AGE RELATED CHANGES IN HISTOMORPHOLOGY OF MEDIUM SIZED MUSCULAR ARTERY

Muhammad Marghoob Khan, Shadab Ahmed Butt, Muhammad Rizwan Bashir Kiani

Army Medical College, National University of Sciences and Technology (NUST) Islamabad

ABSTRACT

Objective: The aim of this study was to contribute to the knowledge of histomorphometric changes which are associated with increasing age in local population, with the experience obtained in the dissection on cadavers.

Study Design: Cross-sectional comparative study

Place and Duration of Study: The study was carried out at the department of Anatomy, Army Medical College Rawalpindi in collaboration with Forensic departments of various medical institutes where cadavers were brought for autopsy, spanning from 15 Feb 2010 to 15 Aug 2010.

Material and Methods: A total of forty cadavers from local population (Punjab and Khyber Pakhtunkhwa) were dissected and specimen (Common hepatic artery) (CHA) were obtained. Two age groups, one below the age of forty years (1 to 39 years) and the other above the age of forty years (40 to 70 years) were made. The specimen were processed and stained with Hematoxylin and Eosin. Using a microscope with 10 X objective, micrometry was done and data of intima thickness (IT), media thickness (MT) and intima media thickness (IMT) was noted.

Results: After comparing the two age groups, statistically significant difference was found between the IT (p value <0.01). The mean values of media failed to attain any statistical difference. No statistically significant difference was found in the IMT of the two age groups.

Conclusion: Increase in itima thickness was found while MT and IMT did not show any statistical difference.

Keywords: Adventitia, Hematoxylin and Eosin, Intima, Media.

INTRODUCTION

The decline of various physiological body processes is age related. Chronological age and biological ages are not necessarily the same[1]. Vascular ageing and hence the estimates of arterial function have been found to be related closely to the chronological age as Thomas Sydenham cited by Vlachopoulos said, "A man is as old as his arteries"[2].

The arterial system consists of the larger elastic arteries and the medium size muscular arteries. Histologically, the arterial wall consists of three concentric layers-the tunica adventitia, tunica media, and tunica intima. There is a single layer of endothelial cells between the blood and the vessel wall. The relative ratio of these three varies not only with the size of the artery but also with the age of the subject. In addition large arteries are rich in elastin and collagen while on the other hand muscular arteries are rich in vascular smooth muscle[3]. The criteria for normal thickness is expressed quantitatively as the intima: media ratio. The ratio may vary from about 0.1 to 1.0 or more in normal arteries of humans[4]. Histologically muscular and elastic arteries have been studied extensively by various researchers using different arterial models like carotid, vertebral, aorta and coronary arteries especially emphasizing on IMT[5]. IMT, endothelial function, and arterial stiffness are certain measures of arterial function and are not only associated but also prognostic of cardiovascular events in adults. Due to this, interest has been focused on various interventional, pharmacologic, and mechanical forms of therapy for the treatment of atherosclerotic and aneurismal lesions affecting various small and large arteries. Techniques and devices such as dilating balloons, perfusion catheters, lasers, atherectomy devices, stents, intravascular ultrasound, have been used or are under study for future use. Many of these techniques and devices require an understanding of histologic and pathologic features of the arteries and diseases which affect them. In normal human

Correspondence: Dr Muhammad Marghoob Khan, Army Medical College, Rawalpindi
Email: marghoobkhan786@yahoo.co.uk
Received: 07 Oct 2013; Accepted: 06 Jan 2014

arteries, the amount of smooth muscle and fibroelastic tissue in the intima are a function of age[6,7]. With the passage of time, in healthy youth there is slight increase in IMT and arterial stiffness increases, but there is no change in endothelial function from 5 to 20 years of age. On the other hand, in youth with cardiovascular risk factors there are larger increases in IMT and arterial stiffness, and reductions in endothelial function compared with healthy youth[8]. Histologic changes occur in intima, media and adventitia, which begin after birth and continue afterwards. Nevertheless these changes are influenced by factors such as hypertension, diabetes melliatus, diet etc[9]. The effects of ageing on the arterial tree are heterogeneous and the mechanical properties of the blood vessels vary depending on the artery being studied[3]. Although studies are performed on various other arteries, little or no data is available regarding use of common hepatic artery (CHA) so we used CHA as medium sized muscular artery in our research to see effects of aging.

