Research Article

Evaluation of Structural Adaptation of the Brachial Artery to Buckling Load In Relation To Elbow

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Background: Medium-sized arteries of the limbs facing buckling changes at the areas of crossing major joints that affect the diameter and shape of the artery lumen with subsequent reduction in the blood supply to the targeted tissue. The intima and media of the human arteries have a hemodynamic self-regulatory mechanism to overcome this effect. This study was conducted to detect any structural changes in the walls of the brachial artery as an example of medium-sized arteries in relation to the Alpha-SMA activity opposite the elbow creases compared to non-bucking exposed arteries.
Materials and methods: Three serial specimens (at elbow crease, 1 cm above, and 1 cm below) were taken from brachial arteries of the left upper limbs belonging to ten female cadavers. The vessel diameter at the three levels were measured by Digimatic Caliper then processed to histological staining protocol. The paraffin sections were stained with H&E, Masson's trichrome (MT), and anti-α-smooth muscle actin. The whole arterial wall thickness and the differential thickness of the intima and the media were obtained after considering the magnification scale. The intima-media thickness ratio (IMT) were calculated. The distribution of the bluish stained collagen fibers in Masson trichrome stain and the expression of the brownish stained fibers in the Alpha-SMA sections were measured by image analysis. One way Anova test was used to test relationships between selected variables.
Results: The diameters of the brachial artery at the three studied levels showed no significant variations. The morphometric analysis of the intima/media thicknesses ratio of the studied levels showed weak significance. The area percentage of the Alpha-SMA and the collagen fibers in Masson trichrome stain shows weak significant variations between the three studied levels.
Conclusion: Although, our study could not prove evident histopathological changes. The study proved the presence of morphometric variations in the arterial wall structure in relation to elbow joint crease.
Key words: brachial artery, medium sized arteries, arterial wall structure.

INTRODUCTION

The walls of arteries are complex and dynamic structures continually adapting to their local chemical and mechanical environments. Peripheral arteries in particular are under continuous mechanical loads from blood pressure, blood flow, tissue tethering, and body movement. To accommodate their functions, the extracellular matrix proteins (ECM) within the walls of arteries varied in chemical nature and distribution. The dissemination and the structural pattern of elastin fibers subserve the specific functional properties, including mechanic-sensing, control of external forces, mechanical properties of the vascular wall, cellular positioning, and communication between cells. Of further significance, these processes are adapted as regard physiologic factors as limb movements, aging, and pathological conditions as atherosclerosis, diabetes mellitus, and hypertension.

Medium-sized arteries of the limbs can suffer buckling changes at the areas of crossing major joints. Buckling affects the diameter and shape of the artery lumen with subsequent reduction in the blood supply to the targeted tissue. The continuously changing artery lumen shape alters blood flow and exposes the arterial wall to exhaustion and stress, which increase the possibility to develop arterial thrombosis. As a defense mechanism, the intima and media of the human arteries have a hemodynamic self-regulatory mechanism to overcome this effect. However, this mechanism is weakened or decompensated by aging and some pathological condition as atherosclerosis, hypertension or diabetes.

The intima of the medium-sized arteries is a biologically functional layer that serves as an interface between the thrombogenic media and the blood. In the healthy arteries, the intima constitutes of a single layer of endothelial cells resting on a thin basal membrane. There is a considerable degree of interaction between its viable components and its ECM as a part of the vessel wall adaptation to mechanical forces. The net result is a change in the distribution and adhesion of the smooth muscle cells and the ECM components. Despite the importance of the ECM structure of the arteries, relatively little attention has been paid to variations in its structure.

The media being the middle layer of the arteries is structured to resist the high loads in the circumferential direction. It consists of a complex network of smooth muscle cells, elastin, and bundles of collagen fibrils. However, the pattern of elastin fibers loses the organization toward the periphery, and the smooth muscle is consistently circumferentially and coherently aligned. The particular structural orientation of the elastin, collagen, and smooth muscles in either intima or adventitia was reported to be close to the circumferential organization in the media.

