كالتالى:

• المجموعة الاولى: (المجموعة الضابطه للفئران البالغه) تكونت من عشرة من الفئران البالغة تم تقسيمها بالتساوي إلى:

المجموعة الفرعية (1أ): لم تتناول اية أدوية

المجموعة الفرعية (1ب): أعطيت 3,6 مللي من زيت السمسم (المذيب لفيتامين ه) يوميا لمدة 6 أسابيع.

• المجموعة الثانية: (المجموعة الضابطة للفئران المسنة) تكونت من ستة من الفئران المسنة تم تقسيمها بالتساوي الى:

المجموعة الفرعية (2أ) لم تتناول اية ادوية

المجموعه الفرعية (2ب) أعطيت 3.6مللي من زيت السمسم يوميا لمدة 6 اسابيع.

• المجموعة الثالثة: (مجموعة الفئران المسنه المعالجة بفيتامين ه) تكونت من عشره من الفئران المسنة التي اعطيت 300 مجم من فيتامين ه المذاب في زيت السمسم يوميا لمدة 6 اسابيع.

في نهاية التجربة تم التضحية بالحيوانات واستخراج الشريان الاورطى الصدري وصبغه بكل من الصبغات الاتيه : هيماتوكسلين وايوسن، اورسين ، وثلاثي الكروم. كما اجريت دراسة مناعية بإستخدام الاجسام المضادة للاكتين. و التى أُجريت عليها دراسات قياس الشكل المظهرية. أظهرت النتائج ان للشيخوخة تأثيرات عديدة على تركيب شريان الاورطى الصدري للفئران ، و قد تمثلت هذه التغيرات في : وجود مناطق سميكة في بطانة الشريان بالتناوب مع اخرى غير منتظمة وفقدان الترتيب الخطي للخلايا مع تراكم حبيبات ذات لون بني داكن في الطبقة البطانة وماتحتها. اما بالنسبة للطبقة الوعائية الوسطى فقد احتوت على مناطق تنكسية مع وجود مناطق من الإنفصال كما إكتسبت بعض خلايا العضلات الملساء نواة ذات شكل غير معتاد والبعض الاخر احتوى على اكثر من نواة واحدة. وقد شوهدت ايضا مجموعة من الخلايا المرتبة طوليا موجودة مابين الطبقة البطانة والطبقة الوسطى. بينما ظهرت الالياف المرنة متباعدة مع مناطق متعددة من التفكك وأخرى من الالتصاق. كما كانت هناك زيادة ملحوظة في الكولاجين على حساب خلايا العضلات الملساء. و لوحظ ايضا منطقة تجيب خارجي في جدار الوعاء. فيما يخص الطبقة الخارجية للوعاء الدموي فقد تكونت من نسيج ضام متفرق ورقيق ومفصول على نطاق واسع. و قد كشفت العينات المصبوغة بالاجسام المضادة للاكتين عن مناطق متعددة من خلايا العضلات الملساء ضعيفة التفاعل معها.من ناحية اخرى اظهر شريان الاورطى الصدري للفئرن المسنه التي تم علاجها باستخدام فيتامين ه ان طبقة البطانه والخلايا المكونه لها اصبحت منظمه نسبيا مع وجود عدد قليل غير منتظم.كما أظهرت الطبقة الوسطى بعض المناطق التنكسيه لكن دون وجود انفصال، وبعض من خلايا العضلات الملساء مازالت تحتوي على نواة ذات شكل غير معتاد، كما استمر وجود مجموعات الخلايا المرتبة طوليا بين طبقة البطانة والطبقة الوسطى. و أظهرت الالياف المرنة وحزم الكولاجين تحسنا واضحا حيث انها اصبحت تقريبا منتظمه بغض النظر عن بعض الفراغات بين حزم الكولاجين. و قد تكونت الطبقة الخارجية من نسيج ضام متموج كثيف مع بعض مناطق الانفصال. و كشفت العينات المصبوغة بالاجسام المضادة للاكتين أن معظم خلايا العضلات الملساء قوية التفاعل مع وجود قلة ضعيفة التفاعل.

الاستنتاج: أوضح العمل الحالي أن الشيخوخة كان لها تأثير ضار على الهيكل النسيجي للشريان الأورطي الصدري فى الفئران البيضاء. و قد لوحظ أن التغيرات المرتبطة بالعمر تؤثر بشكل كبير على الطبقة البطنية والوسطى وبدرجة أقل على الطبقة الخارجية. من ناحية أخرى، وجد أن الفئران المسنة التي تم علاجها بفيتامين ه أظهرت تحسنا واضحا في أنسجة جدار الأوعية الدموية الخاصة بهم إما بتحسين بعض التغيرات أو تلاشي الأخرى تماما. وبالتالي ينصح باستخدام فيتامين ه للمسنين من اجل تحسين وظيفة الاوعية الدموية.