MATERIAL AND METHODS

This cross-sectional comparative study was carried out at the department of Anatomy, Army Medical College Rawalpindi in collaboration with Forensic departments of King Edward Medical College Lahore, Allama Iqbal Medical College Lahore and Khyber Medical College Peshawar, where cadavers brought for autopsy were used. The study was carried out from 15 Feb 2010 to 15 Aug 2010. Inclusion criteria was any cadaver of local population, of any age preferably of eighteen to sixty years of age and of any sex. Any cadaver which showed any abnormality such as signs of trauma, surgical scars on abdomen and chirrosis was excluded. Cadavers were divided in two age groups, group A included cadavers of age below forty years (less than 39 years) and group B included cadavers above the age of forty years (40 years and above). Forty cadavers were included in the study, 20 in each group through non probability convenient sampling The dissection, data and specimen collection from autopsy cases were performed in compliance with the recommendations of the Ethical Committee of Center for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi. Consent was taken from relatives of the deceased.

The cadavers used in study were kept in cold storage room at +2C to +4C until the time of autopsy / dissection. The time lapsed between death and autopsy was between four and fifteen hours. Data was noted on Proforma. IT was defined as the distance from the lumen-vessel interface to the interface between the internal elastic lamina and the media, IMT as the distance from the lumen-vessel interface to the interface between the media and the external elastic lamina, and MT was calculated as the difference between IMT and IT. Three measurements were obtained, averaged and mean of these three measurements was used in subsequent analyses. Specimen taken from the CHA for histological examination were observed under the microscope after fixation, embedding, sectioning and staining with hematoxylin and eosin (H & E). Changes in IT, MT and IMT in relation to age were noted by Micrometry. Intima and media thickness were measured in micrometers (μm) under 10 X objective using ocular micrometer. Photography for histologic specimen was carried out in the department of Pathology by Olympus professional digital camera (model DP-21), 2MP with 4X optical and electronic zoom.

Data had been analyzed using SPSS version 16. Descriptive statistics were used to describe the data. Mean and Standard Devaiation (SD) were calculated for quantitative variables. Independent samples t-test was used to compare quantitative variables between both the groups. A *P*- value <0.05 was considered as significant.

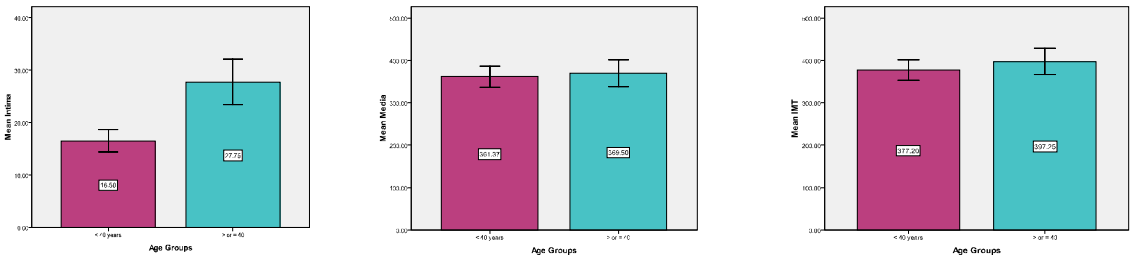
RESULTS

Twenty cadavers were included in each group. There were 17 (85%) males in group A while in group B 18 (90 %) were males. The Average IT was 16.5 ± 4.7 um, (range : 8.3 um – 25 um) in group A and it was 27.7 ± 8.6 um (range : 11.6um - 55um) in group B (*P* value <.001), (Fig 1, a). Average MT in group A was 361.3 ± 54.4 um, (Range : 285 um - 493.3 um) (figure 2, c) and it was 369.5 ± 71 um (Range :

240 um – 488 um) in group B. It failed to attain any statistical difference (*P* value = 0.687), (Fig 1, b). Average IMT in group A was 377.2 ± 55 um, (range : 305 um – 516 um) and it was 397.2 ± 70.2 um, (range : 266.6 um - 513.3 um) in group B. No statistically significant difference was found in the IMT of the two age groups (*P* value = 0.322), (Fig 1, c). Linear increase in thickness of IT, MT and IMT was not found in any of the groups.