Alpha-smooth muscle actin (Alpha-SMA) is an important smooth muscle marker of activity present in the myofibroblasts of the arterial wall. Alpha-SMA is an isoform of the vascular smooth muscle actins, typically contributing to vascular motility and contraction. Its activity plays a key role in the development of the fibrotic response. In an activated state, myofibroblasts cease to proliferate and start to synthesize large amounts of extracellular component proteins. The expression of Alpha-SMA correlates with the activation of myofibroblasts. The activity of Alpha-SMA is proofed to be affected by physiological tissue remodeling and by fibrotic diseases.

The standard evaluation of transverse sections of the vessels is crucial in the diagnosis of diseases. The increased prevalence of peripheral arterial diseases necessitates the need to study the detailed structural variations in the peripheral arteries. Studying the cross-sectional morphological changes of the carotid vessels is abundant in literature because of its relation to stroke development, however, peripheral arteries studies on the other hand are scarce.

This study aims to emphasize the main structural changes (if any) in the walls of the arteries of the upper limb as an example of medium-sized

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arteries in relation to the Alpha-SMA activity opposite the elbow creases compared to non-bucking exposed arteries.

MATERIAL AND METHODS

Ten females' human cadavers were applied to the study. The cadavers were obtained from the morgue of the Anatomy Department, Faculty of Medicine, Taibah University, Al Madinah Almonawarah Saudi Arabia. The research was carried out following the standard anatomical dissection techniques. Before each procedure, a thorough visual external inspection was performed to exclude specimens with deformations or traces of trauma or surgical procedures. Furthermore, limbs demonstrating visible anatomical variations of the arterial pattern not associated with the brachial artery or any of its branches were excluded from further analysis.

The cadavers are preserved in the standard preservative (formalin 10%) and kept in the cadaver fridge for a known duration (1±4 years). The cadavers were placed in a supine position with their upper limbs abducted to 90° to straighten their arteries and extend their elbow and wrist joints. The elbow creases were identified and marked with pertinent ink pens. The cadavers left upper limbs were dissected to expose the brachial arteries for 5 centimeters above and below the elbow crease and bifurcations of the brachial artery into radial and ulnar branches are identified.

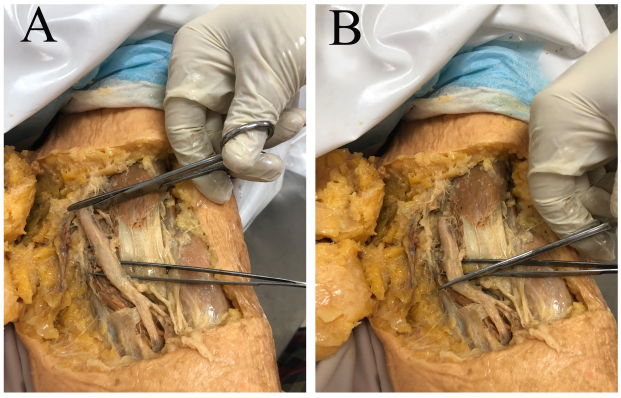


Figure 1: Dissection of the brachial artery

The mark of the elbow crease was labeled on the brachial artery and a 2-centimeters segment of the brachial artery was cut carefully perpendicular to its longitudinal access with the elbow crease mark in its middle. The artery segment was sectioned perpendicular to its longitudinal access midway between its beginning and termination and at 1-cm intervals and preserved in formalin solution (formalin 10%) before further procedures. The obtained 3 serial sections were processed to histological staining protocol. The paraffin sections were cut at 4 µm thickness preserved in paraffin sections and processed for routine staining with H&E, (Sigma Aldrich, Product Number: HHS16 and E4009), Masson's trichrome (MT) (Sigma Aldrich, Product Number: HT15) (Sigma Aldrich, Product Number: 365548) for detection of tissue fibrosis and anti-α-smooth muscle actin (Anti-Actin, smooth muscle Antibody, clone ASM-1/1A4, Product Number: A2547, Sigma Chemical Co., St. Louis, MO, USA) immune stain for detection of myofibroblast activity.

