



# Age Related Histomorphological Changes in Aorta of Pig (*Sus scrofa*)

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## ABSTRACT

The present study was conducted during 2022 for a period of ten months (January to October) in the Department of Veterinary Anatomy, in collaboration with ICAR-AICRP on pigs, C.V.Sc Tirupati, Andhra Pradesh, India on aorta of pigs (*Sus scrofa*). A total of 18 samples of thoracic and abdominal aorta were collected irrespective of sex of the animal. The samples were divided into three groups based on their age i.e., Group I (up to six months), Group II (seven months to one year) and Group III (above one year) and in each group six animals were studied for recording age-related histomorphological changes. In histomorphological study it was observed that the walls of both thoracic and abdominal aorta were made up of three layers viz., tunica intima, tunica media and tunica adventitia. The tunica intima was made up of endothelium, subendothelial tissue and internal elastic membrane. The thickness of tunica intima increased with advancement of age in both thoracic and abdominal aorta due to increase in the number of musculo-elastic layers. The thickness of tunica media increased with advancement of age of the pigs i.e., from group-I to group-II and decreased in group-III pigs in both thoracic and abdominal aorta. The thickness of tunica adventitia of thoracic aorta was less, when compared to tunica adventitia of abdominal aorta. The separate external elastic membrane was not identified in the aorta since it was merged with the adjacent elastic fibres. In the tunica adventitia numerous small blood vessels and few nerve fibres were observed.

**KEYWORDS:** Aorta, tunica intima (TI), tunica media (TM), tunica adventitia (TA)

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**Data Availability Statement:** Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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## 1. INTRODUCTION

Cardiovascular disease (CVD) stands out as a significant concern among the many chronic conditions fueling aging-driven “epidemic,” remaining the primary cause of death in modern societies (LaRocca et al., 2017). Cardiovascular diseases (CVDs) are a group of disorders that impact the heart (heart failure and coronary heart disease) and blood vessels (cerebrovascular disease and peripheral artery disease). About 17.9 million people die from CVDs each year, making them the leading cause of death worldwide (Wang and Magliano, 2025). Several theories have been proposed to explain the age-related changes in various organs and systems, including the gene mutation theory, collagen accumulation, wear and tear, and autoimmune theories (Abu-Dief et al., 2016). The onset of age-related vascular dysfunction raises the risk of cardiovascular disease and other long-term age-related conditions like Alzheimer’s and chronic renal disease (Rossman et al., 2018).

In the cardiovascular system, the main age-related changes are within the heart, heart valves and the vascular system which lead to clinical disorder. One of the key factors influencing cardiovascular risk is ageing, which is linked to gradual changes in the composition and operation of the cardiovascular system, mainly the aorta (Ohyama et al., 2018). The aorta is a major blood vessel that carries blood from the heart to the rest of the body. Its structure relies on a combination of elastic fibers, collagen, smooth muscle cells, and ground substance. As age increases, changes in these components affect the size and function of the aorta, leading to its stiffening. This stiffening contributes to an increase in blood pressure, a common characteristic of aging (Sheppard, 2019). The elastic properties of the aorta are changed in several conditions, such as pregnancy, advanced age, physical conditioning, atherosclerosis, arterial hypertension, diabetes mellitus, heritable disorders of connective tissue, congestive heart failure, smoking etc. (Divya, 2015).

With advancing age, the human aorta shows progressive changes in the structure and the function which include the luminal diameter of aorta, whole length of the aorta, thickness, the microstructural components which include collagen, elastin and smooth muscle cells. The thickness of the tunica intima and tunica media in the aortic wall increases with the advancement of age. This aortic wall thickening reduces the elasticity of the vessel. There is decrease in the number of elastic fibres and smooth muscle cells in the tunica media and an increase in the amount of collagen fibres as the age increases (Komutrattananont et al., 2019). Elastin production in mammals is minimal after the neonatal period, elastic fibers are constantly subjected to aging-related factors throughout life. In contrast, collagen in the media layer is produced by fibroblasts, and its content

increases as an individual age (Gequelim et al., 2019). Ageing causes the aorta to dilate and thin, which raises intramural wall tension. This causes load carrying from weakening elastin to shift onto firmer collagen fibres that leads to aortic stiffening (Pierce et al., 2022).