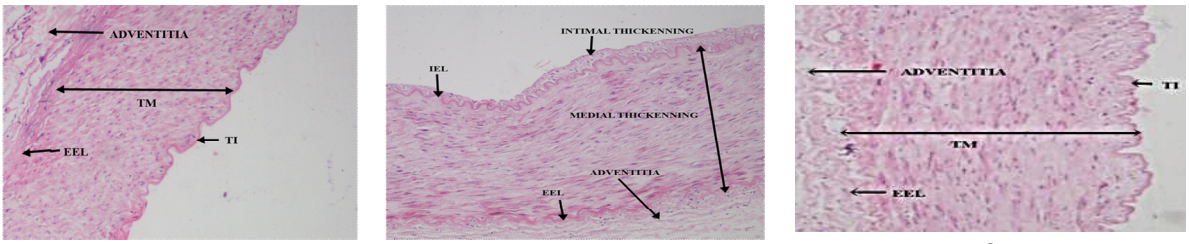
DISCUSSION

Significant increase in IT was observed in older age group (>=40 years) as compared to younger age group (<40 years) (Fig 2, b), which are in accordance with other studies but MT and IMT failed to attain any significance difference statistically[10,11]. However the mean values of older age group showed an increase in MT and IMT as well as marked increase in IT. In our study the maximum value of mean IT was 25 um, MT was 493.3 um (Fig 2, c) and IMT was 516.6um in cadavers of age group, one to thirty nine years. Similarly in the age group forty years and above, the maximum mean value of IT was 55 um, MT was 460 um and IMT was 513.3um. In both the groups a linear increase was noted in the mean values of IT, MT and IMT with few exceptions. In a study carried out by Naoki et al, the histological thickness of the intima was 120 ± 45 µm in 56 specimens, and that of the media was 258 ± 71 µm in 57 specimens which was considerable less than our study. The difference existed as the author carried a comparison between histologic studies and ultrasound findings and second the ages of the cadavers nor the staining methods were described in their study. In addition there existed differences between results obtained through histologic specimen and results obtained through research conducted by ultrasound. IMTs determined by light microscopy were consistently smaller (shrinkage rate of diameter was 88%) than those determined by ultrasonography[12]. The IT, MT and IMT of various elastic and muscular arteries especially carotid, cerebral and coronary arteries had been extensively studied using conventional high-resolution vascular ultrasound imaging (HRU) and has been used as a surrogate marker for the progression of atherosclerotic disease[13,14]. Nevertheless, the spatial resolution of HRU systems appears insufficient for more detailed imaging of different arterial wall layers and for the



a b c

Figure-1: Comparison of intima thickness a, media thickness b, and intima media thickness c of muscular arteries in the two age groups.



a b c

Figure-2: C (10x, H & E).

examination of the peripheral vasculature[15]. In a study it was observed that there is intimal and medial thickening and increased vascular stiffness. In addition he also showed that as aging occurs, smooth muscle cells (SMCs) progressively migrate from the tunica media and accumulate into the tunica intima[9]. Smooth muscle cells in intima were also observed in our study.

Fiscer and Fieman, (1990) showed in their studies that carotid arteries are frequently a seat of atherosclerotic lesions but there is asymmetry between the left and right carotids involvement, despite both being exposed to identical "systemic" risk factors[16]. Local factors, mainly the geometry have been hypothesized to play a role[17]. Bilato and Crow (1996) in their studies found that the diameter of the vessels tends to increase, and thickening of intimal and medial layers is often observed. These changes in the normal architecture of the vessel wall, could be referred as "vasculopathy of aging", are likely to be the consequence of adaptive mechanisms to maintain normal conditions of flow, resist mechanical stress and/or wall tension. Many of these features are similar to the histological findings of the vessels affected by atherosclerotic process. In old age studies conducted on coronary arteries showed that coronary arteries become tortuous, the luminal diameter increases, the media thins, and calcific deposits increase[18]. In addition, calcium deposits in coronary arteries in the elderly do not correlate with severity of atherosclerotic disease[7]. Medium sized muscular arteries such as carotid arteries showed that carotid intimal thickness (CIMT) increases threefold between ages 20–90 years. Excess IMT at a given age predicts silent coronary artery and other arterial diseases and that these will be accelerated in presence of known cardiovascular risk factors. It has been shown that pharmacological interventions with statins and angiotension converting enzyme inhibitors and life style changes can slow the progression of intima medial thickness[3]. In our study we did not take into account the risk factors affecting the IT, MT and IMT, which different studies have shown, to be associated with increased in IMT and smoking[18], hypercholesterolemia and hypertension[19]. In addition Persson et al., 1994 concluded that IMT was significantly thicker in groups with higher estimated multifactorial cardiovascular risk, indicating that IMT reflects a vascular process which is related to known cardiovascular risk factors[20].