A Digimatic Caliper (Mitutoyo Corporation, Kawasaki, Kanagawa, Japan) was used to measure the vessel diameter at the 3 levels. The stained sections were examined and photographed under an Olympus BX-50 microscope (Olympus, Tokyo, Japan) supplied with Olympus DP-71 digital CCD camera controlled by Olympus AnalySIS FIVE software. The whole arterial wall thickness and the differential thickness of the intima and the media were obtained for a total of 10 sections per cadaver (total number of 40 sections) after considering the magnification scale. The thickness of the intima and the media perpendicular to the lumen at 4 locations in the H&E sections. Each measurement was taken twice, and the mean of both measurements was accepted as the result.

The intimal thickening and medial thickness were measured using the image pro-plus software and the values were expressed in micrometers.

The intima-media thickness ratio (IMT) were calculated according to and tabulated. The distribution of the bluish stained collagen fibers in Masson trichrome stain and the expression of the brownish stained fibers in the Alpha-SMA sections above and below the creases were measured by image analysis.

The area percent of colored fibers was estimated by optical density in similar levels using Leica Q Win standard (digital camera CH-9435 DFC 290, Germany). For all measures, 10 non-overlapping fields in each paraffin block for each cadaver were examined at X400 magnification and photographed.

The lamp intensity, camera exposure, and camera gain were kept constant throughout the examination. The photographs were analyzed for positive staining using an Image Analyzer with a measuring frame area = 786,432.0 µm2. Morphometry was carried out at the Image Analysis Unit, Anatomy Department, Faculty of Medicine, Taibah University.

One way Anova test was used to test relationships between selected variables. The significance level adopted in the analysis was p =0.05. The calculations were made with Statistics A software, version 10.0 PL.

RESULTS

Hematoxylin and eosin staining of the arterial wall sections (Fig.2) shows the characteristic 3 wall components; intima, media, and adventitia.

The intima is the inner layer and is very thin and formed primarily of a single layer of flattened endothelial cells aligned along the long axis of the artery and resting on a thin basal membrane. The basal membrane is formed of collagen and elastin fibers.

The media is the thickest middle layer-containing network of bundles of collagen fibrils, elastin, and smooth muscle cells organized in circumferential lamellar units.

The adventitia is the outermost layer. It is a typical fibrous perivascular tissue consist of fibroblasts and fibrocytes immersed in ground-matrix and collagen fibers organized in thick unorganized dispersed bundles.

We could not detect any obvious histological variations between the 3 studied levels.