The histological alterations observed in the aorta of aging pigs provide insights into the progressive stiffening of the vessel, the development of atherosclerotic plaques, and the loss of structural integrity. These changes, including thickening of the intima, fragmentation of elastin fibers in the media, and increased fibrosis in the adventitia, are associated with a decrease in aortic compliance and impaired endothelial function. Understanding the specific histomorphological changes that occur in the aging aorta of pigs can help to elucidate the underlying mechanisms of age-related cardiovascular diseases, such as hypertension, aneurysms, and aortic dissections. But the studies on age related changes in thoracic and abdominal aorta of pig are scarce. In view of its importance in organ transplantation especially heart in human, the detailed study on aorta is very much essential. Therefore, the present work had been envisaged to know the age-related histomorphological changes aorta of pigs.

## 2. MATERIALS AND METHODS

The present study was conducted during 2022 for a period of ten months (January to October) in the Department of Veterinary Anatomy, in collaboration with ICAR-AICRP on pigs, C.V.Sc Tirupati, Andhra Pradesh, India. The collected samples were divided into three groups based on their age i.e., Group I (up to six months), Group II (seven months to one year) and Group III (above one year) and in each group six animals were studied. The fresh tissue samples were fixed in 10% Neutral Buffered Formalin. Later these samples were processed for paraffin sections (Singh and Sulochana, 1997). About 5 µm thick paraffin sections were obtained from each tissue sample and it was subjected to routine and special histological staining methods i.e., Haemotoxylin and Eosin for routine histomorphology, Verhoeff’s method for elastic fibres, Masson’s trichome for collagen fibres, Wilder’s method for reticular fibres and Bielschowsky method for nerve fibres (Singh and Sulochana, 1997) (Table 1).

## 3. RESULTS AND DISCUSSION

In the present study, the aorta of pig was a large elastic type of artery and the wall of both thoracic (Figure 1, 2 and 3) and abdominal aorta (Figure 4) was made up of three layers i.e., tunica intima, tunica media and tunica adventitia. The thickness of thoracic aorta was higher than the abdominal aorta. The thickness of aorta reduced gradually from thoracic

Table 1: Histomorphological stains used for paraffin sections of thoracic and abdominal aorta

Sl. No.	Method	Purpose	Source
1.	Haematoxylin and Eosin method	Routine histological observations	Singh and Sulochana, 1997
2.	Masson's Trichrome method	Collagen fibers	Singh and Sulochana, 1997
3.	Wilder's method	Reticular fibers	Singh and Sulochana, 1997
4.	Verhoeff's method	Elastic fibers	Singh and Sulochana, 1997
5.	Bielschowsky method	Nerve fibers	Singh and Sulochana, 1997

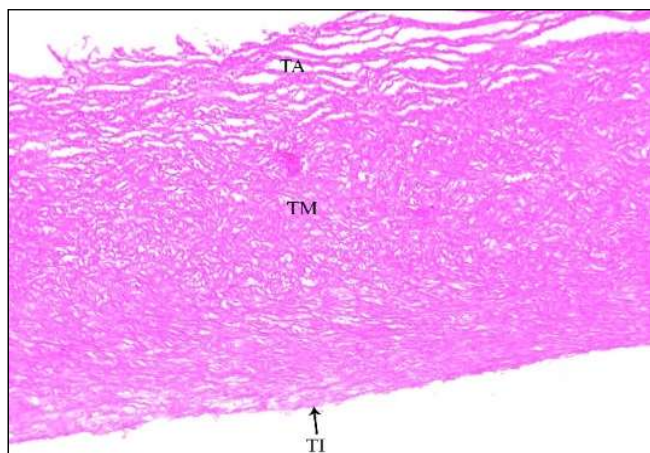


Figure 1: Photomicrograph of thoracic aorta of pig of group-I showing tunica intima (TI), tunica media (TM) and tunica adventitia (TA). Haematoxylin and Eosin ×100

