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Original Article

Functional Adaptability of the Tunica Media of the Atriopulmonary Junction

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Abstract

Introduction: The histoarchitecture of the tunica media of the Atriopulmonary Junction (APJ) has not been extensively studied and yet the tunica media is the backbone, which provides the structural strength for optimum function. This study therefore strives to unravel the structural components of the tunica media of the APJ.

Methods: The study design was a descriptive cross-sectional study. Histological studies were done on tissues from the APJ of 20 hearts using light microscopy. The proportion of elastic fibers in the tunica media of the APJ was determined using the point intercept method by dropping a grid in ImageJ software.

Results: The smooth muscle cells were concentric and more prominent in males extending the entire extent of the tunica media compared to females where they were confined to the intimal side of tunica media. The collagen bundles were mainly longitudinal. The elastic fibers showed a diverse pattern of organization and side and sex differences in orientation and quantity.

Conclusion: Gender and regional variations in the histology of the tunica media of the pulmonary veins suggest a mechanism for controlling hemodynamic forces at the APJ.

Keywords: Tunica media; Atriopulmonary junction

Introduction

The Atriopulmonary Junction (APJ) is an interface between the Left Atrium (LA) and the Pulmonary Veins (PVs) [1]. It is subjected to various forces including hemodynamic stress of the blood as it flows from the lungs to the left atrium; translational forces of the lung through the pulmonary veins; and retrograde pressure forces during systolic contractions of the left atrium [2]. The tunica media of a blood vessel consists of smooth muscle, elastic fiber and collagen bundles [1], but there is paucity of information regarding the histoarchitecture of the APJ. This study was therefore designed to investigate the functional adaptability of the tunica media of the APJ in the face of the volume of forces that it withstands.

Materials and Methods

Ethical approval was sought from the Kenyatta National Hospital-University of Nairobi Ethics and Research Committee before commencement of the study. Permission and requisite authority was obtained from the administration of Chiromo Funeral Parlor for acquiring histological tissues from autopsy specimens. Twenty hearts were used for histomorphology with 10 being from male and 10 from female subjects for a fairer representation. Since a large proportion of the victims of cardiovascular disease are from the adult age group [3], hearts from subjects ranging from 20-40 years were used as study specimens. All specimens were from individuals of black Kenyan descent.

Heart specimens from both sexes and of adult age group (>18 years) were included in this study. With the help of the pathologist,

heart specimens with any observable pathology or congenital defects were excluded. Autopsy specimens that had stayed for more than 48 hours *post mortem* were also excluded from histological study.

Data collection procedure

At autopsy, with the help of the pathologist, a Y-incision was made from the acromio-clavicular joints to the xiphoid process. The thoracic cage was opened by incision of the costal cartilages. The sternum was then removed carefully and pericardium was incised longitudinally to expose the heart. The hearts were categorized according to sex and longitudinal and transverse sections of APJ were cut. This allowed regional histoarchitectural changes from the LA, APJ, and proximal part of PV to be studied. The tissues were fixed for a period of 72 hours in 10% formal saline after which they were dehydrated in ethyl alcohol of increasing concentrations, starting with 70% alcohol to absolute alcohol (1hour in each solution). Toluene was used as a clearing agent for 1.5 hours followed by wax impregnation for 12hours at 58°C. After tissues were embedded in freshly molted wax, 7 micron thick sections were prepared using a sledge microtome (Leica Model SM2400, Leica Microsystems, Nussloch GmbH, Germany). These were then floated in warm water at 37°C to enhance spreading upon which they will be fixed on slides using egg albumin. The slides were left to dry in an oven at 38°C for 12 hours. Dewaxing was done using xylene and the segments rehydrated using xylol (prepared from xylene and isopropyl column), absolute alcohol and descending concentrations of alcohol to 70% then running water. The sections were stained with Masson's Trichrome (MT) stain to demonstrate connective tissue and myocardial sleeve
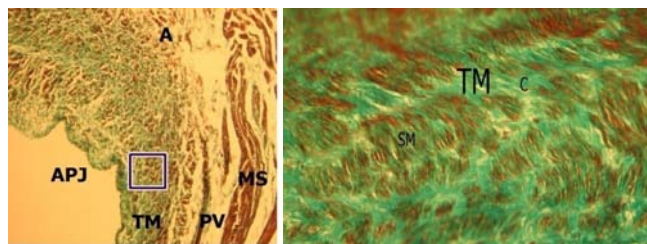
Figure 1A,1B (Inset): Photomicrograph of the longitudinal section of right superior atriopulmonary junction in a 33 year old male. Note the densely packed Collagen Bundles (C) with abundant Smooth Muscle cells (SM) extending deep into the Tunica Media (TM) of the Atriopulmonary Junction (APJ).
PV: Pulmonary Vein; MS: Myocardial Sleeves; C: Collagen bundles; A: Atrium (MT X40, inset: x400).
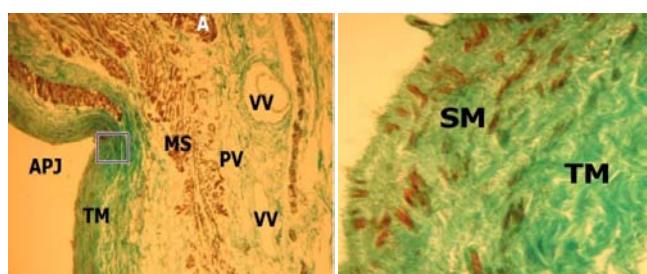