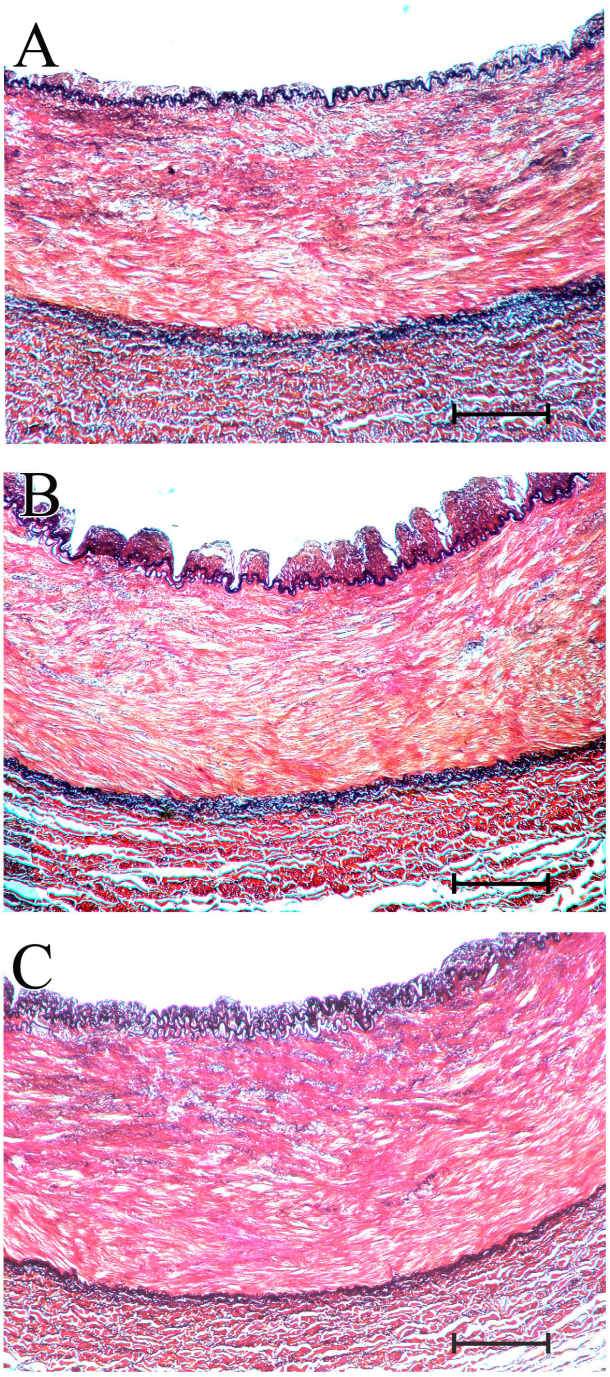


Figure 2: Hematoxylin and eosin section of the brachial artery segment showing the 3 layers of the arterial wall. The intima is thrown into folds with the endothelium resting on the internal elastic lamina. The media is thick and compared to concentrically arranged layers of smooth muscles with interposed elastic

Specific staining of the collagen bundles by Masson trichrome staining cleared the characteristic concentric alignment of the fibers in the intima, media, and adventitia of the arterial wall in the studied 3 levels also without detectable variations (Fig.3).

Alpha-SMA staining revealed the brown color staining of the smooth muscle fibers in the intima and media and absence in the adventitia of the three levels (Fig.4).