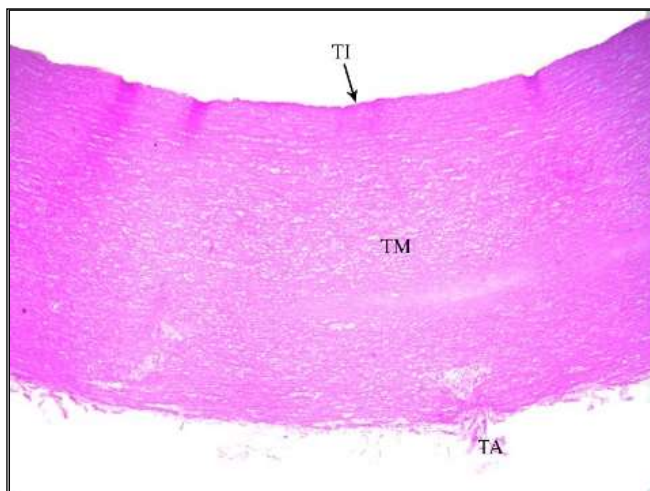


Figure 2: Photomicrograph of thoracic aorta of pig of group-II showing tunica intima (TI), tunica media (TM) and tunica adventitia (TA). Haematoxylin and Eosin ×40

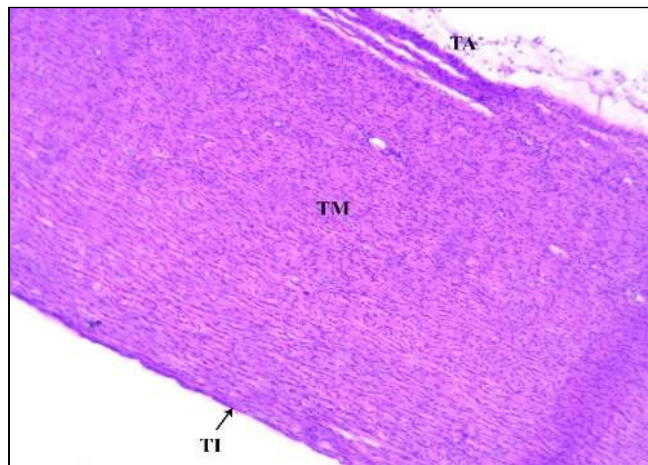


Figure 3: Photomicrograph of thoracic aorta of pig of group-III showing tunica intima (TI), tunica media (TM) and tunica adventitia (TA). Haematoxylin and Eosin ×100

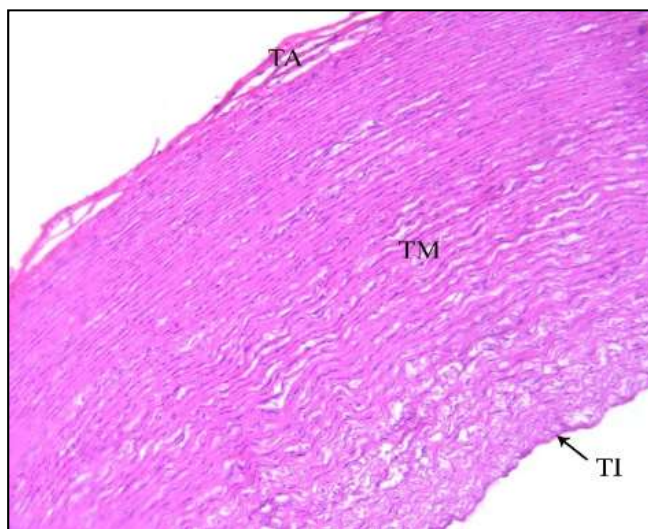


Figure 4: Photomicrograph of abdominal aorta of pig of group-I showing tunica intima (TI), tunica media (TM) and tunica adventitia (TA). Haematoxylin and Eosin ×100

to abdominal region or towards its termination.

The tunica intima of aorta consisted of endothelial layer and subendothelial tissue. The endothelium consisted of single layer of flattened squamous cells. The subendothelial connective tissue consisted of smooth muscle cells which were longitudinally arranged with few elastic and collagen fibres similar observations were also made by Greep (1954) in humans and Dellmann and Carithers (1997) in aorta of large animals.

The internal elastic membrane of aorta was indistinct as it merged with the surrounding elastic lamellae of the tunica media (Figure 5 and 6). Similar findings were also made by Greep (1954) in humans and Eurell and Frappier (2006) in large animals. Further, Ham (1969) in humans observed