CONCLUSION

The results of our study was similar to other studies performed on intima, media and intima media thickness in relation to age on different arteries. However no data was available regarding the age related changes on hepatic artery.

REFERANCES

1. Bulpitt, C.J., Shipley, M.J., Broughton, P.M.G. The assessment of biological age. A report from the department of environment study. Ageing Clin Exp Res. 1994; 6181-191.
2. Vlachopoulos, C., Alexopoulos, N., Stefanadis, C. Aortic Stiffness: Prime time for integration into clinical practice? Hellenic J Cardiol. 2010; 51: 385-390.
3. Jani, B., Rajkumar, C. Ageing and vascular ageing, Postgrad Med J. 2006; 82(968): 357-362.
4. Stary, H.C. Macrophages, macrophage foam cells, and eccentric intimal thickening in the coronary arteries of young children. Atherosclerosis. 1987; 64: 91-108.
5. O', D.H., Polak, J.F., Kronmal, R.A., Manolio, T.A., Burke, G.L., Wolfson, S.K. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med. 1999; 340: 14-22.
6. Vlodaver, Z., Edwards, J.E: Pathology of coronary atherosclerosis. Prog Cardiovasc Dis. 1971; 14: 256-274.
7. Waller, B.F., Morgan, R. The very elderly heart. Cardiovasc Clin. 1987; 18: 361-410.
8. Tell, G.S., Howard, G., McKinney, W.M. Risk factors for site specific extracranial carotid artery plaque distribution as measured by B-mode ultrasound. J Clin Epidemiol. 1989 ; 42: 551-559.
9. Fernhall, B., Agiovlasitis, S. Arterial function in youth: window into cardiovascular risk. J Appl Physiol. 2008; 105: 325-333.
10. Neufeld, H.N., Schneeweiss, A. Coronary Artery Disease in Infants and Children. Lea & Febiger. 1983; 1-28.
11. Yildiz, O. Vascular smooth muscle and endothelial functions in aging. Ann N Y Acad Sci. 2007; 1100: 353-60.
12. Vaudo, G., Schillaci, G., Evangelista, F., Pasqualini, L., Verdecchia, P., Mannarino, E. Arterial wall thickening at different sites and its association with left ventricular hypertrophy in newly diagnosed essential hypertension. Am J Hypertens. 2000; 13: 324-331.
13. Fisher, M., Fieman, S. Geometric factors of the bifurcation in carotid atherogenesis. Stroke. 1990; 21: 267-271.
14. Caplan, L.R., Baker, R. Extracranial occlusive vascular disease. Stroke. 1980; 11: 63-66.
15. Hirokawa, N.N., Satoh, K.K., Hori, M., Saitoh, M., Nishida, M.,·Hata, F., Nishi, M., Hareyama, M. Wall composition analysis of the human hepatic artery by intravascular ultrasound. Jour Med Ultrasonics. 2008; 35: 107–111
16. Baldassarre, D., Amato, M., Bondioli, A., Sirtori, C.R., Tremoli, E. (2000). Carotid artery intima-media thickness measured by ultrasonography in normal clinical practice correlates well with atherosclerosis risk factors. Stroke. 2000; 31: 2426-30.
17. De Groot, E., Hovingh, G.K., Wiegman, A. Measurement of arterial wall thickness as a surrogate marker. Circulation. 2004; 109(23): 33-8.
18. Urbina, E.M., Williams, R.V., Alpert, B.S., et al. Noninvasive assessment of subclinical atherosclerosis in children and adolescents: recommendations for standard assessment for clinical research: a scientific statement from the American Heart Association. Hypertension. 2009; 54: 919-50.

19. Bilato, C., Crow, M.T. Atherosclerosis and the vascular biology of aging. Aging (Milano). 1996; 8(4): 221-34.
20. Tell, G.S., Howard, G., McKinney, W.M. (1989). Risk factors for site specific extracranial carotid artery plaque distribution as measured by B-mode ultrasound. J Clin Epidemiol. 1989; 42: 551-559.
21. Salonen, R., Salonen, JT. Determinants of carotid intima-media thickness: a population-based ultrasonography study in eastern Finnish men. J Int Med. Atherosclerosis. 1991; 229: 225-231.
22. Persson, J., Formgren, J., Israelsson, B., Berglund, G.. Ultrasound-determined intima-media thickness and atherosclerosis. Direct and indirect validation Arterioscler Thromb Vasc Biol. 1994; 14: 261-264.