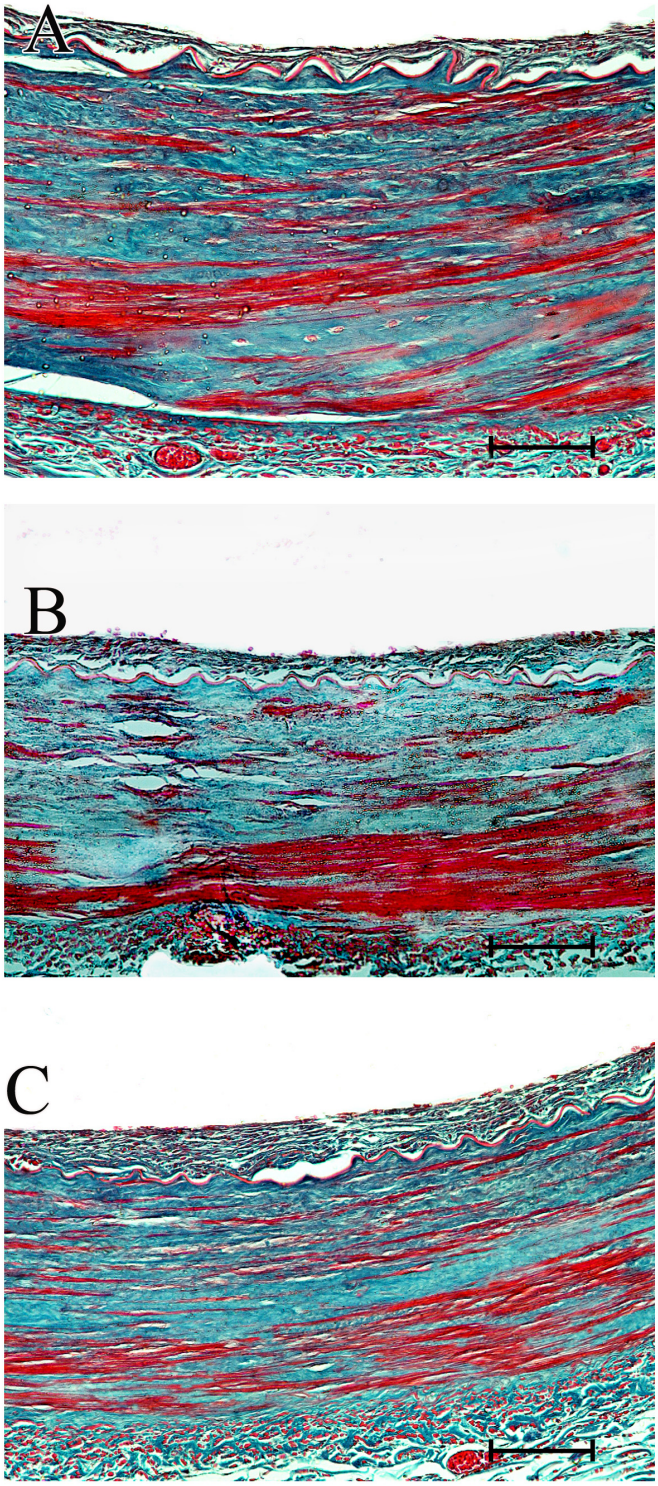


Figure 3: Masson trichrome stain of the 3 sections of the brachial artery showing the characteristic concentric alignment of the bluish stained collagen fibers in the intima, media, and adventitia of the arterial wall.

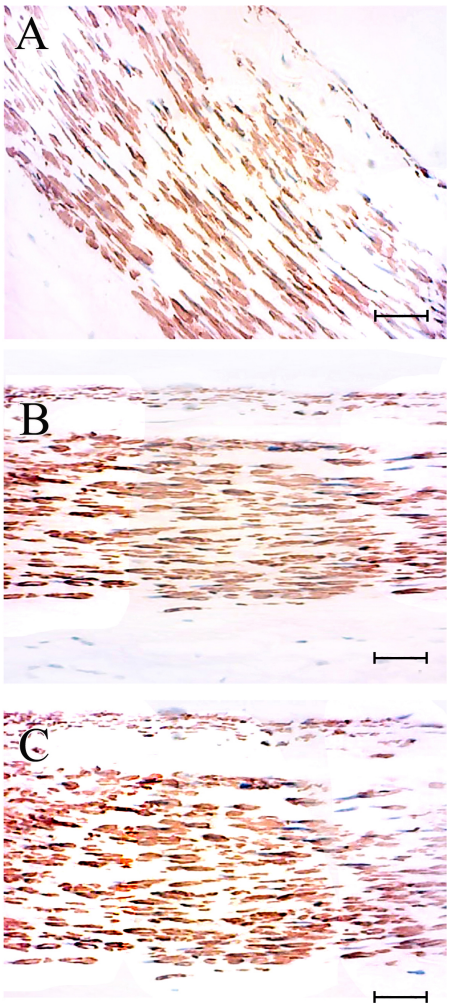
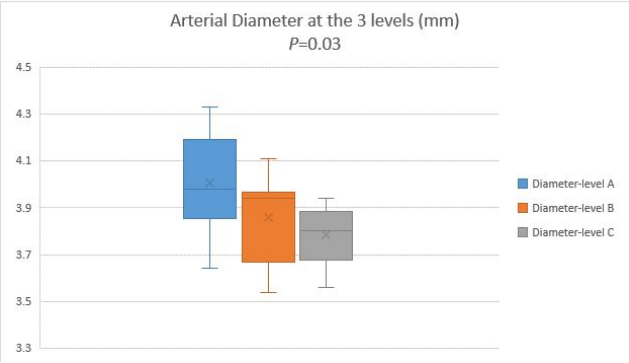


Figure 4: Alpha-SMA staining of the sections of the brachial artery. The brownish stained smooth muscle fibers appear as concentric layers in the intima. The statin is more clearly identified in the thick media and absents from the adventitia.