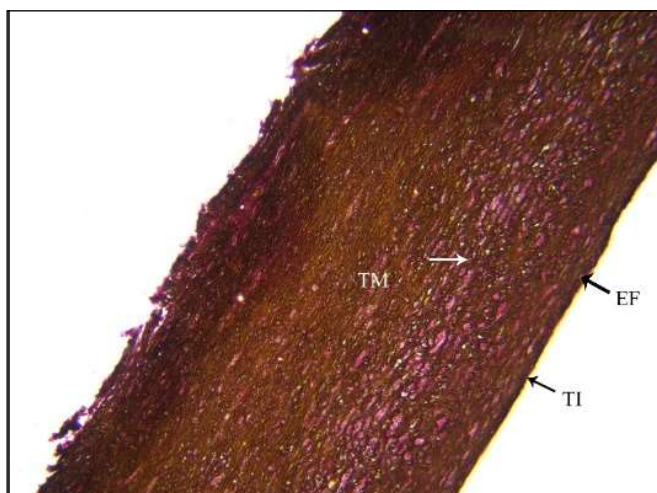


Figure 5: Photomicrograph of thoracic aorta of pig of group-II showing elastic fibres (EF) in tunica intima (TI) and tunica media (TM). Verhoeff's stain  $\times 100$

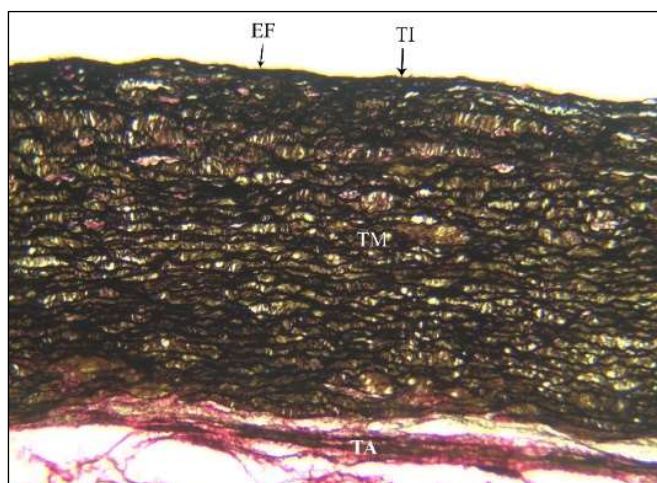


Figure 6: Photomicrograph of thoracic aorta of pig of group-III showing elastic fibres (EF) in tunica intima (TI) and tunica media (TM). Verhoeff's stain  $\times 40$

that the elastic fibres in the tunica media were similar to the internal elastic lamina of tunica intima, whereas Copenhagen and Johnson (1958) observed only one internal elastic lamina in four months human fetus in late gestation and splitting of internal elastic membrane into two or more layers after birth. Similar to the present finding, Cormack (1987) reported that the internal elastic lamina in humans was difficult to observe because of large number of elastic fibers in tunica intima and adjacent layer.

The thickness of the tunica intima and the structure of the aortic wall gradually changed from the Group-I to Group-III. The maximum thickness of tunica intima of thoracic aorta was observed in group-III  $25.01 \pm 0.72 \mu\text{m}$ , followed by group-II i.e.,  $23.26 \pm 0.39 \mu\text{m}$  and in group-I it was  $22.36 \pm 0.31 \mu\text{m}$  (Table 2).

The maximum thickness of tunica intima of abdominal

Table 2: Showing average thickness of different layers in the wall of thoracic aorta in group-I, group-II and group-III

Sl. No.	Group-I	Group-II	Group-III
Tunica intima	$22.36 \pm 0.31 \mu\text{m}$	$23.26 \pm 0.39 \mu\text{m}$	$25.01 \pm 0.72 \mu\text{m}$
Tunica media	$749.35 \pm 62.73 \mu\text{m}$	$1448.26 \pm 42.06 \mu\text{m}$	$1268.55 \pm 96.65 \mu\text{m}$
Tunica adventitia	$120.22 \pm 5.35 \mu\text{m}$	$59.07 \pm 4.94 \mu\text{m}$	$99.44 \pm 10.21 \mu\text{m}$
Total wall thickness	$975.70 \pm 66.75 \mu\text{m}$	$1527.43 \pm 40.81 \mu\text{m}$	$1393.29 \pm 97.18 \mu\text{m}$

Table 3: Showing average thickness of different layers in the wall of abdominal aorta in group-I, group-II and group-III