Figure 2A, 2B (Inset): Photomicrograph of a longitudinal section of the right superior atriopulmonary junction in a 27 year old female. Note the Smooth Muscle Cells (SMs) are oriented in varying directions and are confined to the intimal zone of the Tunica Media (TM) in the Atriopulmonary Junction (APJ).
A: Atrium; PV: Pulmonary Vein; VV: Vasa Vasora; NV: Nervi Vasora; MS: Myocardial Sleeve; C: Collagen bundles (MT X40, inset: x400).

organization and Weigert's resorcin fuschsin with van Gieson counterstaining (WT) to demonstrate elastic fibers. Mounting of the cover slips onto specimens was done using D.P.X mountant (Unilab Kenya).The slides were examined with a light microscope (Leica model BME, Leica Microsystems, Nussloch GmbH, Germany) at x40, x100, x400 magnifications and the histological features were recorded on data sheets.The slides were photographed using a Zeiss™ digital photomicroscope (Carl Zeiss AG, Oborkochen, Germany) at various magnifications and were then downloaded for further computer analysis. The photographs were edited using Photoscape Image Editor ™ version 3.5. The proportion of elastic fibers in the tunica media of the APJ was determined using the point intercept method by dropping a grid in ImageJ software:

$$100 \times \frac{\text{Total pixels occupied by elastic fibers}}{\text{Total pixels occupied by tumica media}}$$

Results

Salient features of the tunica media of the APJ were flattened fusiform shaped smooth muscles amidst dense regular connective tissue consisting of collagen bundles and elastic fibers. The Smooth Muscle (SM) cells were concentric and more prominent in males (Figure 1) compared to females (Figure 2). Whereas the SM cells occupied almost the entire extent of the tunica media in the males (Figure 1A, 1B), in females they were confined to the intimal side of the tunica media (Figure 2A, 2B). The collagen bundles were mainly longitudinal but changes in orientation were observed towards the

Table 1: Percentage of elastic fibers in tunica media of the atriopulmonary junction.

	Anterior wall of junction (%)		Posterior wall of atriopulmonary junction (%)	
	Male	**Female**	**Male**	**Female**
LS	66.5	32.2	75.5	51.6
LI	55.7	41.7	66.7	30.5
RS	55.1	27.0	72.1	48.6
RI	48.6	27.3	76.2	33.3

LS: Left Superior Atriopulmonary Junction; LI: Left Inferior Atriopulmonary Junction; RS: Right Superior Atriopulmonary Junction; RI: Right Inferior Atriopulmonary Junction.

adventitial side of the tunica media as they became less densely packed (Figure 1, 2).