The comparison diameters of the brachial artery at the three studied levels showed no significant variations (graph 1 & Tab.1).

Table 1: The brachial artery diameter in the three studied levels in mm

Groups	Count	Average	P-value
Diameter-level A	10	4.007	0.030012
Diameter-level B	10	3.86	
Diameter-level C	10	3.785	



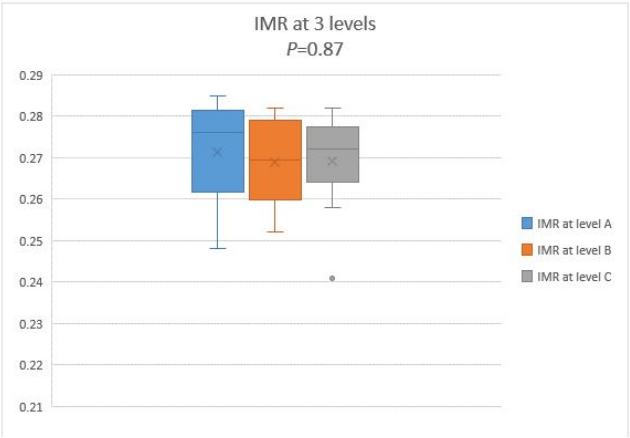
Graph 1: The brachial artery diameter in three studied levels in mm. Data are shown as the median, interquartile range (box), and mean (whiskers); P-value for the trend is shown in the graph.

The morphometric analysis of the intima/media thicknesses ratio of the studied levels showed weak significance (graph 2 & Tab.1). The morphometric measurement of the area percentage of the Alpha-SMA and the collagen fibers in Masson trichrome stain shows weak significant variations between the three studied levels (Tab. 1 & Graph 3, 4).

Table 2: intima/ media ratio in the three studied levels of the brachial artery.

Groups	Count	Average	P-value
IMR at level A	10	0.2714	0.876975
IMR at level B	10	0.2689	
IMR at level C	10	0.2691	

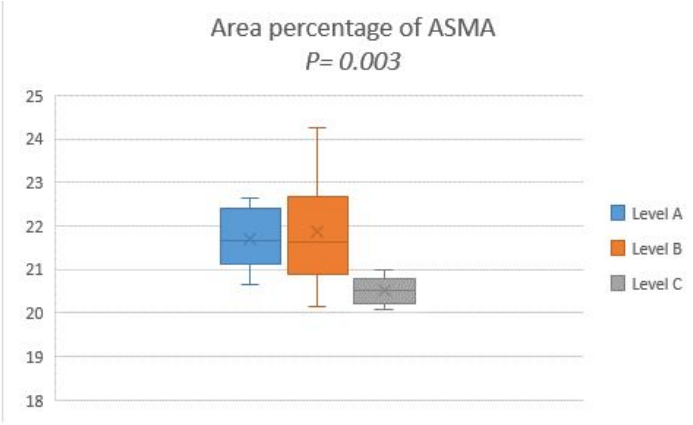
Histological findings of the routine histology revealed the structure of the brachial artery with characteristic intimal structure thrown into folds of endothelium resting on the internal elastic lamina. The media is thick and compared to concentrically arranged layers of smooth muscles with interposed elastic fibers. The adventitia contains collagen fibers with dispersed elastic fibers in-between (Fig.2). Masson trichrome stain of the 3 sections of the brachial artery showing the characteristic concentric uniform alignment of the bluish stained collagen fibers in the intima, media, and adventitia of the arterial wall (Fig. 3). Alpha-SMA staining of the sections of the brachial artery. The brownish stained smooth muscle fibers appear as concentric layers in the intima. The statin is more clearly identified in the thick media and absents from the adventitia.



Graph 2: intima/ media ratio in the three studied levels of the brachial artery. Data are shown as the median, interquartile range (box), and mean (whiskers); P-value for the trend is shown in the graph

Table 3: Area percentage of Alfa Smooth Muscle Actin in the three studied levels.