Sl. No.	Group-I	Group-II	Group-III
Tunica intima	$25.28 \pm 0.28 \mu\text{m}$	$26.33 \pm 1.11 \mu\text{m}$	$27.41 \pm 2.20 \mu\text{m}$
Tunica media	$595.05 \pm 19.91 \mu\text{m}$	$902.71 \pm 39.52 \mu\text{m}$	$892.05 \pm 65.79 \mu\text{m}$
Tunica adventitia	$128.29 \pm 8.72 \mu\text{m}$	$83.93 \pm 3.90 \mu\text{m}$	$108.02 \pm 4.95 \mu\text{m}$
Total wall thickness	$747.27 \pm 22.11 \mu\text{m}$	$1228.68 \pm 33.61 \mu\text{m}$	$1017.21 \pm 60.32 \mu\text{m}$

aorta was observed in group-III  $27.41 \pm 2.20 \mu\text{m}$ , followed by group-II i.e.,  $26.33 \pm 1.11 \mu\text{m}$  and in group-I it was  $25.28 \pm 0.28 \mu\text{m}$  (Table 3).

The thickness of tunica intima increased with advancement of age in both thoracic and abdominal aorta of the animal. The thickness of tunica intima of abdominal aorta was more compared to the thickness of tunica intima of thoracic aorta in all the three age groups of pigs. Similar observations were made by Cormack (1987) and O'Rourke (2007) in human aorta. The above observations suggested that due to increase in thickness of intima the lumen of the aorta gradually reduced from thoracic to abdominal regions.

The number of elastic fibres were more in tunica intima in group-I and group-II and they gradually become replaced with collagen fibres in group-III as observed by Copenhagen and Johnson (1958) in humans. These findings suggested that the elasticity of aorta gradually decreased with the advancement of age.

In the aorta the tunica media formed the bulk of wall like in coronary arteries as reported by Eurell and Frappier (2006) in large animals and Divya (2015) in pigs. The tunica media predominantly consisted of concentric fenestrated laminae of elastic fibres along with smooth muscle cells and collagen fibres. Similar findings were also reported by Greep (1954) in humans, Dellmann and Carithers (1997) in large animals,

Bruel and Oxlund (2002) in rat, Jeppesen and Skydsgaard (2005) in porcine, Ogeng'o et al. (2010) in goat and Rao et al. (2016) in aorta of man and ruminants.

The maximum thickness of tunica media of thoracic aorta and abdominal was observed in group-II i.e.,  $1448.26 \pm 42.06 \mu\text{m}$ ,  $902.71 \pm 39.52 \mu\text{m}$ , followed by group-III i.e.,  $1268.55 \pm 96.65 \mu\text{m}$ ,  $892.05 \pm 65.79 \mu\text{m}$  and group-I i.e.,  $749.35 \pm 62.73 \mu\text{m}$ ,  $595.05 \pm 19.91 \mu\text{m}$  (Table 2 and 3) respectively.

The thickness of tunica media increased with advancement of age of the pigs i.e., from group-I to group-II and decreased in group-III pigs in both thoracic and abdominal aorta. This might be due to gradual increase of collagen fibres in tunica media of aorta. Similar observations were made by Cormack (1987) in thoracic and abdominal aorta of humans. Further, Ham (1969) observed increased thickness of tunica media in adulthood than in childhood. In contrary, O'Rourke (2007) in human aorta observed that the media did not appreciably thicken with age, individual elastin lamellae thin and became separated by increasing amounts of non-load-bearing material. In pigs, the tunica media of thoracic aorta was thicker than abdominal aorta in all the three age groups. It indicates thickness of aorta gradually decreased from thoracic region to the abdominal region.

The elastic fibres of the tunica media were arranged in concentric layers. The number of elastic laminae in the wall of both thoracic (Figure 5 and 6) and abdominal aorta (Figure 7) increased with advancement of the age from pigs of group-I and group-II. Further, in the present study thinning, splitting, fraying and fragmentation of elastic lamellae was observed with advancement of age. Yang and Kohnken (2021) reported similar observations in aorta of humans and dogs. But in group-III their number reduced gradually and replaced by collagen fibres as reported by Dao et al. (2005) aorta of rat. This indicated that the elasticity of the aorta is gradually decreased with the advancement of age the animal.

In the present study the spaces between the elastic laminae increased (Figure 8) and they appeared less wavy and also the number of smooth muscle cells decreased. Yang and Kohnken (2021) reported similar observations in aorta of humans and dogs. Further, many collagen fibres were noted between the smooth muscle cells and the elastic fibres. The above findings suggestive of gradual increase in thickness of aorta. These findings are similar to the observations of Fritze et al. (2012) in human aorta.