The elastic fibers showed a diverse pattern of organization with both circular and longitudinal orientations observed. The elastic fibers displayed side and sex differences in orientation and quantity. The proportion of elastic fibers was greater in males than in females for both the anterior and posterior walls of the APJ (Table 1). Other differences were noted in orientation of the elastic fibers whereby males mainly had circular elastic lamellae in the posterior wall (Figure 3) and longitudinal elastic fibers (Figure 5) in the anterior wall. Females had a predominantly longitudinal disposition of the elastic fibers in both the posterior walls (Figure 4) and anterior walls (Figure 6). The elastic fibers in the tunica media also increased in thickness and became more filamentous towards the adventitia (Figure 3).

Discussion

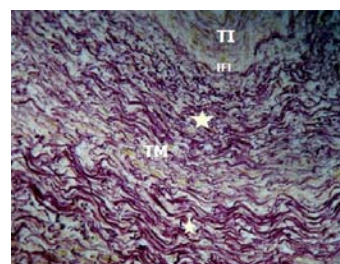In tandem with previous reports [2], the tunica media was

Figure 3: Photomicrograph of a transverse section of the posterior wall of the left superior atriopulmonay junction of a 27-year-old male. Note the presence of concentric elastic fibers (★) in the Tunica Media (TM), which increase in fiber thickness and become longer filaments towards the adventitia.
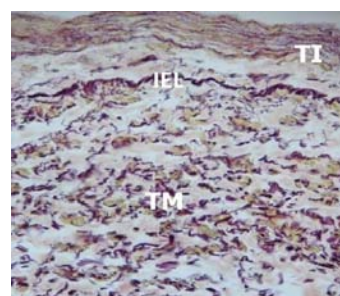TI: Tunica Intima; IEL: Internal Elastic Lamina (WE X400).

Figure 4: Photomicrograph of transverse section of the posterior wall of the left superior atriopulmonary junction in a 25 year old female showing preponderantly longitudinal elastic fibers (in the Tunica Media (TM).
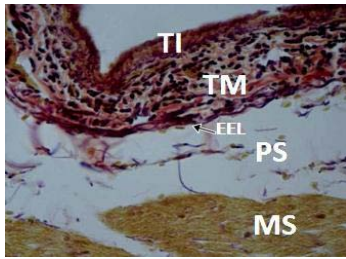TI: Tunica Intima; IEL: Internal Elastic Lamina (WE X400).

Figure 5: Photomicrograph of a transverse section of the anterior wall of the left superior atriopulmonary junction in a 27 year old male showing relatively fewer elastic fibers in the Tunica Media (TM) with predominantly longitudinal dispositions.
TI: Tunica Intima; EEL: External Elastic Lamina; PS: Perimuscular Sheath; MS: Myocardial Sleeves (WE X400).

Figure 6: Photomicrograph of transverse section of the anterior wall of the Left superior atriopulmonary junction in a 25 year old female with elastic fibers in the Tunica Media (TM) oriented in varying directions but a predominance of longitudinal arrangement.
TM: Tunica Media; MS: Myocardial Sleeve (WE X400).

observed to have concentric smooth muscle fibers interspersed in dense connective tissue consisting of collagen bundles and elastic fibers. An additional finding in the present study was the existence of gender and side differences in the fiber orientation and density.

A circular pattern of orientation of smooth muscle cells was observed, and noted to be similar to that in the aorta. This supports the theory of pulsatile flow of blood in the PV, previously considered as passive conduits [4]. Contraction of these cells provides the propulsive force for the maintenance of a constant pulsatile stream of blood from the lungs to the atria. This is comparable to the pulsatile nature of blood flow in the aorta that has been attributed to the presence of smooth muscle cells [5]. Furthermore, the involvement of these smooth muscle cells in instigating myogenic response at the junction is important for the local regulations of blood flow and generation of basal vascular tone cannot be overlooked [6].

There were sex variations noted in the content of the smooth muscle cells in the tunica media. The more densely packed smooth muscles in the tunica media in males than in females may be a result of differential exposure to testosterone in utero [7]. Testosterone has been linked to inducing vascular smooth muscle migration and its relative abundance in utero for males, which is also maintained postnatally, may explain the densely packed nature of smooth muscle cells in the male specimens compared to female [7].