Groups	Count	Average	P-value
level A	10	21.71	0.003782
level B	10	21.873	
level C	10	20.526	



Graph 3: Area percentage of Alsfa Smooth Muscle Actin in the three studied levels

DISCUSSION

The local flow at the site of arterial bending is expected to change the hemodynamic mechanism controlling the arterial blood flow. The continuously changing mechanism under both neutral and dynamic conditions is presumed to be accompanied by structural changes. Arteries must remain patent and stable under changing loads. It has been well documented that slight changes in the mechanical stresses are important under physiological conditions as vascular cell biology and vascular function, and under pathological conditions such as atherosclerosis. Changes in structural compositions as elastin deficiency due to a genetic defect, flow increase, or other vascular diseases can lead to weaker walls and/or tortuous arteries.

Although our study revealed a non-significant change in the diameter of the brachial artery above and below the elbow crease, this finding is predicted, as the structural changes can be limited to the ultrastructural level. Even the routine histopathological examination of arterial sections by Hxe and eosin, Masson Trichrome, and Alpha-SMA could not prove obvious structural changes in the studied sections, however, the morphometric analysis revealed considerable variations as regards the area percentage of the Alpha-SMA and the Masson trichrome stained fibers.

The intima/media thickness parameter is a validated indicator of the arterial wall structure under both physiologic and pathological conditions or as a predictor of early structural changes. Although this ratio is often estimated by non-invasive techniques as ultrasound studies or by various radiological facilities, our study used direct histopathological measurements, which are presumed to be more accurate. The resulted non-significant change in the ratio can be referred to either non-change in the thickness of both intima and media at the studied 3 levels or concomitant change in both thicknesses.

Our study measured the Alpha-SMA activity as a marker of vessel wall exposure to stress. Alpha-SMA encoded by the ACTA2 gene is an isoform of the vascular smooth muscle actins, typically expressed in the vascular smooth muscle cells contributing to vascular motility and contraction. The potential roles of SMA are reported to play a role in the development of vasculopathies as it resents in the microfilament bundles of vascular smooth muscles and exerts contractile functions. It has been linked to arterial tones and activation of myofibroblasts. The forceful contractile property of myofibroblasts is attributable to the stress fibers containing Alpha-SMA. It is postulated that pathological changes in the arterial wall components and extracellular matrix which are determinants of contractility, dispensability, and elasticity are Alpha-SMA dependents. In experimental homozygous Alpha-SMA knock-out mice, deficits of vascular contractility and reduced basal blood pressure were noted, implicating the important role of Alpha-SMA in maintaining vascular tone and prevention of arterial wall stiffness. However, the intimal myofibroblasts undergo homogeneous concentric diffuse hyperplasia under arterial stress conditions with increased collagen fibers in the ECM with interposed smooth muscle fibers.

The orientation, proportion, and close interconnection between the elastic and collagen fibrils, elastic laminae, and smooth muscle cells within the media is fundamental to maintain proper arterial action. This structured arrangement gives the media an ability to resist high loads in the circumferential direction difference between central and peripheral arteries as the elastin pattern loses its organization toward the periphery so that the laminated architecture of the media is hardly present in muscular arteries.

Our study revealed a non-significant increase in the collagen fibers area between the 3 studied levels. Collagen is the main load-carrying element in arterial walls. It is relatively inextensible and serves as a (stiff) reinforcing structural element. The most common collagen in blood vessels is Type I. The function and integrity of arteries are maintained by the tension in collagen fibers. The structural arrangement of collagen causes the arterial wall and its layers to be anisotropic.

The importance of studying the detailed structural variations in the arterial walls increases with the demand for the progressive increase in organ transplantation. The availability of recent technological studies adds to the importance of these studies.

CONCLUSION

This is the first study to investigate the presence of possible structural variations in the arterial wall in relation to joint creases. Although, our study could not prove evident histopathological changes. The study proved the presence of morphometric variations in the arterial wall structure in relation to elbow joint crease.

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