In aorta few blood vessels were observed in the outer layer of tunica media but in the inner layer i.e., towards intima blood vessels are sparse. Olabu et al. (2011) also reported blood vessels in the tunica media of goat aorta especially in outer layer close to the adventitia.

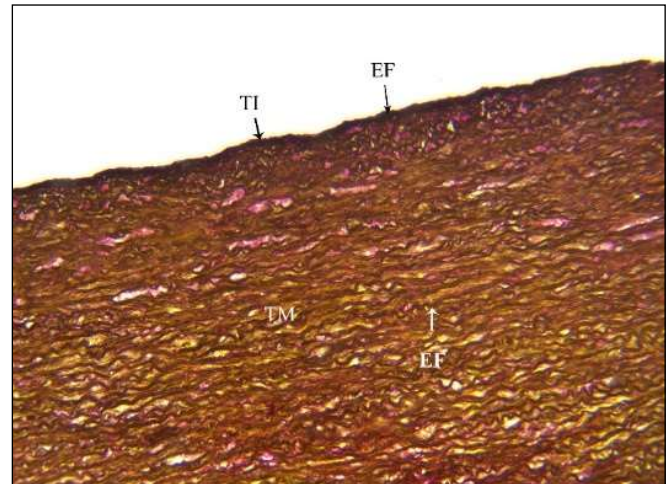


Figure 7: Photomicrograph of abdominal aorta of pig of group-I showing elastic fibres (EF) in tunica intima (TI) and tunica media (TM). Verhoeff's stain  $\times 100$

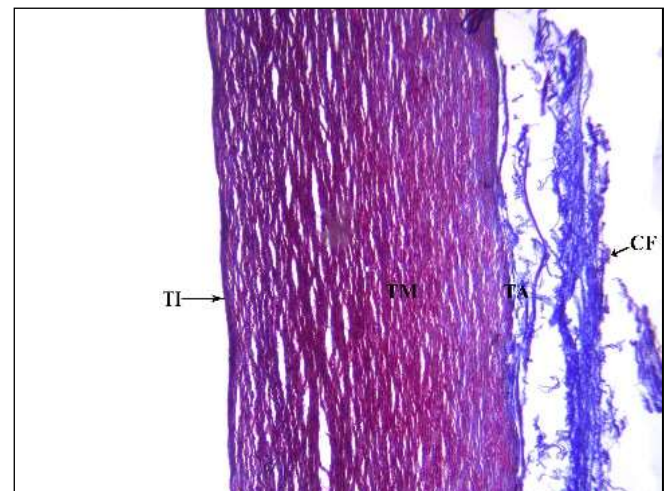


Figure 8: Photomicrograph of thoracic aorta of pig of group-II showing loosely arranged smooth muscle fibres in tunica media (TM) and collagen fibres (CF) in tunica adventitia (TA). Masson's Trichrome  $\times 40$

In aorta the tunica adventitia was a thin coat of loose connective tissue and it was predominantly consisted of longitudinally arranged collagenous fibres (Figure 9 and 10) with few elastic fibres (Figure 11) and smooth muscle cells. Similar observations were made by Copenhaver and Johnson (1958) in humans, Dellmann and Carithers (1997) in aorta of large animals, Eurell and Frappier (2006) in large animals, Rao et al. (2016) in aorta of man and ruminants, Copenhaver et al. (1971) reported that the fibrillar collagen was the major constituents of tunica intima, media and adventitia in human aorta. Cormack (1987) in humans, Gibbons and Shadwick (1989) in aorta of lower vertebrates and mammals noted similar findings.

The maximum thickness of tunica adventitia of thoracic



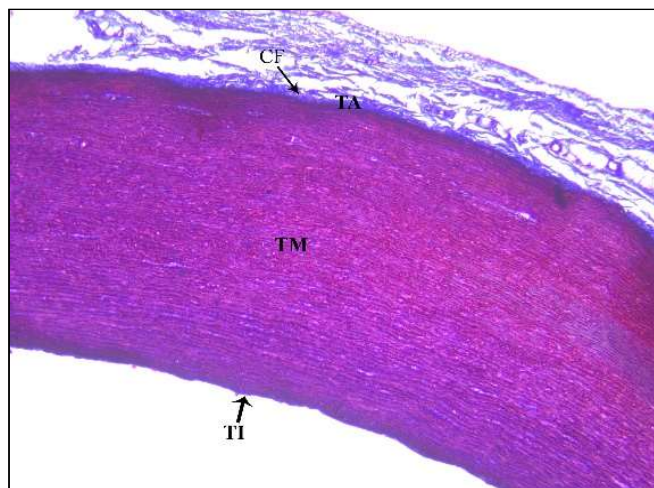


Figure 9: Photomicrograph of thoracic aorta of pig of group-II showing collagen fibres (CF) in tunica adventitia (TA). Masson's Trichrome ×40