The abundant presence of elastic fibers in the tunica media accords unique physiological properties to particular anatomical sites where they are found. Elastic fibers possess rubber like properties; they can stretch easily and rapidly, and can return to their prestressed state with minimal loss of energy, thereby providing elasticity and stretchability to tissue [8]. The elastic fibers of the APJ are comparable to those found in elastic arteries. It is, therefore, not surprising that the pulmonary venous system should contribute upto 60% of the compliance of the pulmonary vascular bed [9]. Previous studies done on the aorta have attributed the elastic lamellae to be involved in the windkessel function whereby during systole, a fraction of the cardiac output is accommodated to be released in diastole when the elastic fibers relax [10]. Although reports of such a phenomenon are largely unreported for other vessels, the APJ has been linked to capacitance functions which seem comparable to the windkessel mechanism of the aorta. Past observations seem to support this view, whereby an increase in the left atrial pressures were seen to cause a decrease in the resistance of the pulmonary venous system primarily due to
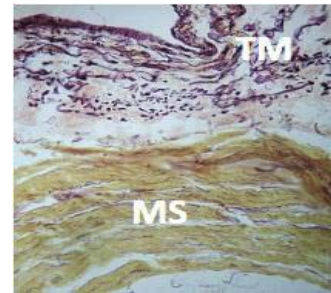
distension forces contributed by elastic fibers [11]. The reverse is noted when the left atrial pressure decrease, and the elastic recoil facilitates the blood flow to the LA.

Another possible function of the elastic fibers may be to act as shock pads at the APJ. Lung capillary outflow is largely protected from the pressure events that occur in the left atrium [12]. This may be as a result of the elastic fibers absorbing the retrograde pressure pulsations originating during systolic contractions of the left atrium. Presence of circular elastic fibers at junctions is commonly reported; where they instill resilience and compliance as is the requirement for structures subjected to incessant distension and contraction [13]. These circular elastic fibers were more common on the posterior wall compared to the anterior wall, further being greater in the male specimens. This infers that the posterior wall is the main component in maintaining the capacitance function and this function is more enhanced in males. The higher elastic content of the junction in males may be a functional adaptation in response to the greater hemodynamic stresses owing to the higher blood pressure in males [14]. The elastic fibers in females and in particular the anterior wall of males showed a predominantly longitudinal disposition, which may allow for a change in the length of the PVs corresponding to the lung movements during respiration. This may be the reason why the PVs are not torn off from the heart at the APJ, when subjected to pull forces during inspiration. The predominance of the longitudinal arrangement in females may be a function of the differential breathing patterns adopted by the different sexes. It is known that males exhibit predominantly abdominothoracic breathing pattern, whereas females are seen to mainly have a diaphragmatic or thoracoabdominal pattern [15]. The more rapid action of the rib cage muscles in thoracoabdominal breathing [16] facilitates greater lung movements in the vertical plane in females, predisposing them to more shearing forces at the APJ. The predominantly longitudinal elastic fiber arrangement in females may therefore be an adopted characteristic to allow for a change in length corresponding to their lung movements during respiration.

Collagen seems to be the main load bearing component of the junction responsible for structural integrity and tensile strength [17]. The compact and regular arrangement of collagen fibers in the tunica media serves to increase the ability of the junction to better withstand hemodynamic stresses. The present study demonstrated a concurrent presence of elastic and collagen fibers in the tunica media of the APJ suggesting integrative structural and functional interactions.

Previous studies have reported on the synergistic functions of elastic and collagen fibers whereby low transmural pressure is mainly borne by elastic fibers, engaging collagen fibers only as transmural pressures rise [18]. This may augment functional adaptability of the junction to hemodynamic stresses by preventing one fiber system from being overworked by bearing all the strain forces. It is thus plausible that the biomechanical properties of the APJ will be discordant with Hooke's Law since the more the junction is subjected to stretch forces the more it will tend to resist. This is supported by Apter (1967), who attributed the resultant non-linear compliance of blood vessels, to the relative indistensibility of the collagen fibers.

Conclusion

In conclusion, the tunica media of the atriopulmonary junction is well endowed structurally to withstand the myriad of forces attacking the interface. Gender and regional variations in the histology of the tunica media of the pulmonary veins suggest a mechanism for controlling hemodynamic forces at the atriopulmonary junction.

Acknowledgement

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