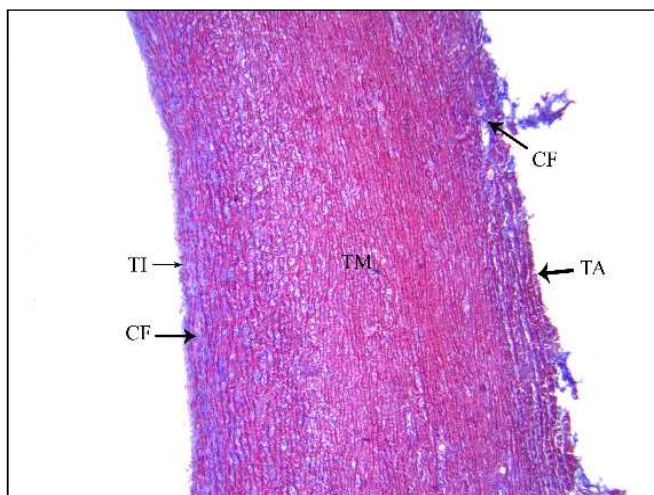


Figure 10: Photomicrograph of thoracic aorta of pig of group-III showing collagen fibres (CF) in tunica intima (TI) and tunica adventitia (TA). Masson's Trichrome ×100

and abdominal aorta was observed in Group- III i.e.,  $99.44 \pm 10.21 \mu\text{m}$ ,  $108.02 \pm 4.95 \mu\text{m}$  followed by Group-II i.e.,  $59.07 \pm 4.94 \mu\text{m}$ ,  $83.93 \pm 3.90 \mu\text{m}$  and Group- I  $120.22 \pm 5.35 \mu\text{m}$  and  $128.29 \pm 8.72 \mu\text{m}$  (Table 2 and 3) respectively. The thickness of tunica adventitia of thoracic aorta was less, when compared to the thickness of tunica adventitia of abdominal aorta. This greater thickness of tunica adventitia in adult animals is mainly due to increased number of collagen fibres.

In the present study, the external elastic membrane was merged with the adjacent elastic fibres present at the junction of tunica media and tunica adventitia and it was not clearly demarcated. The elastic fibres and collagen fibres and smooth muscle fibres were intermingled. Similar observations were made by Cormack (1987) in humans and

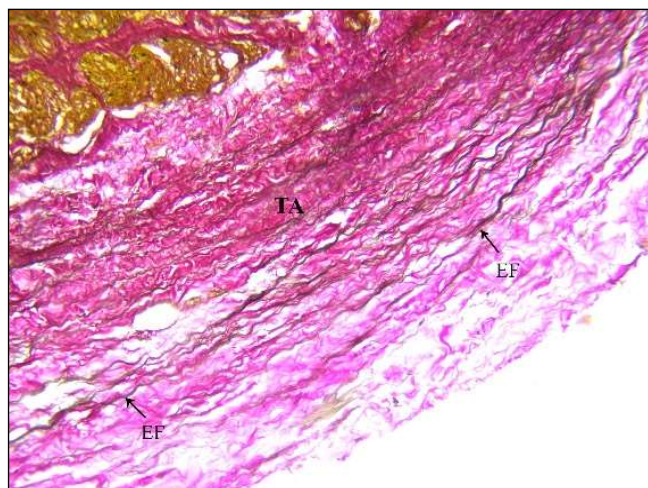


Figure 11: Photomicrograph of abdominal aorta of pig of group-II showing elastic fibres (EF) in tunica adventitia (TA). Verhoeff's stain ×100

Eurell and Frappier (2006) in large animals.

A number of small blood vessels were observed in that tunica adventitia (Figure 12). This indicated that the outer layer of aorta of both thoracic and abdominal regions was highly vascular. Ham (1969) observed similar findings in

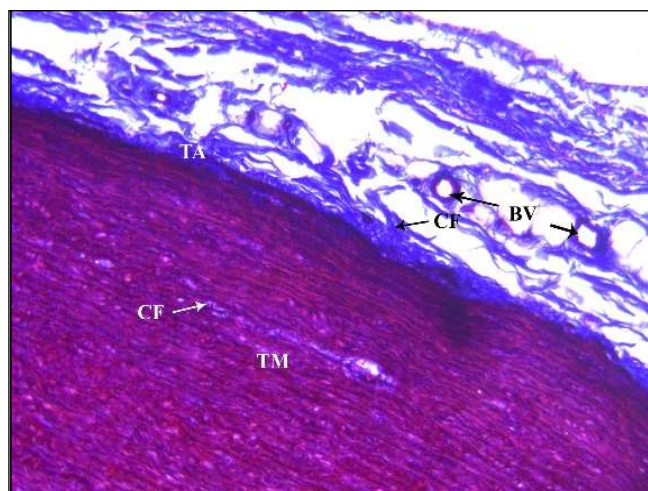


Figure 12: Photomicrograph of abdominal aorta of pig of group-I showing collagen fibres (CF) in tunica media (TM) and tunica adventitia (TA) and blood vessel. Masson's Trichrome ×100

aorta of humans, Jaeger (1964) in bovine and Divya (2015) in aorta of pigs.

In all the three age groups, the elastic fibres were more in thoracic aorta and less in abdominal aorta, but in the abdominal aorta collagen fibers content is more than that of thoracic aorta. It must be because the thoracic aorta must pump blood to the caudal parts of the body and it requires more elasticity and tensile strength. Similar observations

were made by O'Rourke (2007) in human aorta, Tonar et al. (2015) in porcine aorta reported greatest elastin fraction in the descending thoracic aorta and it decreased proximally towards the aortic arch and distally towards the abdominal aorta. He also reported reduced collagen fraction from ascending aorta and aortic arch towards descending aorta. With advancement of age the number of elastic fibres reduced and gradually became replaced with collagen fibres. Therefore, with advancement of age in the animal the diameter of the aorta is increased with reduced elasticity and it becomes stiffer with decreased vascular compliance as opined by Sokolis (2007) in aorta of pigs and Morrison et al. (2009) in human aorta.

The maximum thickness of wall of thoracic aorta was observed in Group-II i.e.,  $1527.44 \pm 40.81 \mu\text{m}$  followed by Group-III i.e.,  $1393.30 \pm 97.18 \mu\text{m}$  and Group-I i.e.,  $975.70 \pm 66.75 \mu\text{m}$ . Similarly, the maximum thickness of wall of the abdominal aorta was observed in Group-II i.e.,  $1228.69 \pm 33.61 \mu\text{m}$  followed by Group-III i.e.,  $1017.21 \pm 60.32 \mu\text{m}$  and Group-I i.e.,  $747.27 \pm 22.11 \mu\text{m}$  (Figure 13).

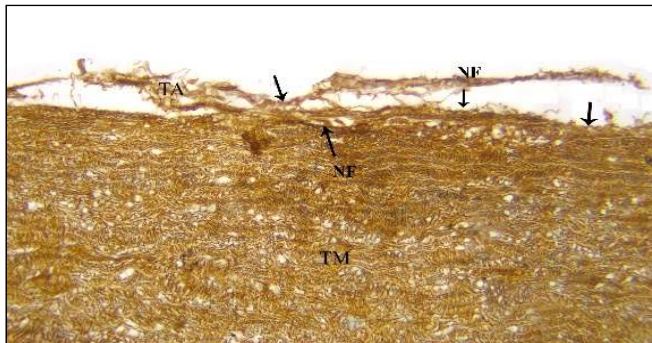


Figure 13: Photomicrograph of thoracic aorta of pig of group-III showing nerve fibres (arrows) in tunica adventitia (TA). Bielschowsky method  $\times 100$

#### 4. CONCLUSION

The walls of both thoracic and abdominal aortae comprised three layers: tunica intima, tunica media, and tunica adventitia. The tunica intima, consisting of endothelium, subendothelial tissue, and internal elastic membrane, thickened with age due to increased musculo-elastic layers, with greater thickness in the abdominal aorta. The tunica media, the thickest layer, contained smooth muscle cells, elastic, and collagen fibers, thickening with age due to more elastic laminae, later replaced by collagen. The tunica adventitia, a thin connective layer, showed more collagen fibers in the abdominal aorta, lacking a distinct external elastic membrane.

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